

**DEVELOPMENT OF THE CHEMICAL CONTROL OF  
BREATHING IN THE NEWBORN**

A thesis submitted for the degree of  
**Doctor of Philosophy**

by

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To my parents and my brother for all their love and support.

And to club kids everywhere.

The memory of sunrise at MANUMISSION and outside at SPACE has  
been frequently recalled whilst writing this thesis.

### *Abstract*

The neonate must establish control of its vital functions after birth to meet the metabolic demands of an extra-uterine environment. Respiratory control is of particular importance, and both the peripheral and central components of it have been shown to mature postnatally over several weeks. It is generally accepted that the increase in hypoxia sensitivity of the peripheral chemoreceptors occurs over the first 2 postnatal weeks, whilst the maturation of central mechanisms may take longer. The newborn is vulnerable to hypoxic and asphyxic periods until these control mechanisms are fully mature.

The work in this thesis has concentrated on the development of peripheral respiratory control in the newborn human and lamb. Non-invasive testing of respiratory chemoreflexes in the newborn infant was performed by alternating breaths of air and a hypoxic gas mixture and measuring the respiratory response. There was no significant maturation of the response between *ca.* 2 days and 2 months of age. These results suggest that the resetting of hypoxia chemosensitivity occurs very soon after birth.

The respiratory response to a chemical stimulus may be influenced by other control mechanisms. I investigated the interaction between chemo- and mechano- respiratory chemoreflexes in healthy term infants by the additional use of the end-inspiratory occlusion test. The respiratory response to a chemical stimulus did not show a reflex prolongation in  $t_E$  for an increase in  $V_{Ti}$ , indicating that the respiratory chemoreflex was not influenced by Hering-Breuer reflex control of breathing. I did however find evidence for a negative correlation between chemo- and mechanoreflexes. This suggests that some infants may maintain a strong mechanoreflex until chemoreflexes are fully developed.

Finally, to obtain information on the postnatal increase in  $CO_2$  chemosensitivity, direct nerve recordings were made in newborn lambs. There was a postnatal increase in steady state  $CO_2$  sensitivity, which was greater at a lower  $PaO_2$ . In older lambs this increased  $CO_2$  sensitivity was due to a significant stimulus interaction between  $O_2$  and  $CO_2$  that was not present in younger lambs. A new method was developed for measuring dynamic  $CO_2$  sensitivity of the arterial chemoreceptors, so that their response could be related to the chemoreflexes in human neonates. Dynamic  $CO_2$  chemoreceptor responses were found in lambs of all ages, and this response was independent of age and  $PaO_2$ . This provides new information on a mechanism which may be vital for the newborn to control ventilation in relation to metabolism. It has implications for understanding the causes of respiratory failure in the newborn and for SIDS.

***Personal Statement***

Except as acknowledged in Chapter 3, the work presented in this thesis was performed solely by the candidate and is original.



.....  
Nicole A Calder



.....  
Certified by supervisor Professor Mark Hanson

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***Abbreviations***

ABP	Arterial blood pressure
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
BPD	Bronchopulmonary dysplasia
BVR	Biphasic ventilatory response
CA	Carbonic anhydrase
Ca <sup>2+</sup>	Calcium ion
cAMP	Cyclic AMP
Cl <sup>-</sup>	Chloride ion
CLD	Chronic Lung Disease
CPG	Central pattern generator
CSF	Cerebrospinal fluid
CSN	Carotid sinus nerve
d	day
DNP	Dinitrophenol
DRG	Dorsal respiratory group
ECoG	Electrocorticogram
EEG	Electroencephalogram
EELV	End expiratory lung volume
EIPA	eythlisopropyamiloride
<i>f</i>	Respiratory frequency
FCCP	<i>p</i> -trifluoromethoxy-phenylhydrazone
FEV <sub>1</sub> /FVC	Volume of forced expiration in the first second of expiration as a function of forced vital capacity
Fico <sub>2</sub>	Fractional inspired carbon dioxide
Fio <sub>2</sub>	Fractional inspired oxygen
FRC	Functional residual capacity
H <sup>+</sup>	Hydrogen ion
H <sub>2</sub> CO <sub>3</sub>	Carbonic acid
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
HBIR	Hering-Breuer inflation reflex
HCO <sub>3</sub> <sup>-</sup>	Bicarbonate ion
hr	hour
Hz	Discharge frequency; 1 Hertz= 1 cycle per second
I.A.	Intraarterial
I.V.	Intravenous
K <sup>+</sup>	Potassium ion
Li <sup>+</sup>	Lithium ion

mRNA	Messenger ribonucleic acid
mV	Millivolts
Na <sup>+</sup>	Sodium ion
NH <sub>4</sub> Cl	Ammonium chloride
nm	nanometres
NREM	Non-rapid eye movement sleep
NTS	Nucleus tractus solitarius
°C	degrees Celsius
P <sub>A</sub> CO <sub>2</sub>	Partial pressure of carbon dioxide in alveolar gas
P <sub>a</sub> CO <sub>2</sub>	Partial pressure of carbon dioxide in arterial blood
P <sub>A</sub> O <sub>2</sub>	Partial pressure of oxygen in alveolar gas
P <sub>a</sub> O <sub>2</sub>	Partial pressure of oxygen in arterial blood
P <sub>CO<sub>2</sub></sub>	Partial pressure of carbon dioxide
P <sub>ET</sub> CO <sub>2</sub>	Partial pressure of end-tidal carbon dioxide
P <sub>ET</sub> O <sub>2</sub>	Partial pressure of end-tidal oxygen
pH	Log <sub>10</sub> hydrogen ion concentration in a solution
pH <sub>i</sub>	Intracellular pH
PNEU	Pneumotachometer; integrated airflow signal
P <sub>O<sub>2</sub></sub>	Partial pressure of oxygen
PSR	Pulmonary stretch receptor
REM	Rapid eye movement sleep
S.E.M.	Standard error of the mean
SD	Standard deviation
SIDS	Sudden Infant Death Syndrome
SLN	Superior laryngeal nerve
T <sub>b</sub>	Core body temperature
t <sub>E</sub>	Expiratory time
t <sub>I</sub>	Inspiratory time
t <sub>Tot</sub> = t <sub>I</sub> + t <sub>E</sub>	Breath duration
TTX	tetrodotoxin
V <sub>E</sub> = V <sub>ti</sub> . f	Ventilation
VLM	Ventrolateral medulla
VRG	Ventral respiratory group
V <sub>te</sub> /t <sub>E</sub>	Mean expiratory flow
V <sub>te</sub>	Expiratory tidal volume
V <sub>ti</sub> /t <sub>I</sub>	Mean inspiratory flow
V <sub>ti</sub>	Inspiratory tidal volume
wk	week
µm	micrometres

## 1.0 INTRODUCTION

### *Overview of thesis*

This thesis is concerned with the development of respiratory control in the newborn, with attention particularly focused on the peripheral chemoreceptors. My experiments have been performed in sleeping human infants, awake human adults and anaesthetized newborn lambs. I have looked at two components of the respiratory chemoreflex in these experiments. First, in the studies in babies and in adults I measured the respiratory response to an inspired hypoxic stimulus non-invasively. These experiments investigated maturation of the respiratory chemoreflex, but have not allowed any component of the chemoreflex response to be studied in isolation. Secondly, in the studies in newborn lambs I have measured the afferent response of the chemoreceptors to an alveolar / arterial stimulus at the level of the carotid body. The method used on newborn infants had a direct influence on the experimental design for assessing the carotid chemoreceptor response to CO<sub>2</sub> in newborn lambs, i.e. the inspired stimulus alternated on a regular (single breath) basis and was repeated over time. These observations have allowed me to make some conclusions on the response of the carotid body to CO<sub>2</sub>, and the changes in CO<sub>2</sub> sensitivity which occur with age. I feel that these conclusions are of relevance to understanding the causes of respiratory failure, including SIDS in the infant.

I have written this thesis so that, to a certain extent, the individual Chapters 'stand alone'. I felt that this was appropriate due to the different aspects of peripheral respiratory reflexes that I have investigated in this thesis, and the variation in my experimental methods. Consequently, the introduction and discussion sections for these Chapters are quite substantial, with less emphasis placed on the overall introduction (Chapter 1) and general discussion (Chapter 8).

In this section, Chapter 1, I introduce several of the areas that are central to this thesis and review the current state of knowledge. I have focused Chapter 1 on the chemoreceptors, with reference to the research performed in neonatal babies and animals aimed at understanding the development of respiratory control, and its implications in health and disease. In Chapter 2 I have assembled the various methods that are recurrent in my experiments, with additional method sections being included in Chapters 4 and 7. In Chapter 3 I have investigated the development of hypoxia chemosensitivity in newborn babies after the first week of life. In Chapter 4 I have considered another afferent reflex involved in control of breathing, namely the Hering-Breuer reflex and its

interaction with chemoreflexes in the newborn period. In Chapter 5 I have assessed the adult chemoreflex respiratory response to the method I have employed in newborn babies. In Chapters 6 and 7 I have measured the steady state and dynamic chemoreceptor responses to CO<sub>2</sub> in anaesthetized newborn lambs. In Chapter 8, the general discussion, I summarize the findings of my experiments and discuss their implications for future research.

## 1.1 Aspects of ventilatory control I: An overview

The placenta is the organ for gaseous exchange *in utero*, and the fetus receives oxygenated blood from the mother via the placenta. The fetus also returns de-oxygenated blood to the placenta for the mother to re-oxygenate. The newborn must manage its own ventilation in an extra-uterine environment by gaseous exchange across the lung to meet the demands of oxygen consumption and metabolism. Some of the strategies for respiratory control develop postnatally (this is dealt with in more detail in section 1.5), and many of the experiments in this thesis address the time course of the attainment of respiratory control in the newborn period.

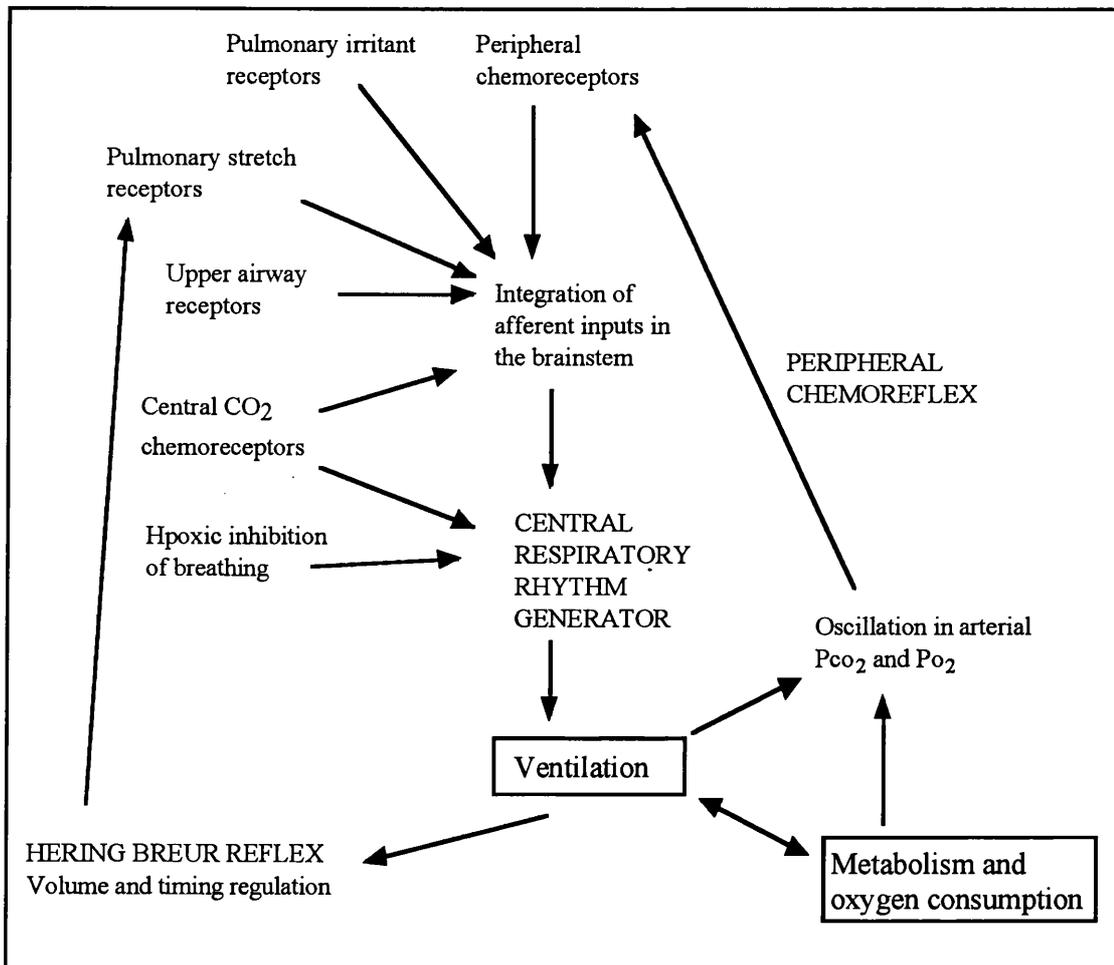


Figure 1.1.i Flow diagram of ventilatory control

The respiratory control system can be divided into several main areas:

- (a) The neural generation of a rhythmic respiratory pattern
- (b) Chemoreceptor control of breathing that responds to arterial blood gas status
- (c) Mechanoreceptor control of breathing that reflexly influences the relationship between volume and timing, and controls the patency of the upper airway.

In figure 1.1.i I have illustrated how these factors interact to manage ventilation and meet oxygen requirements. I will briefly review the generation of respiratory rhythm and mechanoreceptor control of breathing in this section. Chemoreceptor control of breathing is dealt with in the next section, as this forms the background to much of the work in this thesis.

### 1.1.1 The neural generation of a rhythmic respiratory pattern

Much of the current knowledge on the neural generation of a rhythmic respiratory pattern comes from studies on respiratory-related neurones in the cat and rat. Several areas of the brainstem contain neurones that possess an intrinsic respiratory-related firing pattern, and are referred to as respiratory neurones or respiratory-related neurones (for review see von Euler, 1986; Bianchi, Denavit-Saubie & Champagnant, 1995). Collectively these respiratory neurones form the *central pattern generator* (also referred to as *central rhythm generator*). The respiratory CPG shows a spontaneous periodic pattern of discharge and functions automatically throughout life.

There are three main types of respiratory neurones, related to the phase of respiration during which they are excited; inspiratory, post-inspiratory (termination of inspiration and passive expiration) and expiratory neurones (active expiration) (Richter, Ballantyne & Remmers, 1986; Richter, Ballanyi & Schwarzacher, 1992; Bianchi et al, 1995). Early-inspiratory and post-inspiratory neurones are most important in generating respiratory rhythm, that is determining the "on-switch" and "off-switch" of inspiratory and expiratory activity, and are characterized by a rapid onset and decline of excitability (Richter et al, 1992). They are modified by synaptic feedback from expiratory neurones to determine respiratory pattern (Richter et al, 1992). Respiratory neurones are located in two main sites in the brain stem, the dorsal (DRG) and ventral respiratory groups (VRG) of the medulla. The DRG corresponds to the ventrolateral region of the nucleus tractus solitarius (NTS). The VRG corresponds to a bilateral column of neurones located in the general region of the nucleus ambiguus, that extends between the bulbospinal and bulbopontine border (von Euler, 1986; Bianchi et al, 1995).

Some studies in neonatal rats have used isolated brain stem-spinal cord preparations *in vitro*, whereby peripheral afferents and suprapontine inputs are eliminated in order to locate sites of respiratory rhythm generation (Onimaru & Homma, 1987; Onimaru, Arata & Homma, 1987, 1988, 1989; Smith, Ellenberger, Ballanyi, Richter & Feldman, 1991). Pre-inspiratory neurones in the rostral ventrolateral medulla (RVLM) have been suggested as a primary generator of the respiratory rhythm (Onimaru & Homma, 1987; Onimaru et al, 1987; Onimaru et al, 1988). Electrical stimulation of the RVLM induces firing of these pre-inspiratory neurones and resets respiratory rhythm (Onimaru et al, 1988). These pre-inspiratory neurones demonstrate "pace-maker" properties which continue to discharge rhythmically after bathing in low calcium solutions, indicating that the depolarization necessary to reach threshold was most likely to be due to release of intracellular  $Ca^{2+}$  stores. Furthermore, discharge frequency increased when the pH was lowered, indicating the chemoreceptive properties of the cells (Onimaru et al, 1989). One particular region of the VRG that has been identified as important for the generation of respiratory rhythm is the "pre-Botzinger complex" (Smith et al, 1991). In these experiments, brain stem slices from the neonatal rat were studied *in vitro* for the location of neurones that generated respiratory rhythm. In the intact slices, neurones with pacemaker like properties were identified. When the pre-Botzinger complex was removed by microdissection, the respiratory related discharge was abolished. Onimaru et al. (1989) and Smith et al. (1991) conclude that in the newborn rat, respiratory rhythm is generated from a network of these pre-inspiratory neurones that show a pacemaker activity, and that the VRG is an important region for this.

Respiratory-related neurones have also been found in the rostral pons. Collectively known as the pontine respiratory group (PRG), tonically discharging neurones are found in the Kolliker Fuse (KF) nucleus and the nucleus parabrachialis medialis (NPBM) (for review see Biachi et al, 1995).

### 1.1.2 Mechanoreceptor control of breathing

Mechanoreflex control of breathing is mediated by the vagus and superior laryngeal nerve (SLN) and is important in the newborn for the maintenance of lung inflation and patency of the upper airway. The Hering-Breuer inflation reflex (HBIR) is mediated by slowly adapting stretch receptors and acts to shorten inspiration when lung volume is rapidly increased above the normal tidal volume range (Breuer, 1868; Hering, 1868; Clark & von Euler, 1972). The resulting prolongation of expiratory time is commonly referred to as apnoea. The HBIR will also prolong expiration if inflation (or maintenance of lung volume) occurs during the expiratory phase (for review see

Trippenbach, 1981). The reflex inhibition of inspiration is stronger in animals than in adult man (Widdicombe, 1961), and in the newborn the HBIR plays an important role in the control of ventilation and maintenance of respiratory pattern (Johnson, 1986; Fedorko, Kelly & England, 1988).

The Hering-Breuer deflation reflex shortens expiration and initiates the beginning of the next inspiration if a volume of air is removed from the lungs (Dawes & Mott, 1959; Tsubone, 1986; Trippenbach, 1994), and is likely to be mediated by rapidly adapting stretch receptors (Koller & Feller, 1970; Trippenbach, 1981). This may play a protective role in the newborn when the chest wall is still highly compliant and hence prevents collapse of the lungs. Rapidly adapting stretch receptors, also referred to as irritant receptors, are also believed to be involved in the generation of "Head's paradoxical reflex" in which large lung inflations produce an increase in respiratory frequency (Widdicombe, 1954). This is important for the newborn at birth when inflating the lungs (Cross, Klaus, Tooley & Weisser, 1960). The Hering-Breuer reflexes are further reviewed in Chapter 4.

The SLN is a mixed nerve, containing both sensory and motor fibres, and is important for the regulation of upper airway patency, particularly in the newborn (Trippenbach, 1981; Lagercrantz, Milerad & Walker, 1991). Upper airway receptors are responsive to flow, pressure, temperature and "respiratory drive" (Sant'Ambrogio, Mathew, Fisher & Sant'Ambrogio, 1983; Ukabam, Knuth & Bartlett, 1992). Negative pressure in the upper airway, generated during inspiration, has an inhibitory effect on phrenic activity and is a potent stimulus for stimulating the dilator muscles of the upper airway, thus maintaining airway patency (Mathew, Abu-Osba & Thach, 1982a, 1982b; Thach, Menon & Schefft, 1989). Ventilation in the newborn is also strongly affected by stimulation of trigeminal receptors in the nares and face, and produces a slowing of respiratory frequency and augmentation of tidal volume when stimulated (Fleming, Levine & Gonclaves, 1982; Dolfen, Dufty, Wilkes, England & Bryan, 1983; Ramet, Paraud, D'Alleat, Dehan & Gaultier, 1990).

## **1.2 Aspects of ventilatory control II: The chemoreceptors**

The chemoreceptors are responsible for the chemical control of breathing. They play a homeostatic role in maintaining arterial pH and  $P_{CO_2}$  and matching ventilation to meet demands in oxygen consumption and carbon dioxide production. There are two main groups of chemoreceptors, central chemoreceptors and peripheral arterial chemoreceptors.

### 1.2.1 Chemoreceptor response to chemical stimuli

The peripheral chemoreceptors respond to a fall in  $\text{PaO}_2$  with an increase in chemoreceptor discharge. Similarly, a rise in  $\text{PaCO}_2$  or a fall in pH will increase discharge of both the peripheral and central chemoreceptors (for review see Gonzalez, Almaraz, Obeso & Rigual, 1994). In the adult cat,  $\text{CO}_2$  and  $\text{O}_2$  interact multiplicatively at the carotid body to increase chemoreceptor discharge (Fitzgerald & Parks, 1971). That is,  $\text{CO}_2$  sensitivity is greater at lower compared to higher  $\text{PaO}_2$ s. There is evidence to suggest that this is not the case for the aortic chemoreceptors (Fitzgerald, 1976), nor the neonatal rat (Pepper, Landauer & Kumar, 1995) nor kitten (Carroll, Bamford & Fitzgerald, 1993). Interaction between  $\text{CO}_2$  and  $\text{O}_2$  is also discussed in relation to my experiments in Chapter 6 (see section 6.4.6). The mechanisms of chemoreception to natural stimuli are discussed in section 1.4.

The carotid chemoreceptors are stimulated by nicotine and acetylcholine (see Gonzalez et al, 1994; Prabhakar, 1994). Although nicotinic receptors are found within the carotid body, Mulligan & Lahiri (1987) have suggested that the effect is via stimulation of ganglionic-nicotinic receptors. Acetylcholine can have a dual action on chemoreceptor discharge. Stimulation of nicotinic receptors increases discharge and stimulation of muscarinic receptors decreases discharge, so the resultant effect is dominated by the relative populations of the receptors (see Gonzalez et al, 1994 for review). Chemoreceptors are also stimulated by cyanide. Cyanide stimulates the chemoreceptors by blocking cytochrome c oxidase and reducing  $\text{O}_2$  availability (Jones, Bickar, Wilson, Brunori, Colosimo & Sarti, 1984). Other metabolic poisons e.g. dinitrophenol (DNP) and oligomycin, disrupt the electron transport system of oxidative phosphorylation and increase chemoreceptor discharge (Mulligan & Lahiri, 1981, Gonzalez et al, 1994). Cyanide and DNP, like hypoxia, promote the release of dopamine from the carotid body (Obeso, Almaraz & Gonzalez, 1989). Cyanide reduces ATP in the carotid body, but dinitrophenol does not, however it increases glucose consumption which suggests the production of ATP by glycolysis (Obeso et al, 1989).

### 1.2.2 Central chemoreceptors

Carbon dioxide is the most important stimulus for the central chemoreceptors in the drive to breathe (for review see Bruce & Cherniack, 1987; Coates, Li & Nattie, 1993). In the absence of peripheral chemoreceptor afferent input, there is a respiratory response to hypercapnia but not to hypoxia (Gemmil & Reeves, 1933; Dumke, Schmidt & Chiodo, 1941). The central chemoreceptors have been estimated to contribute 50-

90% of the total ventilatory response to hypercapnia (Bruce & Cherniack, 1987). There is a delay in response time of the central chemoreceptors to chemical stimulation associated with the equilibration of CO<sub>2</sub> in the extracellular fluid of the brain. The CO<sub>2</sub> content of arterial blood will determine the movement of CO<sub>2</sub> across the blood-brain barrier. Ions will not pass easily across the blood-brain barrier, so after CO<sub>2</sub> has diffused across the bi-lipid membrane of the blood-brain barrier, it is hydrated to form H<sub>2</sub>CO<sub>3</sub> by carbonic anhydrase (CA). This rapidly disassociates to form H<sup>+</sup> and HCO<sub>3</sub><sup>-</sup>, and it is the concentration of H<sup>+</sup> that is widely accepted to be the stimulus for the central chemoreceptors. There are various estimates for the delay in response time to CO<sub>2</sub> of central chemoreceptors; between *ca.* 6sec in newborn piglets using the dynamic end-tidal forcing technique to measure ventilation (Wolsink, Berkenbosch, DeGoede & Olievier, 1991, 1993) to 6-10sec in the adult cat by directly measuring the fall in extracellular pH after a change in alveolar gas (Eldridge, Kiley & Millhorn, 1984; Kiley, Eldridge & Millhorn, 1985). Kiley et al. (1985) showed that airway occlusion in anaesthetized cats produced a rise in alveolar and arterial CO<sub>2</sub>, which in turn lowered medullary extracellular pH with 6-10sec delay, and increased phrenic nerve activity. In contrast, infusion of 100% CO<sub>2</sub> or acid buffer directly into the cerebrospinal fluid (CSF) had a minimal effect on medullary extracellular pH, and no effect on respiration. There is a linear relationship between the change in arterial P<sub>co2</sub> and medullary extracellular pH, however the relationship between the respiratory response to a change in arterial P<sub>co2</sub> or extracellular pH is curvilinear, and takes at least 5min to reach a steady state (Eldridge et al, 1984). Furthermore, Eldridge et al. (1984) recorded on the surface of the medulla oscillations in extracellular pH related to respiration at slow respiratory frequencies.

It had been assumed from work earlier this century that the central chemoreceptors were located superficially on the ventrolateral medulla (VLM) (Mitchell, Loeschcke, Massison & Severinghaus, 1963; Schlaefke, See & Loeschcke, 1970; for review see Bruce & Cherniack, 1987). It was subsequently suggested that chemosensitive neurones were also located deeper in the VLM (Cozine & Ngai, 1967; Issa & Remers, 1992). Issa & Remers (1992) found in the isolated neonatal rat brainstem-spinal cord *in vitro* preparation that chemoreceptive cells could be identified in a column along the length of the VLM to a depth of 100-350µm. Neurones sensitive to H<sup>+</sup> were located to a depth of 1-3mm at two sites *in vivo* in the cat, the VLM and the NTS (Kogo & Arita, 1990). Dean, Bayliss, Erickson, Lawing & Millhorn (1990) also located chemosensitive cells in the NTS. They made intracellular recordings from neurones in the NTS of rat transverse brain slices *in vitro*, and found that a population of neurones were depolarized or increased their discharge frequency to CO<sub>2</sub> in the presence of synaptic blockade. This indicates that the cells recorded were in fact inherently chemo-sensing. Coates, Li &

Nattie (1993) concluded from their experiments in anaesthetized cats and rats, that many sites in the brain stem contained neurones that were chemosensitive. They used acetazolamide, an inhibitor of carbonic anhydrase, to stimulate small areas of the brain stem and found that there was an increase in phrenic nerve activity when injections were made to a depth of 800 $\mu$ m in the VLM, within the vicinity of the NTS and within the vicinity of the locus coeruleus.

### 1.2.3 Peripheral arterial chemoreceptors

Two groups of peripheral arterial chemoreceptors have been identified and their function in controlling respiration extensively investigated: the carotid chemoreceptors and the aortic chemoreceptors.

#### 1.2.3.a Carotid chemoreceptors

The carotid chemoreceptors have been widely investigated in terms of their contribution to respiratory control since their discovery (Heymans & Bouckaert, 1930; Heymans, Bouckaert & Dautrebande, 1930). They are the main organs involved in the sensing of arterial  $P_{O_2}$ , and together with the central chemoreceptors, provide information on arterial  $P_{CO_2}$  and pH to the central respiratory control centre to adjust ventilation to meet a change in arterial blood gases. They also initiate the dominant peripheral chemoreflex.

The carotid body is a paired organ that is found in the region of the common carotid artery bifurcation, however there are variations in the precise location between species. For instance in the lamb, there is no division of the common carotid artery into internal and external carotid arteries, and the carotid body is located at the origin of the occipital artery (Blanco, Dawes, McCooke & Hanson, 1984a). The weight of the carotid body varies between species, and is in the range of 100's  $\mu$ g (for review see Pallot, 1987; Gonzalez et al, 1994).

The carotid body receives its blood supply from the carotid body artery, which originates from the common carotid artery. There are a few veins by which blood is drained from the carotid body which terminate in the internal jugular vein or one of its branches. The carotid body has the highest blood flow of any organ in the body, estimated at 2000ml/min/100g weight compared to 60ml/min/100g weight in the brain (de Burgh Daly, Lambertsen & Schweitzer, 1954). This high flow ensures that during periods when  $P_{O_2}$  is reduced, for instance during hypoxaemia, that  $O_2$  delivery to the

carotid body is not compromised. This has also led to the suggestion that the oxygen content of the carotid body is great, however with such a great flow, arteriovenous O<sub>2</sub> difference is small. In addition, the high flow should ensure that PaO<sub>2</sub> and PaCO<sub>2</sub> are closely equilibrated with tissue Po<sub>2</sub> and Pco<sub>2</sub>, which is important in terms of the chemo-sensing function.

The carotid body receives innervation from the superior cervical ganglion by the gangliomerular nerves. Stimulation of the gangliomerular nerves reduces blood flow to the carotid body (de burgh Daly et al, 1954; Purves, 1970), however this innervation does not appear to be crucial in the chemo-sensing of blood gases (see section 6.4.2.d). The carotid body also receives innervation from the glossopharyngeal nerve. The carotid sinus nerve sends afferent discharge to the NTS in the brainstem. Measurement of carotid sinus nerve discharge is one method used to detect the sensitivity of the carotid chemoreceptors to arterial Po<sub>2</sub>, Pco<sub>2</sub> and pH. This has been performed both *in vivo* (see section 3.1.1 and 6.1) and *in vitro* (see section 3.1.2 and 6.1). Sensitivity of the chemoreceptors to these stimuli has also been assessed by measuring the ventilatory response to an inspired stimulus (see section 3.1.3, 6.1 and 7.1). This is an appropriate method to assess chemosensitivity to Po<sub>2</sub> and Pco<sub>2</sub> non-invasively in newborn infants.

The carotid bifurcation is also involved in the monitoring of blood pressure and the baroreceptors transmit this information via the carotid sinus nerve to the NTS to maintain homeostasis. As this thesis is primarily concerned with chemoreflexes, I have not reviewed blood pressure homeostasis. An extensive review of the role of the peripheral chemoreceptors in cardiovascular control can be found in Marshall (1994). Further detail on the structure of the carotid body is found in section 1.3.

### 1.2.3.b Aortic chemoreceptors

The aortic chemoreceptors are located in the region of the aortic arch and are innervated by axons of the vagus nerve. They are the second most important peripheral chemoreceptors after the carotid chemoreceptors. In the cat and dog, there is a distinctly identifiable aortic nerve which runs with the vagus, referred to as the depressor nerve which can be isolated at the junction with the SLN near the nodose ganglion (Schmidt & Comroe, 1940; Hanson, Rao & Torrance, 1979). In the dog, Comroe (1939) found receptors in one of the small arteries leaving the aorta that responded to injections of cyanide, and identified a nerve leaving in this region that led to the vagodepressor trunk. In the sheep, the aortic chemoreceptor afferents run in the depressor nerve, but occur in bundles in the vagus (Kumar & Hanson, 1989). In the aortic region, the blood supply

could come from several vessels arising from the brachiocephalic artery, aorta, coronary artery and pulmonary artery (Schmidt & Comroe, 1940).

Several workers have reported that the response of the aortic chemoreceptors differs from the carotid chemoreceptors (Paintal & Riley, 1966; Sampson & Hainsworth, 1972; Fitzgerald, 1976; Hanson et al, 1979). Carotid chemoreceptors in the adult typically show an increased CO<sub>2</sub> sensitivity at a lower PaO<sub>2</sub> (Fitzgerald & Parks, 1971; refer also to section 6.4.6). Fitzgerald (1976) did not observe a multiplicative interaction between O<sub>2</sub> and CO<sub>2</sub> from aortic chemoreceptor recordings, rather that there was an additive effect between O<sub>2</sub> and CO<sub>2</sub>. At lower O<sub>2</sub> levels the slope of the CO<sub>2</sub> response curve (i.e. CO<sub>2</sub> sensitivity), was greater for carotid chemoreceptors but not for aortic chemoreceptors. The aortic chemoreceptors are less sensitive to hypoxia and hypercapnia compared to the carotid chemoreceptors (Fitzgerald, 1976; Hanson et al, 1979; Lahiri, Nishino, Mulligan & Mokashi, 1980b; Lahiri, Mokashi, Mulligan & Nishino, 1981a; Lahiri, Mulligan, Nishino, Mokashi & Davies, 1981b). Lahiri et al. (1980b, 1981b) found that the response to CO<sub>2</sub> of the aortic chemoreceptors was only a fraction of the response of the carotid chemoreceptors, and suggested that the low CO<sub>2</sub> sensitivity was the reason for a relatively 'blunted' response to hypoxia. These observations reiterated those of Fitzgerald (1976) that there is a lack of multiplicative interaction between O<sub>2</sub> and CO<sub>2</sub> for aortic chemoreceptors.

Hanson et al. (1979) observed marked variation in the response of the aortic chemoreceptors to steady state CO<sub>2</sub>. In 70% of recordings the steady state sensitivity was not significantly greater than zero, indicating that chemoreceptor discharge was not greater at higher etCO<sub>2</sub>s. Hanson et al. (1979) also measured the dynamic chemoreceptor response to CO<sub>2</sub> and found that this was greater than the steady state response to CO<sub>2</sub> in 83% of recordings (this form of chemoreceptor response to a rapid change in CO<sub>2</sub> is dealt with in more detail in section 1.4 and 7.1). They determined the dynamic chemoreceptor response to CO<sub>2</sub> by 2 breath oscillations in inspired CO<sub>2</sub>, and analysed discharge as a function of time, correlated to the high and low etCO<sub>2</sub> values. The dynamic responses of the aortic chemoreceptors to CO<sub>2</sub> were greater than previously measured for carotid chemoreceptors.

Lahiri et al. (1980b) recorded from aortic and carotid chemoreceptors simultaneously in anaesthetized cats to measure the relative latencies to changes in arterial blood gas, intravenously administered drugs known to excite the chemoreceptors and changes in arterial blood pressure. Carotid chemoreceptors responded more rapidly to hypoxia and hypercapnia induced by inhalation, and the rate of change in discharge was also greater for the carotid chemoreceptors. They also noted that carotid chemoreceptors responded

to a rapid change in  $PET_{CO_2}$  with either an undershoot or overshoot in discharge before reaching a steady level, in contrast to the aortic chemoreceptors which responded more slowly (refer to section 1.4 and 7.1). This contrasts with the findings of Hanson et al. (1979) who found a greater dynamic response of the aortic chemoreceptors to  $CO_2$ . This is most likely due to the methodological differences between the two studies. The criteria of absence of an undershoot or overshoot to a rapid change in  $CO_2$  may be insufficient to conclude that there is no adaptation to the stimulus, or dynamic chemoreceptor response to  $CO_2$  (this is also discussed in relation to my experiments in section 7.1). Lahiri et al. (1980b) found that the response to I.V. infusion of nicotine, cyanide and doxapram was more rapid for the carotid compared to the aortic chemoreceptors. The aortic chemoreceptors responded more rapidly to a change in systemic arterial blood pressure however, when hypotension was produced by inflation of a balloon placed in the thoracic inferior vena cava. Lahiri et al. (1980b) concluded that relatively poor circulation/perfusion of the aortic bodies was the cause for the delay in response time of the aortic chemoreceptors, and that they were more important in the monitoring of blood pressure and flow.

## 1.2.4 Other chemoreceptors

Chemoreceptor tissue has also been found in the neck, thorax and abdomen (Coleridge, Coleridge & Howe, 1970; Howe, Pack & Wise, 1981; Easton & Howe, 1983). Little is known on the role these receptors may play in respiratory control, and I have not discussed them in detail in this thesis. One group of chemoreceptors that are established to be functionally important to the newborn are the laryngeal chemoreceptors.

### 1.2.4.a Laryngeal chemoreceptors

The laryngeal receptors respond to chemical stimuli by initiating a reflex apnoea, bradycardia, decreased cardiac output, increased vascular resistance and redistribution of blood flow (Lee, Stoll & Downing, 1977; Sutton, Taylor & Lindeman, 1978; Groggaard, Kreuger, Lindstrom & Sundell, 1986). Apnoea can be elicited by stimulation of the laryngeal chemoreceptors with water, sucrose, urea, milk and electrolytic stimulation in newborn animals (Sutton et al, 1978; Boggs & Bartlett, 1982) and in human infants (Davies, Koenig & Thach, 1989). The laryngeal chemoreflex is mediated by the SLN, and the reflex response is abolished by section of the SLN (Sutton et al, 1978).

The laryngeal chemoreflex response diminishes with age (Lee et al, 1977; Sutton et al, 1978; Groggaard, Lindstrom, Stahlman, Marchal & Sundell, 1982; Marchal, Crance & Arnould, 1986). Lambs during the first postnatal week showed a greater apnoeic response to water stimulation than lambs aged 2-4wks, and this delayed effect was attributed to poor carotid chemoreceptor maturation in younger animals (Groggaard et al, 1982; Marchal, Corke & Sundell, 1982). The laryngeal chemoreflex response is also affected by sleep state, the apnoeic response being greater in quiet and active sleep compared to wakefulness, and producing arousal in quiet sleep (Groggaard et al, 1982; Marchal et al, 1986). The laryngeal chemoreflex response could play a protective role during feeding to prevent the aspiration of milk into the lungs, and has also been implicated in the link between apnoea of prematurity and SIDS (Downing & Lee, 1975; see also section 1.6).

### 1.3 Structure of the carotid body

There is a vast amount of literature on the structure of the carotid body and I have taken the detail in this section from reviews by Eyzaguirre & Fidone (1980), Heath & Smith (1985), Pallot (1987) and Gonzalez, Almaraz, Obeso & Rigual (1994). The carotid body is made up of two types of cells: **type I cells** or glomus or chemoreceptor cells, and **type II cells** or sustentacular cells. The two different cell types form cell clusters that are separated from each other by varying amounts of connective tissue. Type II cells partially surround the type I cells, and separate them from the capillary wall. One third to one quarter of the carotid body is made up of capillaries and venules, and as such is a great deal more vascularized than the brain, which accounts for the high perfusion of the carotid body (Gonzalez et al, 1994). Arteriovenous shunting has also been described in terms of blood flow, thus venous flow is a sum of the blood leaving the tissue beds and that diverted from the tissue via the arteriovenous shunts. Blood vessels of the carotid body are innervated by sympathetic nerves arising from the superior cervical ganglion.

The cell clusters are innervated by sensory nerve fibres of the carotid sinus nerve that penetrate the connective tissue, and terminate on the type I cells. The nerve fibres divide frequently and become unmyelinated as they approach the cell clusters. Myelinated fibres are 1-10 $\mu\text{m}$  in diameter and conduct at 4-53 m/sec, unmyelinated fibres are 0.1-1.3 $\mu\text{m}$  in diameter and conduct at 0.5-2.0 m/sec (Eyzaguirre & Fidone, 1980). Nerve endings vary greatly and range between small boutons with less than 1 $\mu\text{m}^2$  surface contact with the type I cell, to large calyx-shaped nerve endings with up to 10 $\mu\text{m}^2$  surface contact (Gonzalez et al, 1994). Nerve endings contain large numbers of clear cored vesicles and a lesser number of dense cored vesicles. Nerve fibres that

terminate on one type I cell, may branch up to 10-20 times to terminate on other type I cells. The nerve terminal is separated from the type I cells by a cleft of *ca.* 20-30nm. A schematic diagram of the cell cluster is shown in figure 1.3.i.

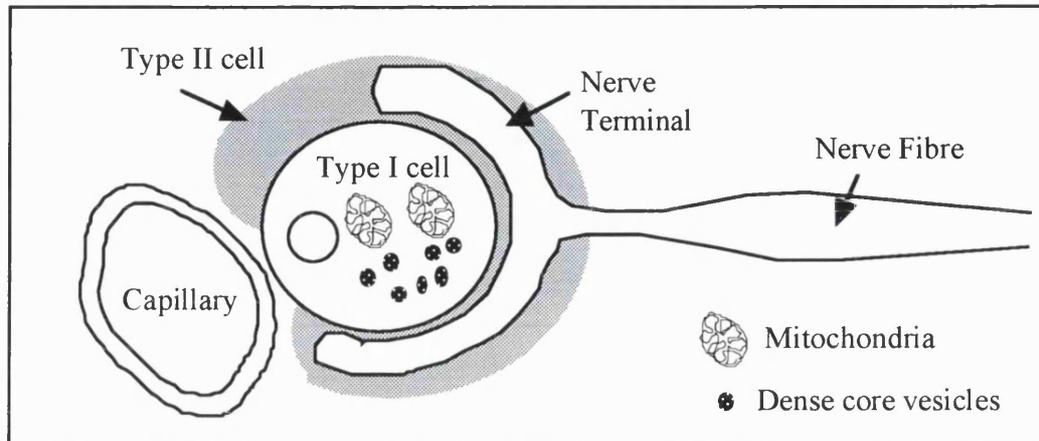


Figure 1.3.i Schematic diagram of carotid body, showing type I cell, type II cell, capillary, nerve terminal and nerve fibre.

### 1.3.1 Type I cells

Type I cells are more numerous in the cell clusters, are spheroidal in shape and are up to 10-20  $\mu\text{m}$  in diameter (Eyzaguirre & Fidone, 1980; Pallot, 1987). They are identified by a clear round nucleus and a distinctive granular cytoplasm, which is due to the presence of dense core vesicles (20-30 nm). They have characteristics similar to those of secretory cells:- well developed mitochondria, endoplasmic reticulum, Golgi apparatus and dense cored vesicles. Type I cells can have finger like process that project from them up to 40 $\mu\text{m}$  (Gonzalez et al, 1994).

Mitochondria are abundant in the cytoplasm of type I cells and can form dense clusters or aggregates. They are usually rodlike in appearance and have diameters of 0.2 $\mu\text{m}$  and lengths of 1.5 $\mu\text{m}$ . The well developed Golgi apparatus gives rise to the dense core vesicles which contain calcium binding sites and appear to play an exocytotic role in the release of catecholamines (Eyzaguirre & Fidone, 1980; Gonzalez et al, 1994). Immature dense core vesicles at the level of the Golgi apparatus do not appear to contain catecholamines. The dense core vesicles range in size from 35-200nm and are similar, though generally smaller, to those contained in adrenal medullary cells. Both the size and abundance of dense core vesicles varies between species. Other abundant cell organelles include centrioles, microtubules, lysosomes and vacuoles. Clear core vesicles

are also found in the cytoplasm of type I cells and are suggested to contain acetylcholine.

### 1.3.2 Type II cells

Type II cells are elongated and are restricted to the periphery of cell clusters. They do not completely surround the type I cells, and so part of the type I cells are exposed to the perivascular space. Thus it is unlikely that they provide a diffusion barrier between type I cells and blood vessels as previously suggested by de Kock & Dunn (1964). Type II cells vary in size, but are approximately 13 x 4µm (Heath & Smith, 1985). They are similar in appearance to Schwann cells and also ensheath nerve fibres. Relatively little is known of the function of type II cells in the carotid body, although it has been suggested that they have a nutrient role and control local ion concentrations. They phagocytose the debris of nerve endings, in the instance of denervation (McDonald & Mitchell, 1975).

### 1.3.3 Ganglion cells

Ganglion cells of the autonomic nervous system are also found in the carotid body. They are usually round and larger than type I cells, 20-40µm, and have a vesicular nucleus and granulated cytoplasm. Ganglion cells, either singly or in small groups are found in the connective tissue at the periphery of the carotid body near the connection with the glossopharyngeal nerve. They are believed to innervate the blood vessels of the carotid body and help regulate vascular tone (Heath & Smith, 1985).

### 1.3.4 Neurotransmitters and neuropeptides of the carotid body

The carotid body of all species contains catecholamines with dopamine and noradrenaline being most prevalent (Pallo, 1987; Gonzalez et al, 1994). The relative proportions of each appears to differ between species: in cats there are similar amounts of noradrenaline and dopamine; in the rabbit there is about five times as much dopamine as noradrenaline. In the rat, chemoreceptor cells stain positive for tyrosine hydroxylase and DOPA hydroxylase, but much less for dopamine β-hydroxylase (for review see Gonzalez et al, 1994). Tyrosine is converted to DOPA by tyrosine hydroxylase, the rate limiting step, then decarboxylated to dopamine by dopamine β-hydroxylase. Tyrosine hydroxylase is prevalent in the type I cells, and this suggests a strong role for dopamine synthesis and function in the rat carotid body (Prabhakar, 1994). Exogenous dopamine has been shown to increase chemoreceptor discharge in the dog *in vivo*, and in

the rabbit *in vitro*, and also to decrease chemoreceptor discharge *in vivo* in the rabbit, cat and dog, and *in vitro* in the cat (Prabhakar, 1994). Although controversial, it is generally believed that dopamine has an inhibitory effect on chemoreceptor discharge, a view which is supported by an increased chemoreceptor discharge with blockade of dopaminergic receptors (Lahiri, Nishino, Mokashi & Mulligan, 1980a).

The noradrenaline content appears to be little affected by removal of the superior cervical ganglion, indicating that noradrenaline is contained within the chemoreceptor cells. Acetylcholine has been found in chemoreceptor cells of the rat and cat, and there is a high-affinity sodium-dependent uptake mechanism for choline in cat chemoreceptor cells, suggesting that acetylcholine receptors are found in chemoreceptor cells (for review see Gonzalez et al, 1994). Serotonin (5-HT) is also found in the carotid body of rats, cats and humans and is particularly abundant in the chicken. Neuropeptides found in the carotid body include substance P, tachykinin A, cholecystokinin, galanin, neurotensin, bombesin, calcitonin, and atrial natriuretic peptide. For the most part, these neuropeptides are not known to be involved in the process of chemoreception, but their abundance does reflect the complexity of synaptic neurotransmission. A review of the neurotransmitters found in the carotid body and their possible role in chemo-sensing can be found in Prabhakar (1994). I have particularly discussed the relevance of the abundance of dopamine in the type I cells in relation to the current concepts of chemoreception in section 1.4.

## 1.4 Mechanisms of carotid body chemoreception

It is generally assumed that carotid body type I cells are the site for chemoreception. There is also evidence to suggest that different mechanisms exist for the sensing of oxygen and carbon dioxide (for review see Acker, 1994, Buckler & Vaughan-Jones, 1994; Hanson & Kumar, 1994; Gonzalez et al, 1994). Recently it has also been shown that type I chemoreceptor cells are excitable, that is they are depolarized in response to chemical stimuli. The average membrane potential is -20 to -30mV, however there is a wide range between -8 to -80mV ( see Gonzalez et al, 1994). There is evidence for both Na<sup>+</sup> and Ca<sup>2+</sup> voltage-gated channels involved in membrane depolarization and the subsequent release of neurotransmitters in the response to chemical stimuli (see below for detail).

## 1.4.1 Chemotransduction of carbon dioxide

### 1.4.1.a Carbonic anhydrase catalyses the conversion of CO<sub>2</sub> to H<sup>+</sup> and HCO<sub>3</sub><sup>-</sup>

Carbonic anhydrase is essential for the rapid response to CO<sub>2</sub> described by Black, McCloskey & Torrance (1971). They found that the chemoreceptor response to an injection of CO<sub>2</sub>-equilibrated saline was characterized by a rapid rise in discharge frequency over the first second, followed by a decline to the steady state level over the next 10-15sec. The CA inhibitor acetazolamide abolishes the rapid response to CO<sub>2</sub>. This evidence, together with the observations that chemoreceptor discharge oscillated in response to PaCO<sub>2</sub> oscillations (Hornbein, Griffio & Roos, 1961; Leitner & Dejourn, 1968; this is reviewed in detail in section 7.1), suggested that the carotid body showed both a dynamic and a steady state CO<sub>2</sub> sensitivity.

Carbon dioxide is hydrated in the blood by the action of carbonic anhydrase to form carbonic acid (H<sub>2</sub>CO<sub>3</sub>), which rapidly dissociates into hydrogen ions (H<sup>+</sup>) and bicarbonate ions (HCO<sub>3</sub><sup>-</sup>). From the inhibition of CA function in the carotid body, it was discovered that intracellular pH (pH<sub>i</sub>), and not extracellular pH as previously thought, was the stimulus for CO<sub>2</sub> chemo-sensing (Hanson, Nye & Torrance, 1981). Carbon dioxide that is carried in the blood will diffuse into type I cells from the extracellular space and acidify intracellular pH. A comparison of two different types of CA inhibitors, acetazolamide which rapidly crosses cell membranes and benzolamide that does not, showed that acetazolamide abolished the adaptation to CO<sub>2</sub>. Benzolamide only partially abolished it (Hanson et al, 1981). Histological evidence of rat type I cells has confirmed the presence of CA intracellularly (Ridderstrale & Hanson, 1985; Nurse, 1995), and it was also located in red blood cells. It was not found in cell bodies of the petrosal ganglion or superior cervical ganglion. Thus, it became apparent that the ability of the type I cell to regulate pH<sub>i</sub> was crucial to the chemotransduction of CO<sub>2</sub> (Peers & Buckler, 1995 for review).

### 1.4.1.b Ability of the carotid body type I cell to regulate intracellular pH

Buckler, Vaughan-Jones, Peers & Nye (1991b) measured the pH<sub>i</sub> of type I cells of neonatal rat carotid bodies using a pH-sensitive dual emission fluoroprobe, carboxy-seminaphthorodoafluor 1 (see Buckler & Vaughan-Jones, 1990 for full detail). Type I cells bathed in buffered CO<sub>2</sub>-HCO<sub>3</sub><sup>-</sup> saline (physiological conditions) showed a pH<sub>i</sub> of *ca.* 7.2-7.3 (Buckler et al, 1991; Wilding, Cheng & Roos, 1992). These values were less acidic than those of Biscoe, Duchon, Eisner, O'Neill & Valdeomillos (1989) and He, Wei

& Eyzaguirre (1991) who found  $pH_i$  to be 6.9 and 6.7 respectively. He et al. (1991) found that  $H^+$  ions were not passively distributed across the cell membrane of rat type I cells, and the membrane potential was dependent on the  $H^+$  ions. Intracellular, and not extracellular pH controlled the resting membrane potential of type I cells (He, Wei & Eyzaguirre, 1993). Buckler et al. (1991b) commented that the most likely explanation for the difference in the estimates of  $pH_i$  was the media used to store the cells, and recommended the use of buffered  $CO_2-HCO_3^-$  media to discount the possibility of cell degeneration.

Buckler et al. (1991b) investigated the ability of the type I cells to regulate  $pH_i$  by  $NH_4Cl$  challenges to acidify the cells. Intracellular pH was tightly regulated and the intrinsic buffering capacity ( $\beta$ ) was greater at lower  $pH_i$ s. They investigated the ability of the cell to recover from  $NH_4Cl$  acidification, and found three main cellular mechanisms that allowed regulation of  $pH_i$ . First, an inhibitor of  $Na^+-H^+$  exchange, ethyl isopropyl amiloride, inhibited recovery from the acid challenge (see also Wilding et al, 1992). Similarly, removal of extracellular  $Na^+$  prevented recovery of  $pH_i$ , providing good evidence for the involvement of  $Na^+-H^+$  ion exchange in regulating  $pH_i$ . Secondly, in  $CO_2-HCO_3^-$  buffered media they found evidence for an  $Na^+-HCO_3^-$  ion exchange which was involved in recovery from the acid challenges, and which was inhibited when extracellular  $Na^+$  was removed. Thirdly, when cells were buffered in  $CO_2-HCO_3^-$  media, removal of extracellular  $Cl^-$  caused  $pH_i$  to become alkaline, and was not inhibited by removal of extracellular  $Na^+$ , which suggests a role for  $Cl^- -HCO_3^-$  ion exchange in regulating  $pH_i$ . Two of these mechanisms,  $Na^+-H^+$  ion exchange and  $Na^+-HCO_3^-$  ion exchange, allow  $H^+$  to leave the cell in exchange for  $Na^+$ , thus maintaining electroneutrality and recovery from acidification. The third,  $Cl^- -HCO_3^-$  ion exchange, suggests a possible mechanism for acid to enter the cell.

In addition to the  $Cl^- -HCO_3^-$  ion exchange (Buckler et al, 1991b), two other mechanisms have been proposed for the acidification of type I cells. Wilding et al. (1992) measured  $pH_i$  in adult rat carotid body type I cells and found evidence for a  $K^+-H^+$  ion exchange. Intracellular pH was increased when the extracellular  $[K^+]$  was increased, and decreased when extracellular  $[K^+]$  was decreased, so an exchange of intracellular  $K^+$  for extracellular  $H^+$  may be a route for cell acidification. However there does not appear to be  $K^+-H^+$  ion exchange in neonatal type I cells (Richmond & Vaughan-Jones, 1993). The authors suggested that the results of Wilding et al. (1992) could be explained by contamination of the cell by the techniques used. Stea & Nurse (1991a) also found evidence for a  $HCO_3^-$ -permeable anion exchange in cultured rat carotid body type I

cells. An efflux of  $\text{HCO}_3^-$  would allow for an equivalent influx of  $\text{H}^+$ , and hence cell acidification.

#### 1.4.1.c Intracellular pH of type I cells is dependent on extracellular pH

Buckler, Vaughan-Jones, Peers, Lagadic-Gossman & Nye (1991a) measured the effect of changing extracellular pH by  $\text{Pco}_2$  and  $\text{HCO}_3^-$  on intracellular pH in isolated type I carotid body cells of neonatal rats. They determined that the intrinsic intracellular buffering power was a function of  $\text{pH}_i$ . Changes in  $\text{Pco}_2$  at a constant extracellular pH (by changing  $\text{HCO}_3^-$  also) produced a transient acidification of  $\text{pH}_i$ , but steady state  $\text{pH}_i$  was not affected. Changes in  $\text{HCO}_3^-$  whilst  $\text{Pco}_2$  was held constant, thus producing changes in extracellular pH, led to a changes in  $\text{pH}_i$ . In fact, a linear relationship between extracellular and intracellular pH was determined. As previously mentioned, He et al. (1993) showed that  $\text{pH}_i$  controlled the resting membrane potential of type I carotid body cells. Resting membrane potentials were most negative when  $\text{pH}_i$  was lowest; at a normal extracellular pH of 7.4 the resting membrane potential is *ca.* 55mV (see Gonzalez et al, 1994). These cellular ion exchange mechanisms show the ability of type I cells to control  $\text{pH}_i$  tightly, however it is very sensitive to extracellular pH. So  $\text{pH}_i$  is important and is possibly the first step in acid chemotransduction.

#### 1.4.1.d A fall in intracellular pH induces a rise in intracellular $\text{Ca}^{2+}$ in type I cells

The other important cellular mechanism involved in acid chemotransduction is the rise in intracellular  $\text{Ca}^{2+}$  ( $\text{Ca}^{2+}_i$ ) (for review see Hanson, 1994; Gonzalez et al, 1994; Peers & Buckler, 1995), however there appear to be conflicting ideas on the mechanisms involved. Biscoe & Duchon (1990) measured changes in  $\text{Ca}^{2+}_i$  produced during anoxia in dissociated type I rabbit carotid body cells. They were unable to demonstrate that a fall in extracellular pH produced a rise in  $[\text{Ca}^{2+}]_i$  at normoxic  $\text{Po}_2$ s, but found that there was an interactive effect between  $\text{Po}_2$  and  $\text{Pco}_2$  and the subsequent rise in  $[\text{Ca}^{2+}]_i$ . Anoxia produced a rise in  $[\text{Ca}^{2+}]_i$  that was temperature sensitive, virtually inactive at 17-20°C and greatly increased at 36°C. The rise in  $[\text{Ca}^{2+}]_i$  was unaffected by blockade of  $\text{Na}^+$  and  $\text{Ca}^{2+}$  channels, and was gradually reduced by exposure to  $\text{Ca}^{2+}$ -free bathing solutions. This strongly suggested that the rise in  $[\text{Ca}^{2+}]_i$  was via release from an intracellular store, which the authors believed to be the mitochondria, as the response was inhibited in the presence of a mitochondrial uncoupler (*p*-trifluoromethoxy-phenylhydrazon, FCCP).

In contrast, Buckler & Vaughan-Jones (1993) were able to demonstrate that hypercapnia produced a rise in  $[Ca^{2+}]_i$  in isolated type I cells of neonatal rat carotid body. They observed a rise in  $[Ca^{2+}]_i$  to hypercapnic acidosis (an increase in  $CO_2$  with constant  $HCO_3^-$ ), isohydric hypercapnia (an increase in  $CO_2$  at constant pH) and isocapnic acidosis (constant  $CO_2$ ), which was characterized by a rapid rise which subsequently declined. The rise in  $[Ca^{2+}]_i$  was inhibited in  $Ca^{2+}$ -free bathing solutions, which led the authors to suggest that the rise in  $[Ca^{2+}]_i$  was at least partially dependent on extracellular  $Ca^{2+}$ . The role for extracellular  $Ca^{2+}$  mediating the rise in  $[Ca^{2+}]_i$  was investigated in terms of the voltage-gated  $Ca^{2+}$  channel model, in a subsequent series of experiments performed by Buckler & Vaughan-Jones (1994b). The results of Biscoe & Duchon (1990) and Buckler & Vaughan-Jones (1993) differ slightly, but together they provide good evidence for a fall in  $pH_i$  triggering a rise in  $[Ca^{2+}]_i$ . This is believed to trigger the release of neurotransmitter from the type I cell and is discussed in section 1.4.1.e.

#### *The $Na^+$ - $H^+$ ion exchange and $Na^+$ - $Ca^{2+}$ ion exchange model*

From the ion exchange processes that I have described above, it is possible to see that a fall in  $pH_i$  could lead to a rise in  $[Ca^{2+}]_i$ . A fall in  $pH_i$  means that there is a rise in  $[H^+]_i$ , which can be transported out of the cell by the  $Na^+$ - $H^+$  ion exchange. Thus a rise in  $[Na^+]_i$  occurs, and  $Na^+$  can be transported out of the cell by reversing the existing  $Na^+$ - $Ca^{2+}$  ion exchange, resulting in a net increase in  $[Ca^{2+}]_i$  (for review see Gonzalez et al, 1994). Evidence for a strong  $Na^+$ - $Ca^{2+}$  ion exchange in type I cells has been found by Rocher, Obeso, Gonzalez & Herreros (1991) (this is discussed in section 1.4.1.e). Briefly, they found in type I cells of adult rabbits that the release of dopamine in response to  $CO_2$  was dependent on external  $Ca^{2+}$  influx via the  $Na^+$ - $Ca^{2+}$  ion exchange.

Obeso, Rocher, Fidone & Gonzalez (1992) proposed that the rise in  $Ca^{2+}_i$ , and hence dopamine release, was possibly via the  $Na^+$ - $Ca^{2+}$  co-transporter in adult rabbit type I cells. They observed that in response to both high  $P_{CO_2}$  and low pH, dopamine was released from type I cells. Interestingly, the release of dopamine was dihydropyridine insensitive. Although they found evidence for a role of voltage gated  $Ca^{2+}$  channels in the response to low  $P_{O_2}$  (see section 1.4.2.d), their results suggested that the rise in  $Ca^{2+}_i$  to high  $P_{CO_2}$  and low pH occurred via the  $Na^+$ - $Ca^{2+}$  co-transporter.

#### *The voltage-gated $Ca^{2+}$ channel model*

There is also evidence to suggest that  $Ca^{2+}$  influx occurs via  $Ca^{2+}$  channels following depolarization of the type I cell membrane (Peers, 1990a; Peers & Green, 1991). In this model,  $K^+$  channels are inhibited by acid stimuli, which in turn depolarizes the cell membrane and allows entry of extracellular  $Ca^{2+}$  through voltage gated channels. Peers

(1990a) measured  $K^+$  currents in type I cells of neonatal rats. An increase in extracellular  $[Ca^{2+}]$  enhanced  $K^+$  currents, whilst  $Ca^{2+}$  channel antagonists (selective for L-type  $Ca^{2+}$  channels) suppressed the  $K^+$  current, indicating that  $Ca^{2+}$ -dependent  $K^+$  currents occurred via  $Ca^{2+}$ -activated  $K^+$  channels.  $K^+$  currents were also reduced when extracellular pH was lowered, and increased when extracellular pH was alkaline. Moreover these effects of extracellular pH on  $K^+$  currents were reversible. Peers & Green (1991) found in isolated type I neonatal rat carotid body cells that  $K^+$  currents, and hence  $K^+$  channels, were inhibited by acid stimuli that lowered  $pH_i$ , whilst  $Ca^{2+}$  currents were unaffected by acid stimuli. Similar reports have been made from adult rabbit type I chemoreceptor cells (López-López, Gonzalez, Ureña & López-Barneo, 1989). A reduction in extracellular pH from 7.40 to 7.0 reduced the amplitude of the  $K^+$  currents by *ca.* 20%. These effects were also reversible.

Stea & Nurse (1991a; 1991b) and Stea, Alexander & Nurse (1991) have also found an effect of acid stimuli on  $K^+$  and  $Na^+$  currents in rat type I cells. During voltage clamping of the cell, the amplitude of the outward  $K^+$  current was reduced by a reduction in pH, weak acid solution or acid loading by inactivation of the  $K^+/H^+$  antiporter with nigericin. Nigericin also reduced the amplitude of the  $Na^+$  current. The effects of pH on  $K^+$  and  $Na^+$  currents were reversible. This provides further evidence in the adult for acid stimuli acting on  $K^+$  channels.

Further support for the membrane depolarization hypothesis arises from simultaneous measurement of membrane potentials and the rise in  $[Ca^{2+}]_i$  observed during hypercapnia. Buckler & Vaughan-Jones (1994b) confirmed their previous findings that hypercapnia increased  $[Ca^{2+}]_i$  in neonatal rat type I cells, which was inhibited in  $Ca^{2+}$ -free media and thus attributed to an influx of extracellular  $Ca^{2+}$ . During hypercapnia, the rise in  $[Ca^{2+}]_i$  occurred simultaneously with a depolarization of the membrane. The rise in  $[Ca^{2+}]_i$  was inhibited by voltage clamping to prevent depolarization. Buckler & Vaughan-Jones (1994b) rejected the hypothesis that a rise in  $[Ca^{2+}]_i$  is mediated by  $Na^+-Ca^{2+}$  ion exchange, as replacement of extracellular  $Na^+$  with  $Li^+$  did not prevent the rise in  $[Ca^{2+}]_i$  observed during hypercapnia.  $Li^+$  is membrane permeable, but not transported by the  $Na^+-Ca^{2+}$  ion exchange, so whilst extracellular  $Na^+$  may play a role the regulation of  $[Ca^{2+}]_i$ , the rise observed during hypercapnia is not due to the  $Na^+-Ca^{2+}$  co-transporter. They concluded that hypercapnia elevated  $[Ca^{2+}]_i$  via membrane depolarization and voltage gated  $Ca^{2+}$  entry, possibly by the inhibition of  $K^+$  channels described by Peers & Green (1991).

It is possible that a species difference or maturational difference may account for the different observations of Rocher et al. (1991) and Buckler & Vaughan-Jones (1994b).

This is however the first evidence for membrane depolarization produced by acid stimuli concomitant with an influx of  $\text{Ca}^{2+}$ . Debate continues over the mechanism of  $\text{Ca}^{2+}_i$  elevation seen during acid and hypercapnic stimuli, however in section 1.4.1.e I will discuss the role for intracellular  $\text{Ca}^{2+}$  in mediating neurotransmitter release to acidic stimuli in type I cells.

#### 1.4.1.e A rise in intracellular $\text{Ca}^{2+}$ triggers a release of neurotransmitters

As mentioned in section 1.3.4, dopamine is abundant in the type I cells of several species and is believed to play a role in chemotransduction. Rocher et al. (1991) investigated the role of dopamine release in isolated adult rabbit type 1 cells of the carotid body. Inhibition of the  $\text{Na}^+$  pump with ouabain, or immersion in  $\text{K}^+$  free media, produced a release of dopamine that was dependent on the presence of  $\text{Na}^+$  and  $\text{Ca}^{2+}$  in the media. They used nisoldidine to block voltage-dependent  $\text{Ca}^{2+}$  channels and found that poisoning of the  $\text{Na}^+$  pump still evoked dopamine release. This suggested that the dopamine release occurred by  $\text{Na}^+-\text{Ca}^{2+}$  exchange when  $[\text{Na}^+]_i$  was elevated. Media containing  $\text{CO}_2$ , weak acids or dinitrophenol (DNP; which uncouples oxidative metabolism and produces a rise in  $[\text{H}^+]_i$  by approaching electroneutral equilibrium) also produced dopamine release. The involvement of the  $\text{Na}^+-\text{H}^+$  exchange was also implied as blockade with eythlisopropylamiloride (EIPA) reduced the dopamine release response to DNP or weak acid.

That the  $\text{Na}^+-\text{H}^+$  ion exchange is involved in the dopamine release response to DNP or weak acid could suggest that  $\text{Na}^+-\text{Ca}^{2+}$  exchange does not in fact mediate the rise in  $\text{Ca}^{2+}_i$ . For instance it is possible that the rise in  $[\text{H}^+]_i$  inhibits  $\text{K}^+$ -channels (as described by Peers, 1990a; Peers & Green, 1991) which in turns depolarizes the membrane. This activates the voltage gated  $\text{Ca}^{2+}$  channels and leads to a rise in  $\text{Ca}^{2+}_i$ . This is in part contradicted by the fact that nisoldidine did not affect the dopamine release response. However, Rocher et al. (1991) based part of their conclusions for the presence of  $\text{Na}^+-\text{Ca}^{2+}$  exchange on the response to DNP, which has been shown to produce only a small fall in  $\text{pH}_i$  relative to the much larger increase in  $\text{Ca}^{2+}_i$  (see Peers & Buckler, 1995 for review). Thus, the mechanism for the rise in  $\text{Ca}^{2+}_i$  is still debatable.

Further evidence for the role of dopamine in mediating the excitatory response to hypercapnia comes from Rigual, Lopez-Lopez & Gonzalez (1991a). Cat carotid bodies were excised and mounted in a superfusion chamber and chemoreceptor activity from the carotid sinus nerve and dopamine release were simultaneously recorded. Low pH and high  $\text{CO}_2$  of the perfusate increased chemoreceptor discharge and dopamine release,

whilst low  $[Ca^{2+}]_i$  perfusates reduced the stimulatory effect of low pH on both the dopamine release and chemoreceptor response. This again suggests a role for  $Ca^{2+}_i$  mediating the release of dopamine and in turn the increase in chemoreceptor discharge. Furthermore, the response to  $CO_2$  in the presence of acetazolamide was accompanied by a reduction both in the dopamine release and chemoreceptor discharge. This provided strong evidence for acid stimuli increasing chemoreceptor discharge by neurotransmitter release presumably through an increase in  $Ca^{2+}_i$ , the most likely candidate for the neurotransmitter being dopamine.

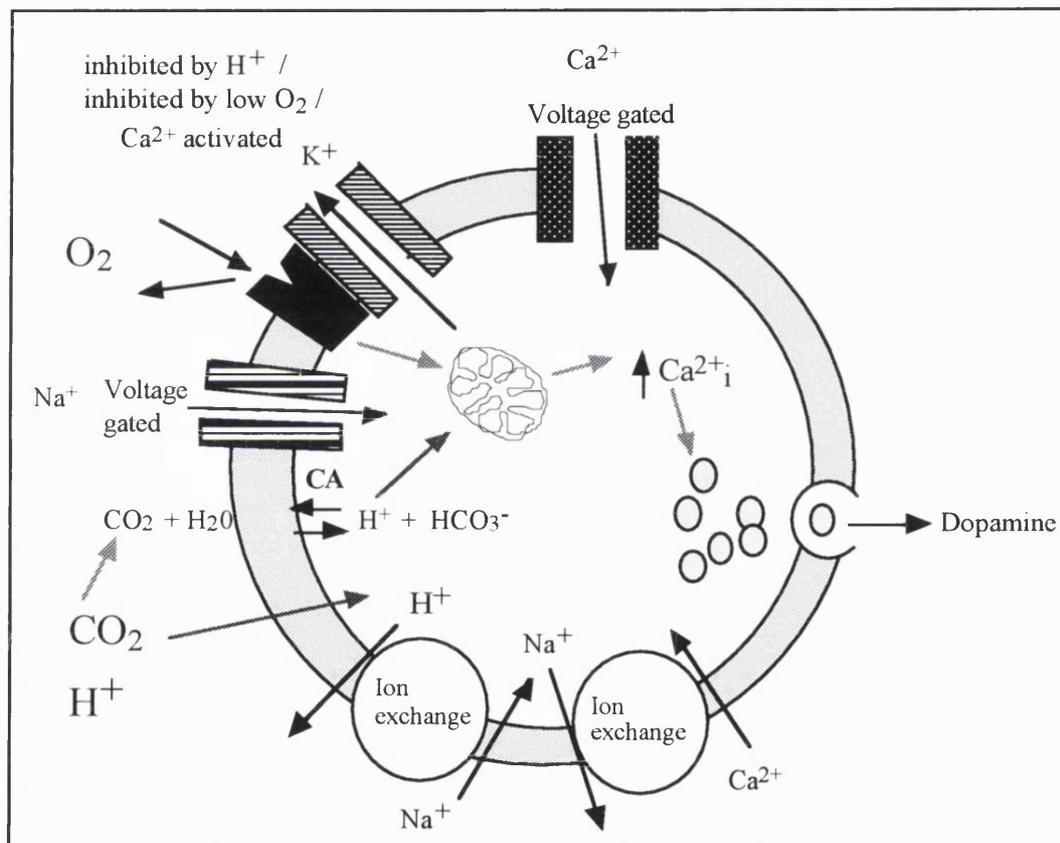


Figure 1.4.1.e.i Schematic representation of acid and oxygen chemotransduction in the carotid body. CA= carbonic anhydrase, mitochondrial release of  $Ca^{2+}_i$ ,  $Na^+$ - $H^+$  ion exchange,  $Na^+$ - $Ca^{2+}$  ion exchange, voltage gated  $Ca^{2+}$  channels, voltage gated  $Na^+$  channels,  $H^+$  and  $O_2$  sensitive  $K^+$ -channels, and neurotransmitter release (dopamine).

In summary, the current theory for the chemotransduction for acid stimuli is a rise in  $Ca^{2+}_i$  produced by a fall in  $pH_i$ . The elevated levels of  $Ca^{2+}_i$  cause neurotransmitter release which produces an increase in chemoreceptor discharge. It is presumed that the neurotransmitter released stimulates nerve endings, however much less is known of this process. Two mechanisms have been proposed to produce the rise in  $Ca^{2+}_i$ : first a

Na<sup>+</sup>-Ca<sup>2+</sup> ion exchange and secondly a voltage gated Ca<sup>2+</sup> influx mediated by inhibition of Ca<sup>2+</sup>-activated K<sup>+</sup> channels. This is illustrated schematically in figure 1.4.1.e.i.

## 1.4.2 Chemotransduction of low oxygen

As in the chemotransduction of CO<sub>2</sub> and acid stimuli, a rise in Ca<sup>2+</sup><sub>i</sub> is crucial in the chemotransduction of hypoxia. Relatively less is known of the hypoxic sensing mechanism that ultimately mediates this rise in Ca<sup>2+</sup><sub>i</sub>. Presently, the popular belief is that O<sub>2</sub> binds to some membrane bound receptor and is possibly linked to O<sub>2</sub>-sensitive K<sup>+</sup> channels. The hypoxia effect on K<sup>+</sup> channels may be linked to the O<sub>2</sub> sensor itself, or release an endogenous substance intracellularly to act on the K<sup>+</sup> channel. The dihydropyridine-independent effect of low P<sub>O<sub>2</sub></sub> on K<sup>+</sup> currents suggests that the O<sub>2</sub> sensor and K<sup>+</sup> channel are likely to be linked. The basic overview of hypoxic chemotransduction is illustrated schematically in figure 1.4.1.e.i.

### 1.4.2.a Hypoxia has a direct effect on K<sup>+</sup> channels

Hypoxia has been shown to have a direct effect on K<sup>+</sup> channels (López-Barneo, Benot & Ureña, 1993). It is postulated that the K<sup>+</sup> channel may be closely associated with a haemoglobin like-O<sub>2</sub> sensor in the cell membrane (López-López et al, 1989; López-Barneo et al, 1993). López-López et al. (1989)(see also López-Barneo, López-López, Gonzalez & Ureña, 1988) measured ionic conductances by whole-cell patch clamp in dissociated type I cells from adult rabbits. Hypoxia (P<sub>O<sub>2</sub></sub> 10mmHg) reduced the K<sup>+</sup> current without effect on the Na<sup>+</sup> or Ca<sup>2+</sup> currents. The reduction in K<sup>+</sup> current was graded with respect to hypoxia between 70 and 120mmHg, and was reduced to a maximum of 35% of normoxic levels when P<sub>O<sub>2</sub></sub> was 10mmHg. Furthermore the hypoxia effect was independent of Ca<sup>2+</sup><sub>i</sub> and ATP. Voltage clamping of the cell membrane was able to initiate large potentials in K<sup>+</sup>, Na<sup>+</sup> or Ca<sup>2+</sup> indicating that they were voltage gated, and the amplitude of the K<sup>+</sup> potential was reduced in hypoxia.

Since this pioneering work of López-López et al. (1989), further investigations have been made on the nature of this hypoxia sensitive K<sup>+</sup> channel (Ganfornina & López-Barneo, 1991, 1992). Ganfornina & López-Barneo (1991) showed in dispersed type I cells from adult rabbits that K<sup>+</sup> channels were inhibited by hypoxia and independent of the dihydropyridines, confirming the findings of López-López et al. (1989). In a subsequent series of experiments, Ganfornina & López-Barneo (1992) characterized three different types of voltage gated K<sup>+</sup> channels in type I cells from adult rabbits; Ca<sup>2+</sup> activated- K<sup>+</sup> channels of large conductance, K<sup>+</sup> channels of small conductance and

P<sub>o</sub><sub>2</sub>-sensitive K<sup>+</sup> channels of intermediate conductance. It was the P<sub>o</sub><sub>2</sub>-sensitive K<sup>+</sup> channels that were of greatest interest and also the most abundant. The probability of the P<sub>o</sub><sub>2</sub>-sensitive K<sup>+</sup> channels being open was greatly increased at low P<sub>o</sub><sub>2</sub>s. It was also found that the activation of these channels was not dihydropyridine (ATP and GTP) dependent. Together with the observations of López-López et al. (1989), this work provided evidence for a voltage gated, Ca<sup>2+</sup> independent- K<sup>+</sup> channel in the adult rabbit carotid body.

It is interesting that evidence obtained from neonatal rats suggests the presence of a voltage gated Ca<sup>2+</sup> dependent- K<sup>+</sup> channel, that is sensitive to low P<sub>o</sub><sub>2</sub> (Peers, 1990b). Whole-cell patch clamp techniques were used in isolated neonatal rat type I cells to evoke depolarization and measure K<sup>+</sup> currents. Under normoxic conditions, outward K<sup>+</sup> currents were observed and these were reduced during hypoxia (P<sub>o</sub><sub>2</sub> 25 torr). The hypoxic suppression of K<sup>+</sup> currents was substantially reduced by exposure to Ca<sup>2+</sup> channel blockers but there was some residual current, indicating the predominance of a Ca<sup>2+</sup>-dependent voltage-gated K<sup>+</sup> channel but also the presence of a Ca<sup>2+</sup>-independent voltage-gated K<sup>+</sup> channel. Peers (1990b) suggested that the effects of hypoxia were selective for the Ca<sup>2+</sup>-dependent voltage-gated K<sup>+</sup> channel. Speculation arises as to whether this is a species or maturational difference.

In fetal rabbits, (Delpiano & Hescheler, 1989; Hescheler, Delpiano, Acker & Pietruschka, 1989) large outward K<sup>+</sup> currents have also been recorded. It is suggested that these voltage gated K<sup>+</sup> currents are also involved in O<sub>2</sub> chemo-sensing in the fetal rabbit, but further work is necessary to determine their response to chemical stimuli.

#### **1.4.2.b Current theories of the modulation of K<sup>+</sup> channel activity by hypoxia**

Several theories have been suggested to explain the mechanism behind the hypoxic inhibition of K<sup>+</sup> channels. The inhibition of K<sup>+</sup> channels is believed to be the first step in O<sub>2</sub> sensing due to the speed of the response (for review see Gonzalez et al, 1994). The speed at which hypoxia inhibits K<sup>+</sup> channels has been estimated by comparing the effect of tetrodotoxin (TTX) to block Na<sup>+</sup> channels. TTX blocks Na<sup>+</sup> channels and inhibits Na<sup>+</sup> current within a few hundred milliseconds. The hypoxic inhibition of K<sup>+</sup> current occurs more quickly than the TTX inhibition of Na<sup>+</sup> current (for review see Gonzalez et al, 1994). One possibility is that hypoxia involves the generation of cyclic AMP (cAMP).

Cyclic AMP has been shown to rise in type I cells during hypoxia (Wang, Cheng, Yoshizaki, Dinger & Fidone, 1991a; Dinger, Wang, Chen, Wang, Hanson, Stensaas &

Fidone, 1993). Incubation of rabbit carotid bodies in hypoxic solutions *in vitro* elevated cAMP levels in proportion to the severity of the hypoxia (Wang et al, 1991a). Carotid bodies studied 10d after CSN denervation also showed elevated basal levels of cAMP, and the hypoxic response was also increased. Furthermore, these elevated levels of cAMP observed during hypoxia have been located by immunohistochemical techniques to occur in the type I cell (Wang, Stensaas, de-Vente, Dinger & Fidone, 1991b). Interestingly, cAMP has also been shown to have an effect on K<sup>+</sup> currents in rabbit type I cells (López-López JR, De-Luis DA & Gonzalez, 1993). As previously demonstrated the outward K<sup>+</sup> current was inhibited by low P<sub>O</sub><sub>2</sub>, and cAMP similarly reduced the amplitude of this current. The effect was not voltage dependent and a reduction of the K<sup>+</sup> current was also observed by the action of forskolin, which accelerates the action of adenylate cyclase in the formation of cAMP. These observations provide compelling evidence for a rise in cAMP observed during hypoxia in mediating the inhibition of K<sup>+</sup> channels.

An alternative theory proposed is that K<sup>+</sup> channels are inhibited by hypoxia via an effect on NAD(P)H oxidase (Cross, Henderson, Jones, Delpiano, Hentschel & Acker, 1990; Acker, Bolling, Delpiano, Dufau, Gorlach & Holtermann, 1992). Kummer & Acker (1995) have demonstrated immunohistochemically the presence of NAD(P)H oxidase in type I cells from humans, guinea pigs and rats. It is proposed that H<sub>2</sub>O<sub>2</sub> can be generated in the type I cell during hypoxia by an electron transferring chain involving the cytochrome b (Cross et al, 1991; Acker et al, 1992). The cytochrome is reduced by hypoxia, thus it does not take part in energy production, rather it is suggested that cytochrome b (as part of the NAD(P)H oxidase) is an O<sub>2</sub> sensor (Acker et al, 1992). In this model, NAD(P)H oxidase produces H<sub>2</sub>O<sub>2</sub> during hypoxia, which is presumed to be reduced to H<sub>2</sub>O by glutathione oxidase, with a concomitant increase in the reduced glutathione. It is the elevation of glutathione that is suggested to directly inhibit the K<sup>+</sup> channels (for review see Peers & Buckler, 1995). Currently it is not known which of these models is most likely in the sensing of low O<sub>2</sub>.

#### 1.4.2.c Hypoxia leads to an increase in intracellular Ca<sup>2+</sup> in type I cells

##### *Evidence for the hypoxia-induced rise in Ca<sup>2+</sup><sub>i</sub> arising from an intracellular store*

As described in section 1.4.1.d, Biscoe & Duchon (1990) measured the rise in Ca<sup>2+</sup><sub>i</sub> during anoxia in dissociated type I cells of adult rabbit carotid body. They suggested that this release of Ca<sup>2+</sup> was from an intracellular store, which they believed to be the mitochondria. Cyanide, which disrupts the electron transport system in oxidative metabolism, and FCCP, which uncouples mitochondrial metabolism by abolishing the proton gradient across the inner mitochondrial membrane, also produced a rise in Ca<sup>2+</sup><sub>i</sub>

supporting their theory that the mitochondria was the site of  $\text{Ca}^{2+}$  release. During anoxia there was an increase in voltage gated  $\text{K}^+$  conductance with no change in overall membrane conductance, suggesting an activation of  $\text{K}^+$  channels by hypoxia.

In a further series of experiments in adult rabbits, Duchen & Biscoe (1992a, 1992b) provided further evidence for the role of the mitochondria in the response to  $\text{O}_2$  and a correlation between changes in mitochondrial metabolism and  $\text{Ca}^{2+}_i$ . Duchen & Biscoe (1992a, 1992b) used autofluorescence to measure mitochondrial function and membrane potentials. They measured NAD(P)H autofluorescence during anoxia, and found graded increases in NAD(P)H autofluorescence, which reflected an increase in the NAD(P)H / NAD(P) ratio. During oxidative metabolism, NAD(P)H is converted to NAD(P), and donates two electrons to the electron transport system which in turn produces ATP. A relative increase in NAD(P)H / NAD(P) suggests less oxidative metabolism, a consequence of hypoxia. NAD(P)H levels were especially affected below a  $\text{P}_{\text{O}_2}$  of 60mmHg, and this was important because it demonstrated a change in mitochondrial function over the physiological range implicating a possible role in  $\text{O}_2$  sensing. Hypoxia raised  $\text{Ca}^{2+}_i$  and they demonstrated that this change was not the cause for an increase in NAD(P)H: hypoxia induced changes in NAD(P)H were still observed in  $\text{Ca}^{2+}$ -free media, suggesting that it was released from an intracellular store (Duchen & Biscoe, 1992a).

They also investigated mitochondrial membrane potential using the fluorescence agent Rhodamine 123 (Duchen & Biscoe, 1992b). Depolarization results in a quenching of Rhodamine 123, and an increase in the fluorescence signal. The mitochondrion maintains a large negative membrane potential due to the proton motor force of the inner mitochondrial membrane, and this is responsible for the production of ATP (for review see Mitchell, 1985). Anoxia, cyanide and FCCP all increased the fluorescence signal. Similar to their observations of the effect of hypoxia on NAD(P)H, graded reductions in  $\text{P}_{\text{O}_2}$  below *ca.* 60mmHg produced a graded depolarization of the mitochondrial membrane potential. They provided evidence which showed that the rise in  $\text{Ca}^{2+}_i$  and mitochondrial depolarization were closely linked. Firstly, the rise in  $\text{Ca}^{2+}_i$  is temperature-sensitive (Biscoe & Duchen, 1990) as was the depolarization during hypoxia, which was greater at warmer temperatures (Duchen & Biscoe, 1992b). Secondly, the graded hypoxia-induced changes in  $\text{Ca}^{2+}_i$  occurred over a similar range to the membrane depolarizations. Thirdly, oligomycin (which blocks the action of the membrane bound ATP synthesising enzyme) raised  $\text{Ca}^{2+}_i$ , suggesting alterations in ATP production could link the changes in  $\text{Ca}^{2+}_i$  and mitochondrial membrane potential.

*Evidence for the hypoxia-induced rise in  $Ca^{2+}_i$  arising from an extracellular store*

Other workers have suggested that the hypoxia-induced rise in intracellular  $Ca^{2+}$  is not from mitochondrial release or some other intracellular store. In adult rabbits type I cells, Obeso et al. (1992) investigated the role of voltage-dependent  $Ca^{2+}$  channels mediating the rise in  $Ca^{2+}_i$ . High extracellular  $K^+$  released dopamine (this is discussed further in section 1.4.2.d), and this was 95% dependent on extracellular  $Ca^{2+}$ . Furthermore, dihydropyridine antagonists reduced the dopamine release by 90-100%, indicating the process was energy dependent, and this was further shown by a potentiation of dopamine release with dihydropyridine agonists. The use of the channel blocker nisoldipine, selective for L-type  $Ca^{2+}$  channels, also reduced the effect on dopamine release by high extracellular  $K^+$ . They concluded that voltage-gated  $Ca^{2+}$  channels sensitive to dihydropyridines were the main route of entry for  $Ca^{2+}$  influx. However the residual release of dopamine in high extracellular  $K^+$  after blockade of L-type  $Ca^{2+}$  channels suggested a possible role, albeit smaller, for the  $Na^+-Ca^{2+}$  ion exchange.

Buckler & Vaughan-Jones (1994a) measured membrane potentials and  $Ca^{2+}_i$  from isolated neonatal rat type I cells. They showed that graded reductions in  $P_{O_2}$  between 160 and 0 torr produced graded increases in  $Ca^{2+}_i$ . The increase in  $Ca^{2+}_i$  was virtually abolished by exposure to  $Ca^{2+}$ -free media, which led them to suggest that the rise in  $Ca^{2+}_i$  observed during hypoxia was mediated by influx of extracellular  $Ca^{2+}$ . Furthermore, they showed by blockade of  $Ca^{2+}$  channels with nicardipine (selective for L-type channels), that the hypoxia-induced rise in  $Ca^{2+}_i$  was reduced by 67%. The non-selective  $Ca^{2+}$  channel blocker,  $Ni^{2+}$ , reduced the hypoxia-induced rise in  $Ca^{2+}_i$  by 77%. Together this evidence suggested a strong role for voltage-gated  $Ca^{2+}$  channels in the rise of  $Ca^{2+}_i$ . They also observed that type I cells were depolarized during hypoxia and this occurred simultaneously with a rise in  $Ca^{2+}_i$ . When the type I cells were voltage clamped close to resting membrane potential (-40 to -60mV), cells were no longer depolarized during hypoxia, however some residual rise in  $Ca^{2+}_i$  was observed. The rise was slower and reached a lower level compared to un-clamped conditions, and was accompanied by an inward current into the cell. The authors concluded that hypoxia depolarized the membranes of type I cells probably by affecting the  $K^+$  channels, which in turn activated voltage-gated  $Ca^{2+}$  channels and led to a rise in  $Ca^{2+}_i$ . Whilst this was the main mechanism, they could not rule out the contribution some other  $Ca^{2+}$  channel made to the rise in  $Ca^{2+}_i$  which could be the  $Na^+-Ca^{2+}$  ion exchanger described in the adult rabbit (Obeso et al, 1992). They discard Biscoe & Duchen's (1990) theory that the mitochondria were responsible for the release of  $Ca^{2+}_i$ , but do not deny the possible role of the mitochondria in  $O_2$  sensing.

#### 1.4.2.d Hypoxia-induced rise in intracellular $\text{Ca}^{2+}$ leads to neurosecretion

As mentioned in section 1.4.2.c, Obeso et al. (1992) observed that elevation of  $\text{Ca}^{2+}_i$  led to the release of dopamine. Furthermore, low  $\text{P}_{\text{O}_2}$  (0-49 torr) caused a release of dopamine and this was 95% dependent on extracellular  $\text{Ca}^{2+}$ . The hypoxia-induced release of dopamine was blocked by the action of nisoldipine, selective for L-type  $\text{Ca}^{2+}$  channels, by 79% at 49 torr and 20% at 0 torr. Again, they observed that the hypoxia-induced release of dopamine was dihydropyridine sensitive. That the release of dopamine to low  $\text{P}_{\text{O}_2}$  was dihydropyridine sensitive, contrasted with their observations that dopamine release in response to high  $\text{P}_{\text{CO}_2}$ /low pH was not dihydropyridine sensitive, a finding which lent further support to the presence of  $\text{Na}^+$ - $\text{Ca}^{2+}$  ion exchange. They concluded that  $\text{Ca}^{2+}$  influx in moderate hypoxia was predominantly via the voltage-gated  $\text{Ca}^{2+}$  channels, however in severe hypoxia  $\text{Ca}^{2+}$  influx also occurred via the  $\text{Na}^+$ - $\text{Ca}^{2+}$  ion exchange.

Thus it appears that the neurotransmitter responsible for the transduction of low  $\text{O}_2$  is dopamine, as is the case for the transduction of acid stimuli or  $\text{CO}_2$ .

#### 1.4.3 The bicarbonate hypothesis

The bicarbonate hypothesis has been proposed to explain the interaction between  $\text{CO}_2$  and  $\text{O}_2$  that occurs at the carotid chemoreceptor level. It also explains the observed difference between the transient chemoreceptor response to  $\text{CO}_2$  and the steady state chemoreceptor response (Torrance, 1976; Torrance, Bartels & McLaren, 1993). As I have mentioned previously, multiplicative interaction occurs between  $\text{CO}_2$  and  $\text{O}_2$ , so that at lower  $\text{P}_{\text{O}_2}$ s the sensitivity to  $\text{CO}_2$  is increased (Fitzgerald & Parks, 1971). This was the case for the steady state response to  $\text{CO}_2$ . The dynamic response to  $\text{CO}_2$  is different. Black et al. (1971) showed that the carotid chemoreceptor response adapts to a rapid increase in  $\text{CO}_2$ . Furthermore, Band & Wolff (1978) showed that the transient chemoreceptor response to  $\text{CO}_2$  was in fact from a family of parallel response curves, that were applicable for any  $\text{PaO}_2$  or  $\text{PaCO}_2$ . This was further confirmed by Kumar, Nye & Torrance (1988) who showed that the slope of the transient chemoreceptor response to an oscillation in inspired  $\text{CO}_2$  was steeper than the steady state chemoreceptor response and independent of the mean level of  $\text{PaCO}_2$ . Hence, they were parallel at different  $\text{PaCO}_2$  levels (this is also discussed in section 7.1). So from the family of transient response curves, and the fan of steady state chemoreceptor response curves, it can be inferred that the chemoreceptor response to  $\text{CO}_2$  increases briskly at first and then adapts to the steady state level. This adaptation is great in

hyperoxia and much less in hypoxia (Torrance et al, 1993). The bicarbonate hypothesis has been used to explain these findings.

Acid-sensing hypothesis for the method of chemoreception was first proposed by Winder (1937) who suggested that nerve endings were sensitive to acid and that during hypoxia they became exposed to lactic acid via glycolysis. Torrance's bicarbonate hypothesis suggests that  $\text{CO}_2$  and  $\text{O}_2$  converge at a common pH, and that  $\text{CO}_2$  changes pH by its hydration to  $\text{H}^+$  and  $\text{HCO}_3^-$  by carbonic anhydrase, and that  $\text{O}_2$  changes pH by some membrane bound oxygen sensing mechanism. As discussed in section 1.4.1.a, intracellular pH is the stimulus for acid chemotransduction (Hanson et al, 1981). Adaptation has been described in terms of a pumping mechanism to maintain intracellular pH in the type I chemoreceptor cells. That the hydration of  $\text{CO}_2$  by CA, to  $\text{H}^+$  and  $\text{HCO}_3^-$ , is rapid has been mentioned and it is supposed that  $\text{pH}_i$  is  $\text{P}_{\text{O}_2}$  dependent. Whilst a fall in  $\text{pH}_i$  can occur rapidly by the action of CA, the pumping of  $\text{H}^+$  out of the cell to regulate  $\text{pH}_i$  occurs more slowly (Torrance et al, 1993). The chemoreceptor response to a change in  $\text{pH}_i$  is brisk and linear, but the extrusion of  $\text{H}^+$  from the cell by the pump will result in adaptation. During hyperoxia when adaptation is great, the time taken to reach a steady level can be related to the greater work required by the pump to lower  $[\text{H}^+]_i$ . During hypoxia, the action of the pump is less. The hypothesis assumes that there is a linear relationship between the response of nerve endings and  $\text{pH}_i$  or  $\text{CO}_2$ , thus in the steady state  $\text{pH}_i$  must be held constant as is  $\text{CO}_2$  and  $\text{HCO}_3^-$ .

It is less clear how the level of  $\text{P}_{\text{O}_2}$  determines the degree of adaptation. The relationship between  $\text{P}_{\text{O}_2}$  and chemoreceptor discharge is hyperbolic. In normoxia and hyperoxia, the chemoreceptor response curve is flat so that a brisk increase in  $\text{CO}_2$  would be compensated for by intense adaptation. In hypoxia the slope of the hyperbola is greater and the adaptation to  $\text{CO}_2$  is less. At present it is not known how the degree of adaptation is determined in relation to the  $\text{P}_{\text{O}_2}$ .

Torrance et al. (1993) proposed that a membrane bound oxygen sensing mechanism somehow stabilizes the response to  $\text{CO}_2$  and that interaction between the stimuli could be explained in terms of convergence at some  $\text{pH}_i$ . It is also feasible that the regulation of  $\text{Ca}^{2+}_i$  is a means by which interaction could occur, as both hypoxia and hypercapnia raise  $\text{Ca}^{2+}_i$ . Thus, interaction between the stimuli could be explained in terms of convergence at some  $[\text{Ca}^{2+}_i]$ . One possible mechanism for  $\text{Ca}^{2+}$  influx is by membrane depolarization and the activation of voltage gated  $\text{Ca}^{2+}$  channels (Buckler & Vaughan-Jones, 1994a, 1994b). If for instance hypoxia inhibits  $\text{K}^+$  channels as suggested by

Peers & Green (1991), and during hypercapnia the influx of  $\text{Ca}^{2+}$  occurs either by voltage gated  $\text{Ca}^{2+}$  channels or  $\text{Na}^+$ - $\text{Ca}^{2+}$  ion exchange, then interaction between  $\text{CO}_2$  and  $\text{O}_2$  could occur by activation of more voltage gated  $\text{Ca}^{2+}$  channels. Adaptation could be viewed in terms of maintaining a constant level of  $\text{Ca}^{2+}_i$ . During hyperoxia either less  $\text{Ca}^{2+}$  influx occurs, or  $\text{Ca}^{2+}$  contributing to the rise in  $\text{Ca}^{2+}_i$  due to transient increases in  $\text{CO}_2$  is pumped out. Biscoe & Duchon (1990) did find evidence for an interactive effect between  $\text{CO}_2$  and  $\text{O}_2$  in elevating  $\text{Ca}^{2+}_i$ . As discussed above, their evidence led them to speculate that  $\text{Ca}^{2+}$  was released intracellularly from the mitochondria.

There is some evidence to suggest that hypoxia does not result in acidification of  $\text{pH}_i$ , however the reports are contradictory. Garcia-Sancho, Giraldez & Belmonte (1978) observed little or no change in  $\text{pH}_i$  during 10%  $\text{O}_2$  exposure in slice preparations of carotid bodies studied *in vitro*. Iturriaga, Rumsey, Lahiri, Spengel & Wislon (1992) measured  $\text{pH}_i$  and chemosensory discharge of cat carotid bodies in a superfused preparation. They did not find a fall in  $\text{pH}_i$  during hypoxia ( $\text{P}_{\text{O}_2}$  52 torr), however chemoreceptor discharge was increased. Wilding et al. (1992) did not observe a change in  $\text{pH}_i$  in superfused rat type I cells with exposure to 2%  $\text{O}_2$ . Mokashi, Ray, Botre, Katayama, Osanai & Lahiri (1995) found that cultured type I cells from cat and rat carotid bodies did not show a significant fall in  $\text{pH}_i$  in response to hypoxia (from 130 to 1-2 torr), however  $\text{pH}_i$  fell in response to a fall in extracellular pH. Other workers contradict this finding. He, Wei & Eyzaguirre (199b) found that in 60% of cell clusters from cultured cat carotid bodies, a reduction in  $\text{P}_{\text{O}_2}$  from 300 to 120 torr reduced  $\text{pH}_i$ . This  $\text{P}_{\text{O}_2}$  level in this preparation was not sufficiently low to be termed hypoxia, although  $\text{P}_{\text{O}_2}$  in the cells was not known. Neither was it possible to differentiate between type I cells and sustentacular cells. Pang & Eyzaguirre (1993) found in cultured clusters and isolated glomus cells from rat and cat carotid bodies that hypoxia ( $\text{P}_{\text{O}_2}$  2-30 torr) either increased or decreased  $\text{pH}_i$ , but usually lowered it.

If it is the case that hypoxia does not result in acidification of  $\text{pH}_i$ , then clearly  $\text{O}_2$  and  $\text{CO}_2$  cannot interact at some common pH, and the bicarbonate hypothesis needs some modification. In this respect the findings of Biscoe & Duchon (1990) are very interesting. Could  $\text{O}_2$  and  $\text{CO}_2$  interact by controlling  $\text{Ca}^{2+}_i$ ? Several mechanisms have been proposed for the elevation in  $\text{Ca}^{2+}_i$ , as I have outlined above, and it is interesting to speculate that the phenomenon described by the 'bicarbonate hypothesis' may not be due to a convergence at some pH, but in fact at some  $\text{Ca}^{2+}_i$ .

## 1.5 Maturation of peripheral chemoreceptor function

For many years there was debate over the role of the arterial chemoreceptors in the fetus. With the discovery that fetal breathing was not under reflex control, it was questioned if indeed the peripheral chemoreceptors were active *in utero*. There is now evidence to show that the fetus does respond to a reduction in PaO<sub>2</sub> below 25mmHg and also to a rise in PaCO<sub>2</sub>. A role for the chemoreceptors has also been found in the cardiovascular response to hypoxia.

At birth, the peripheral chemoreceptors are not required for the onset of breathing. Postnatally the chemoreceptors reset their sensitivity to oxygen and carbon dioxide, and the afferent limb of the chemoreflex plays an important role in the regulation of breathing in the newborn. Numerous studies have investigated the postnatal maturation of chemoreceptor sensitivity to hypoxia, however less is known of the postnatal changes in CO<sub>2</sub> sensitivity. Some of these studies are discussed in this section, and further detail is given in section 6.1 and 7.1. Chapters 6 and 7 are dedicated to the investigation of the chemoreceptor response to CO<sub>2</sub> in the newborn lamb, and as the literature is relevant to the experiments I have performed, I have incorporated discussion of it into these Chapters in preference to Chapter 1.

### 1.5.1 Peripheral chemoreceptors in utero: The fetal response to hypoxia and hypercapnia

The fetal sheep has been used extensively to study fetal breathing and the chemoreflex response to hypercapnia and hypoxia (Barcroft & Barron, 1937a, 1937b; for review see Harding, 1994). In the fetal sheep full term is 147d, and fetal breathing movements (FBM) have been reported from as early as 40d (Barcroft & Barron, 1937a, 1937b). In this section I will briefly discuss fetal breathing movements *in utero*, and the current knowledge of their role and origin. Also, I will briefly discuss the homeostatic role of the peripheral chemoreceptors *in utero*.

#### 1.5.1.a General characteristics of FBM

The fetal breathing (commonly referred to as FBM) was described by Dawes, Fox, Leduc, Liggins & Richards (1972) as "*rapid irregular breathing, consisted of bursts of activity of very much higher frequency (1-4Hz) lasting from a few seconds to an hour; the inspiratory movements were irregular both in the rate and depth*". Early in gestation FBM are nearly continuous, however over the course of gestation the

occurrence of FBM decreases and in the late gestation fetus, breathing movements are episodic (Maloney, Adamson, Brodecky, Cranage, Lambert & Ritchie, 1975). FBM are not essential for gaseous exchange, rather they are important in terms of the anatomical development of the lung (Alcorn, Adamson, Maloney & Robinson, 1980; Fewell, Lee & Kitterman, 1981). Section of the phrenic nerve abolishes FBM, and results in pulmonary hypoplasia and atrophy of the diaphragm (Alcorn et al, 1980; Fewell et al, 1981). In contrast, bilateral vagotomy does not affect the incidence, size or frequency of FBM indicating that they are not under mechanoreflex control (Dawes et al, 1972; Boddy, Dawes, Fisher, Pinter & Robinson, 1974). However the vagus does mediate the stimulation of FBM in response to a reduction in intra-tracheal pressure (Ponte & Purves, 1973). FBM occur during low voltage ECoG or rapid eye movement sleep (REM), and are virtually absent during high voltage ECoG (analogous to non-rapid eye movement sleep, NREM) (Dawes et al, 1972). The episodic nature of FBM that becomes apparent in mid to late gestation is related to the differentiation of the ECoG into low and high voltage states (Dawes et al, 1972; Ioffe, Jansen, Russell & Chernick, 1980). Hypoxia inhibits the occurrence of FBM (Snyder & Rosenfeld, 1937; Boddy et al, 1974) and hypercapnia stimulates them (Boddy et al, 1974; Bowes, Wilkinson, Dowling, Ritchie, Brodecky & Maloney, 1981; Jansen, Ioffe, Russell & Chernick, 1982; Koos & Sameshima, 1988). Denervation of the carotid sinus nerves showed that the peripheral arterial chemoreceptors were not necessary for the genesis of FBM *in utero* (Jansen, Ioffe, Russell & Chernick, 1981; Koos & Sameshima, 1988; Moore, Parkes, Nijhuis & Hanson, 1989). Thus, FBM did not appear to be under any reflex control as neither section of the vagi, or of the carotid sinus nerve affected their occurrence.

#### **1.5.1.b Reduction in the incidence of FBM during hypoxia: an appropriate response of the fetus to a decrease in oxygen availability**

The reduction in the incidence of FBM during hypoxia is well documented (Snyder & Rosenfeld, 1937; Boddy et al, 1974; Jansen et al, 1981). This finding was surprising to early investigators of fetal breathing because the neonate was known to increase ventilation in response to hypoxia, at least in the initial stage of the ventilatory response (Cross & Warner, 1951; Cross & Oppé, 1952). However, it subsequently became apparent that this was an appropriate response for the fetus if oxygen availability was reduced.

Parer (1980) observed that fetal oxygen consumption fell by more than half during acute maternal hypoxia in the sheep and that the fall was proportional to the degree of hypoxia. Furthermore Natale, Clewlow & Dawes (1981) showed that fetal hypoxaemia was associated with a reduction in forelimb movement, in keeping with the conservation

of oxygen consumption observed by Parer (1980). Blanco, Dawes & Walker (1983) found that during hypoxia in the fetal sheep the hind limb reflex was depressed. Electrical stimulation of the sciatic nerve produced a reflex response during normoxia which was greatest in high voltage ECoG (NREM sleep). During fetal hypoxia (PaO<sub>2</sub> 12mmHg) the reflex was depressed, but not when the spinal cord had been sectioned at L1-2. This showed not only that hypoxia was associated with a decrease in body movements, but that the inhibition was mediated by the influence of the brainstem on spinal pathways.

### **1.5.1.c Central origin of fetal breathing movements**

Barcroft & Karvonen (1948) found that the fetal sheep was unresponsive to CO<sub>2</sub> before 43-49d gestation, but at 60-69d it induced breathing movements. In contrast, Bowes et al. (1981) found that there was no increase in CO<sub>2</sub> responsive between 105d and 138d. Hypercapnia increases the occurrence of FBM and also increases their frequency and the pressure generated in tracheal fluid by the breathing movement (Jansen et al, 1982; Koos & Sameshima, 1988). Moreover, hypercapnia increases the incidence of low voltage ECoG (REM sleep). This evidence led to speculation that FBM were of a central origin.

Dawes, Gardner, Johnston & Walker (1983a) performed brainstem transections in the upper pons in fetal sheep at 118-123d gestation and measured responses to hypoxia after recovery. Two types of transections were made:- caudally through the upper pons or the colliculi, or rostrally through the caudal hypothalamus or anterior commissure/suprachiasmatic nucleus. They hypothesized that the episodic nature of FBM in late gestation was related to the differentiation of sleep states into low and high voltage ECoG, and that high voltage ECoG directly inhibited FBM; performing brainstem transection would remove the descending inhibition on FBM. They found that both rostral and caudal transections dissociated FBM from ECoG, however only the caudal sections consistently increased the incidence of FBM so that it become continuous. Hypoxia caused an increase in the incidence of FBM in the caudally transected fetuses, but not in those transected rostrally. Carotid sinus nerve section performed in 2 fetuses at the time of caudal section did not affect the stimulation of FBM, however they were not vagotomized. This led the authors to speculate that the area associated with the hypoxic inhibition of breathing in intact fetuses was suprapontine.

Brainstem transection experiments were repeated by Gluckman & Johnston (1987) and Moore et al. (1989). Gluckman & Johnston (1987) performed lesions in the midbrain

and upper pons in fetal sheep 119-121d gestation using an electrode positioned stereotaxically and passed a current of 18mA continuously for 20-30sec. Lesions in the upper pons removed the inhibition of FBM observed during hypoxia in intact fetuses, but did not produce continuous breathing. Lesions that were not performed bilaterally, or were more caudal, did not remove the hypoxic depression of FBM. Gluckman & Johnston (1987) concluded that the integrity of a discrete locus in the lateral pons, and at the level of the cerebellar peduncles, was required for the hypoxic depression of FBM. Moore et al. (1989) chemodenervated, and vagotomized fetuses (123-137d) to remove any input from the carotid and aortic chemoreceptors. Brainstem transections were performed in the upper pons, through a craniotomy in the occipital bone, but histological evidence was not provided to locate the exact site. They found that 3 of 8 fetuses continued to breathe during hypoxia, despite peripheral chemoreceptor denervation, and concluded that the peripheral chemoreceptors were not necessary for the inhibitory effect of hypoxia on FBM.

In a further study in fetal sheep (119-1221d gestation), Johnston & Gluckman (1993) bilaterally lesioned (3-10mA for 45-60sec) the lateral pons with stereotaxic coordinates. In 14 of 29 fetuses, bilateral lesions in the rostral lateral pons removed the hypoxic inhibition of FBM. Hypoxia inhibited FBM in 15 fetuses, and the site of the lesion was later determined histologically to not encompass the rostral lateral pons bilaterally. In the 14 fetuses in which the lesions were 'successful', hypoxic responses after peripheral chemodenervation (carotid sinus nerve section and vagotomy) or sham operation were measured. A stimulation of FBM was observed in sham operated fetuses (n=4) during hypoxia, and chemodenervation abolished this stimulatory effect (n=6). Johnston & Gluckman (1993) concluded that the stimulation of FBM observed after lesions in the rostral lateral pons during hypoxia was mediated by the peripheral chemoreceptors and that this was evidence for tonic chemoreceptor-mediated influences on FBM. They speculated that the earlier work of Dawes et al. (1983a) failed to remove the input from the aortic chemoreceptors, and hence carotid sinus nerve section alone was insufficient to abolish the stimulation of FBM. Furthermore, they commented that Moore et al. (1989) were unable to test the effectiveness of their transections, because they were performed at the same time as CSN section and vagotomy. They also questioned the 'completeness' of the chemodenervation.

In the neonate during hypoxia, there is an initial stimulation of breathing mediated by the peripheral chemoreceptors (Martin-Body & Johnston, 1988; see also section 3.1.3.a). After *ca.* 2min there is a fall in ventilation believed to be mediated by the same mechanism that produces an inhibition of breathing in the fetus (Williams & Hanson, 1989; Martin-Body, 1988; see also section 3.1.3.a). More recently, there is preliminary

evidence to suggest that a site rostral to the pons may be involved in the hypoxic depression of breathing (Ackland, Waites, Noble & Hanson, 1995; Waites, Ackland, Noble & Hanson, 1995). Ackland et al. (1995) measured the ventilatory response to acute isocapnic hypoxia in neonatal rabbits (28-35d). Ackland et al. (1995) electrolytically lesioned the midbrain bilaterally and found that the hypoxic depression of breathing was abolished. They were subsequently able to identify their sites of lesion histologically, and confirmed that they were made in the red nucleus. Microinjections of L-glutamate in the red nucleus also had an inhibitory effect on respiratory output (Waites et al, 1995). The authors concluded that cell bodies in the red nucleus played a key role in the inhibitory effect on breathing. Future work is needed to elucidate the mechanisms involved.

#### 1.5.1.d Are the peripheral arterial chemoreceptors functional in utero?

These observations on the effect of respiratory stimuli on the occurrence of FBM led to speculation on the role of the arterial chemoreceptors in the control of fetal breathing. Early evidence for the role of the arterial chemoreceptors *in utero* was conflicting. Cross & Malcolm (1952) recorded carotid chemoreceptor activity from two fetal lambs 7-8d before term. They concluded "*the patterns of response of the chemoreceptor fibres were found to be similar to those in adult animals*" and that chemoreceptor discharge was abolished upon administration of 100% O<sub>2</sub>. No detail was given on the levels of hypoxia used. Biscoe, Purves & Sampson (1969) recorded carotid chemoreceptor activity from fetal lambs aged 130-147d (n=8), but were unable to find any chemoreceptor activity in lambs (n=4) aged 120-125d. They found spontaneous chemoreceptor activity in the carotid sinus nerve to be sparse. In the older lambs, chemoreceptor discharge increased in response to umbilical cord occlusion but no response was seen when PaO<sub>2</sub> increased from 30 to 90mmHg. A small increase in discharge was seen in 3 fetuses in response to potassium cyanide, but not to sodium cyanide intra-arterial injections, neither was there a response to nicotine injections. There was a small increase in discharge with electrical stimulation of the sympathetic nerve supply to the carotid body. Biscoe et al. (1969) concluded that the fetal carotid chemoreceptors were relatively insensitive to chemical stimuli.

Jansen, Purves & Tan (1978) measured chemoreceptor discharge in fetal lambs within 5d of term. They found chemoreceptor responses in 8 of 20 fetuses to sodium cyanide, lactic acid, hypoxia, hypercapnia and sympathetic stimulation. Chemoreceptor activity increased with umbilical cord occlusion and they commented that this was quantitatively similar to that of the adult cat. When carotid bodies were removed from fetal lambs and studied *in vitro* using a superfused preparation (Eyzaguirre & Lewin,

1961), chemoreceptor discharge was plentiful. Jansen et al. (1981) concluded that there was an increase in hypoxia sensitivity that occurred around that time of birth and speculated that a re-distribution of blood flow in the carotid body may play a role in this maturation. They ruled out the possibility that sympathetic innervation of the carotid body was responsible for the change in hypoxia sensitivity.

Further evidence was obtained by Blanco et al. (1984a) that showed spontaneous chemoreceptor discharge (to a maximum of 5Hz) was present at PaO<sub>2</sub> 25mmHg in fetal lambs from as early as 90d gestation (90-143d). Discharge increased by 200-500% as PaO<sub>2</sub> was reduced to *ca.* 10mmHg. Thus, it is likely that the lack of response observed by Biscoe et al. (1969) to hypoxia (PaO<sub>2</sub> 30mmHg) was due to a relatively higher PaO<sub>2</sub>. Blanco et al. (1984a) found that chemoreceptor discharge was also increased when CO<sub>2</sub>-equilibrated saline was injected retrogradely into the lingual artery, or when the umbilical cord was occluded. These results provided good evidence for the carotid chemoreceptors being active *in utero*. Blanco et al. (1984a) also showed in one fetus aged 135d, from recordings of the vagus nerve, that aortic chemoreceptors were responsive to umbilical cord occlusion.

Aortic chemoreceptor activity has been recorded in the fetal sheep (Ponte & Purves, 1973). Chemoreceptor activity (0.9-4.5Hz) was present at normal fetal blood gas values (PaO<sub>2</sub> 22-25mmHg; PaCO<sub>2</sub> 39-45mmHg), and increased by *ca.* 40% when PaO<sub>2</sub> was lowered to 13-17mmHg. Aortic chemoreceptors increased discharge in response to NaCN and nicotine, and discharge was also doubled with umbilical cord occlusion. The aortic chemoreceptors exert an effect on cardiovascular control in the fetus. Dawes, Lewis, Milligan, Roach & Talner (1968) injected NaCN into the left atrium of fetal sheep and concluded that the cardiovascular response was mediated via the aortic chemoreceptors, which they later confirmed in an additional series of experiments. The rise in arterial blood pressure and femoral vasoconstriction observed during hypoxia in the fetus was not abolished by carotid sinus nerve section, but was abolished by vagotomy (Dawes, Duncan, Lewis, Merlet, Owen-Thomas & Reeves, 1969). Although the authors proposed a role for the aortic chemoreceptors in fetal cardiovascular control, more recent studies implicate the carotid chemoreceptors as the main afferent pathway for the peripheral vasoconstriction (Giussani, Spencer, Moore, Bennet & Hanson, 1993; see section 1.5.1.e).

The central chemoreceptors are also responsive to CO<sub>2</sub> *in utero*. Hypercapnia will stimulate FBM even in chemodenervated fetal sheep (Koos & Sameshima, 1988). Hohimer, Bissonnette, Richardson & Machida (1983) made ventriculocisternal perfusion of mock CSF with variations in HCO<sub>3</sub><sup>-</sup> concentrations. Perfusions with a low

$[\text{HCO}_3^-]$  increased the incidence of FBM and perfusions with a high  $[\text{HCO}_3^-]$  decreased the incidence of FBM, indicating that the central chemoreceptors could exert a tonic influence on FBM.

### 1.5.1.e Role of chemoreceptors in cardiovascular control in the fetus

Although the peripheral chemoreceptors do not play a role in the genesis of FBM in the intact fetus, they do play an important role in fetal cardiovascular control. The fetal cardiovascular response to an acute hypoxic insult is characterized by bradycardia, a gradual increase in arterial blood pressure and peripheral vasoconstriction (Giussani et al, 1993; Bartelds, van Bel, Teitel & Rudolph, 1993; for review see Giussani, Spencer & Hanson, 1994). The carotid chemoreflex mediates the peripheral vasoconstriction to acute isocapnic hypoxia in the fetus (Giussani et al, 1993). The aortic chemoreceptors appear to play a negligible role in the initiation of the reflex response (Bartelds et al, 1993).

## 1.5.2 Resetting of carotid body chemosensitivity postnatally

### 1.5.2.a Changes in arterial blood gas

It is perhaps relevant to consider the changes in arterial blood gas around the time of birth to understand the stimuli behind the process of chemoreceptor resetting, and these changes in  $\text{PaO}_2$  and  $\text{PaCO}_2$  from the time of birth are reviewed by Adamson (1991). For the fetus, a  $\text{PaO}_2$  of 25-35mmHg and a  $\text{PaCO}_2$  of 42mmHg are within the normal range (Rurak, 1994). In the human infant,  $\text{PaO}_2$  is *ca.* 22mmHg and  $\text{SaO}_2$  is *ca.* 60% after the first minute of air breathing.  $\text{PaCO}_2$  remains constant at 50mmHg, or may increase slightly (Adamson, 1991). At 10 minutes after birth,  $\text{PaO}_2$  is *ca.* 60mmHg and  $\text{PaCO}_2$  is approaching 40mmHg by 30min (Adamson, 1991). Thereafter,  $\text{PaO}_2$  continues to rise more slowly over the first postnatal week.

### 1.5.2.b Evidence for chemoreceptor resetting

#### *Carotid chemoreceptor resetting of hypoxia sensitivity*

There is now substantial evidence documenting the postnatal increase in hypoxia sensitivity of the carotid body. In this section I will review some of the literature for chemoreceptor resetting obtained from **direct** nerve recordings in neonatal animals both *in vivo* and *in vitro*. I have reviewed this literature in further detail in section 3.1.1 and 3.1.2 as it is pertinent to the experiments in Chapter 3. **Indirect** evidence has arisen

from numerous ventilatory studies in the newborn animal and human infant and is reviewed in section 3.1.3.

With the discovery that the chemoreceptors were not involved in the genesis of FBM, nor were they involved in the inhibition of FBM, speculation arose as to their function *in utero* and at the time of birth. Biscoe & Purves (1967) measured carotid chemoreceptor activity in anaesthetized lambs (n=9) from birth to 5d. They found that discharge was increased when breathing 10% O<sub>2</sub>, and reduced during 100% O<sub>2</sub> breathing. They compared in three lambs aged 18hr, 2.5d and 5d the fall in discharge during 100% O<sub>2</sub> breathing and the time taken to reach 90% of the maximal response. All three showed a fall in discharge of *ca.* 80% which was of *ca.* 16sec duration, and the youngest lamb showed the most rapid response. Their observations led them to conclude that hypoxia sensitivity of the carotid chemoreceptors was mature at birth and they discarded the hypothesis proposed by Miller & Smull (1955) that chemoreceptor reflexes increased in strength postnatally. It is perhaps of note to consider that the 5d old lamb is still relatively insensitive to hypoxia, so it comes of little surprise that the fall in discharge during hyperoxia was similar at 18hr and 5d. In this respect it may have been more useful to compare the response to hypoxia with age, but no data was given to investigate this possibility. Further work went on to discount these initial findings.

As previously mentioned, Blanco et al. (1984a) showed in fetuses that chemoreceptor discharge increased when PaO<sub>2</sub> was reduced below 25mmHg, the cord was occluded or when CO<sub>2</sub> equilibrated saline was injected into the lingual artery. In contrast no spontaneous chemoreceptor discharge was recorded on the day of birth in normoxia, or when the PaO<sub>2</sub> was lowered. There was however a response to CO<sub>2</sub>. On the second postnatal day, spontaneous chemoreceptor discharge was recorded and the steady-state P<sub>o2</sub> response curves for the neonatal lambs were displaced to the left of the those measured in the adult. This was the first direct evidence from nerve recordings that the large increase in arterial P<sub>o2</sub> at birth 'silenced' the chemoreceptors. This led further strength to Miller & Smull's (1955) proposal. Their observations of the respiratory response to hypoxia in newborn infants led them to suggest that the hypoxic chemoreflex was weak at the time of birth and increased postnatally, a process that is now referred to as chemoreceptor 'resetting'.

Further evidence for the process of postnatal chemoreceptor resetting to hypoxia has been obtained *in vivo* in the newborn kitten (Marchal, Bairam, Haouzi, Crance, Di Giulio, Vert & Lahiri, 1992a; Carroll, Bamford & Fitzgerald, 1993), and *in vitro* in the neonatal rat (Kholwadwala & Donnelly, 1992; Pepper, Landauer & Kumar, 1995). These studies have shown, as did Blanco et al. (1984a), that the chemoreceptor P<sub>o2</sub>

response curve is shifted to the left in younger animals studied around the time of birth. The increase in hypoxia chemosensitivity associated with resetting can be attributed to a rightward shift of the curve towards the adult range with increasing postnatal age.

Blanco, Hanson & McCooke (1988) showed that hyperoxia produced by artificial ventilation for a period of 24-31hrs in fetal lambs *in utero* initiated the process of chemoreceptor resetting to hypoxia. After ventilation, fetal lambs were delivered by caesarean section and chemoreceptor recordings made from few fibre preparations. Hyperoxic ventilated lambs showed a greater level of chemoreceptor discharge compared to normoxic ventilated lambs for any given PaO<sub>2</sub>. This demonstrated the importance of an increase in PaO<sub>2</sub> in resetting of chemoreceptor sensitivity to hypoxia.

#### *Carotid chemoreceptor resetting of carbon dioxide sensitivity*

Until recently, much less evidence has been available on the postnatal resetting of chemoreceptor activity to CO<sub>2</sub>. This was the objective of my experiments in Chapters 6 and 7, and I have reviewed the literature in these Chapters. Briefly, Marchal et al. (1992a) provided preliminary evidence for reduced carotid chemoreceptor CO<sub>2</sub> sensitivity in kittens less than 10d compared to kittens older than 10 days. They described a chemoreceptor CO<sub>2</sub> response curve for kittens less than 10d that was displaced to the right of kittens older than 10d. Responses were only recorded in 100% O<sub>2</sub>. Carroll et al. (1993) also measured chemoreceptor CO<sub>2</sub> responses in kittens aged 1wk, 4wk and 8wks. They measured chemoreceptor responses at three P<sub>o2</sub> levels and report an increase in the CO<sub>2</sub> chemoreceptor response with age. They also comment that the increase in discharge at 1wk and 4wks is due to an upward shift of the CO<sub>2</sub> chemoreceptor response curve, and that no multiplicative interaction between CO<sub>2</sub> and O<sub>2</sub> was observed. Pepper et al. (1995) recorded single fibre chemoreceptor activity *in vitro* in adult rat and rat pups aged 5-7d. With increasing hypercapnia, the P<sub>o2</sub> response curve was shifted to the right in the adult but not in the neonate, once again indicating that there was no CO<sub>2</sub>-O<sub>2</sub> interaction in younger animals.

These observations *in vitro* are in agreement with those made from chemoreceptor recordings *in vivo*. The problems associated with these experiments are discussed in section 6.1. To address some of the issues not covered by these experiments I have measured steady state chemoreceptor responses to CO<sub>2</sub> at four different PaO<sub>2</sub> levels over a narrow range of postnatal ages (see section 6.3).

### 1.5.2.c Aortic chemoreceptors

Kumar & Hanson (1989) showed that the aortic chemoreceptors also reset their sensitivity to hypoxia postnatally. Single fibre aortic chemoreceptor activity was recorded in lambs *in vivo* at 1-4d (n=15) and 10-19d (n=15). The  $P_{O_2}$  response curve for older lambs was displaced to the right of younger lambs. Older lambs were also unable to sustain an increase in chemoreceptor discharge below  $PaO_2$  25-30 mmHg.

There is evidence to suggest that aortic chemoreceptors may play a more important role in the respiratory response to  $CO_2$  and  $O_2$  when the influence of the carotid chemoreceptors has been removed. Williams & Hanson (1989) measured the chemoreflex respiratory response at 5d and 10d to alternate breaths of air and  $F_{iO_2}$  0.16 in lambs that had undergone carotid sinus nerve section on postnatal day 1-2. They found that the chemoreflex respiratory response was small in CSN sectioned lambs on day 5 compared to sham-operated lambs, but by day 10 there was some degree of reflex response to the alternations. They concluded that this was due to a compensation of the aortic chemoreceptors.

Smith & Mills (1980) found a similar role for the aortic chemoreceptors after removal of carotid body function in cats. After ligation of the carotid body artery and section of the carotid sinus nerve cats were allowed to recover for up to 315d. CSN section abolished the ventilatory response to hypoxia acutely. An improvement was observed 93-111d after the initial surgery with the ventilatory response reaching 70% of the pre-operative level. By 260-315d there was complete recovery of the hypoxic ventilatory response, and the authors concluded that the respiratory chemoreflex was mediated by the aortic chemoreceptors as the hypoxic ventilatory response was abolished after bilateral vagotomy. This recovery of the peripheral chemoreflex to hypoxia in the cat was greater than reported in the pony (Bisgard, Forster, Orr, Buss, Rawlings & Rasmussen, 1976) or human (Honda, Watanabe, Hasegawa, Myojo, Takizawa, Sugita, Kimura, Hasegawa, Kuriyama, Saito, Satomura, Katsuki & Severinghaus, 1976; Honda, Watanabe, Hashizume, Satomura, Hata, Sakakibara & Severinghaus, 1979). So, in several species there is evidence for a compensation in the contribution aortic chemoreceptors make to the peripheral chemoreflex control of breathing.

### 1.5.3 Mechanisms of chemoreceptor resetting

Although relatively little is known on the mechanisms of chemoreceptor resetting, the role of dopamine has been strongly implicated. It has been shown in newborn lambs

that chemoreceptor resetting can not be attributed to a postnatal change in carotid body vasculature, and hence it is unlikely to be due to an increase in carotid body blood flow (Moore, Clarke, Hanson, Daly & Ead, 1991). Demonstration of carotid chemoreceptor hypoxia resetting *in vitro* confirms that it is not due to external influences (Kholwadwala & Donnelly, 1990; Pepper et al, 1995). I will discuss the evidence for a possible role of dopamine in hypoxia chemoreceptor resetting.

Hervonen, Kanerva, Korkala & Partanen (1972) showed in carotid bodies of newborn rats using formaldehyde-induced fluorescence that the concentration of catecholamines on postnatal day 1 was high, and that it was reduced at 2wks. Dawes, Hanson, Holman & McCooke (1983b) measured the catecholamine content in carotid bodies removed from newborn lambs at <24hr (n=2) and at 8-9d (n=4) and found some interesting preliminary observations. The concentration of adrenaline was greatly reduced from 24hr to 8-9d, and the concentration of dopamine metabolite 3,4- dihydroxyphenylacetic acid (DOPAC) greatly increased. They did not however observe a change in the concentration of dopamine.

Hertzberg, Hellstrom, Lagercrantz & Pequignot (1990) measured catecholamine content of rat carotid bodies and correlated these observations with the postnatal increase in hypoxia chemosensitivity. The respiratory chemoreflex to 100% O<sub>2</sub> breathing was absent on the day of birth, relatively weak at 3d and greater at 7d. Dopamine and noradrenaline content were measured by high performance liquid chromatography in fetuses the day before birth, at 6-12 hrs after birth and at 7d. Dopamine increased substantially from fetal levels to the first postnatal day, but fell to half of this by day 7. There was a decrease in the turnover rate of dopamine and noradrenaline between 0-6hrs and 6-12hrs after birth. Noradrenaline also increased between the day before birth in the fetus and the fourth postnatal day. These observations led the authors to suggest that the increase in hypoxic sensitivity was at least partly related to the decrease in dopamine synthesis and release. This fitted with previous observations that dopamine had an inhibitory effect on baseline ventilation and the hypoxic ventilatory response in the newborn (Maycock, Standaert, Guthrie & Woodrum, 1983) and in the adult (Bisgard, Forster, Klein, Manohar & Bullard, 1980). Furthermore, exogenous dopamine had been shown to inhibit chemosensory discharge in the adult (Bisgard, Mitchell & Herbert, 1979; Lahiri & Nishino, 1980) and in the newborn kitten (Marchal, Bairam, Haouzi, Hascoet, Crance, Vert & Lahiri, 1992b). In addition, dopamine antagonists have been shown to increase chemoreceptor discharge and potentiate the response to hypoxia and hypercapnia (Bisgard et al, 1979; Lahiri et al, 1980).

Blanco et al. (1988) showed that artificial ventilation of fetal lambs *in utero* with 100% O<sub>2</sub> initiated resetting of hypoxia chemosensitivity. Holgert, Hokfelt, Hertzberg & Lagercrantz (1995) investigated the effects of postnatal oxygenation on mRNA levels of dopamine and tyrosine hydroxylase in neonatal rat carotid bodies. Fetal carotid bodies showed high levels of dopamine and tyrosine hydroxylase mRNA on the day prior to birth, which was substantially reduced on postnatal day 1, and a further reduction was observed by postnatal day 7. The turnover in dopamine mRNA is high at the time of birth, after which it rapidly decreases. This provided even stronger evidence for the postnatal reduction in carotid body dopamine content mediating resetting of hypoxia chemosensitivity. At present, this hypothesis is the most likely to explain chemoreceptor resetting, however the mechanism initiating rapid dopamine turnover around the time of birth, and its subsequent fall is unknown.

#### 1.5.4 Implications of chronic hypoxia on resetting of chemosensitivity

Chronic hypoxia has been shown to blunt the chemoreflex respiratory response of newborns to hypoxia. In newborn rats, chronic hypoxia from the day of birth (Fio<sub>2</sub> 0.13-0.15) abolished the initial increase in ventilation observed during steady state hypoxia (Eden & Hanson, 1987b). Normoxic rats showed a typical biphasic ventilatory response to hypoxia, whereas chronically hypoxic rats showed only a depression of ventilation (the biphasic ventilatory response is discussed in more detail in Chapter 3). In newborn kittens, exposure to chronic hypoxia from the day of birth (Fio<sub>2</sub> 0.13-0.15) blunted the chemoreflex response to two breath alternations in Fio<sub>2</sub> between 0.21 and 0.14 (Hanson, Kumar & Williams, 1989). Normoxic kittens over the same period (days 1 to 14) showed a maturation of the hypoxic respiratory response. In carotid bodies of newborn rats studied *in vitro*, chemoreceptor responses were measured at 5wks in chronically hypoxic rats (Fio<sub>2</sub> 0.12) and normoxic controls (Landauer, Pepper & Kumar, 1995). Chronically hypoxic rats did not show a postnatal increase in hypoxia sensitivity in contrast to controls, however they were responsive to CO<sub>2</sub>. Furthermore, chronic hypoxic exposure interfered with the development of CO<sub>2</sub>-O<sub>2</sub> interaction observed in normoxic rats but not in chronically hypoxic rats.

In this respect it is interesting that Hertzberg, Hellstrom, Holgert, Lagercrantz & Pequignot (1992) have demonstrated elevated levels of dopamine in the carotid bodies of newborn rats exposed to 100% O<sub>2</sub>. Hertzberg et al. (1992) compared the development of the chemoreflex response to hyperoxia in rats born and reared in hypoxia (Fio<sub>2</sub> 0.12-0.14) and normoxic rats. The turnover rates of dopamine were also compared between the two groups. As expected, the chemoreflex response to 100% O<sub>2</sub> was blunted in

chronically hypoxic rats compared to normoxic rats. Chronically hypoxic rats also sustained a high increase in dopamine turnover, which was reduced when the hypoxic period was terminated. Normoxic rats show a postnatal decline in the turnover rate of dopamine. The authors suggested that the blunted chemoreflex respiratory response was due to the elevated levels of dopamine release from the carotid body.

Wach, Bee & Parer (1989) also proposed a role for elevated dopamine levels mediating the blunted respiratory chemoreflex. Adult rats were exposed to chronic hypoxia ( $F_{iO_2}$  0.10) for 2-3wks, after which time peripheral chemoreflex function was tested by acute hypoxic exposure (reduction in  $F_{iO_2}$  from 0.21 to 0.10) and compared to control rats. Chronically hypoxic rats showed reduced respiratory response to acute hypoxia, an effect which was partially reversed by administration of the dopamine antagonist, domperidone. After the antagonist, acute hypoxic ventilatory responses were not significantly different between the two groups. In a further series of experiments Bee & Pallot (1995) showed that the characteristic changes associated with chronic hypoxic exposure were apparent after an 8d period of acute hypoxia. Initially, the increase in ventilation during acute hypoxia was associated with a fall in dopamine carotid body content on days 1, 2 and 4. However, by day 8 dopamine content was increased and the ventilatory response to a hypoxic challenge reduced compared to normoxic control rats. Thus, the rats exhibited signs of chronic hypoxaemia.

Dopamine appears to be the neurotransmitter involved in neurosecretion from type I cells and transduction of chemical stimuli to nerve endings (see section 1.4.1.e). It is clear that exogenously applied dopamine exerts an inhibitory effect on respiratory control, however some observations appear to be paradoxical on first inspection. Dopamine has an inhibitory effect on ventilation (Bisgard et al, 1980; Maycock et al, 1983) and chemoreceptor discharge (Bisgard et al, 1979; Lahiri & Nishino, 1980; Marchal et al, 1992b), but will potentiate the carotid body chemoreceptor response to hypoxia and hypercapnia (Lahiri et al, 1980a). Marchal et al. (1992b) proposed that this could be explained by large releases of dopamine from type I cells, during hypoxia or hypercapnia, stimulating dopaminergic receptors on nerve endings and hence increasing chemoreceptor discharge. Whilst in contrast, relatively low levels of dopamine could stimulate dopaminergic receptors on type I cells and be involved in autoregulation to produce an inhibitory effect on chemoreceptor discharge. Dopamine is released from type I cells after the elevation in  $Ca^{2+}_i$  produced by low oxygen or high  $P_{CO_2}$ /low pH. Thus, its role as a sensory neurotransmitter can be seen from its release from type I cells in response to respiratory stimuli.

The hypothesis of Hertzberg et al. (1992), that chronic hypoxia sustains the release of dopamine from the carotid body, fits with the observations of exogenously applied dopamine on ventilation and chemoreceptor discharge. Dopamine may be part of the mechanism by which chronic hypoxia interferes with chemoreceptor hypoxia resetting, if chronic hypoxia stimulates dopamine release from the carotid body, which in turn depresses chemoreceptor responses and respiratory reflexes. Chronic hypoxia may have fatal consequences on respiratory control for the newborn, and the possible link between sudden infant death syndrome (SIDS) and chronic hypoxaemia is discussed in section 1.6.4.

## 1.6 Why study respiratory control in the newborn?

Fetal and neonatal physiology is an area in which there is interest in both the normal developmental processes that occur prior to, and after birth, and also the deviation from this during disease. In particular, neonatal respiratory physiology has warranted extensive research to reduce the incidence of infant mortality and morbidity. Newborns are prone to periods of apnoea, particularly when they are born prematurely. There is also the need to unravel the mystery of SIDS, and although research until now has been successful in reducing the incidence of SIDS, the mechanisms which fail and underlie this unexpected death remain to be elucidated.

### 1.6.1 Onset of continuous breathing at birth

In the extra-uterine environment, the fetal fluid-filled lung becomes inflated with air, and the newborn must begin to make continuous breathing. The fluid that is secreted by the fetal lung is important for lung development, however this must be reabsorbed to enable the newborn to breathe air. Absorption of lung fluid occurs at birth by an active transport process of sodium ions across the epithelium into the interstitial fluid and is mediated by the rise in adrenaline in the blood of the fetus during labour (Walters, 1994). In the sheep, the lung becomes sensitive to the action of adrenaline towards the end of gestation and is controlled by cortisol and triiodothyronine ( $T_3$ ). The time required to remove this lung fluid varies between 6-24hrs (Chernick, 1977a).

Surfactant is secreted *in utero* at *ca.* the 30th gestational week in the human (Froh & Ballard, 1994), and is necessary after birth to lower the surface tension at the air-liquid interface of the alveoli to prevent alveolar collapse. The surface tension of the alveoli are determined by Laplace's equation:  $P=2T/r$  where P is the distending pressure, T is the surface tension and r is the radius of an alveolus, thus, surfactant will reduce the

pressure needed to keep the alveoli open and prevents the collapse of the smaller alveoli. This enables functional residual capacity to be established, without which the newborn would need to generate large inflation pressures on each inspiration to fill the lung. Surfactant reduces surface tension to such an extent that the elastic recoil of the lung is balanced by the equal and opposite force of the recoil of the chest wall (Ramsden, 1994).

A rise in pulmonary blood flow is also necessary to manage effective ventilation/oxygenation postnatally, and this is possible with the closure of the ductus arteriosus that occurs at birth. The closure of the ductus is in part related to the rise in PaO<sub>2</sub> at birth which desensitizes the ductus to the dilatory properties of circulating prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) (Smith & McGrath, 1993, 1994), and to a fall in the concentration of circulating PGE<sub>2</sub> in the hours prior to birth (Clyman, Mauray, Roman, Rudolph & Heymann, 1980). The peripheral chemoreceptors are not required for the onset of continuous breathing at birth (Jansen et al, 1981). However they do play an important role in the regulation of breathing in the neonate as peripheral chemodenervated animals suffer mortality in the first few postnatal weeks (Hofer, 1984; Bureau, Lamarche, Foulon & Dalle, 1985b; Donnelly & Haddad, 1990; see also section 4.4.6).

The first breath is taken within seconds of birth and is mixed with the fluid still in the lung to create a foam (for review see Adamson, 1991). Subsequent breaths over the next 15-30min will gradually reduce this foam by replacing the lung fluid with air. These initial breaths taken by the newborn are different to those of regular respiration and are referred to as gasps. Dawes et al. (1972) described gasping in the fetal lamb as "single brief relatively deep inspiratory efforts recurring irregularly at a slow rate (e.g. 1-3/min)". Gasping caused much greater falls in tracheal pressure (-75 mmHg) than those associated with FBM (ca. 10 mmHg mid-gestation) and were more prolonged. Gasping is necessary to generate a large inspiratory force to overcome the problems of viscosity of lung fluid (which is ca. 100 times more viscous than air; Chernick, 1977b) and surface tension of the alveoli. The transpulmonary pressure necessary to begin lung inflation is ca. 20-25cm H<sub>2</sub>O for a newborn baby, and the pressure generated on the first breath may be as much as 40-100cm H<sub>2</sub>O (Chernick, 1977a). These high transpulmonary pressures are probably aided in part by a partially closed glottis (Chernick, 1977b). Guntheroth & Kawabori (1975) noted that gasping in the adult dog and monkey was much more effective at restoring PaO<sub>2</sub> than in the neonate due to the fluid filled lung at birth, and with one or two gasps there was an increase in oxygen saturation when the circulation was functional. However, they comment that the gasp is more durable in the newborn and will persist for up to 30min after failure of the cardiovascular system.

The initial changes in respiration are very rapid, however changes in ventilation continue over the next few days to complete the transition from intermittent fetal breathing to continuous breathing in the newborn (Fisher, Mortola, Smith, Fox & Weeks, 1982). Several factors have been identified as crucial to the onset of breathing in birth, most importantly an increase in CO<sub>2</sub> and a reduction in body surface temperature.

The increase in CO<sub>2</sub> that occurs with clamping of the umbilical cord has been recognized as an important factor in the regulation of breathing at birth. Clamping of the umbilical cord *in utero* initiates gasping in the fetus (Barcroft & Karvonen, 1947; Dawes et al, 1972), similar to the response when the ewe receives anoxia (Barcroft & Karvonen, 1947). In the fetal goat, clamping of the umbilical cord *in utero* produced gasping efforts after the first minute, which were followed by more regular gasps at 0.5-1.0 sec intervals and continued for 5-8min before cessation (Towell & Salvador, 1974). In exteriorized fetal monkeys, clamping of the umbilical cord initiated gasping after *ca.* 3min with no apparent improvement of oxygen saturation due to the presence of lung fluid (Guntheroth & Kawabori, 1975). Regular respiration was not established for 30min until there was an increase in oxygen saturation, and the incidence of gasping decreased as regular breaths increased in frequency and volume.

Blanco, Martin, Hanson & McCooke (1987) showed that CO<sub>2</sub> (PaCO<sub>2</sub> 66-86mmHg) produced a stimulation of FBM when fetal lambs were hyperoxic and at 40°C in a saline bath before cord clamping, thus demonstrating that CO<sub>2</sub> alone provides a stimulus for the onset for regular respiration. Adamson, Richardson & Homan (1987) similarly found that umbilical cord occlusions *in utero* in fetal sheep initiated gasping at 1-2min. The fetuses had access to hyperoxic air supplied via the trachea, so that gasping was associated with a rise in PaO<sub>2</sub> and continued during high-voltage ECoG. Furthermore, this was reversed when the cord was unclamped which decreased breathing activity. It has been shown by Pagtakhan, Faridy & Chernick (1971) using cross-circulation techniques in exteriorized fetal lambs, that the initiation of breathing associated with cord clamping is due to the change in blood gases and not to the cord clamping *per se*. When PaCO<sub>2</sub> was less than 40mmHg, breathing was not stimulated until PaO<sub>2</sub> was reduced below 8mmHg, however at normal fetal PaCO<sub>2</sub>s breathing was stimulated at 8-10mmHg. In a similar series of experiments Woodrum, Parer, Wennberg & Hodson (1972) partially exteriorized fetal lambs and perfused the head and neck with carotid arterial loops. They found that reductions in PaO<sub>2</sub> or increases in PaCO<sub>2</sub> stimulated FBM, and they suggested a role for the carotid chemoreceptors responding to changes in arterial blood gas which contributed to the onset of breathing at birth.

Barcroft & Karvonen (1947) described that when fetal sheep (139-147d) were delivered into a warm saline bath, and their noses were exposed above the level of the bath, the onset of respiration occurred. It started as gasps, and only became rhythmic after the umbilical cord had been tied. Similarly, immersion of the fetal snout in ice-cold water will stimulate breathing movements (Dawes, 1968). *In utero*, cutaneous cooling of the fetus leads to continuous FBM (Gluckman, Gunn & Johnston, 1983). The cold stimulation had a stimulatory effect on cutaneous thermoreceptors, but not on core thermoreceptors. This has been associated with a shift of CO<sub>2</sub> response curve to the left (Moss, Mautone & Scarpelli, 1983). Cold stimulation has also been demonstrated to override hypocapnia and hypoxia in the initiation of continuous breathing movements *in utero* (Blanco et al, 1987).

Extra-corporeal membrane oxygenation (ECMO) has been used to manipulate fetal blood gases irrespective of the placenta. Experiments in chronically instrumented fetal lambs showed that FBM was not simulated by cord occlusion alone when immersed in warm saline (Blanco, 1994) and that cold stimulation increased fetal breathing activity (Kuipers, Maertzdorf, Kennen, de Jong, Hanson & Blanco, 1992). This suggests cold stimulation is one of the most important factors in the initiation and regulation of breathing at birth.

Hypoxia alone does not initiate regular breathing. Hypoxic fetal lambs held at normocapnia and at 40°C did not make any breathing movements for 120sec after clamping of the cord (Blanco et al, 1978). Over this time PaCO<sub>2</sub> would increase in the fetus, and hence the stimulus for the onset of respiration would be asphyxia and not hypoxia.

There is also some evidence that sensory stimulation in addition to cutaneous cooling can provide a drive for continuous breathing. Barcroft & Barron (1937a, 1937b) report a stimulation of breathing in the fetus at 40-60d in response to touching the face. In contrast, Dawes et al. (1972) found that in fetuses 40-55d it was not possible to be sure that sensory stimulation evoked FBM as tactile stimulation to the nose and face rarely produced inspiratory effort. Other factors proposed to be contributing stimuli to the onset of regulation are removal of the umbilical circulation, light, removal of fluid from the larynx, lung expansion, increased pulmonary flow and increased right ventricular output (Blanco, 1994).

### 1.6.2 Apnoea and prematurity

Understanding the development of respiratory control in the neonatal period is crucial for the care and maintenance of well-being in newborn babies, particularly those born prematurely. Premature infants are prone to prolonged periods of apnoea and periodic breathing (Milner & Greenough, 1988; Butcher-Puech, Henderson-Smart, Holley, Lacey & Edwards, 1985). Apnoea may be central, obstructive or "mixed" and results in a fall in PaO<sub>2</sub> and a rise in PaCO<sub>2</sub>. Central apnoea is described by a total cessation of airflow and respiratory effort, and obstructive apnoea by the cessation of airflow in the presence of respiratory effort. Mixed apnoea is a combination of both, usually initiated by central apnoea, and accounts for somewhere between 20% and 50% of all apnoeas in preterm infants (Butcher-Puech et al, 1985; Miller, Carlo & Martin, 1985; Brazy, Kinney & Oakes, 1987; Milner & Greenough, 1988). Preterm infants are reported to show reduced respiratory chemoreflexes (Rigatto, Brady, Chir & de la Torre Verduzco, 1975b; Gerhardt & Bancalari, 1984) and are also at increased risk for SIDS (for review see Poets & Southall, 1994).

### 1.6.3 Respiratory control and SIDS

Poor respiratory control has been linked to SIDS as a possible cause for death. Abnormalities in the brainstem of SIDS victims suggest a delay in the maturation of respiratory neurones (Takashima & Becker, 1985; Takashima, Mito & Becker, 1985; for review see Becker, 1990; Takashima, Mito & Yamanouchi, 1994), subtle brainstem astrogliosis (which implies neuronal death from some sort of trauma e.g. hypoxia-ischaemia) (Naeye, 1976; Takashima, Armstrong, Becker & Bryan, 1978; Takashima & Becker, 1985) and hypomyelination of neurones in the respiratory centres of the brainstem (Naeye, Olsson & Combs, 1989). These pathologies indicate either that maturation of respiratory control in the brainstem delayed in SIDS victims, or that neurological damage is incurred during the fatality and between the time of death and autopsy.

### 1.6.4 Chronic hypoxaemia and SIDS

There have also been reports of abnormalities in victims of SIDS that involve the peripheral control of respiration. Some SIDS infants have been found to be chronically hypoxaemic (Naeye, Fisher, Ryser & Whalen, 1976; Rognum & Saugstad, 1991) and chronic hypoxaemia has been found to elevate dopamine concentration in rat carotid body (Pallot & Barer, 1982). Reports which suggest abnormal carotid body function in

SIDS victims include structural abnormalities (Naeye et al, 1976; Cole, Lindenberg, Galioto, Howe, DeGraff, Davis, Lubka & Gross, 1979), and elevated levels of dopamine and noradrenaline (Perrin, Becker, Madapallimatum, Cutz, Bryan & Sole, 1984). Other studies have not substantiated this finding, and do not support changes in the structural integrity of the carotid body or catecholamine content in SIDS victims (Dinsdale, Emery & Gaddson, 1977; Lack, Perez-Atayde & Young, 1986). There is still currently debate on the role of the carotid body in the aetiology of SIDS.

More recently there is evidence to suggest that respiratory chemoreflexes to hypoxia are reduced in infants at increased risk of SIDS (Calder, Williams, Smyth, Boon, Kumar & Hanson, 1994b; Katz-Salamon & Lagercrantz, 1994). Infants who have suffered BPD are a group in which the incidence of SIDS is reported to be as much as seven times higher than the population as a whole (Werthammer, Brown, Neff & Taeusch, 1982). These infants have been suggested to be 'borderline hypoxaemic' (Uyboco, Kwiatkowski, Cates, Kavanagh & Rigatto, 1989). In animals it has been shown that chronic hypoxia reduces the gain of respiratory chemoreflexes (Eden & Hanson, 1987b; Hanson, Williams & Kumar, 1989). This information suggests either that chronic hypoxaemia disrupts carotid body function, or that integration of afferent information from the carotid body at the CNS does not produce an appropriate ventilatory response.

Presently it is not clear if there is a causal link between SIDS victims and poor respiratory control. However in the face of rising arterial  $P_{CO_2}$  and falling  $P_{O_2}$  immediately prior to death, one wonders if the arterial chemoreceptors respond to the deterioration in blood gases, and if so why no ventilatory response is initiated. Pathology studies of SIDS victims seems to implicate a maturational delay of neuronal function and a possible role for hypoxia. By studying the normal process for the development of respiratory control in the newborn, and the chemoreflex responses to hypoxia and hypercapnia, researchers hope to move towards a better understanding and prevention of apnoea and infant death. The work in this thesis goes in part to aiding our present knowledge of neonatal respiratory control.

## 1.7 AIMS

My experiments in this thesis fall in to two main sections:- those performed in newborn infants and adults measuring the chemoreflex respiratory response to single breath alternations between air and a mildly hypoxic gas mixture; and those performed in anaesthetized newborn lambs measuring the carotid chemoreceptor response to  $CO_2$ . Thus I have investigated the peripheral respiratory chemoreflex from two perspectives,

first the respiratory response to an imposed hypoxic stimulus, and secondly the afferent input from the carotid chemoreceptors to the brainstem to a change in to a CO<sub>2</sub> stimulus.

The non-invasive measurement of respiratory chemoreflexes in newborn infants was necessary to build on the information obtained previously using the alternate breath method with a hypoxic stimulus. There were two main questions arising from previous work:-

*Question 1:* Is the resetting of peripheral arterial chemoreceptor to hypoxia in newborn infants complete by the end of the first week, as suggested by the observations of Williams, Smyth, Boon, Hanson, Kumar & Blanco (1991) (see section 3.1 for review)? This was the question I investigated in Chapter 3.

*Question 2:* Is there an influence of mechanoreflex control in the newborn on the respiratory response to an imposed chemical stimulus? This was the question I investigated in Chapter 4.

In addition to Question 1 and further to the findings in Chapter 3, a third question arose which was important for the interpretation of the development of the respiratory chemoreflex response to hypoxia.

*Question 3:* Was the chemoreflex respiratory response to a single breath alternating stimulus, between air and a mildly hypoxic gas, greater in human adults than in newborn infants? These experiments were necessary to complement Question 1. Previously, there had been no experiments in the adult using the same stimulus I used in newborn infants, nor was the method of analysis comparable to other alternate breath techniques used by other workers. I addressed this question in Chapter 5.

There were two questions that followed from my work investigating the development of the hypoxic chemoreflex. In the literature there was a great deal of information on the carotid chemoreflex response, and the respiratory chemoreflex response to hypoxia in the fetus and newborn but it appeared that the response to CO<sub>2</sub> had been essentially neglected. There was virtually no information available on the development of the chemoreflex response to CO<sub>2</sub>. So there were two series of experiments that I performed to investigate the development of the peripheral chemoreflex response to CO<sub>2</sub>, dedicated to measuring carotid chemoreceptor responses to provide direct information on the afferent input to the brainstem.

*Question 4:* Was there a postnatal maturation of the steady state chemoreceptor response to CO<sub>2</sub>, similar to the postnatal resetting of chemoreceptor sensitivity to hypoxia described by Blanco et al. (1984a)? This question was addressed by the experiments performed in Chapter 6.

*Question 5:* In section 1.4, I reviewed Torrance's bicarbonate hypothesis and the current ideas about the way in which CO<sub>2</sub> is detected by the carotid body. In the adult, we know that there is a dynamic sensitivity to CO<sub>2</sub> that is greater than the steady state sensitivity. There had been no previous attempts to measure dynamic sensitivity to CO<sub>2</sub> in the neonate. From Torrance's bicarbonate hypothesis, if dynamic sensitivity to CO<sub>2</sub> is independent of the background level of oxygen, then there should be a dynamic sensitivity to CO<sub>2</sub> present from birth, and it should change little with resetting of hypoxia sensitivity. In Chapter 7 I have measured the carotid chemoreceptor dynamic response to CO<sub>2</sub> directly, and investigated any postnatal changes in this sensitivity.

## 2.0 METHODS

### 2.1 Measurement of the respiratory response of healthy term infants at two postnatal ages to breath-by-breath alternations in Fio<sub>2</sub>.

#### 2.1.1 Subject Selection

Healthy newborn infants were recruited from the postnatal wards at University College Hospital, London and The Royal Berkshire Hospital, Reading. Parents were recruited with the use of an information sheet (see Appendix 1). Local ethical committee approval and written parental consent were obtained (see Appendix 2). Infants delivered either vaginally or by caesarean section were studied, as it has been shown previously that there is no significant difference in response between the two groups (Williams et al, 1991).

A total of 33 infants were studied in the first few postnatal days of life. Three of the infants included in the final analysis were studied at Royal Berkshire Hospital in Reading and the data recording performed by Dr Bridget Waites. Of the 33 infants studied, only 13 repeat studies were completed successfully. The most common reason for failure was the inability to achieve a quiet sleep state in these infants. In addition, some infants were excluded from analysis if the minimum of two test and control runs were not recorded, and some mothers failed to keep their appointments for repeat studies.

#### 2.1.2 Experimental Conditions

The initial studies were performed on the postnatal wards before mothers and babies were discharged. The study was repeated in the ante-natal clinic when mothers and babies returned for their 6th week postnatal check-up for infants delivered by caesarean section. For infants delivered vaginally, the study was repeated at *ca.* 6 weeks in a room on the labour ward.

The infants were settled after a feed, and recording began when they were in quiet sleep as judged behaviourally (Precht, 1974). Ambient temperature ranged between 23-28°C on the postnatal wards and 23-25°C when the studies were repeated. Infants were wearing a one piece towelling babysuit at the time of study, and were covered with

a single blanket. They were studied in the lateral or supine position and remained in a single position for the duration of the study.

The experimental set-up is shown in figure 2.1.2.i. Breathing was measured by inductance plethysmography (Respirtrace Corp., Ardsley, NY, USA) and calibrated by the method of Sackner, Watson, Belsito, Feinerman, Suarez, Gonzalez, Bizousky, & Krieger (1989) to derive scaling factors for relative contributions the ribcage and abdominal signals made to the sum signal (see section 2.1.5 for description of calibration method). The sum signal was passed to a BBC Master 128 Acorn microcomputer (BBC, UK) for off-line analysis. In a previous study the sum Respirtrace signal was calibrated using a pneumotachometer for 6 infants at several time points throughout the protocol, and the sum Respirtrace signal remained highly correlated (range  $r = 0.96 - 0.99$ ) to the pneumotachometer signal (Williams, 1990).

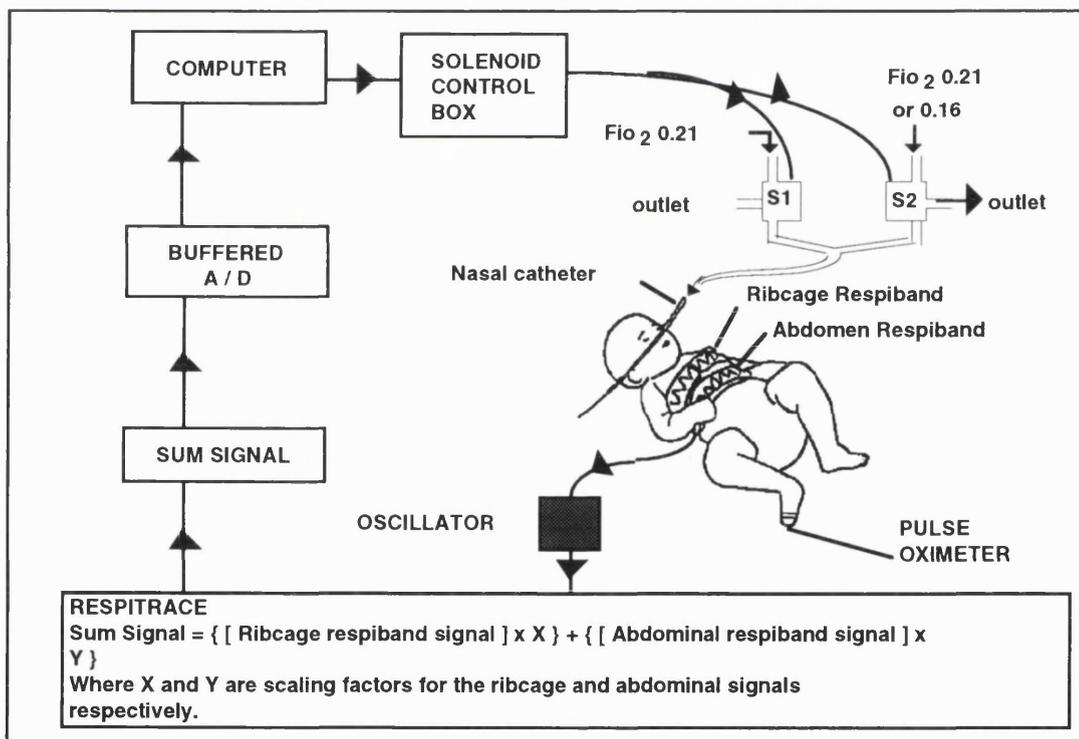


Figure 2.1.2.i Diagram of experimental set-up for the alternate breath test

Inspired gas was humidified and supplied to the infant at a rate in excess of minute ventilation, i.e. at 2.0-2.5l/min, via a nasal catheter (no. 1615, Salter Labs, Arvin, CA, USA) attached via a Y-connector to two gas delivery lines. The composition of gas in each delivery line was set using rotameters connected to cylinders of medical grade air ( $\text{Fio}_2$  0.21) and gas with an  $\text{Fio}_2$  of 0.16, balance  $\text{N}_2$  (pre-calibrated; British Oxygen Company special gases, UK). Delivery of inspired gas through a pair of 3-way

solenoid-operated valves was controlled by the computer, which switched between them at the start of each expiration. During test runs, breath-by-breath alternations of air and an  $F_{iO_2}$  of 0.16 were delivered for up to 100 breaths. During control runs air was delivered in both gas delivery lines. Oxygen saturation was monitored throughout the procedure by a pulse oximeter operating in the beat-to-beat mode (Nellcor N200, Nellcor Inc., Hayward, CA, USA). Saturation was not recorded, but used as a visual safety check. It never fell below 92%.

### 2.1.3 Experimental protocol

Infants were studied in the lateral or supine position and measurements began after quiet sleep was established. The order of test and control runs was randomised between infants. A minimum of two test and two control runs was necessary for the data to be included in the analysis. The maximum duration of each run was 100 breaths or 3min.

### 2.1.4 Data analysis

#### 2.1.4.a Analysis of chemoreflex respiratory responses

From the sum Resptrace signal the computer measured for each breath  $V_{ti}$ ,  $V_{te}$ ,  $t_I$  and  $t_E$ , and calculated  $f$ ,  $V_{ti}/t_I$ ,  $V_{te}/t_E$ ,  $t_I/t_{Tot}$  and  $V_{ti} \cdot f$ . Breaths were always analysed in pairs, and the breath-by-breath percentage alternation calculated for each respiratory variable. The difference between a pair of consecutive breaths expressed as a percentage of the mean of the two breaths gave the breath-by-breath percentage alternation. Each breath was always compared to the immediately preceding breath and the percentage alternation plotted cumulatively with respect to breath number, reversing the sign (+ or -) for every second alternation. This is illustrated schematically in figure 2.1.4.a.i for breaths analysed simply in terms of  $V_t$ . For the first breath, there is no breath-by-breath % alternation, and hence the cumulative plot starts at zero. For the first pair of breaths, breath one is subtracted from breath two and expressed as a percentage of the mean of the two breaths, and then plotted at the time of breath two. The same is done for the second pair of breaths, i.e. breath two compared to breath three, except that the sign of the alternation (in this case negative) is reversed. The second alternation is plotted cumulatively with the first alternation, and with increasing breath number the alternation builds up. Hence, a regular alternation produced a consistent deviation in the cumulative plot of alternations from the baseline. When the alternation in the respiratory variable is regular, i.e. in response to the breath-by-breath changes in  $F_{iO_2}$ , the cumulative alternation becomes highly linear.

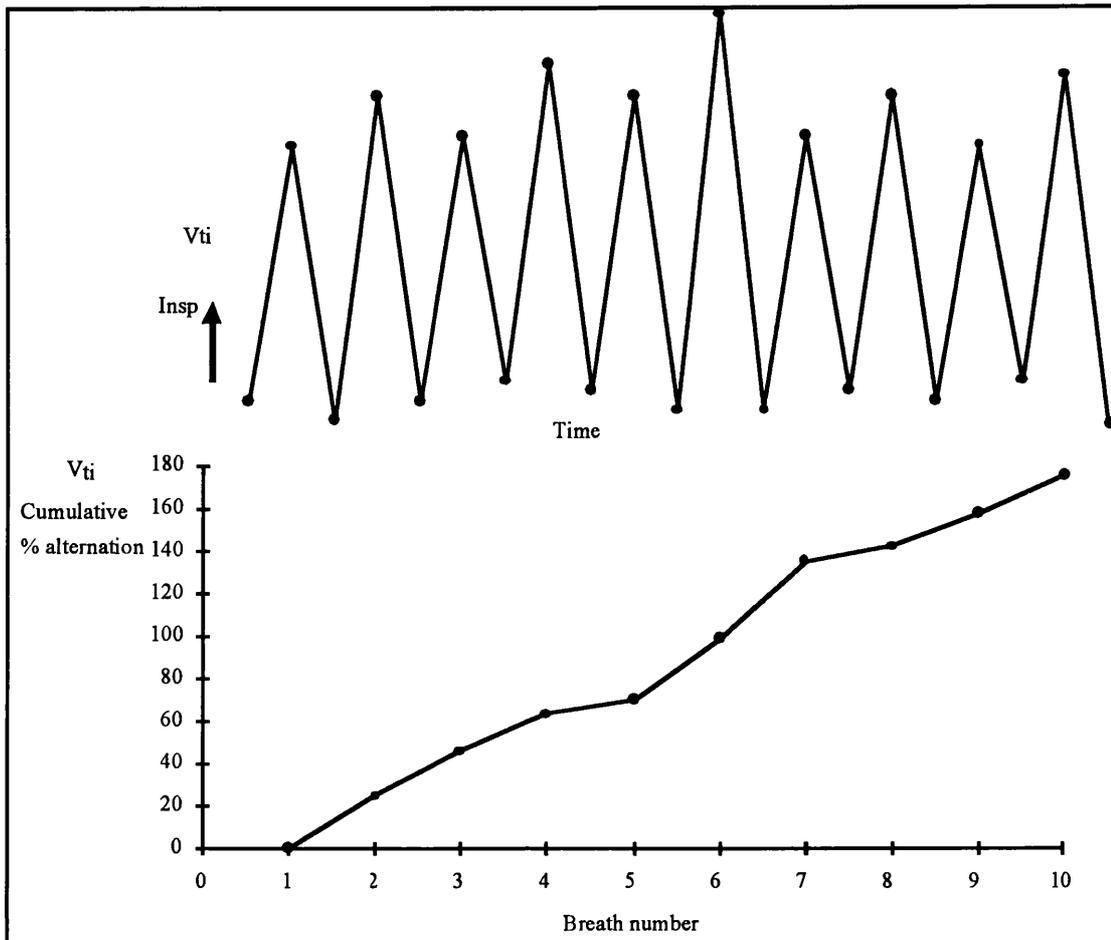


Figure 2.1.4.a.i Breath-by-breath alternation in  $V_{ti}$  (above) and formation of cumulative % alternation by reversing sign (+ or -) of ever other alternation (below).

Regression analysis was used to describe the cumulative alternation. The slope of the regression line was used to determine the strength of the chemoreflex response. The absolute value of the slope (irrespective of its sign) indicated the mean breath-by-breath percentage alternation. The absolute value was used because it was not possible to say definitively which breath was responding to the hypoxic stimulus. Although lung to carotid body delay could be estimated at 2-3sec (Black & Torrance, 1971; Black, McCloskey & Torrance, 1971), newborn infants breathe too rapidly to calculate which breath responded to the hypoxic stimulus. Therefore, it was not possible to say that a negative slope fitted to an alternation related to a decrease in that respiratory variable, for example a decrease in  $t_E$  in response to the hypoxic stimulus.

An example of a control (top) and test (bottom) responses is shown in figure 2.1.4.a.ii for a 4 day old infant. The breath-by-breath alternations are plotted cumulatively with respect to breath number for respiratory variables  $V_{ti}$ ,  $t_E$  and  $t_I$ . The slope of the regression line fitted to the test response indicates the mean breath-by-breath % alternation. The chemoreflex response in figure 2.1.4.a.ii showed a mean breath-by-

breath alternation of 13.7% for  $V_{ti}$ , and 9.9% for  $t_I$ . This contrasts with the control response which showed a breath-by-breath alternation of 0.6% for  $V_{ti}$  and 2.2% for  $t_I$ . In this particular infant, the breath-by-breath alternation in  $t_E$  was similar for control and test, so that it would be very unlikely that the alternation in  $t_E$  was significant (for further details see section 2.1.4.b).

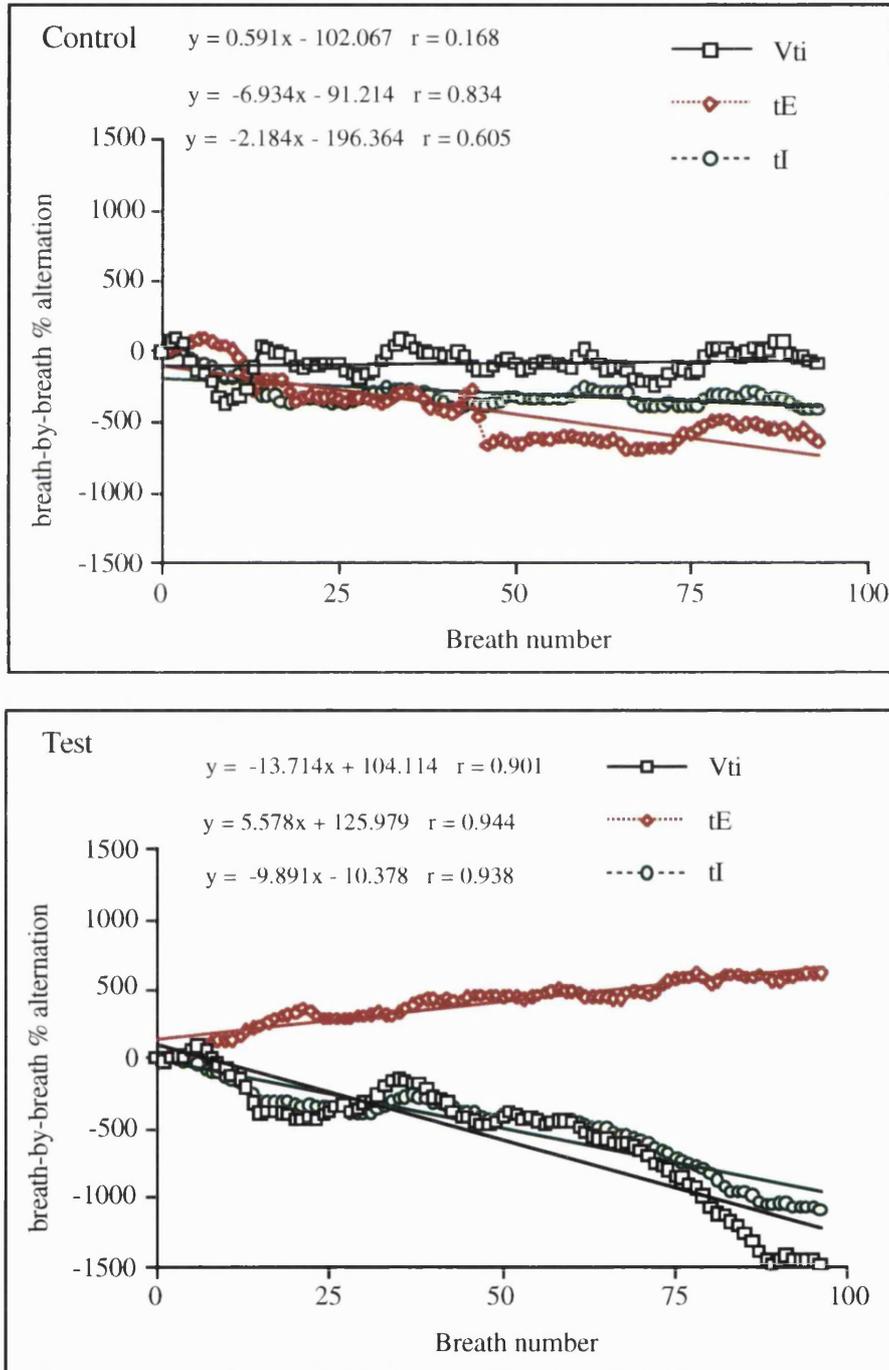


Figure 2.1.4.a.ii Control (top; breath-by-breath alternations in  $F_{iO_2}$  between 0.21 and 0.21) and test (bottom; breath-by-breath alternations in  $F_{iO_2}$  between 0.21 and 0.16) responses in a 4 day old infant. Squares= $V_{ti}$ , diamonds= $t_E$ , circles= $t_I$ . Responses are described by regression analysis. The slope indicates the mean breath-by-breath % alternation.

Chemoreflex responses were compared with respect to age in two ways, either when control and test responses were averaged for each infant, or when all individual test responses were used for each infant. Test responses were compared to control using Wilcoxon matched pairs test (when responses were averaged for each infant) or Mann-Whitney U test (when more than one response per infant was used) ( $P < 0.05$ ).

#### 2.1.4.b Assessment of significant chemoreflex respiratory responses

An infant was classified as a responder if at least one of the respiratory components of the chemoreflex response was significantly different from control. Two or three control responses from each of the 13 subjects were used to approximate a normal distribution ( $n > 30$ ). Parametric statistics were not used to test for significant alternations, rather this was a new method developed to determine those infants showing significant alternations, subsequently being determined responders. It was important to establish a critical value for test responses, such that a response greater than the critical value would be deemed as showing a significant alternation. The critical value was determined as the 95th percentile (based on the normal distribution  $z > 1.645$ ) from all control responses. Test responses showed a significant alternation if they occurred above the 95th percentile of the control distribution for the group, and were at least twice as great as the largest control response for that individual. Tests had to be at least twice as great as control so that it was certain that the alternation was significant. Table 2.1.4.b.i shows the critical values for each respiratory variable.

Respiratory variable	$V_{ti}$	$V_{te}$	$t_I$	$t_E$	$f$	$V_{ti}/t_I$	$V_{te}/t_E$	$t_I/T_{tot}$	$V_E$
Critical value: Study 1 (n=33)	3.27	4.85	3.06	3.61	2.62	3.50	5.00	2.77	2.98
Critical value: Study 2 (n=33)	4.26	3.74	3.84	3.02	2.58	3.11	3.52	2.67	2.66

Table 2.1.4.b.i Critical values (% breath-by-breath alternation) for determining significant alternations for infants studied at two postnatal ages.

#### 2.1.5 Calibration of equipment

To use Resptrace as a measure of tidal volume, it is necessary to derive scaling factors for the ribcage (RIB) and abdominal (ABD) Resptrace signals for their contribution to the sum, where X is the scaling factor for the ribcage signal and Y is the scaling factor for the abdominal signal.

$$\text{SUM signal} = (\text{RIB} \cdot X) + (\text{ABD} \cdot Y)$$

Thus, changes in the sum signal will be proportional to changes in tidal volume when the scaling factors are appropriate. I have used the method of Sackner et al. (1989), which describes a single-posture method during natural breathing, to calculate these scaling factors.

The calibration method of Sackner et al. (1989) is based on the assumptions of Konno & Mead (1967). They demonstrated that the respiratory system could be approximated to two degrees of freedom. So, any change in volume measured at the mouth can be approximated to a change in the volumes at the ribcage and abdomen. Konno & Mead (1967) calibrated the ribcage and abdominal signals by producing only one degree of freedom, that is when the mouth is occluded. Hence any change in volume at the ribcage is equal and opposite to the volume change at the abdomen (i.e.  $\Delta RIB \approx -\Delta ABD$ ). This process is commonly referred to as the isovolume method, but is limited by the fact that it requires co-operation from the subject to voluntarily produce changes in RIB and ABD volumes when the mouth and nose are occluded.

Sackner et al. (1989) developed a method based on the standard deviations of RIB and ABD volumes during 5min of natural breathing. They observed that breath to breath changes occurred in RIB and ABD volumes for breaths of the same  $V_t$ , and furthermore that these variations ( $vRIB$  and  $vABD$ ) fitted a normal distribution. If the subject breathes at constant  $V_t$  the standard deviation is zero. Thus, they solved the proportional scaling factor ( $K$ ) based on the equation-

$$K \approx -SD(\Delta vABD)/SD(\Delta vRIB).$$

Clearly, it is impossible for  $V_t$  to remain constant in a voluntarily breathing subject, so they reasoned that "*collection of a large number of breaths with exclusion of those breaths with large deviations from the mean sum might provide an approximation for a constant  $V_t$  to solve the equation*". They found that this assumption held when the method was compared to the isovolume method, and that the most consistent proportional scaling factor was calculated from 3-10min of breathing when breaths outside 0.6 -1.0 SD of the mean were discarded.

For the purpose of my experiments, if more than 50% of the total breaths during a 2-3min calibration period were within 0.5 SD of the mean, the proportional scaling factor was deemed significant. The RIB signal was arbitrarily scaled as 1.0, and the ABD signal scaled accordingly. These scaling factors hold for one postural position but must be reapplied if there is a change in posture. All my experiments were performed in the same postural position (lateral or supine) for that subject.

In a previous study performed by Williams (1990) the Resptrace sum signal was calibrated using a pneumotachometer for 6 infants at several time points throughout the protocol, and the Resptrace sum signal remained highly correlated (range  $r = 0.96 - 0.99$ ) to the pneumotachometer signal. Thus, I have made the assumption based on these observations that for a 20-30min recording period, the changes in the Resptrace sum signal are proportional to changes in tidal volume. I was not able to validate this assumption using a pneumotachometer for infants.

## **2.2 Measurement of chemo- and mechanoreflexes during quiet breathing in the newborn infant**

### **2.2.1 Subject Selection**

As outlined in section 2.1.1. This study was designed to assess the feasibility of measuring both respiratory chemo- and mechanoreflexes in the same infant. Information about the study was circulated to 225 mothers on the postnatal wards at University College Hospital (see Appendix 3). 58 agreed to participate in the study and written consent was obtained from one or both parents (see Appendix 2). It was only possible to obtain complete results from 17 of the 58 infants who participated in the study (see table 2.2.1.i). The most common reason for failure was the difficulty in obtaining a period of quiet sleep that allowed both the chemo- and mechanoreflex components of the study to be completed.

### **2.2.2 Experimental Conditions**

The experimental conditions for the alternate breath test are outlined in section 2.1.2. Details of end-inspiratory occlusions are described in section 4.2.2.

### **2.2.3 Data Analysis**

#### **2.2.3.a Analysis of phase relationships between alternating respiratory variables**

The pattern of the chemoreflex response during test runs was analysed for alternating respiratory variables  $V_{Ti}$  and  $t_E$ . Two test responses were used for each infant. When more than two control and two test runs were recorded, responses were selected on the basis of behavioural observations, the length of the run, and the linearity of the response. The slope of the regression line fitted to the chemoreflex respiratory

response was used to determine the phase relationships between the alternating respiratory variables. A response was classified as "in phase" when  $V_{ti}$  and  $t_E$  were either increasing or decreasing together in response to the hypoxic alternations, or "out of phase" when one variable was increasing and the other was decreasing. Chemoreflex responses were classified as being "in phase", "out of phase" or "mixed" (i.e. one each of "in phase" and "out of phase").

<b>Summary of parental acceptance</b>	
Numbers of parents asked to participate	225
Numbers of parents consenting to study	58
Numbers of parents refusing consent	167
Number of successfully completed studies	17
<b>Reasons for withholding consent:</b>	
Not interested/sceptical of research	59
Said no on basis of problems with baby sleeping or slightly jaundiced	27
Rapid discharge and mother felt insufficient time	26
Father withheld consent after mother agreed	20
Worried about test disturbing baby	12
Poor understanding of study due to language difficulty	5
Long/difficult birth	6
Mother nervous/upset	5
Participating in other research	3
Supportive of research but not wanting to be personally involved	3
<b>Summary of practical application</b>	
Number of successfully completed studies	17
Number of incomplete studies	41
<b>Reasons for failure to complete study:</b>	
Inability to achieve satisfactory sleep state	20
Only possible to complete alternations or occlusions	13
Mother changed mind and not wish to continue	6
Technical problems	2

Table 2.2.1.i Summary of recruitment and success rate for study.

### 2.2.3.b Analysis of chemoreflex respiratory responses

Two control and two test runs were analysed for each infant, and the average was used for further analysis. In the instance where there were more than 2 control and 2 test runs recorded, runs were selected on the basis of behavioural observations, the length of the run, and the linearity of the response. The mean breath-by-breath percentage alternation (the slope of the response derived by regression analysis) was calculated for control and test runs as described in section 2.1.4. Average test responses were compared to mean control responses by Wilcoxon matched pairs test ( $P < 0.05$ ).

### 2.2.3.c Assessment of significant chemoreflex respiratory responses

A subject was classified as a responder if at least one of the respiratory components of their chemoreflex response was significantly different from control. Two control runs from each of the 17 subjects were used to approximate a normal distribution ( $n > 30$ ). Test responses were significant if they occurred above a critical value, which was determined as the 95th percentile of the control distribution for the group (based on the normal distribution  $z > 1.645$ ), and were at least twice as great as the largest control response for that individual. Critical values are shown in table 2.2.3.c.i.

Respiratory variable	$V_{ti}$	$V_{te}$	$t_I$	$t_E$	$f$	$V_{ti}/t_I$	$V_{te}/t_E$	$t_I/T_{tot}$	$V_E$
Critical value	7.49	10.46	6.07	7.08	5.87	7.07	13.51	5.05	7.84

Table 2.2.3.c.i Critical values (% breath-by-breath alternation) for determining significant alternations in infants assessed for mechanoreflex responses to end-inspiratory occlusion.

### 2.2.3.d Analysis of end-inspiratory occlusions

End-inspiratory occlusions were measured by the method of Rabbette, Fletcher, Dezateux, Soriano-Brucher & Stocks (1994). Further detail is given in section 4.2.4.

## 2.3 Measurement of chemoreflex respiratory response to a breath-by-breath alternation in $F_{iO_2}$ in adult man

### 2.3.1 Subject Selection

20 adults were recruited from University College London (Ethical committee approved). Subjects were provided with a written information sheet and written consent was obtained (Appendix 4 and 5). They were also required to complete a questionnaire (Appendix 6).

### 2.3.2 Experimental Conditions

The experimental set-up is shown in figure 2.3.2.i. Subjects were studied whilst awake and seated, and were instructed to breathe through the nose. They were allowed to read and background music was present throughout the experiment (*ca.* 1 1/2-2hrs duration). Respiration was measured by inductance plethysmography (Respirace, Ardsley NY,

USA), and the ribcage and abdominal signals were calibrated (Sackner et al, 1989) to derive the scaling factors for their relative contribution to the sum signal.

Inspired gas composition was set using a mass spectrometer (Airspec 2000, CASE Medical Ltd., UK). The inlet system allows a very small continuous flow of sample gas into the analyser, which analyses up to 8 gases in 20msec and each gas is updated every 20 msec. The mass spectrometer was used to sample end-expiratory gases at the nose.

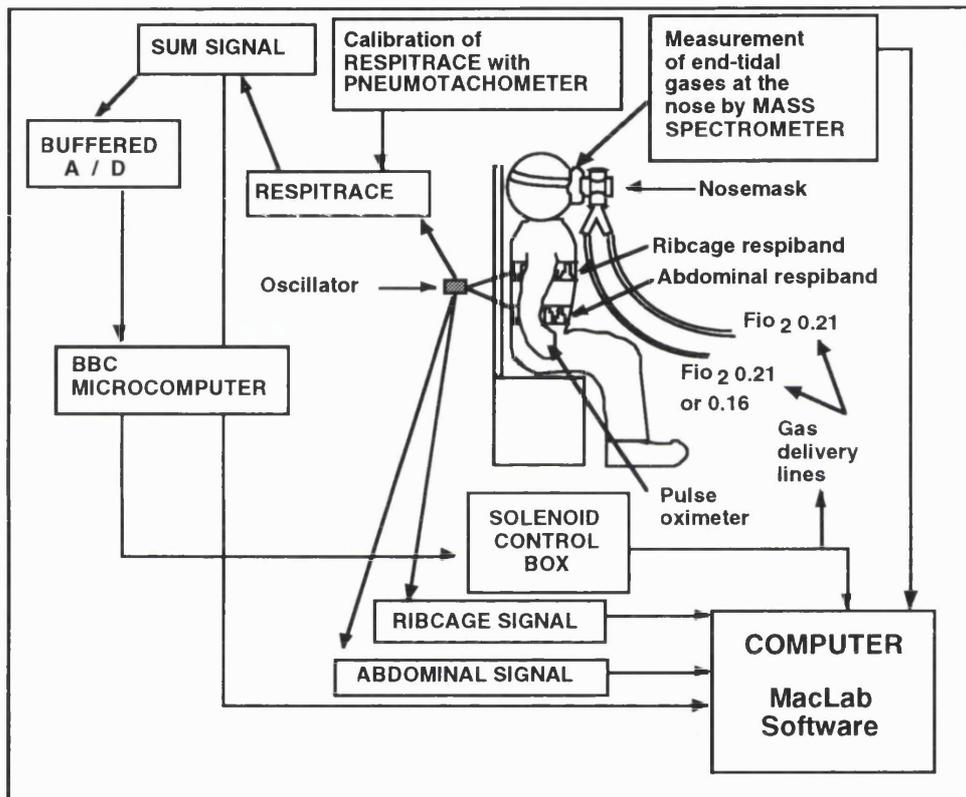


Figure 2.3.2.i Experimental set-up for measurement of adult respiratory chemoreflexes.

A BBC microcomputer measured tidal volume ( $V_{Ti}$  and  $V_{Te}$ ), inspiratory time ( $t_I$ ) and expiratory time ( $t_E$ ), calculated  $f$  (frequency),  $V_{Ti}/t_I$ ,  $V_{Te}/t_E$ ,  $t_I/T_{tot}$  and  $V_{Ti}/f$  for each breath. The computer controlled two 3-way solenoids which switched inspired gas between one of two lines on a breath-by-breath basis at the start of each expiration. Gases were delivered via a nose mask to the subject (Respironics Inc., UK; dead space=110 ml estimated by water displacement) at 25l/min, and flow was balanced in both gas delivery lines by a pneumotachometer (size 0, PK Morgan, UK; max flow rate=30 l/min, dead space=4-7ml). MacLab software on a Macintosh Quadra 650 was used to record the abdomen, ribcage and sum Respitrace signals, end-tidal oxygen ( $etO_2$ )

and carbon dioxide (etCO<sub>2</sub>) concentrations, and the switch signal used to trigger the solenoids. Pulse oximetry (Nellcor N200, Nellcor Inc., Hayward, CA, USA) was used to display heart rate and oxygen saturation (SaO<sub>2</sub>) throughout the experiment, and SaO<sub>2</sub> never fell below 93%.

### 2.3.3 Experimental Protocol

The subjects were blind to the experimental protocol which was randomised between subjects. Six of the 20 subjects were aware of the purpose of the experiments. Three of each control and test runs were performed in each subject, each up to four min duration. The protocol conditions were as follows:

- (i) Control, no nose mask: The subject breathed normally without wearing the nose mask whilst the solenoids alternated in the background.
- (ii) Control, wearing facemask: The subject breathed via the nose mask and inspired gas was switched on a breath-by-breath basis between 2 gas delivery lines each containing Fio<sub>2</sub> 0.21.
- (iii) Test, 21-16: Inspired gas was switched on a breath-by-breath basis between 2 delivery lines containing Fio<sub>2</sub> 0.21 and Fio<sub>2</sub> 0.16
- (iv) Test, 26-16: Inspired gas was switched on a breath-by-breath basis between 2 delivery lines containing Fio<sub>2</sub> 0.26 and Fio<sub>2</sub> 0.16

### 2.3.4 Data Analysis and Statistics

#### 2.3.4.a Exclusion of unsuitable responses

Six subjects were excluded from analysis because their end-expiratory gas signals indicated that they were breathing through the mouth, and their etO<sub>2</sub> signal did not alternate in response to the breath-by-breath alternations in Fio<sub>2</sub> (see figure 2.3.4.a.i).

#### 2.3.4.b Comparison of controls with and without a nose mask

For each subject, control runs wearing a nose mask, and control runs without a nose mask, were analysed separately and averaged. To observe the effect of wearing a nose mask on the baseline values for V<sub>ti</sub>, t<sub>I</sub>, t<sub>E</sub> and *f*, as well as the chemoreflex respiratory response (mean percentage breath-by-breath alternations), the respiratory

variables were compared between the two different types of control runs using Wilcoxon matched pairs ( $P < 0.05$  was used for statistical significance).

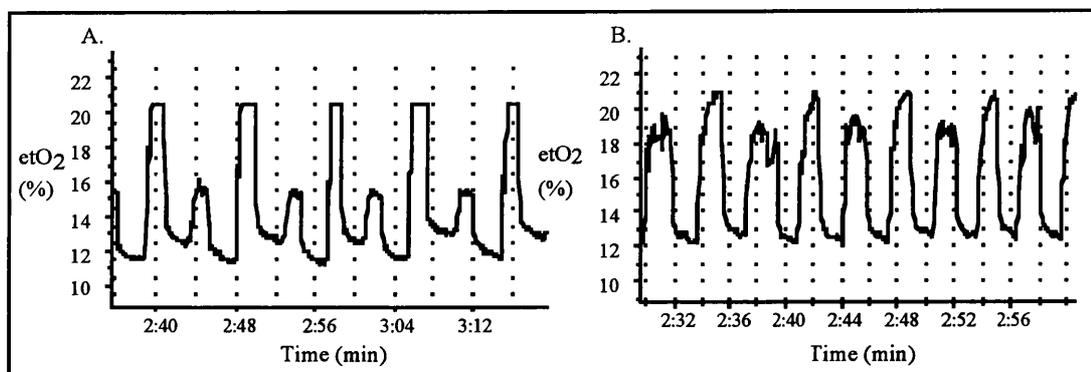


Figure 2.3.4.a.i End-tidal oxygen concentration (etO<sub>2</sub>) during breath-by-breath alternations in Fio<sub>2</sub>. Subject A shows clear changes in etO<sub>2</sub> in response to the alternations in Fio<sub>2</sub> between 0.21 and 0.16. Subject B is breathing through the mouth and nose causing a dilution of the inspired stimulus, and no clear alternation in etO<sub>2</sub> is observed.

### 2.3.4.c Analysis of chemoreflex respiratory responses

Chemoreflex responses were analysed as described in section 2.1.4.a. Control (with nose mask) and test responses were averaged for each adult. To allow comparison of chemoreflex responses between adults, the average control response was subtracted from the average test response. This gave an index of the chemoreflex respiratory response that was independent of the range of control responses between adults. Unlike data analysis in sections 2.1.4.a and 2.2.3.b, I did not compare averaged control and test responses for adults because of the large variation in control responses between individuals. I reasoned that the chemoreflex response was in fact the increase in the percentage alternation from control to test responses. So, I favoured the data analysis to detect significant alternations and responders that I have developed in this thesis.

### 2.3.4.d Assessment of significant chemoreflex responses

It was important to define which adults were responding to the alternations in Fio<sub>2</sub> in terms of showing a significant alternation in the chemoreflex respiratory response. A subject was classified as a responder if at least one of the respiratory components of their chemoreflex response was significantly different from control. Three control runs from each of the 14 subjects were used to approximate a normal distribution based on the assumption that  $n > 30$ . Test responses were significant if they occurred above a

critical value, which was determined as the 95th percentile of the control distribution for the group (based on the normal distribution  $z > 1.645$ ), and were at least twice as great as the largest control response for that individual. Critical values are shown in table 2.3.4.d.i.

Respiratory variable	$V_{ti}$	$V_{te}$	$t_I$	$t_E$	$f$	$V_{ti}/t_I$	$V_{te}/t_E$	$t_I/T_{tot}$	$V_E$
Critical value	8.38	9.21	10.35	10.06	7.84	6.12	12.89	8.81	8.41

Table 2.3.4.d.i Critical values (% breath-by-breath alternation) for determining significant alternations in adult subjects.

### 2.3.4.e Comparison of adult and infant chemoreflex responses

An average test and control response was calculated for each subject. The average control response was subtracted from the average test response for each respiratory variable. This was to compensate for the greater variation observed during control runs in the adult and between different adults, and the difference in sleep state between the adult and infant experiments. Adult responses were compared to infants for each respiratory variable by Mann-Whitney U test ( $P < 0.05$  was used for statistical significance).

### 2.3.5 Calibration of equipment

The mass spectrometer was calibrated using gas of a known composition from a pre-calibrated cylinder (15.4 %  $O_2$ , 9.63%  $CO_2$ , 4.82 % Ar, 70.15%  $N_2$ ; BOC Ltd., UK). The pneumotachometer volume signal, derived from the integrated airflow signal (size 0, PK Morgan, UK; max flow rate=30 l/min, dead space=4-7ml), was calibrated using known volumes of air from a graduated syringe.

The Resptrace sum signal was calibrated to derive scaling factors for relative contributions the ribcage and abdominal signals made to the sum signal (by the method of Sackner et al. (1989; see section 2.1.5 for description of calibration method). The Resptrace sum signal was calibrated into mls by the subject breathing through a small piece of tubing (*ca.* 10cm length, 1cm diameter) attached to a pneumotachometer. Pneumotachometer and sum Resptrace signals were recorded simultaneously on the MacLab from the pneumotachometer and sum Resptrace signals which allowed the calculation of the ratio between the two signals (see figure 2.3.5.i). Five breaths were compared and the ratio between the two signals averaged so that a scaling factor could

be calculated. Using this scaling factor the sum Respitrace signal was calibrated in ml. Comparison between the two signals, pneu (ml) and sum signal (ml), is done in the form of a ratio in figure 2.3.5.i, pneu:sum  $1.04 \pm 0.05$  (mean  $\pm$  S.E.M.). There was variation between breaths in the pneu:sum ratio, which approximates to 1:1 but deviates to either side. Presumably this variation is caused by a slight inaccuracy of the Respitrace, and may be due to a slow drift in baseline. Alternatively, this may be due to a poor signal from the Respibands if they are ill fitting or move slightly, or if the calibration factor to assess the relative contribution each of the two bands makes to the sum signal is slightly incorrect. It can be seen from figure 2.3.5.i that the profile for the breath shape is more clearly defined by the pneumotachometer, and changes in mean inspiratory flow are immediately more obvious. It was not possible to build the pneumotachometer into the nose mask, and so it was necessary to measure breathing by Respitrace. Furthermore, this was the methodology used in the infants and I wanted to use the same method in adults. Respitrace has one great advantage for measuring breathing non-invasively, in that it is easy for both the subject and operator. Thus slight discrepancies in calibrated tidal volume measurements were a limitation tolerated for the purpose of this study.

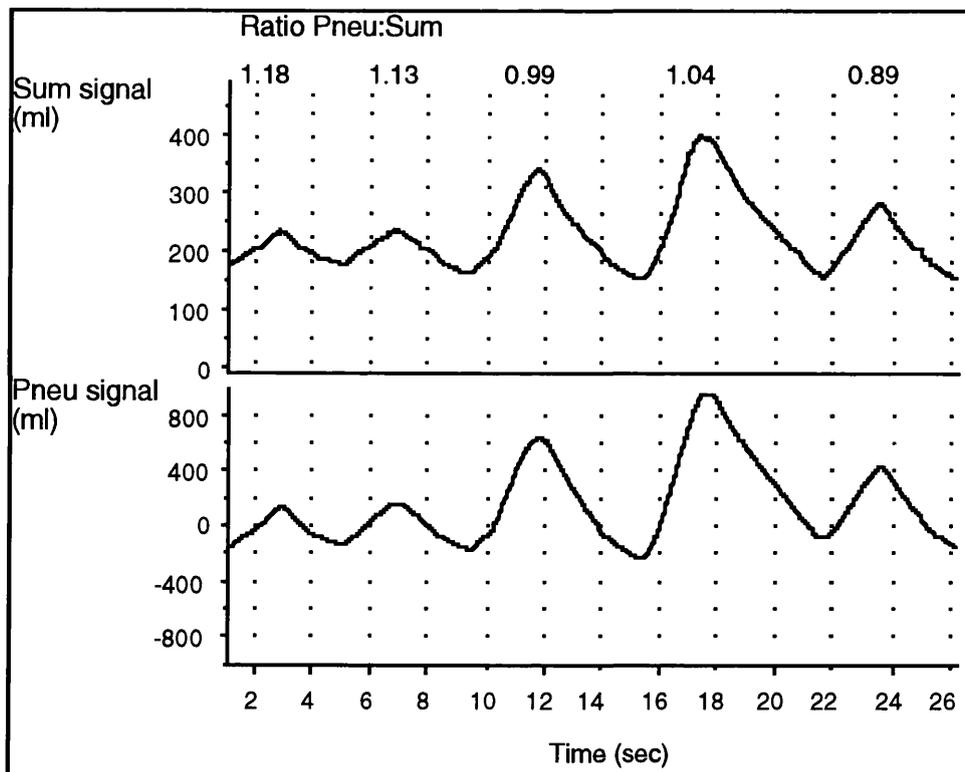


Figure 2.3.5.i Calibration of sum Respitrace (sum) signal from the pneumotachometer (pneu) signal. Shown at the top are the ratios between the signals for each breath. The sum Respitrace signal is expressed in ml by calculating a scaling factor in ml/V between pneumotachometer and sum Respitrace signals.

Independent of the calibration of the sum signal into mls, it was most important that the sum Resptrace signal remained proportional to the tidal volume signal as measured from the pneumotachometer. The Resptrace sum signal was compared to the pneumotachometer signal for proportionality at the beginning and end of the experiment. Figure 2.3.5.ii shows in one subject that the sum Resptrace signal had remained proportional to the pneumotachometer over the course of the protocol. The correlation coefficient between the sum and pneumotachometer signals before the experiment was  $0.95 \pm 0.01$  (14 subjects; mean + S.E.M), and  $0.97 \pm 0.02$  (12 subjects; mean + S.E.M) at the end.

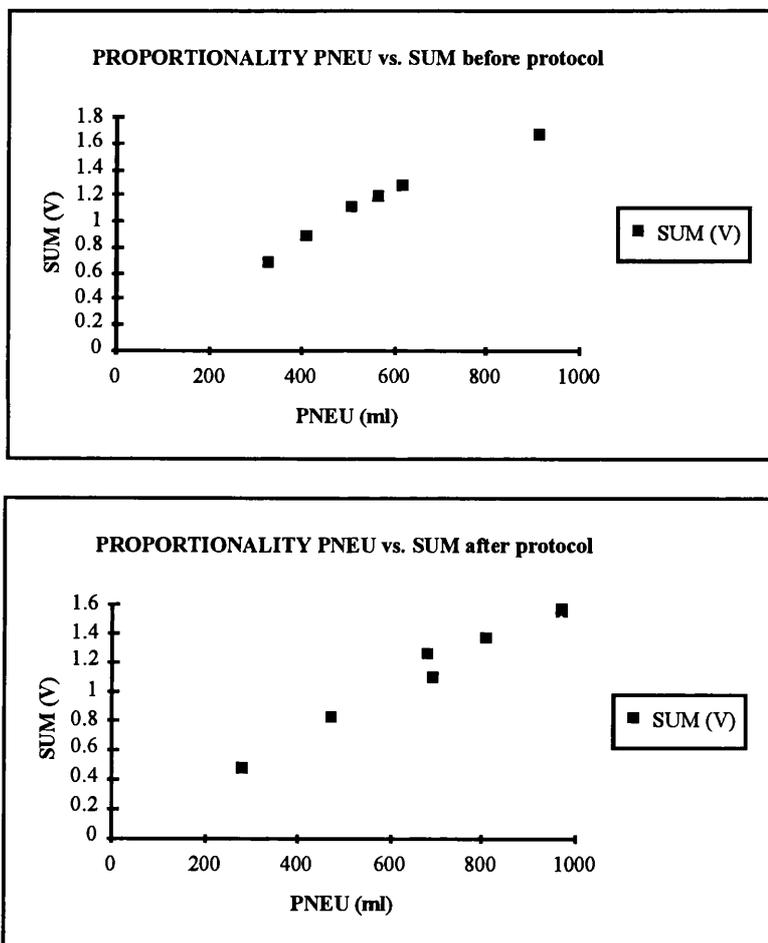


Figure 2.3.5.ii Calibration of pneumotachometer and sum Resptrace signal in one subject before (top) and after (bottom) the protocol.

## 2.4 Measurement of carotid chemoreceptor responses to CO<sub>2</sub> in the newborn lamb

### 2.4.1 Sheep husbandry

Lambs were transported on postnatal day 2 or later to the sheep holding facilities at Biological Services Department, University College London. Lambs were housed in floor pens for up to 4 days, and were not in isolation for more than 24 hrs. Lambs routinely received 1 ml Neftin (Smith Kline Beecham, UK), administered orally, for the prevention of infection.

Ambient temperature was held at 16°C, humidity at 50% and lighting was fixed on a 12 hour/12 hour day to night cycle. Lambs were bottle fed at regular intervals on a commercially available formula (Volac International Ltd, U.K.) and had access to water *ad libitum*. Food was withheld for up to 12 hrs prior to experiments. All animals were maintained at all times following the Home Office recommendations (Guidelines on the Care of Laboratory Animals and their use for Scientific Purposes, 1987).

### 2.4.2 Animal preparation

Anaesthesia was induced with thiopentone (20mg/kg i.v.) and lambs were intubated with a cuffed endotracheal tube (i.d. 4.0-5.5mm), then artificially ventilated (Sheffield Infant Ventilator Mark 4, East of Oxford, U.K.) and anaesthesia was maintained with 1.5-2.5% halothane (Rhône Mérieux, Ireland). The flow of inspired gases were set using rotameters (Platon, U.K.) and total flow varied between 4.0-7.0l/min. The trachea was dissected out and a ligature passed around it to secure the endotracheal tube firmly in place. End-expiratory gases were sampled from a port in the endotracheal tube by a mass spectrometer (AirSpec 2000, Case Scientific, U.K.). The left femoral vein was catheterized (o.d. 1.34 mm) to allow administration of drugs. The left brachial artery was catheterized (o.d. 1.65 mm) to allow the sampling of arterial blood gas (IL 1306 pH/blood gas analyser, U.K.) and the monitoring of arterial blood pressure (ABP) with a blood pressure transducer (DTX/Plus, Viggo-Spectramed, CA, USA). Blood pressure was maintained in some animals which had low ABP by 5-10ml bolus i.v. 10% dextran (Sigma, MO, U.S.A.) in 0.9% saline as required (Sigma, MO, U.S.A.). Metabolic acidosis was corrected by 3-5ml bolus injection i.v. of 8.4% NaHCO<sub>3</sub> (BDH Chemicals, U.K.) in 0.9% saline as required. Temperature was maintained at 39.5-40.5°C by a homeothermic blanket (CFP 8185, BioScience, U.K.). ECG was recorded using a headstage (NL 100 AK Headstage, Digitimer Ltd., U.K.) and needle electrodes were

placed on either side of the chest and on the right hind leg. The ECG signal was amplified and filtered using Neurolog Systems (Digitimer Ltd., U.K.). Lambs were placed in a stereotaxic frame held in place by ear bars (Kopf Instruments). Chloralose anaesthesia (60-70mg/kg, Sigma, MO, U.S.A.) was administered i.v. and halothane anaesthesia was discontinued. Lambs were paralysed with gallamine (Flaxedil 5mg/kg i.v., May & Baker, U.K.). Adequacy of anaesthesia was established by stability of heart rate and arterial blood pressure, and absence of any change in either of these in response to noxious stimuli. Once stabilized on chloralose anaesthesia, a mid-line incision was made in the neck of the lamb. The skin was pulled up to form a pool and filled with mineral oil, and all subsequent dissection was performed under mineral oil. The left hypoglossal nerve was cut near to its passage under the common carotid artery to facilitate better visibility. The left carotid sinus nerve (CSN) was located and cut at the junction of the glossopharyngeal nerve (see Blanco et al, 1984a). The sheath was removed and fine filaments were dissected from the nerve onto a blackened plate.

CSN activity was recorded from a bipolar stainless steel electrode (NL 100 AK Headstage, Digitimer Ltd., U.K.). Signal conditioning was possible by Neurolog Systems (Digitimer Ltd., U.K.). The CSN signal was amplified by a pre-amplifier (2K or 5K), filtered (low pass ca. 500 Hz, high pass ca. 5000 Hz) and further amplified (100 to 1000). Window height could be set on a spike trigger, so that action potentials that were to be counted could be viewed on an oscilloscope (Medelec, UK) and heard through a speaker via an audio amplifier (Pioneer, Japan). CSN discharge was integrated by a pulse integrator and counted by a period generator in 0.5, 1.0 or 2.0 sec bins.

ABP, ECG, raw and integrated CSN discharge were displayed on an oscilloscope and recorded onto chart (Cardioscript CD 6000, Picker, Germany) in addition to end-tidal oxygen (etO<sub>2</sub>) and carbon dioxide (etCO<sub>2</sub>) concentration. All signals were passed to a pulse code modulator (Sony, Japan) for conversion to video format and then recorded on VHS video tape (Panasonic, Japan), including ventilator signals and gas switching signals. The experimental set-up is shown in figure 2.4.2.i.

Carotid chemoreceptor activity was identified by its randomness, lack of synchrony with the ECG and brisk increase in discharge in response to reducing Fio<sub>2</sub> to nearly zero. Maximal discharge was calculated as the response to 20-30 sec of inspired nitrogen whilst etCO<sub>2</sub> was held constant by adding CO<sub>2</sub> to the inspirate, and integrated CSN discharge was counted by hand over a 10 sec period as shown in figure 2.4.2.ii. Discharge was then normalized to a percentage of maximal discharge (% max).

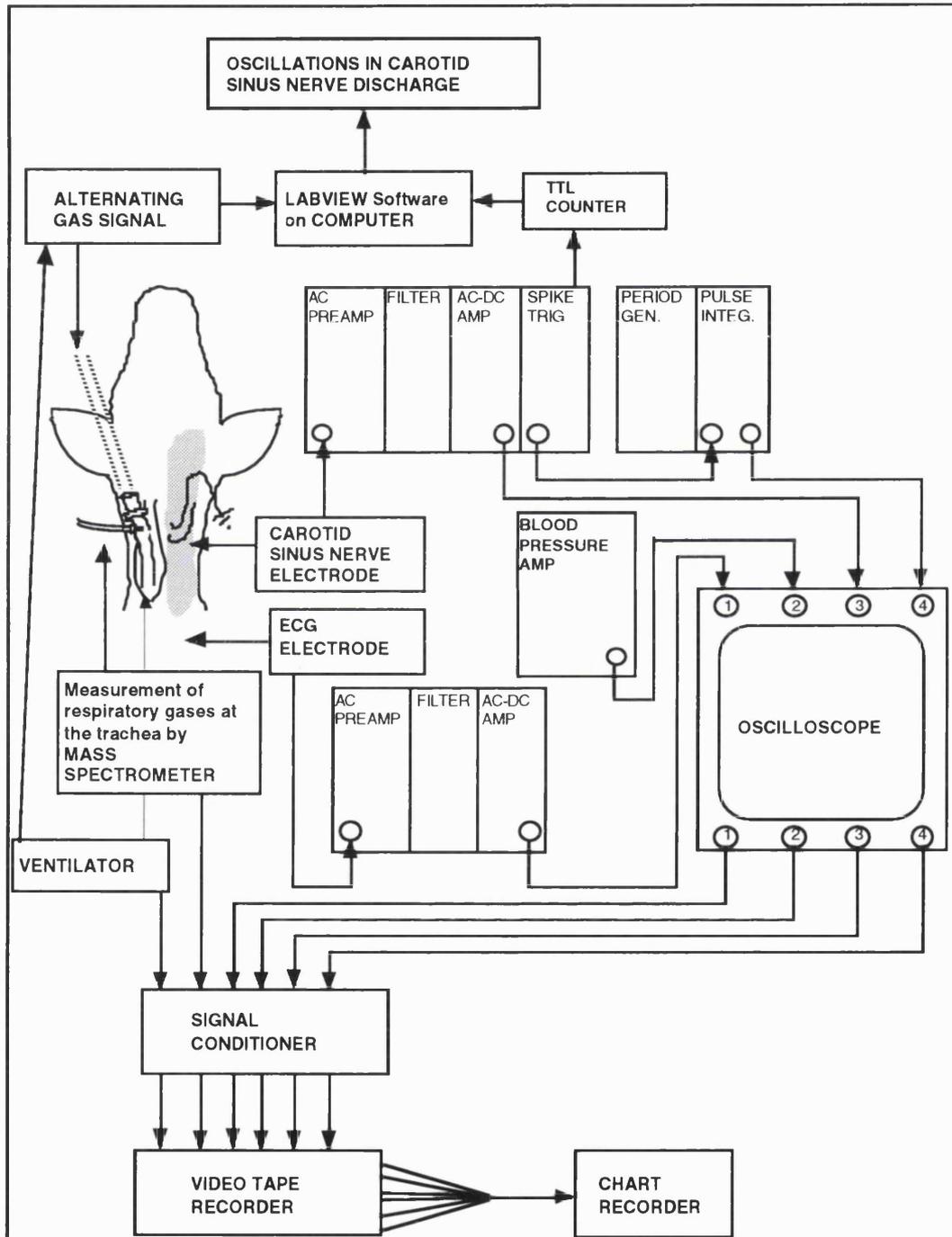


Figure 2.4.2.i Experimental set-up for carotid chemoreceptor fibre recordings.

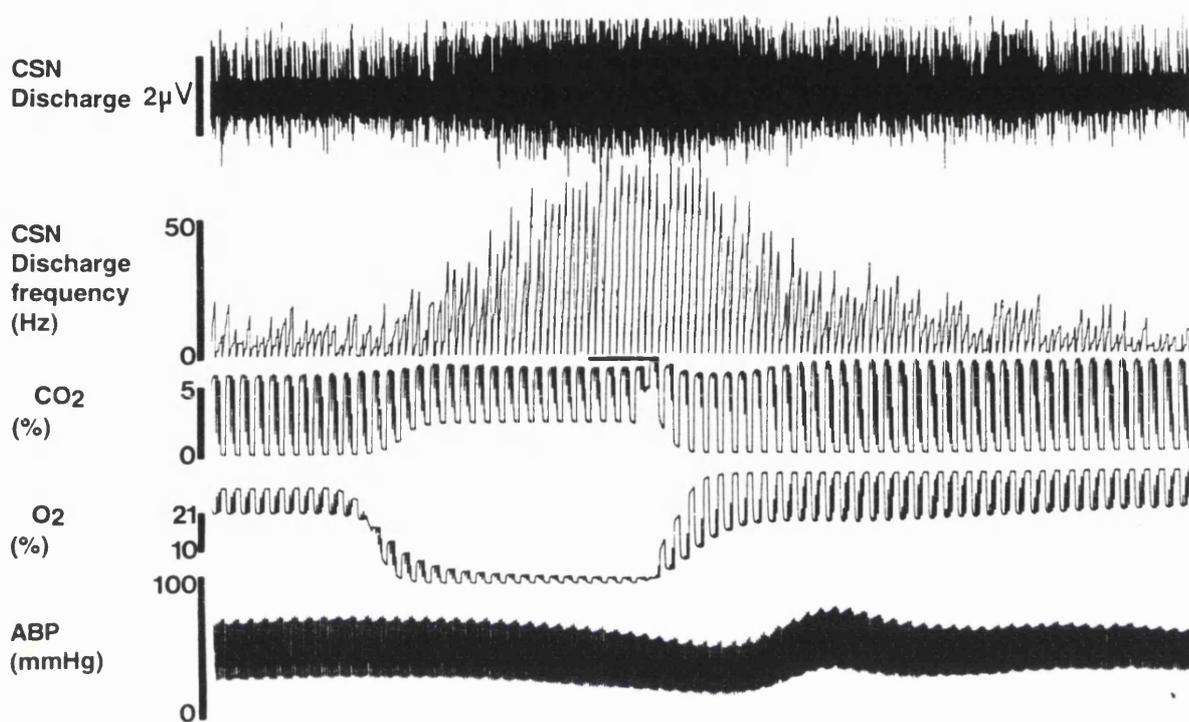


Figure 2.4.2.ii Maximal CSN discharge counted over 10 sec (solid bar).

### 2.4.3 Protocol

The maturation of carotid chemoreceptor steady state responses to  $\text{CO}_2$  was assessed in 43 lambs by producing steps in  $\text{etCO}_2$  by adding  $\text{CO}_2$  to the inspirate (measured by the mass spectrometer). Four levels of  $\text{P}_{\text{O}_2}$  were used:

- (i) hyperoxia (HYP  $\text{P}_{\text{O}_2}$  115-150 mmHg),
- (ii) normoxia (NX  $\text{P}_{\text{O}_2}$  90-105 mmHg),
- (iii) moderate hypoxia (MOD HX  $\text{P}_{\text{O}_2}$  40-60 mmHg) and
- (iv) severe hypoxia (SVHX  $\text{P}_{\text{O}_2}$  20-35 mmHg).

CSN discharge was counted after reaching a steady state (standardized to approx. 3 min after the step change in  $\text{etCO}_2$  was produced) over a period of 20 sec, averaged and expressed as a percentage of maximal discharge. Blood samples were taken at each  $\text{CO}_2$  level for  $\text{PaO}_2$ ,  $\text{PaCO}_2$  and pH analysis.

#### **2.4.4 Data Analysis**

Animals were divided prospectively into three age groups; 2-4d, 5-9d and 10-24d. For each individual fibre, discharge (% max) was plotted against PaCO<sub>2</sub> mmHg for each PaO<sub>2</sub>, and linear regression was used to describe the chemoreceptor CO<sub>2</sub> response curve. The slopes of the linear regression lines were used to compare chemoreceptor CO<sub>2</sub> responses between age groups at a given PaO<sub>2</sub>, and to compare CO<sub>2</sub> responses at any age with different PaO<sub>2</sub> levels by multiple linear regression ( $P < 0.05$  was used for statistical significance). In addition, Mann-Whitney U test ( $P < 0.05$  was used for statistical significance) was used to compare chemoreceptor responses within an age group (to observe the effect of PaO<sub>2</sub>) or within a PaO<sub>2</sub> level (to observe the effect of age).

### **3.0 THE RESPIRATORY RESPONSE OF HEALTHY TERM INFANTS AT TWO POSTNATAL AGES TO BREATH-BY-BREATH ALTERNATIONS IN $F_{iO_2}$ BETWEEN 0.21 AND 0.16**

#### **3.1 Introduction**

The fetus responds to hypoxia by decreasing the incidence of fetal breathing movements (Boddy et al, 1974; see Chapter 1), however this is not due to the relative insensitivity of the arterial chemoreceptors to hypoxia *in utero*. Blanco et al. (1984a) showed in the fetal lamb (90-143d gestation) that the arterial chemoreceptors were active *in utero*. Spontaneous chemoreceptor activity was present at *ca.* 25 mmHg and increased when  $PaO_2$  fell from 22 to 15 mmHg, but was virtually silenced on the day of birth when arterial  $PaO_2$  rose. Carotid chemoreceptor hypoxia sensitivity increased in lambs over the next 2-3 days, and these observations in conjunction with those of other workers, formulated the concept of postnatal 'resetting' chemoreceptor sensitivity to hypoxia. That is, following birth the neonate 'resets' the hypoxia chemosensitivity of the peripheral chemoreceptors to a range appropriate for air breathing. Evidence for the postnatal resetting of chemoreceptor sensitivity to hypoxia has been obtained from direct recordings of carotid sinus nerve (CSN) activity and measurement of the ventilatory response to hypoxia.

#### **3.1.1 Evidence for the postnatal increase in chemoreceptor hypoxia sensitivity from direct recordings *in vivo***

Direct evidence for the process of carotid chemoreceptor resetting *in vivo* has been reported in several different species. An early study by Biscoe & Purves (1967b) recorded carotid chemoreceptor activity to hypoxia in anaesthetized newborn lambs aged 0-5d (n=9) and found that there was no effect of postnatal age on chemoreceptor responses to hypoxia. They concluded that the carotid chemoreceptors were fully matured at birth. All subsequent studies on hypoxia sensitivity of the peripheral chemoreceptors in the neonate disagreed with this early finding. By their own admission, Biscoe & Purves (1967b) remark "*In some experiments, considerable difficulty was encountered in dissecting the sinus nerve to eliminate baroreceptor afferents and the signal-to-noise ratio obtained in the resulting strands was low*". It is possible that hypoxia sensitivity was reduced in some of their preparations by the difficulty of the dissection, or that the counted chemoreceptor activity was in fact noise,

which may account for the lack of a postnatal increase in hypoxia sensitivity in their observations.

Following the work of Blanco et al. (1984a), Hanson, Kumar & McCooke (1987) recorded single or few fibre carotid chemoreceptor activity in lambs age 5d (n=6) and 10d (n=5) and described the hyperbolic  $P_{O_2}$  response curve by equations. They found that the 'half-torr value', (i.e. the  $P_{aO_2}$  needed to produce a 50% decrease in discharge) was significantly different between lambs aged 5 and 10d. The  $P_{O_2}$  response curve was shifted to the right and upwards in older lambs, and they also speculated that the time course of chemoreceptor resetting may be slower in the newborn lambs compared to other species.

A postnatal increase in hypoxia sensitivity of the carotid chemoreceptors has been confirmed in the kitten (Marchal et al, 1992; Carroll et al, 1993). Marchal et al. (1992a) measured single fibre chemoreceptor activity in kittens aged <10d (n=17) and >10d (n=8) at three  $P_{aO_2}$  levels ( $P_{aO_2}$  ca. 330, 100 and 50 mmHg). Chemoreceptor activity was less in the younger kittens at  $P_{aO_2}$  100 and 50 mmHg, but not at 330 mmHg compared to the older kittens. The  $P_{O_2}$  response curve was also displaced upwards and to the right in older kittens. Carroll et al. (1993) measured whole nerve chemoreceptor activity in kittens aged 1, 4 and 8wks and in adult cats at three  $P_{aO_2}$  levels ( $P_{aO_2}$  ca. 400, 80 and 40 mmHg). Baroreceptor activity was removed by thermal and mechanical modification of the CSN (details of the procedure not given), and chemoreceptor activity was expressed as a percentage of baseline discharge (normocapnic normoxia). They found that chemoreceptor responses to hypoxia during normocapnia and hypercapnia were greater in 4wk, 8wk and adult cats compared to 1wk old kittens. Similarly, a shift of the  $P_{O_2}$  response curve upwards and to the right was observed in older kittens, however it is more difficult to interpret the developmental change between 1wk and 4wk due the  $P_{aO_2}$  scale and CSN activity expressed as a percentage of baseline activity (also see section 6.1).

Aortic chemoreceptor sensitivity to hypoxia also resets postnatally and has been demonstrated in the lamb *in vivo* (Kumar & Hanson, 1989). Single fibre chemoreceptor activity was recorded in lambs aged 1-4d (n=15) and 10-19d (n=15), and the response curve for older lambs was displaced to the right of younger lambs. Particularly noted in this study was the inability of older lambs to maintain a sustained increase in discharge below  $P_{aO_2}$  25-30 mmHg.

### **3.1.2 Evidence for the postnatal increase in chemoreceptor hypoxia sensitivity from direct recordings *in vitro***

More recently, evidence for the process of chemoreceptor resetting to hypoxia has been shown *in vitro* in the rat (Kholwadwala & Donnelly, 1992; Pepper et al, 1995). Whole carotid bodies are isolated *in vivo*, removed and suspended in buffered saline *in vitro*, and dilute enzymatic solutions (0.02% collagenase, 0.01% protease) are used to assist in the preparation of the carotid sinus nerve for dissection. Chemoreceptor activity can then be recorded, and single fibre type subsequently confirmed by superimposing triggered action potentials to compare the uniformity of height and shape. Kholwadwala & Donnelly (1992) recorded single fibre carotid chemoreceptor activity from rat pups aged 1-2d, 4-7d, 10-15d and adult rats exposed to anoxia, and found that peak chemoreceptor discharge was greater in adult rats and pups aged 10-15d than rat pups aged 1-2d and 4-7d. Pepper et al. (1995) recorded single fibre chemoreceptor activity *in vitro* in adult rat and rat pups aged 5-7d. With increasing hypercapnia, the  $P_{O_2}$  response curve was shifted to the right in the adult but not in the neonate. These observations are in agreement with those made from chemoreceptor recordings *in vivo*.

### **3.1.3 Ventilatory studies provide evidence for a postnatal increase in chemoreceptor hypoxia sensitivity**

#### **3.1.3.a The ventilatory response to acute hypoxia**

Interest in the ventilatory response of the neonate to isocapnic hypoxia was provoked when the observations of Howard & Bauer (1950) contrasted with observations made in the adult (Dripps & Comroe, 1947). Dripps & Comroe (1947) showed in adult man that ventilation increased when subjects were exposed to 15% oxygen, whilst the findings of Howard & Bauer (1950) demonstrated a fall in ventilation in the newborn infant breathing 12% oxygen. Cross & Warner (1951) went on to demonstrate that the ventilatory response of the newborn baby to hypoxia was in fact biphasic, and it is now well accepted that the neonate responds to acute hypoxia with an initial increase in ventilation (which is often referred to as phase I) followed by a fall in ventilation, (referred to as phase II) that may be to, or to below, the normoxic ventilation level. The biphasic ventilatory response to hypoxia has been reported in the newborn (Cross & Warner, 1951) and preterm infant (Cross & Oppé, 1952), kitten (Schweiler, 1968; Blanco, Hanson, Johnson & Rigatto, 1984b; McCooke & Hanson, 1986), monkey (Woodrum, Standaert, Mayock & Guthrie, 1981; LaFramboise, Standaert, Woodrum & Guthrie, 1981), rabbit (Schweiler, 1968; Grunstein, Hazinski & Schleuter, 1981, Martin-

Body & Johnston, 1988), rat (Eden & Hanson, 1987a) and lamb (Bureau, Foulon, Zinman & Begin, 1984). The initial increase in ventilation is mediated by the peripheral chemoreceptors, as carotid body denervation virtually abolishes the increase in ventilation to acute hypoxia during phase I of the BVR in the neonatal lamb (Bureau, Lamarche, Foulon & Dalle, 1985a). The small residual increase in  $V_E$ , following CSN section, to acute hypoxia has been attributed to the aortic chemoreceptors (Miller & Tenney, 1975; Bureau et al, 1985).

Both the magnitude of the increase in  $V_E$  during phase I of the BVR, and the development of a sustained increase in  $V_E$  to hypoxia have been investigated to assess the postnatal maturation of hypoxia chemosensitivity. The early studies of Cross & Warner (1951) and Cross & Oppé (1952) measured the ventilatory response to 15% oxygen over a wide range of postnatal ages (1d to 40d) and typically showed an increase in  $V_E$  by the second minute of hypoxia breathing. There was however no clear evidence for the effect of postnatal age on this response. Purves (1966d) found that ventilation increased and was sustained (mean increase  $V_E$  *ca.* 10%) during exposure to 10%  $O_2$  for 3min in anaesthetized newborn lambs ( $n=6$ ) before CSN denervation, but the increase in  $V_E$  was abolished after CSN section. Furthermore, the hyperpnoea induced by mild hypoxia (*ca.* 16% oxygen) increased postnatally in another group of unanaesthetized lambs ( $n=27$ ). The increase in  $V_E$  was 10% at 2d, and *ca.* 15% at 8-10d and was sustained for 6-8min, however Purves (1966d) concluded that peripheral chemoreceptors were functional and mature at birth. Similarly, Dawes & Mott (1959) observed a greater increase in  $V_E$  to 10-11%  $O_2$  in older compared to younger newborn rabbits and this was sustained for a longer period in the older animals. Rabbits aged 3-6d showed a mean increase  $V_E$  of 76% which was maintained for 7.5min, whilst rabbits aged 1d showed only a 58% increase in  $V_E$  for less than 3min.

Miller & Smull (1955) measured the respiratory response to 12% oxygen in term and preterm infants shortly after birth and found that the transient increase in ventilation was small or absent. In older infants aged 8-60d they did find a stimulation in breathing during hypoxia and so hypothesized that the chemoreflex was weak at birth and developed with age. This hypothesis was not widely accepted at the time due to evidence that showed the peripheral chemoreceptors were active at birth and it had been suggested that they were also functionally mature (Cross & Warner, 1951; Cross & Oppé, 1952; Purves, 1966c, 1966d). Further work was required before the concept of postnatal resetting of hypoxia chemosensitivity was universally accepted.

Brady & Ceruti (1966) measured the respiratory response to 12% oxygen in infants aged <6d ( $n=33$ ), and repeated the test at 13-15d ( $n=3$ ). In young infants  $V_E$  increased

during the first minute of hypoxia (mean increase *ca.* 10%) and subsequently fell over the next 2min of hypoxia. Older infants showed a much greater increase in  $V_E$  (mean increase *ca.* 20%) which was sustained over the 3 min of hypoxia. This provided strong evidence for the postnatal development of the respiratory response to hypoxia and the increase in hypoxia sensitivity. The increase in hypoxia chemosensitivity may be slower in preterm infants as it has been demonstrated that the increase in  $V_E$  to 15% oxygen is not sustained until 3wks (Rigatto, Brady & de la torre Verduzco, 1975a).

Several other studies in neonatal animals have found that the magnitude of the initial increase in  $V_E$  to hypoxia increases with postnatal age. McCooke & Hanson (1987) showed in conscious kittens that the increase in  $V_E$  during phase I of the BVR increased between day 1 and 14. In newborn lambs, the mean increase in  $V_E$  during hypoxia was 38% at 2d and 47% at 7d (Bureau et al, 1987) and in newborn monkeys the initial increase in ventilation was greater at 21d compared to 1d (Woodrum et al, 1981).

The development of a sustained increase in  $V_E$  to hypoxia is not necessarily the best indication of hypoxia chemosensitivity maturation, as there is evidence to suggest that the mechanism mediating a fall in  $V_E$  is of central origin (Gluckman & Johnston, 1987; Martin-Body, 1988; Martin-Body & Johnston, 1988; Williams & Hanson, 1989). Alternatively, it is possible that a sustained increase in  $V_E$  is brought about when stimulation of the peripheral chemoreceptors to increase  $V_E$  exceeds the depressant effect of hypoxia on breathing, and hence reflects maturity of the peripheral chemoreceptors. Nevertheless, Brady & Dunn (1970) examined the effect of  $CO_2$  in potentiating the respiratory response to hypoxia in newborn infants. In all infants the respiratory response to hypoxia was biphasic, except in the oldest infant (11d) who showed a sustained increase in ventilation.  $CO_2$  inhalation (normocapnia and at two levels of hypercapnia) during hypoxia augmented the initial increase in  $V_E$  (phase I), presumably by an increase in chemoreceptor drive. However, the fall in  $V_E$  that occurred after 2-3min of hypoxia (phase II) was not prevented by  $CO_2$  inhalation.

The persistence of the BVR in the neonate has been shown to be related to the severity of hypoxia as well as to postnatal age. Bureau et al. (1984) found that the ventilatory response of the newborn lamb to  $F_{iO_2}$  0.12 and 0.07 was biphasic at 2d, but only at  $F_{iO_2}$  0.07 at 7d. Eden & Hanson (1985; 1987a) showed in conscious newborn rat pups that at  $F_{iO_2}$  0.15 a BVR was present during hypoxia on days 1 and 3, at  $F_{iO_2}$  0.12 a BVR was present until day 5, and at  $F_{iO_2}$  0.08 a BVR was seen until day 7. Thus, to evoke a BVR to hypoxia with increasing postnatal age the severity of hypoxia must increase, which presumably reflects an increase in hypoxia sensitivity of the peripheral chemoreceptors.

### **3.1.3.b The ventilatory response to hyperoxia in the newborn**

Study of the ventilatory response to hyperoxia in the newborn has also shown a postnatal increase in hypoxia chemosensitivity. Cross & Warner (1951) measured the effect of 100% O<sub>2</sub> for 5min on ventilation in term infants (n=14). They observed a fall in ventilation mediated by a fall in V<sub>t</sub> in the first 1-2min, after which time ventilation increased as V<sub>t</sub> returned to pre-test levels and respiratory rate increased. The effect of postnatal age on the reduction in ventilation was not reported, and only one infant was aged 2d, the rest were aged 5-9d. In a subsequent study, Cross & Oppé (1952) measured the ventilatory response of premature infants (n=20; defined as birthweight ≤ 2.5kg) and term infants (n=30) to 100% oxygen. Preterm infants aged 1-40d showed a mean reduction in ventilation of 16% during the first minute of 100% O<sub>2</sub> breathing when room air was inhaled prior to the test, and this effect was augmented (35% reduction in V<sub>E</sub>) when preterm infants aged 12-45d breathed Fio<sub>2</sub> 0.15 prior to 100% O<sub>2</sub>. In the same study, term infants aged 1-13d (n=31) showed a mean fall in ventilation of 25% in the first minute of 100% O<sub>2</sub> breathing when Fio<sub>2</sub> 0.15 was administered prior to the O<sub>2</sub> test, which was less than the response for preterm infants. Due to the wide range of postnatal ages studied and the lack of information on gestational ages, it is difficult to interpret the effect of postnatal age on hypoxia chemosensitivity in this study, however Cross & Oppé (1952) suggested that their results showed that infants had a more active chemoreflex than adults because the reduction in ventilation to 100% O<sub>2</sub> breathing in the infant was greater than had been reported in the adult (Dripps & Comroe, 1947).

Brady, Cotton & Tooley (1964) went on to investigate the effect on ventilation of 5min of 100% O<sub>2</sub> breathing in a control of room air. Term infants aged 1-57hrs showed a large variation in responses, from no change to a fall in ventilation of 25%, with a mean decrease in ventilation of 10%. This variation was not explicable in terms of postnatal age. The authors agreed with the conclusions of Cross & Oppé (1952) that the peripheral chemoreceptors were functionally mature at birth because a reduction in V<sub>E</sub> to 100% O<sub>2</sub> breathing was evident from infants as young as 1hr after birth.

Rigatto et al. (1975a) showed a non-significant tendency for responses to hyperoxia to increase postnatally in preterm infants. Premature infants were defined by a birthweight of 1000-2000g, although gestation ranged between 28 and 37wks. There was an immediate fall in ventilation in response to 100% O<sub>2</sub> breathing, followed by a rise in ventilation after 1-2min mediated by a significant increase in respiratory frequency. They observed no significant effect of gestation on the ventilatory response, however they did note that the late increase in ventilation during O<sub>2</sub> breathing was

augmented at a greater gestational age, presumably due to maturation of the central chemoreceptors to CO<sub>2</sub> (Rigatto et al, 1975b).

Sankaran, Wiebe, Seshia, Boychuk, Cates & Rigatto (1979) compared the ventilatory response of the preterm infant (n=9) to 5min of 100% O<sub>2</sub> breathing to that of the adult (n=10). When subjects breathed air, the immediate reduction in ventilation was 26% in preterm infants and 10% in adults. However, to correct for a higher resting PaO<sub>2</sub> in adults they received 15% O<sub>2</sub> prior to 100% O<sub>2</sub> breathing to lower PaO<sub>2</sub> comparable to that of preterm infants, and under these conditions the mean fall in ventilation to 100% O<sub>2</sub> was 24%. They concluded that the previously held belief of a greater ventilatory response to 100% O<sub>2</sub>, or hypoxia sensitivity, in preterm infants compared to term infants or adults was false.

Purves (1966c) measured the fall in ventilation during the first minute (in 5sec periods) to 100% O<sub>2</sub> breathing, from 40min after birth to 10d in unanaesthetized newborn lambs (n=8). The mean reduction in V<sub>E</sub> for all lambs was 19% and there was no relation with postnatal age. In a further set of experiments, the respiratory response to 100% O<sub>2</sub> breathing was measured in anaesthetized lambs (n=6) before and after CSN section. The mean fall in V<sub>E</sub> to the test was 22% before, and 5% after CSN section. Although this study did not find a relationship with postnatal age and hypoxia sensitivity, the numbers for these experiments were small. More importantly however, it provided evidence for the role of the carotid chemoreceptors in mediating the fall in V<sub>E</sub> to O<sub>2</sub> breathing.

### **3.1.3.c The ventilatory response to a single breath of oxygen**

The Dejours' test of a single breath of oxygen (Dejours, Labrousse, Raynoud, Girard & Teillac, 1958) is one method that has been widely used, and adapted, to measure the ventilatory response to hypoxia. That peripheral chemoreceptor function was functionally mature at birth in the newborn infant was not universally accepted, and it has been demonstrated in several species using the single breath of oxygen test that hypoxic sensitivity increases postnatally.

Girard, Lacaille & Dejours (1960) measured the ventilatory response to two consecutive breaths of oxygen in term infants aged 1-19hrs (n=10) and 11-180d (n=9). Ventilation for the first 6sec after the start of the O<sub>2</sub> breathing was compared to pre-test, and was reduced by 18% in older infants but unaffected in younger infants. Thus, infants aged 1-19hrs were insensitive to two breaths of oxygen. Aizad, Bodani, Cates, Horvath & Rigatto (1984) measured the fall in ventilation to a single breath of O<sub>2</sub> from

breathing  $F_{iO_2}$  0.16 in term (n=10; mean postnatal age=3d; mean gestation 39wk) and preterm infants (n=10; mean postnatal age=9d; mean gestation= 34wk). During quiet sleep, preterm infants showed a fall in ventilation of 40% whilst term infants showed only a 14% reduction. The fall in ventilation during active sleep was similar in both age groups, and apnoea was a more common feature of the ventilatory response in preterm infants. The increased hypoxia chemosensitivity in preterm infants was presumably due a greater postnatal age, and is similar to the observations of Cross & Oppé (1952). Crance, Becquart, Bouverot & Arnould (1971) used five breaths of  $O_2$  to measure hypoxia sensitivity in term infants. The mean reduction in ventilation for infants aged 3-24hrs was 10%, and for infants aged 1-6d was 22%. Hagan & Gulston (1981) measured the fall in ventilation to a single breath of oxygen in term infants (n=12) during quiet sleep. Infants less than 48hrs showed no change in ventilation, whilst infants aged 48hr-3months showed a mean fall in ventilation of 25% to the single breath of  $O_2$ . Although these studies differ slightly in the number of  $O_2$  breaths delivered to the infants, or the manner in which the fall in ventilation is calculated, they all show a greater fall in ventilation to oxygen breathing in older infants compared to younger infants. This reflects a postnatal increase in hypoxia sensitivity of the peripheral chemoreceptors.

The single breath of oxygen has also been used extensively in other species to demonstrate a maturation of hypoxia chemosensitivity. Purves (1966b) measured the respiratory response to a single breath of 100% oxygen in lambs aged 6hr to 8d (n=11). In lambs younger than 3d there was no change in ventilation in response to the single breath of  $O_2$ , whilst in older lambs ventilation fell by 7-17%. Bureau & Bégin (1982) measured the fall in  $V_E$  in newborn lambs from air to 100%  $O_2$  after 5 and 10sec inhalation, and the nadir response. Lambs showed a mean fall in  $V_E$  of 30% aged 2d, 62% aged 10d and 49% aged 30d (nadir response), and this was significantly less at 2d compared to older lambs. Carroll & Bureau (1987a) measured the decrease in  $V_E$  to repeated episodes of 5 breaths of 100%  $O_2$  during steady state moderate hypoxia ( $F_{iO_2}$  0.08) and found in newborn lambs at 2-3d, the fall was less than at 10-11d.

Williams (1990) measured the respiratory response to two breaths of oxygen in newborn lambs and kittens. Lambs aged 5-6d (n=9) and 10-13d (n=10) showed a significant mean fall in  $V_E$  of 40-50% when measured over the first 3sec after the  $O_2$  test. This was mediated by a significant lengthening of expiratory time, and in the older lambs a reduction in  $V_t$ . Another group of lambs were CSN sectioned on postnatal day 1-2, which abolished the ventilatory response to two breaths of 100%  $O_2$  when measured at 5-6d (n=7) and 10-13d (n=10). The respiratory response to the  $O_2$  test in kittens was measured in the same manner as in the lambs, but after 15min of pre-

exposure to  $F_{iO_2}$  0.14. Kittens aged 1d showed a mean fall in  $V_E$  of *ca.* 50% 6sec after the two breaths of  $O_2$ . Kittens aged between 2 and 6d showed a range of responses between 55-85% reduction in  $V_E$ , and kittens aged 10d, 12d and 24d showed a more consistent reduction in  $V_E$  of 70-80%. In all kittens the fall in  $V_E$  was mediated by a reduction in  $V_t$  and a lengthening of  $t_E$ , particularly in kittens aged 10-14d and 21-27d who showed a prolongation in  $t_E$  of 500-700% compared to control.

In a modification of the early Dejours method, a slightly longer period of 100% oxygen breathing has also been used to measure hypoxia chemosensitivity in newborn infants. Hertzberg & Lagercrantz (1987) found in term infants that the mean decrease in ventilation to 30sec of 100%  $O_2$  at 2-6days was *ca.* 10%. This was greater than infants aged 2-6hrs when no change in ventilation was found in response to  $O_2$  breathing.

#### **3.1.3.d The ventilatory response to a repeated hypoxic stimulus alternating on a single breath basis**

Another method used to test respiratory chemoreflexes to hypoxia, which is the method I have used in my experiments, is the alternate breath test. This technique alternates the inspired oxygen concentration on a repeated basis, typically a breath-by-breath basis. The alternate breath test has been used in kittens, lambs, rats and babies to show a postnatal increase in the respiratory chemoreflex to hypoxia. Its strength is based on the speed at which the stimulus is delivered and the repeated nature of the stimulus. The respiratory response will be mediated by the arterial chemoreceptors as the stimulus is delivered quickly, and the duration too short for the respiratory response to be mediated centrally by chemoreceptive areas. As the alternation is repeated several times throughout a run (up to 100 times), the average alternation for each respiratory variable can be calculated to give a more accurate measure of the respiratory chemoreflex. A stimulus that alternates  $F_{iO_2}$  on a single breath (or two breath basis) will enhance the naturally occurring oscillation in arterial  $P_{O_2}$  via an oscillation in alveolar  $P_{O_2}$  (Haldane & Priestly, 1935; Chilton & Stacey, 1952; DuBois, Britt & Fenn, 1952; Yokota & Kreuzer, 1973). CSN section abolished the ventilatory response to breath-by-breath alternations in  $F_{iO_2}$  in the newborn lamb, which provided evidence for the involvement of the carotid chemoreceptors in the respiratory chemoreflex (Williams & Hanson, 1989).

The development of the technique to deliver alternate breaths of two different oxygen concentrations to assess peripheral chemosensitivity stems from earlier work performed in adult man. Cunningham, Elliott, Lloyd, Miller & Young (1965) performed breath-by-breath oscillations in carbon dioxide (breath-by-breath  $\Delta P_{ACO_2} \approx 10-12$ torr, against a

background of hypoxia;  $PAO_2$  60torr), oxygen (breath-by-breath  $\Delta PAO_2 \approx 20$ torr) and the two together (asphyxia) in adult man ( $n=3$ ). Oscillations were performed for 8-10min, and mean ventilation was compared to steady state ventilatory responses measured at different levels of alveolar  $P_{O_2}$  and  $P_{CO_2}$ . It was found that the oscillations produced no significant effect on the mean level of ventilation. Marsh, Lyen, McPherson, Pearson & Cunningham (1973) went on to demonstrate that during hypoxia ( $PAO_2$  55-66torr), breath-by-breath oscillations in alveolar  $CO_2$  ( $\Delta PACO_2 \approx 10-12$ torr) produced a corresponding breath-by-breath alternation in  $V_E$  of 4.6% in adult man ( $n=6$ ). Mean ventilation was unaffected, and the effect on ventilation was not observed if  $CO_2$  oscillations were performed in hyperoxia. Furthermore, several subsequent studies showed that at least one component of the respiratory chemoreflex alternated on a breath-to-breath basis when alveolar  $P_{CO_2}$  alternated (Ward & Cunningham, 1977a; 1977b; Metias, Cunningham, Howson, Petersen & Wolff, 1981). Metias et al. (1981) alternated  $etCO_2$  by 8-9torr from eucapnia during inspiration in hypoxia ( $etO_2$  50-60torr) which produced a 6.4% alternation in  $V_t$ , 4.7% alternation in  $t_I$  and a 4.7% alternation in  $t_E$ . Alternations were 25% greater when switching of the gases occurred between breaths, i.e. at the start of expiration (analogous to the method I have used).

Hanson, Kumar & Williams (1989) used the alternate breath method for the first time in neonates. They measured the respiratory response to two breath alternations in  $F_{IO_2}$  between 0.21 and 0.16 in newborn kittens ( $n=7$ ) during quiet sleep. Kittens aged 4-8d and 9-14d showed significant breath-by-breath alternations in the individual components of their respiratory chemoreflex in response to the alternations in  $F_{IO_2}$ , in contrast to younger kittens aged 1d and 2-3d that did not show a response. Williams & Hanson (1989) delivered single breath alternations in  $F_{IO_2}$  between 0.21 and 0.16 to newborn lambs ( $n=5$ ) on postnatal days 5-6 and 10-11 and measured the ventilatory response during quiet sleep. Lambs aged 5-6d showed significant alternations in the respiratory components of their chemoreflex, and the response was greater when the test was repeated on the same lambs at 10-11d. A further group of lambs were carotid body denervated (CBD) within 36hrs after birth and subjected to the same protocol ( $n=5$ ). CBD lambs showed no significant alternation in the respiratory response on day 5-6, however a small response had developed by day 10-11. The authors concluded that the response at 10-11d in the CBD sectioned lambs was due to a maturation of the aortic chemoreceptor response, and that the marked reduction in the respiratory chemoreflex produced by CBD was evidence for the predominant role of the carotid chemoreceptors in mediating the response.

In newborn infants, Williams et al. (1991) measured the respiratory response to single breath alternations in  $F_{iO_2}$  between 0.21 and 0.16 during quiet sleep. Infants aged 3-10hrs (n=6) showed a small respiratory chemoreflex with a significant reflex alternation in  $V_t$ . Infants aged 12-24hrs (n=12) showed significant respiratory alternations in  $V_t$ ,  $V_t/t_I$  and  $V_t/f$ , as did infants aged 24-48hrs (n=18). The respiratory chemoreflex was well developed by 5-8d (n=7) with significant alternations in most respiratory variables. A postnatal increase in hypoxia chemosensitivity is inferred from the development of the respiratory chemoreflex to the hypoxia alternations even though the infants were not studied longitudinally, as the time course for chemoreceptor resetting was appropriate from evidence obtained from the direct chemoreceptor recordings in lambs (Blanco et al, 1984a; Kumar & Hanson, 1989).

### **3.1.4 Objective**

The work of Williams et al. (1991) raised an interesting question: was the process of resetting of chemoreceptor hypoxia sensitivity complete in infants aged 5-8d, or did maturation of the respiratory chemoreflex continue beyond the first week of life? There was the suggestion from these experiments that the most dramatic increase in hypoxia chemosensitivity occurred on the first postnatal day, more subtle changes occurring up to the end of the first week. However, no further experiments were performed beyond the first postnatal week to assess the extent to which chemoreflex sensitivity continued to mature. In addition infants were not studied longitudinally, and so inter-subject variation may have influenced the results between different age groups.

The purpose of the experiments in this Chapter was to address the question of whether a further increase in peripheral chemosensitivity occurs beyond the first week of postnatal life. I have measured respiratory chemoreflexes in a group of healthy term infants at two different postnatal ages using the same stimulus employed by Williams et al. (1991). Some of these findings have been published (Calder, Williams, Kumar & Hanson, 1994a).

## 3.2 Methods

### 3.2.1 Subject information

Subject selection is outlined in section 2.1.1. Infant data for the 13 successfully completed studies are given in table 3.2.1.i. Three of the infants were studied at Royal Berkshire Hospital in Reading and the data recording performed by Dr Bridget Waites.

	Gestation (wks)	Birth weight (g)	Postnatal age Study 1 (hrs)	Postnatal age Study 2 (days)
1	38	3140	72	31
2	38	2900	24	62
3	39	3060	48	65
4	38	3140	96	44
5	37	3520	27	43
6	38	3100	32	43
7	38	3780	54	44
8	40	2600	40	59
9	42	3860	70	48
10	42	3720	15	51
11	42	3220	32	54
12	42	3420	17	20
13	42	4340	26	42
Mean $\pm$ SEM	39.7 $\pm$ 0.6	3369 $\pm$ 129	42.5 $\pm$ 6.8	46.6 $\pm$ 3.4

Table 3.2.1.i Subject information. Gestation (wks), birthweight (g), postnatal age study 1 (hrs) and study 2 (days).

## 3.3 Results

### 3.3.1 Baseline values for respiratory variables

Mean values for  $t_I$ ,  $t_E$ ,  $f$  and  $t_I/T_{tot}$  were calculated for control and test responses and the data is given in table 3.3.1.i. It was not possible to calculate mean values for  $V_{tI}$  and  $V_{tE}$  as volumes were not calibrated in terms of mls. No significant differences were found in these variables between control and test runs, at either age or between ages for either control or test runs. There was a small decrease in  $f$  during control runs with age however this was not significant.

		t <sub>I</sub> (sec)	t <sub>E</sub> (sec)	f (breaths/ min)	t <sub>I</sub> /T <sub>tot</sub>
Study 1	Control	0.50 ± 0.02	0.77 ± 0.06	52.0 ± 3.0	0.41 ± 0.01
	Test	0.52 ± 0.02	0.83 ± 0.07	49.8 ± 3.0	0.41 ± 0.01
Study 2	Control	0.54 ± 0.02	0.79 ± 0.04	48.0 ± 2.4	0.42 ± 0.01
	Test	0.53 ± 0.02	0.75 ± 0.03	49.2 ± 1.8	0.42 ± 0.01

Table 3.3.1.i Baseline values for respiratory variables (mean ± S.E.M)

### 3.3.2 Chemoreflex respiratory responses for individual infants

To observe the average change in control and test responses for an individual infant, chemoreflex responses for each respiratory variable were plotted against postnatal age in figures 3.3.2.i-ix. The average was taken of two control and test responses for each infant which allowed comparison between controls and tests with age. Control responses tended to show little change or a small decline in the magnitude of the breath-by-breath % alternation between study 1 and 2. Control responses typically showed breath-by-breath alternations which were less than 10%. In contrast, the magnitude of test responses increased for a small number of infants between study 1 and 2, but appeared to decline for a greater number of infants. There was great variation in the magnitude of test responses between infants, and less so for control responses. It was very difficult to interpret the maturation of chemoreflex responses in this manner as there was so much overlapping between test and control, so other methods of comparison are investigated in section 3.3.3 and 3.3.4.

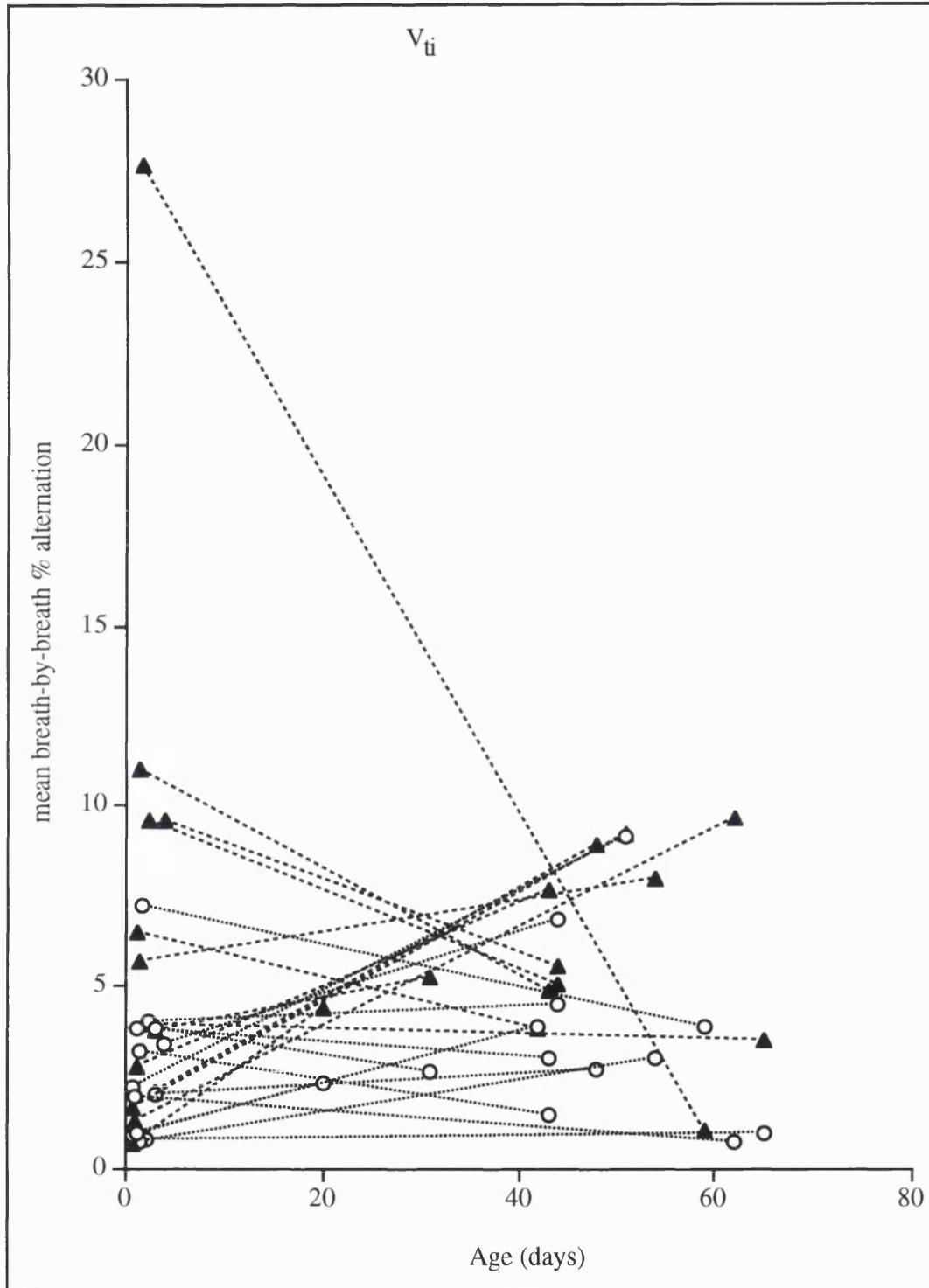


Figure 3.3.2.i  $V_{ti}$  average control (open circles) and test (closed triangles) for infants at two different postnatal ages.

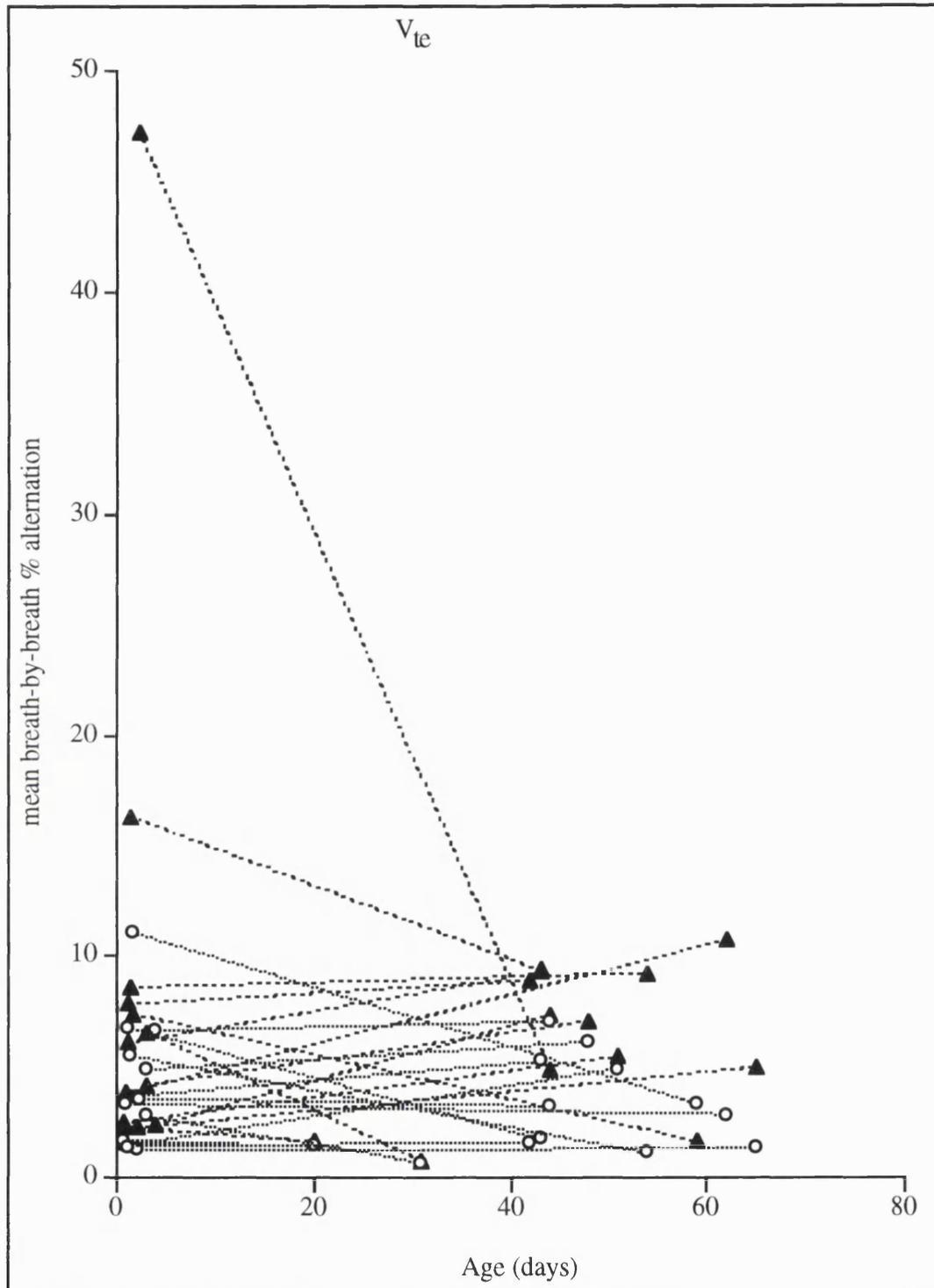


Figure 3.3.2.ii  $V_{te}$  average control (open circles) and test (closed triangles) for infants at two different postnatal ages.

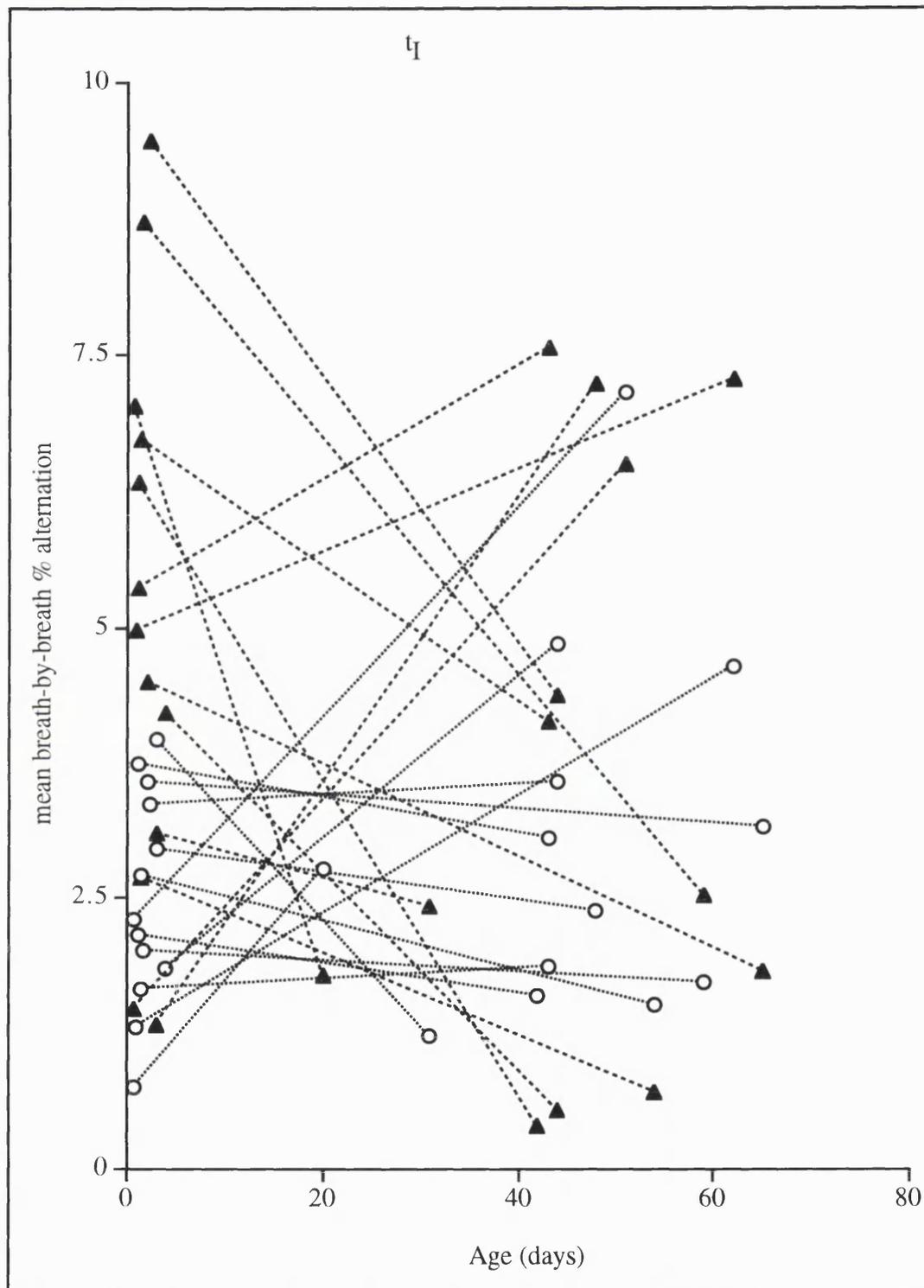


Figure 3.3.2.iii  $t_I$  average control (open circles) and test (closed triangles) for infants at two different postnatal ages.

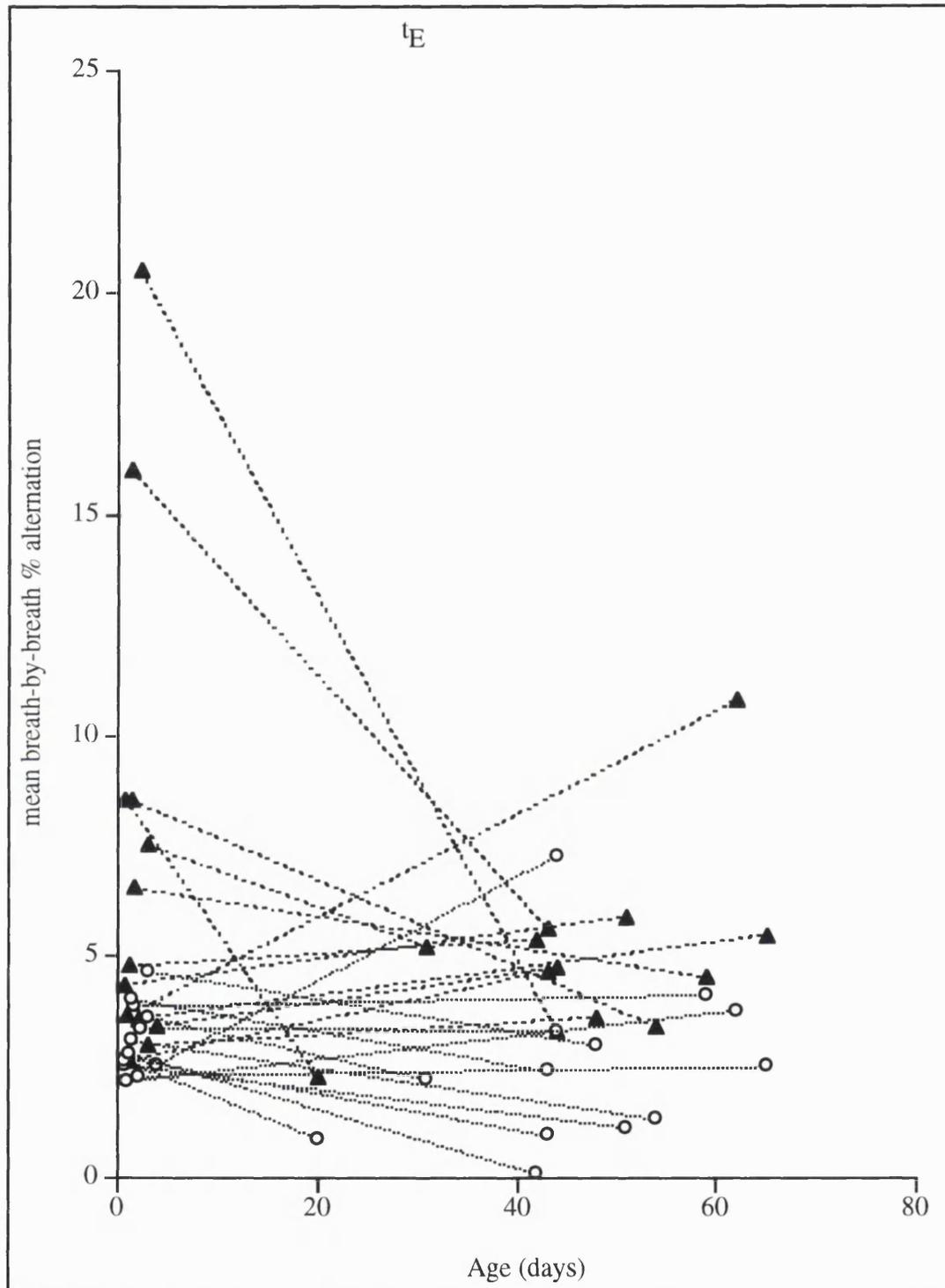


Figure 3.3.2.iv  $t_E$  average control (open circles) and test (closed triangles) for infants at two different postnatal ages.

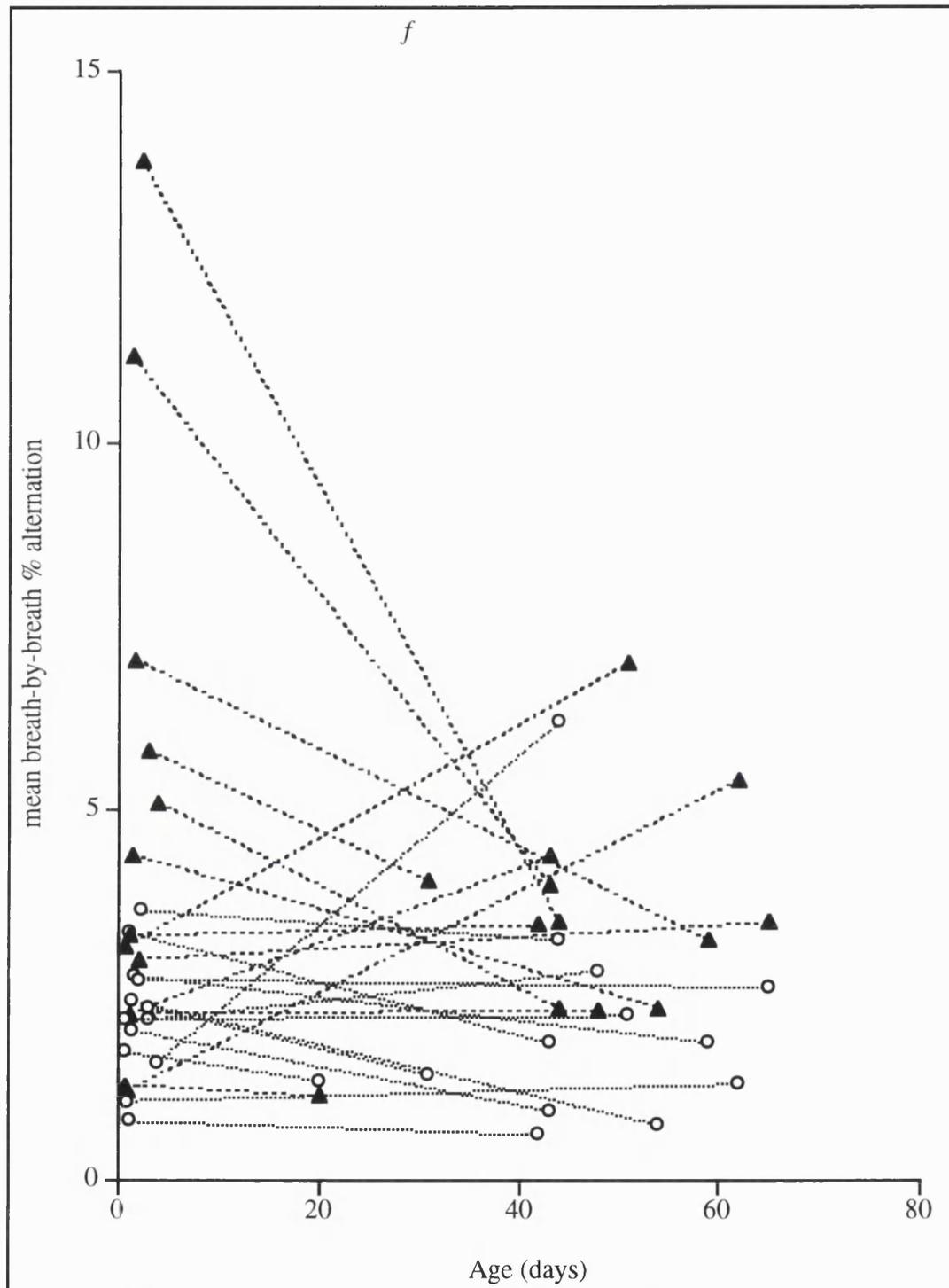


Figure 3.3.2.v *f* average control (open circles) and test (closed triangles) for infants at two different postnatal ages.

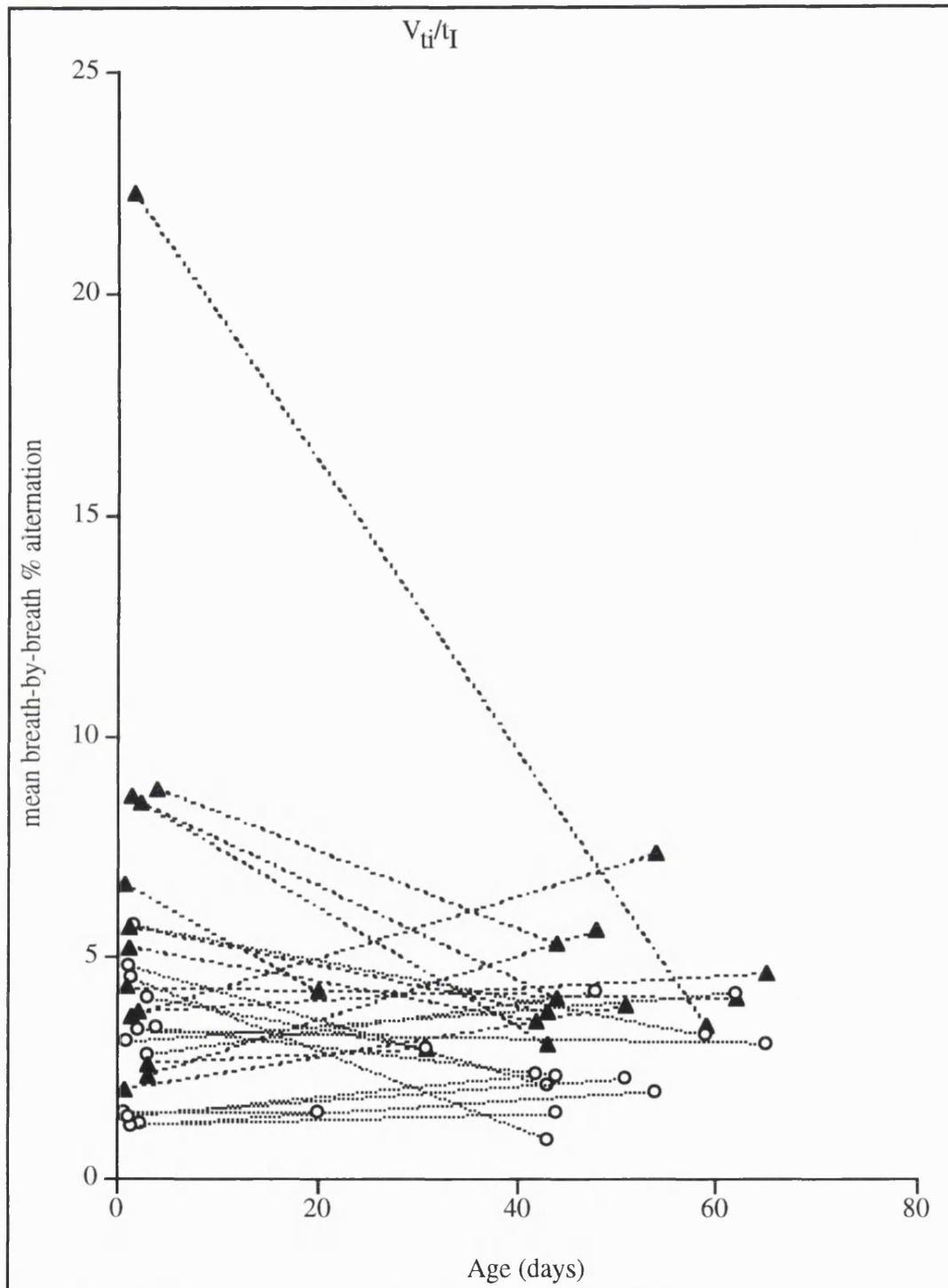


Figure 3.3.2.vi  $V_{ti}/t_I$  average control (open circles) and test (closed triangles) for infants at two different postnatal ages.

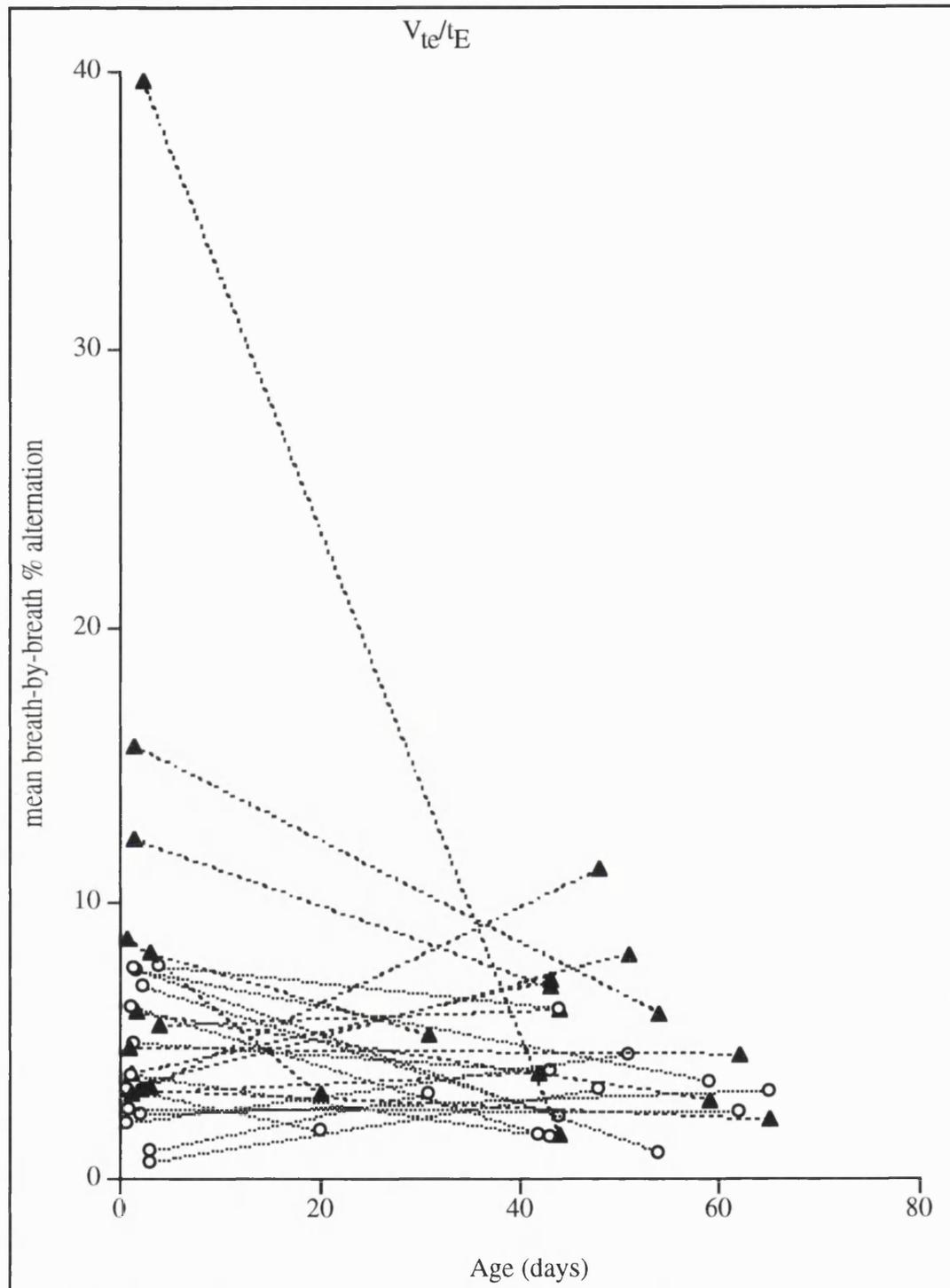


Figure 3.3.2.vii  $V_{te}/t_E$  average control (open circles) and test (closed triangles) for infants at two different postnatal ages.

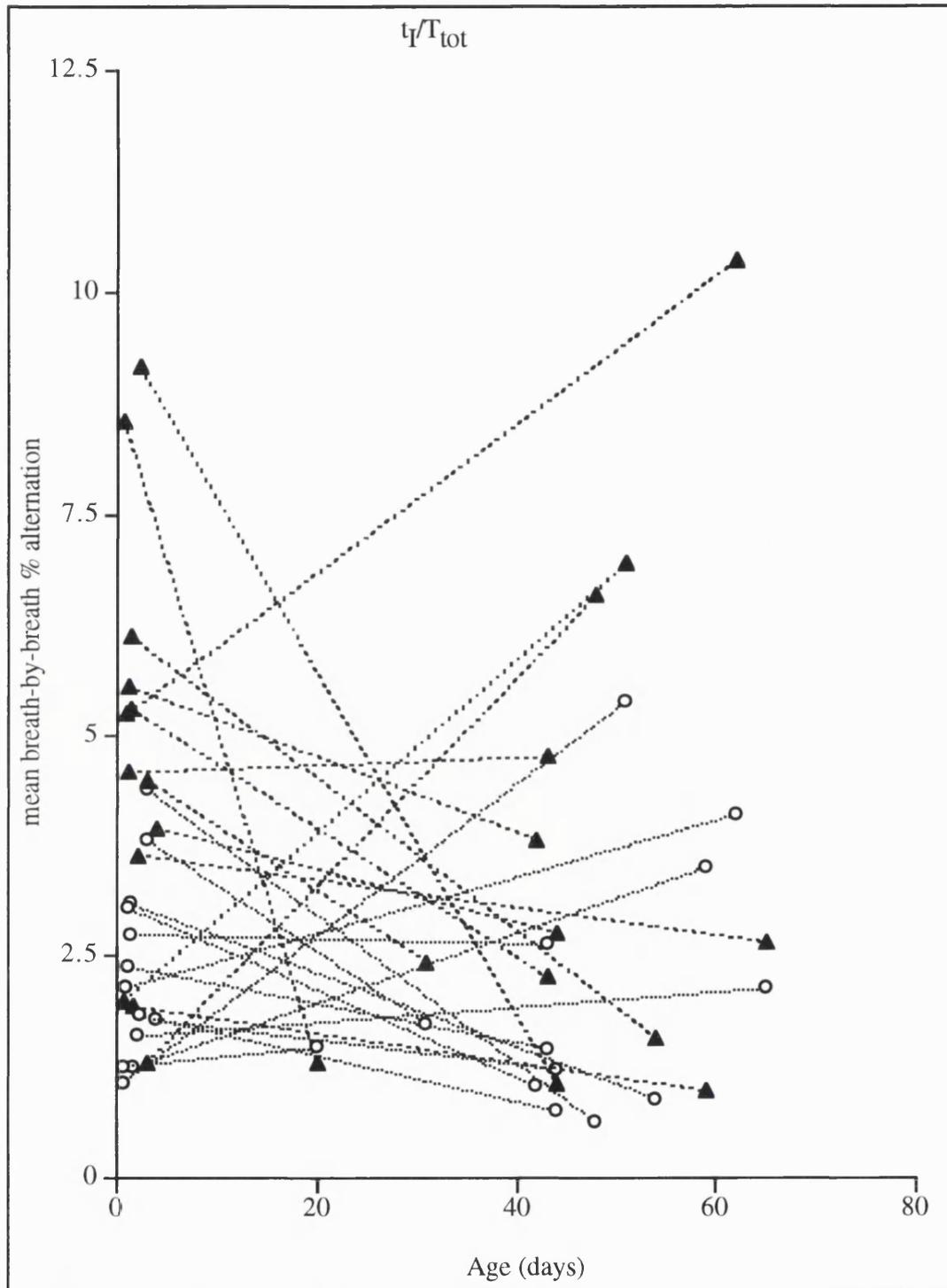


Figure 3.3.2.viii  $t_I/T_{tot}$  average control (open circles) and test (closed triangles) for infants at two different postnatal ages.

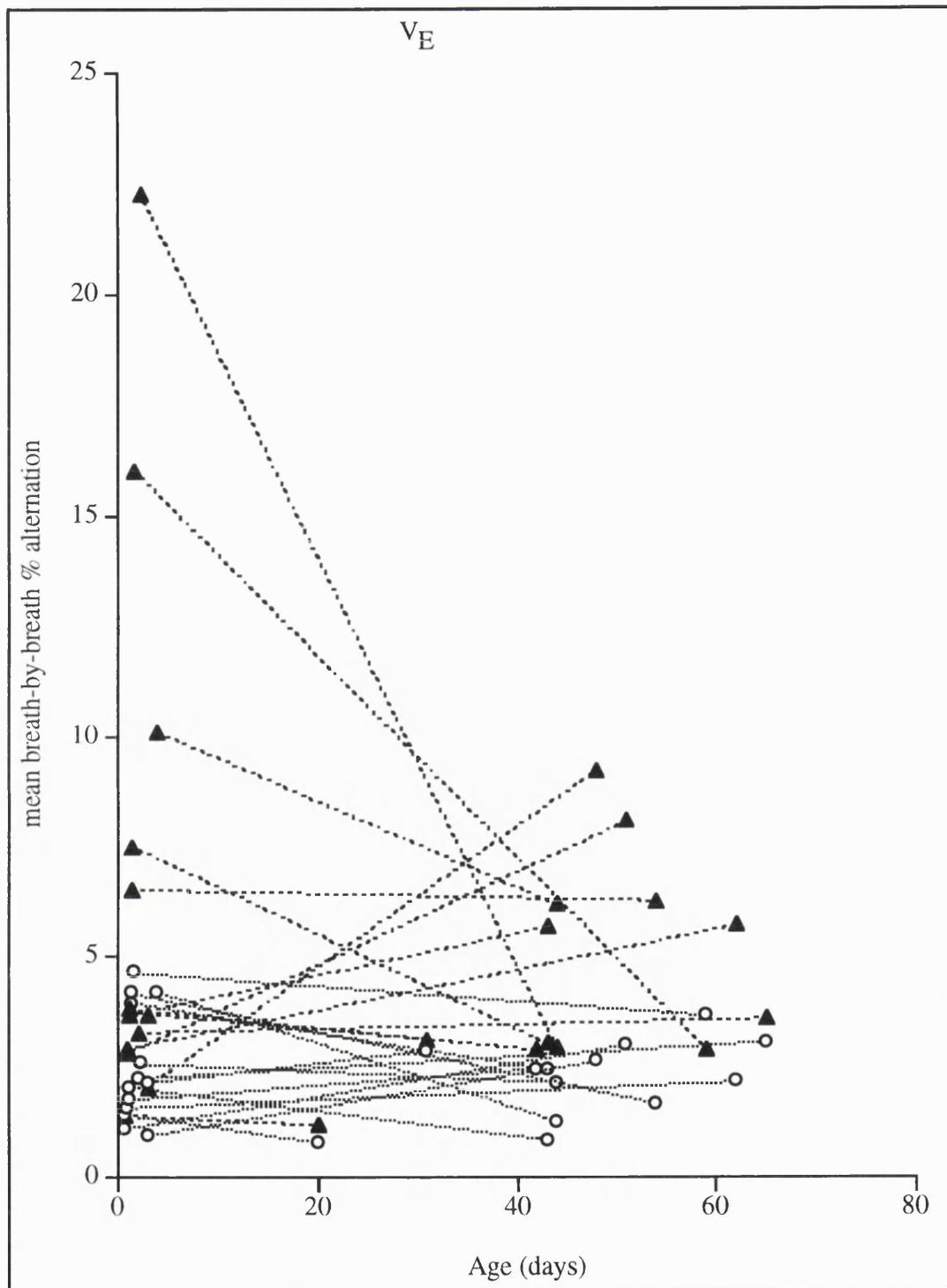


Figure 3.3.2.ix  $V_E$  average control (open circles) and test (closed triangles) for infants at two different postnatal ages.

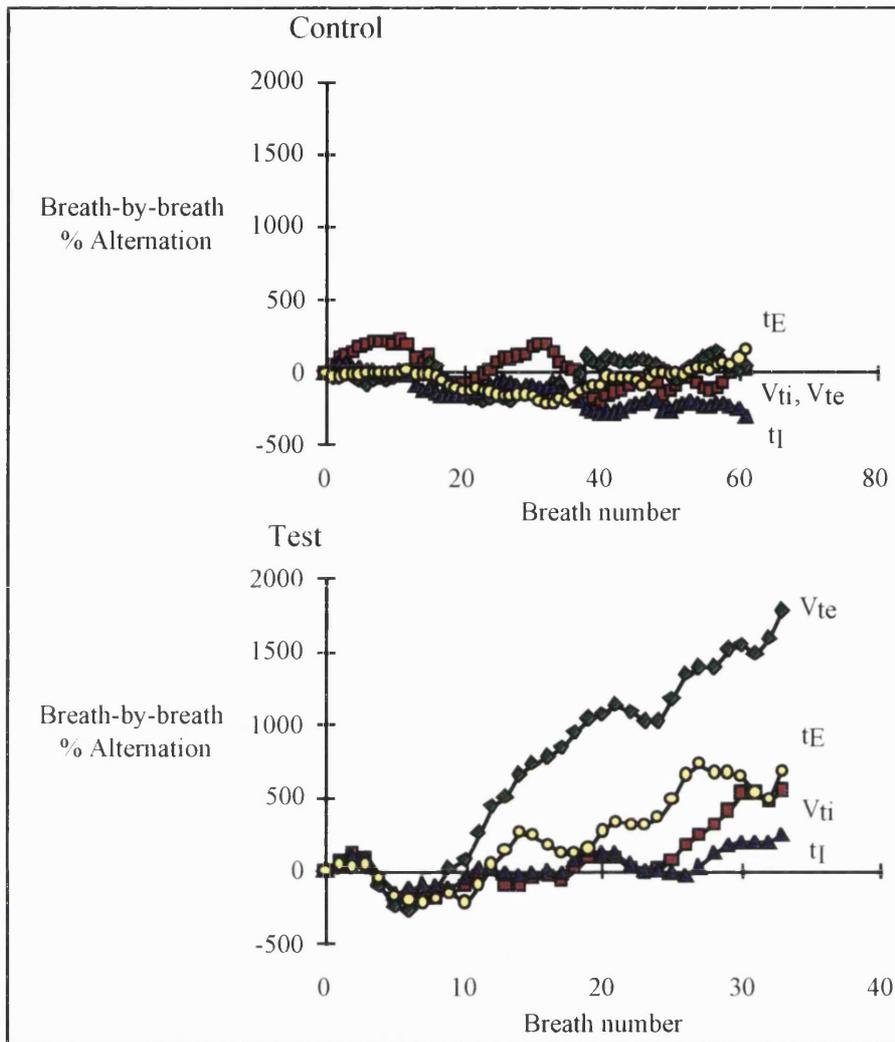


Figure 3.3.2.x Control (top) and test (bottom) chemoreflex responses for one infant aged 2 days. Respiratory variables  $V_{ti}$  (squares),  $V_{te}$  (diamonds),  $t_i$  (triangles) and  $t_E$  (circles) are plotted cumulatively (breath-by-breath % alternation) with respect to breath number.

An example of one infant's chemoreflex response, which showed a larger breath-by-breath alternation during study 1 (figure 3.3.2.x) compared to study 2 (figure 3.3.2.xi) is illustrated below. Test responses at 2 days were significant for alternations in respiratory variables  $V_{ti}$  (11.06%  $r=0.52$ ),  $V_{te}$  (52.54%,  $r=0.96$ ),  $t_E$  (19.72%,  $r=0.79$ ), but not for  $t_i$  (1.22%,  $r=0.65$ ). In contrast, test responses for the same infant aged 44 days were much smaller for alternations in respiratory variables  $V_{ti}$  (3.22%  $r=0.58$ ),  $t_i$  (2.49%,  $r=0.75$ ),  $t_E$  (4.48%,  $r=0.75$ ) and were only significant for  $V_{te}$  (8.11%,  $r=0.84$ ). For control responses, the alternation in respiratory variables were for  $V_{ti}$  (4.21%  $r=0.64$ ),  $V_{te}$  (2.36%,  $r=0.45$ ),  $t_i$  (5.02%,  $r=0.88$ ) and  $t_E$  (1.08%,  $r=0.27$ ) when aged 2 days, and when aged 44 days,  $V_{ti}$  (3.86%  $r=0.87$ ),  $V_{te}$  (2.29%,  $r=0.59$ ),  $t_i$  (3.54%,

$r=0.73$ ) and  $t_E$  (4.45%,  $r=0.82$ ). This infant showed the largest decline in the magnitude of the chemoreflex response between the two studies. There was however little change in the magnitude of the breath-by-breath alternation for control responses.

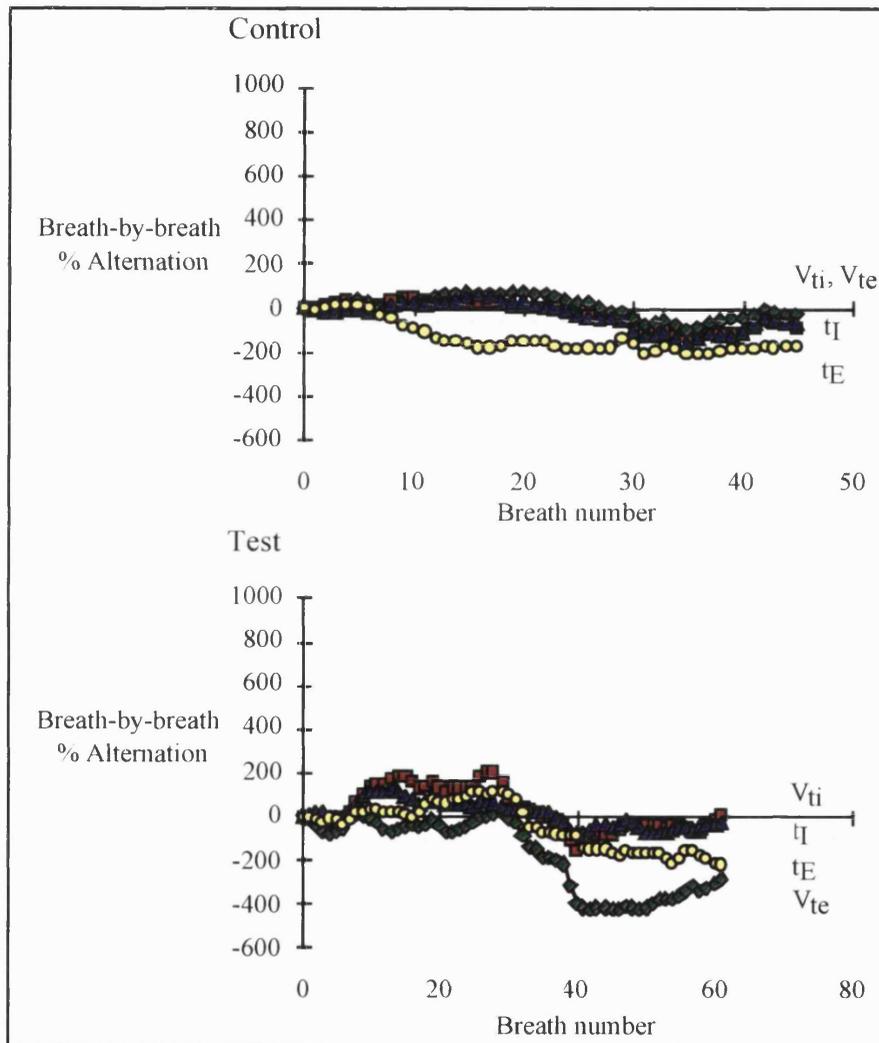


Figure 3.3.2.xi Control (top) and test (bottom) chemoreflex responses for the same infant in figure 3.3.2.x, but aged 44days. Respiratory variables  $V_{ti}$  (squares),  $V_{te}$  (diamonds),  $t_I$  (triangles) and  $t_E$  (circles) are plotted cumulatively (breath-by-breath % alternation) with respect to breath number.

### 3.3.3 Chemoreflex respiratory responses for all infants

Test responses were compared to control for each respiratory variable (see section 2.1.4.a). Control and test mean breath-by-breath % alternations for study 1 and 2 are given in table 3.3.3.i, and these data are also represented graphically in figure 3.3.3.i. For study 1 chemoreflex respiratory test responses were significantly greater than control for  $t_I$ ,  $t_E$ ,  $f$ ,  $V_{ti}/t_I$ ,  $t_I/t_{Tot}$  and  $V_E$ , and for study 2  $V_{ti}$ ,  $V_{te}$ ,  $t_E$ ,  $f$ ,  $V_{ti}/t_I$ ,  $V_{te}/t_E$ ,  $t_I/t_{Tot}$  and  $V_E$  (Mann-Whitney U test,  $P < 0.05$ , as described in section 2.1.4.a) when more than one response per infant was analysed. Responses were also averaged for each infant in table 3.3.3.ii. When test responses were compared to control after averaging for each infant, they were significantly greater for  $t_I$ ,  $t_E$ ,  $f$ ,  $V_{ti}/t_I$ ,  $t_I/t_{Tot}$  and  $V_E$  in study 1, and for study 2  $V_{ti}$ ,  $V_{te}$ ,  $t_E$ ,  $f$ ,  $V_{ti}/t_I$ ,  $V_{te}/t_E$ ,  $t_I/t_{Tot}$  and  $V_E$  (Wilcoxon matched pairs test,  $P < 0.05$ ).

STUDY 1		$V_{ti}$	$V_{te}$	$t_I$	$t_E$	$f$	$V_{ti}/t_I$	$V_{te}/t_E$	$t_I/t_{Tot}$	$V_E$
CONTROL	median	1.82	2.88	1.88	2.66	1.67	2.57	2.39	2.12	1.92
n=13 infants 33 runs	95% confidence limits	1.07- 2.85	1.92- 4.67	0.93- 3.44	1.66- 3.94	1.40- 3.02	1.67- 3.90	1.32- 4.62	1.28- 3.18	1.13- 3.07
TEST	median	2.65	4.59	3.82	6.38	3.15	4.26	5.57	3.50	4.01
n=13 infants 33 runs	95% confidence limits	1.61- 4.78	2.20- 7.56	2.54- 6.22	2.37- 7.17	2.27- 4.62	2.64- 6.96	2.32- 8.64	2.93- 5.06	2.47- 5.70
P value		ns	ns	0.01	0.01	0.02	0.02	ns	0.02	0.01
STUDY 2		$V_{ti}$	$V_{te}$	$t_I$	$t_E$	$f$	$V_{ti}/t_I$	$V_{te}/t_E$	$t_I/t_{Tot}$	$V_E$
CONTROL	median	2.21	2.16	2.61	1.80	1.53	2.52	2.25	1.60	1.53
n=13 infants 33 runs	95% confidence limits	1.31- 4.78	1.00- 4.12	1.79- 3.54	1.01- 3.48	1.15- 2.39	1.16- 3.36	1.67- 2.98	1.00- 2.60	1.11- 3.11
TEST	median	4.25	3.36	2.18	4.48	2.69	4.48	4.02	3.11	3.27
n=13 infants 33 runs	95% confidence limits	3.34- 6.19	1.93- 7.21	1.34- 4.52	2.89- 6.15	1.62- 4.37	2.67- 5.76	1.74- 5.78	1.66- 4.65	2.03- 5.66
P value		0.02	0.04	ns	0.001	0.01	0.01	0.04	0.03	0.01

Table 3.3.3.i. Summary of mean control and test chemoreflex respiratory responses (more than one response per infant; median, 95% confidence limits) to alternate breaths of  $F_{iO_2}$  0.21 (control) and 0.21 or 0.16 (test) at two postnatal ages. Mann-Whitney U test,  $P < 0.05$ . ns=non-significant.

To observe the effect of age on control and test responses, chemoreflex responses for study 2 were compared to those for study 1. There was no significant difference between tests or controls when compared for the effect of age, either when responses were averaged for each infant (table 3.3.3.ii, Wilcoxon matched pairs test,  $P>0.05$ ) or when more than one response was used for each infant (table 3.3.3.i, Mann-Whitney U test,  $P>0.05$ ).

STUDY 1		$V_{ti}$	$V_{te}$	$t_I$	$t_E$	$f$	$V_{ti}/t_I$	$V_{te}/t_E$	$t_I/t_{To}$	$V_E$
CONTROL n=13	median	2.24	3.54	2.29	2.79	2.16	3.08	3.74	2.14	2.12
	95% confidence limits	0.95- 3.84	1.41- 6.58	1.66- 3.57	2.51- 3.85	1.59- 2.75	1.39- 4.51	1.97- 7.50	1.25- 3.08	1.41- 4.16
TEST n=13	median	3.94	6.05	4.98	4.77	3.30	5.23	5.52	4.58	3.68
	95% confidence limits	1.66- 9.58	2.36- 8.57	2.68- 7.03	3.41- 8.56	2.24- 7.07	2.60- 8.65	2.23- 12.28	1.98- 6.13	2.76- 10.10
P value		ns	ns	0.02	0.01	0.02	0.01	0.05	0.02	0.01
STUDY 2		$V_{ti}$	$V_{te}$	$t_I$	$t_E$	$f$	$V_{ti}/t_I$	$V_{te}/t_E$	$t_I/t_{To}$	$V_E$
CONTROL n=13 infants	median	3.04	2.82	2.76	2.40	1.85	2.33	3.05	1.46	2.42
	95% confidence limits	1.49- 4.53	1.32- 5.23	1.58- 4.65	0.95- 3.77	0.93- 2.82	1.47- 3.23	1.54- 3.87	0.89- 3.50	1.26- 2.98
TEST n=13 infants	median	5.27	7.04	2.52	4.76	3.45	3.91	5.17	2.66	3.59
	95% confidence limits	3.81- 8.90	1.62- 9.24	0.71- 7.24	3.40- 5.63	2.32- 4.38	3.46- 5.30	2.84- 7.13	1.29- 6.59	2.88- 6.26
P value		0.03	0.01	ns	0.01	0.04	0.01	0.02	0.02	0.01

Table 3.3.3.ii. Summary of mean control and test chemoreflex respiratory responses (average response for each infant; median, 95% confidence limits) to alternate breaths of  $F_{iO_2}$  0.21 (control) and 0.21 or 0.16 (test) at two postnatal ages. Wilcoxon matched pairs test,  $P<0.05$ . ns=non-significant.

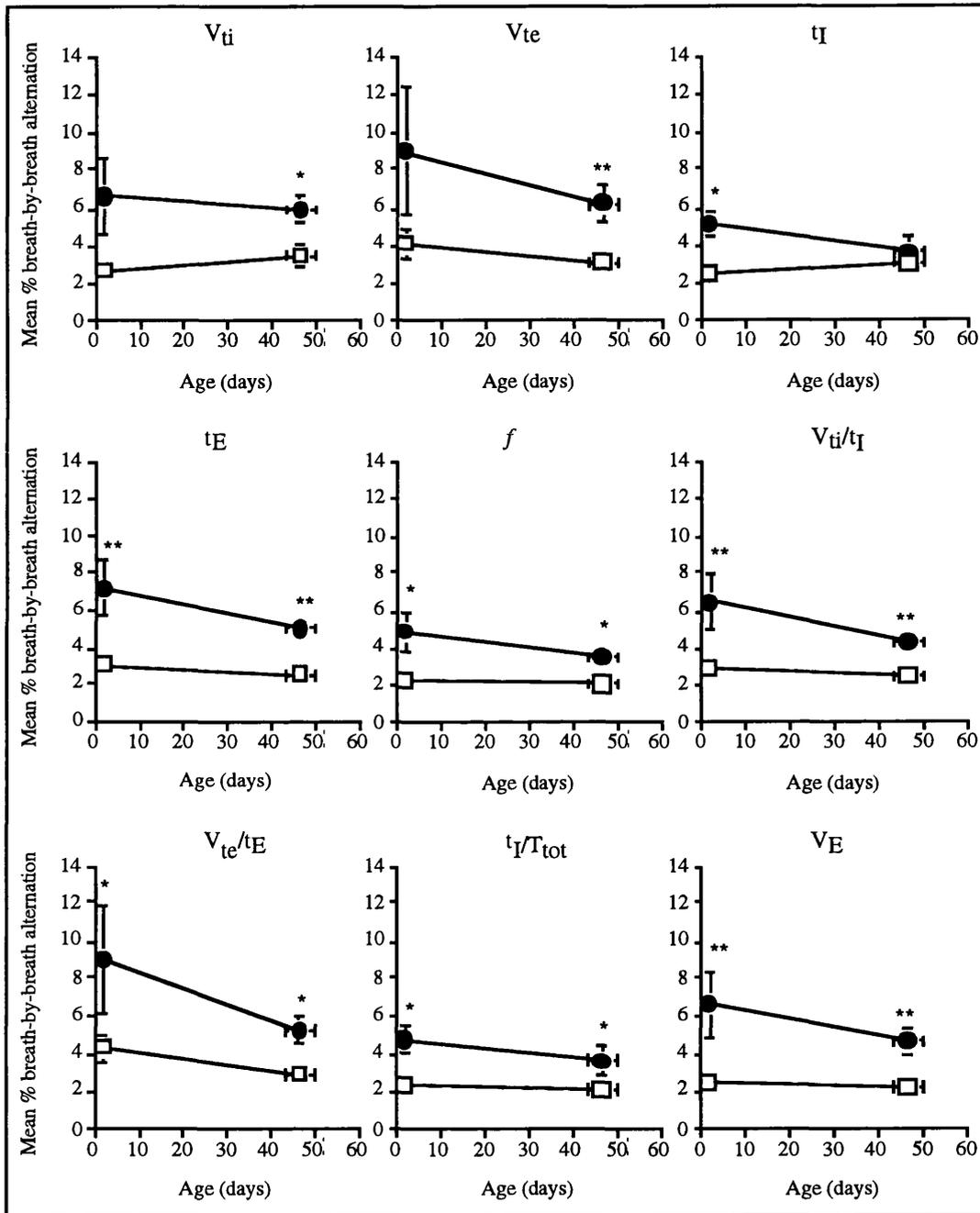


Figure 3.3.3.i Chemoreflex responses (% breath-by-breath alternation; mean  $\pm$  S.E.M) at two postnatal ages. Responses averaged for each infant. Open squares=control responses (breath-by-breath alternations between  $F_{iO_2}$  0.21 and 0.21), closed circles=test responses (breath-by-breath alternations between  $F_{iO_2}$  0.21 and 0.16). \*  $P < 0.05$ , \*\*  $P < 0.01$ , Wilcoxon matched pairs test.

### 3.3.4 Assessment of significant chemoreflex responses for each infant

To assess the number of infants showing a significant chemoreflex respiratory response, test responses were compared to control responses for each infant (see section 2.1.4.b). All 13 infants showed a significant chemoreflex respiratory response at both postnatal ages studied, that is at least one respiratory variable of their chemoreflex showed a significant alternation in response to the breath-by-breath changes in  $F_{iO_2}$ . Details of the total number of infants showing significant chemoreflex responses for each respiratory variable are given in table 3.3.4.i for study 1 and table 3.3.4.ii for study 2. The number of infants showing significant alternations for a given respiratory variable was very similar between the two studies.

Respiratory variable	$V_{ti}$	$V_{te}$	$t_I$	$t_E$	$f$	$V_{ti}/t_I$	$V_{te}/t_E$	$t_I/T_{tot}$	$V_E$
Number of infants showing significant responses (total=13)	7	4	6	6	5	7	7	6	6

Table 3.3.4.i Number of infants showing significant chemoreflex respiratory responses for study 1 to breath-by-breath alternations in  $F_{iO_2}$  between 0.21 and 0.16 for each respiratory variable.

Respiratory variable	$V_{ti}$	$V_{te}$	$t_I$	$t_E$	$f$	$V_{ti}/t_I$	$V_{te}/t_E$	$t_I/T_{tot}$	$V_E$
Number of infants showing significant responses (total=13)	7	8	6	6	8	3	7	4	6

Table 3.3.4.ii Number of infants showing significant chemoreflex respiratory responses for study 2 to breath-by-breath alternations in  $F_{iO_2}$  between 0.21 and 0.16 for each respiratory variable.

Chemoreflex respiratory responses to the hypoxic alternations were plotted for each infant measured at the two postnatal ages in figure 3.3.4.i-ix. Infants showing a significant alternation for a respiratory variable are marked by an asterisk, and the average response is shown for each infant. The average responses were not used to determine significant alternations, rather all test responses were examined for significant alternations. However, for the purpose of illustration the average responses are shown below. Thus, an asterisk response means that the infant showed a significant alternation in one of their test responses, not that the average response was significant. Most infants showed a similar magnitude of chemoreflex response (breath-by-breath % alternation) for study 1 and 2. However three infants, "Mac", "Lon" and "Con" showed a much greater response at the time of study 1 compared to study 2. There was no apparent reason for this observation.

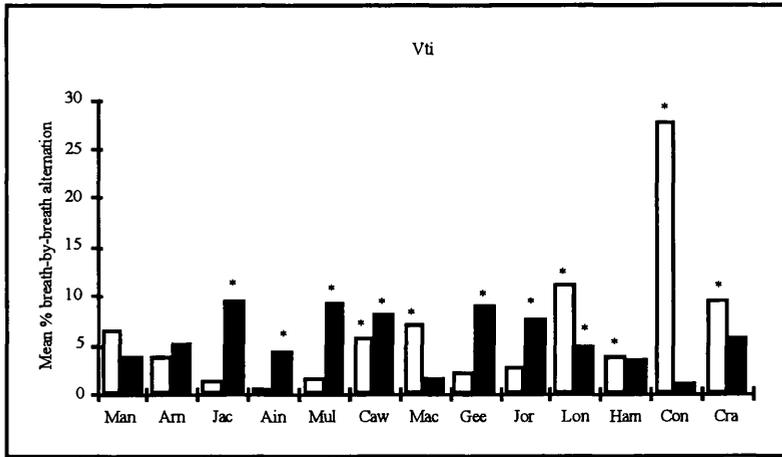


Figure 3.3.4.i V<sub>ti</sub> chemoreflex response (mean % breath-by-breath alternation) to alternations between F<sub>io2</sub> 0.21 and 0.16 at two postnatal ages. Open bars=study 1, Filled bars=study 2. Asterisk denote infants showing significant alternations.

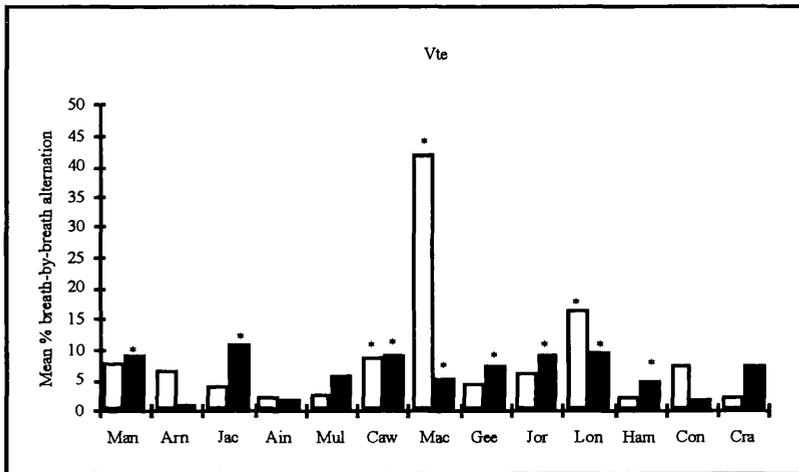


Figure 3.3.4.ii V<sub>te</sub> chemoreflex response (mean % breath-by-breath alternation) to alternations between F<sub>io2</sub> 0.21 and 0.16 at two postnatal ages. Open bars=study 1, Filled bars=study 2. Asterisk denote infants showing significant alternations.

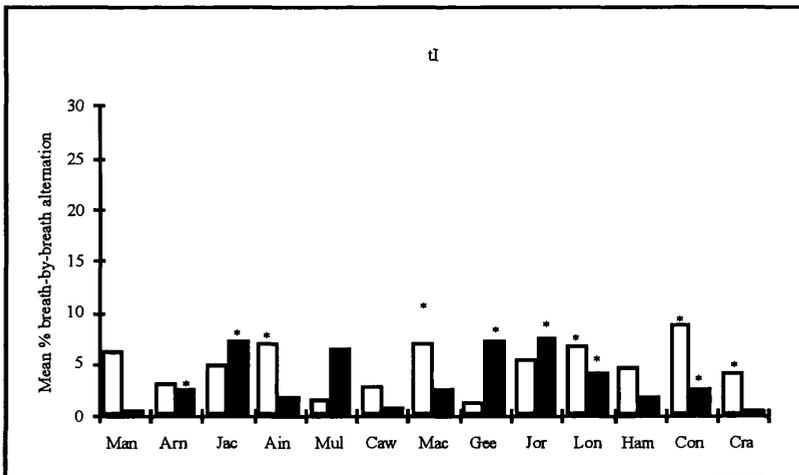


Figure 3.3.4.iii t<sub>i</sub> chemoreflex response (mean % breath-by-breath alternation) to alternations between F<sub>io2</sub> 0.21 and 0.16 at two postnatal ages. Open bars=study 1, Filled bars=study 2. Asterisk denote infants showing significant alternations.

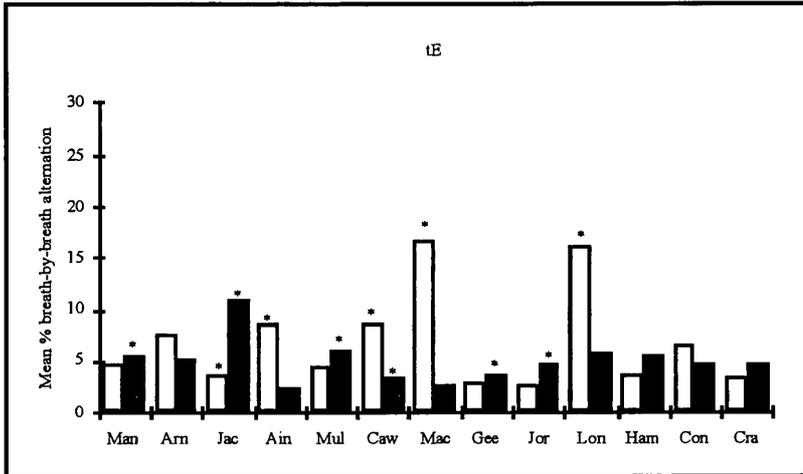


Figure 3.3.4.iv tE chemoreflex response (mean % breath-by-breath alternation) to alternations between Fio<sub>2</sub> 0.21 and 0.16 at two postnatal ages. Open bars=study 1, Filled bars=study 2. Asterisk denote infants showing significant alternations.

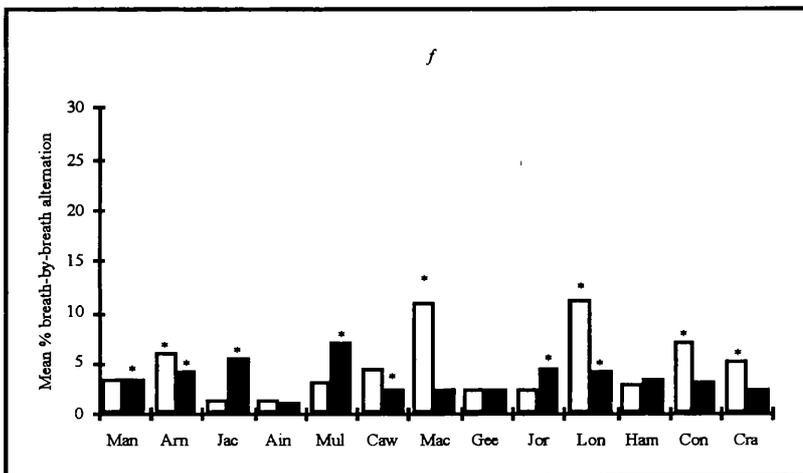


Figure 3.3.4.v f chemoreflex response (mean % breath-by-breath alternation) to alternations between Fio<sub>2</sub> 0.21 and 0.16 at two postnatal ages. Open bars=study 1, Filled bars=study 2. Asterisk denote infants showing significant alternations.

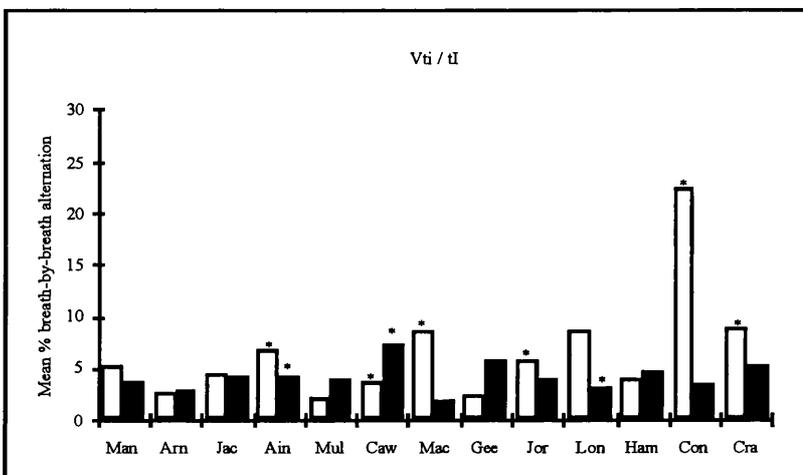


Figure 3.3.4.vi V<sub>ti</sub>/t<sub>I</sub> chemoreflex response (mean % breath-by-breath alternation) to alternations between Fio<sub>2</sub> 0.21 and 0.16 at two postnatal ages. Open bars=study 1, Filled bars=study 2. Asterisk denote infants showing significant alternations.

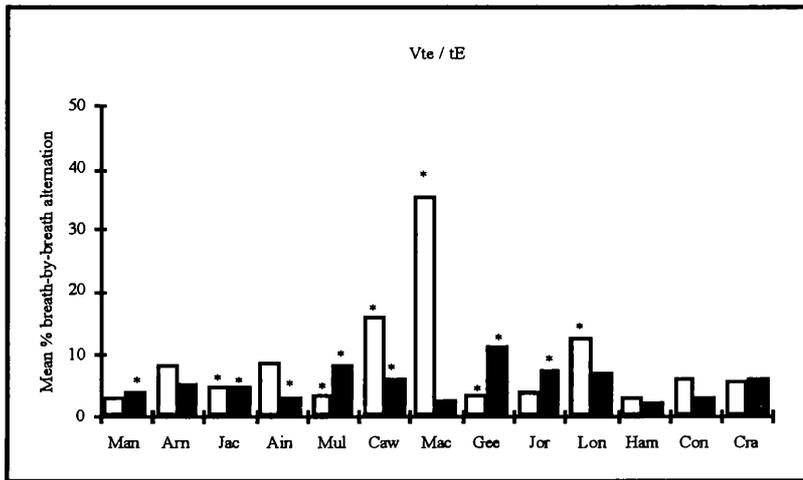


Figure 3.3.4.vii  $V_{te}/tE$  chemoreflex response (mean % breath-by-breath alternation) to alternations between  $F_{iO_2}$  0.21 and 0.16 at two postnatal ages. Open bars=study 1, Filled bars=study 2. Asterisk denote infants showing significant alternations.

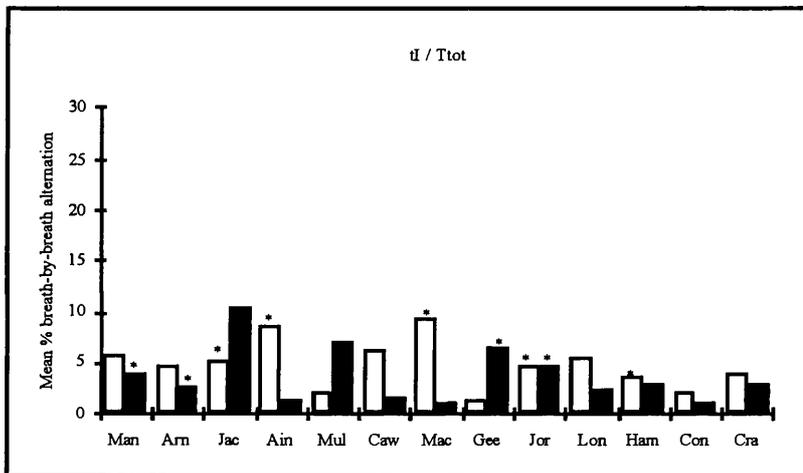


Figure 3.3.4.viii  $tI/T_{tot}$  chemoreflex response (mean % breath-by-breath alternation) to alternations between  $F_{iO_2}$  0.21 and 0.16 at two postnatal ages. Open bars=study 1, Filled bars=study 2. Asterisk denote infants showing significant alternations.

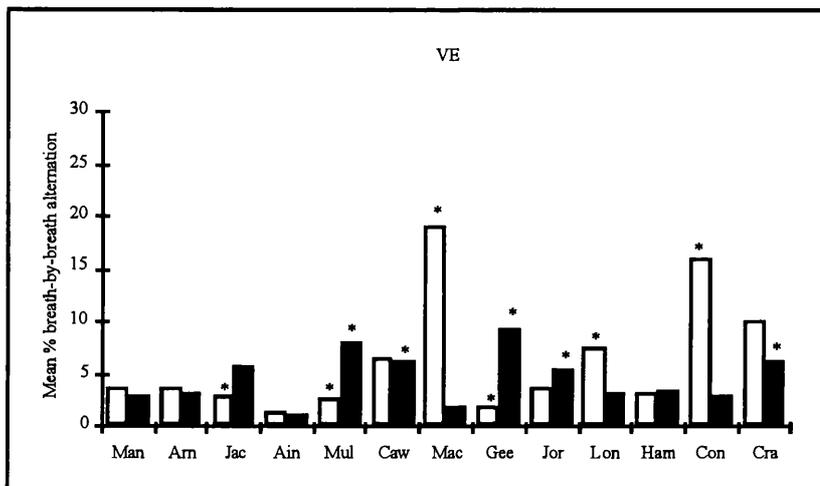


Figure 3.3.4.ix  $V_E$  chemoreflex response (mean % breath-by-breath alternation) to alternations between  $F_{iO_2}$  0.21 and 0.16 at two postnatal ages. Open bars=study 1, Filled bars=study 2. Asterisk denote infants showing significant alternations.

A comparison was also made for each infant of the number of respiratory variables that showed a significant alternation in study 1 and 2 (table 3.3.4.iii). Six infants increased the number of respiratory variables showing a significant alternation, six infants decreased the number of respiratory variables showing a significant alternation and one infant showed no change. These data summarise the results presented in figures 3.3.4.i-ix. As equal number of infants show an increase, and decrease in the number of respiratory variables showing a significant alternation in their chemoreflex response, it is not possible to make any conclusion from analysing the data in this way.

Infant No	1	2	3	4	5	6	7	8	9	10	11	12	13
Study 1	4	3	4	4	2	6	9	2	2	7	2	5	4
Study 2	5	3	7	3	5	7	1	7	8	5	1	2	1

Table 3.3.4.iii Number of significant alternations in respiratory variables for each infant's chemoreflex response at two postnatal ages.

### 3.4 Discussion

#### 3.4.1 Overview

I have shown that a chemoreflex respiratory response to breath-by-breath alternations in  $F_{iO_2}$  between 0.21 and 0.16 was present in newborn infants at  $42.5 \pm 6.8$ hrs and  $46.6 \pm 3.4$ d. (i.e. mean test responses were significantly greater than mean control responses at both postnatal ages). All infants showed a significant alternation in at least one respiratory variable at both postnatal ages studied and were classified as responders.

I have shown that there was no significant increase in the respiratory chemoreflex between *ca.* 24-48hr and 6wks, and test responses were not greater for the group at the older postnatal age. The response for study 1 is appropriate for the 24-48hr age group as measured by Williams et al. (1991), and the response for study 2 similar to that measured at 5-8d. The magnitude of the breath-by-breath % alternation for control responses did not change with age. There was a tendency for the magnitude of the alternation for test responses to fall, however this was not significant. Three infants showed a larger test response at the time of study 1 compared to study 2, but alternations in respiratory variables were still significant at both ages. In summary, I conclude that the increase in hypoxia chemosensitivity occurs over the first two postnatal days in the human.

### **3.4.2 Possible other reasons for not finding a larger chemoreflex responses in older infants**

#### **3.4.2.a Stimulus strength in older infants**

One possibility why I did not observe an increase in hypoxia chemosensitivity could be that the stimulus was inadequate in the older infants. It was not possible to measure end-tidal gas concentrations and so I have no evidence that the stimulus was standardized between infants. In fact, it would be very difficult to detect  $etO_2$  in these infants as the gas delivery was via nasal cannulae. The constant flow of inspired gas would always have an oxygen concentration greater than  $etO_2$ , so it would be impossible to measure breath to breath changes in  $etO_2$ . Even if expiratory gas flow exceeded the flow of inspired gas, there would always be some mixing of the two and hence a dilution of end-tidal  $O_2$ .

I used a standard flow of 2.5l/min of inspired gas delivered by nasal cannulae for all studies. The dead space of the cannulae is only a few mls, and so with a mean  $t_E$  of 0.8sec and flow of 2.5l/min, the dead space that can be cleared during expiration before the baby inspires the next breath is 33mls. Thus, an increase in deadspace is unlikely to account for reduced chemoreflexes in older infants. It is possible that peak inspiratory flow was greater in older infants and so the flow of inspired gas should be greater to meet this larger flow. One way to overcome this problem would be to increase the flow of inspired gas, however I experienced problems with arousal from quiet sleep at flows greater than 2.5l/min and this was also reported by Williams (1990). I found it preferable to maintain a state of quiet sleep, than attempt a greater flow of inspired gas. I was not able to measure  $V_t$  in mls, so I can only estimate in older infants with a mean inspiratory duration of 0.54 sec, and an approximate  $V_t$  of 35mls, that mean inspiratory flow was 3.9l/min. This is greater than the flow of inspired gas I was able to deliver, and so there may have been some dilution of the stimulus at this level in some infants. Of course,  $V_t$  may have been less than 35mls in these infants at  $46.6 \pm 3.4d$  (mean postnatal age  $\pm$  S.E.M.) and so the dilution would be less of a problem. One possibility to overcome this would be to deliver a more severe level of hypoxia (this is discussed also in section 4.4.2.c), however this does raise ethical issues and is generally not acceptable to Ethics Committees or to parents. Furthermore, it is meaningless to deliver a more severe stimulus unless it is possible to measure  $etO_2$  or  $PaO_2$ , because without this information it would not be reasonable to compare the two age groups. Although there is some uncertainty in my results as to the level of hypoxia attained

between infants, and the effect of postnatal age on dilution of the stimulus, at least I am certain that all infants received the same concentration of inspired gas.

Another factor that will determine the amplitude of the oscillation in PaO<sub>2</sub> is a postnatal improvement of gas exchange at the lung. Greater gaseous exchange will augment the amplitude of the PaO<sub>2</sub> oscillation about a given mean level of PaO<sub>2</sub>. In infants it is not possible to measure PaO<sub>2</sub> non-invasively at a rate that would detect breath to breath changes in the amplitude of the oscillation. Neither does measurement of SaO<sub>2</sub> provide information on a rapidly alternating stimulus. If there were an increase in pulmonary diffusing capacity with age, then older infants may have received a greater oscillating stimulus in the blood compared to younger infants. Hence the respiratory response would be greater in older infants. I found that this was not the case, and so my results do not support this idea. However, I cannot rule out this possibility without measurement of breath to breath changes in etO<sub>2</sub>.

The role played by postnatal closure of the ductus arteriosus and a reduction in right to left shunting of blood in the heart may be important. A patent ductus arteriosus in the fetus shunts blood away from the pulmonary circulation to the placenta, via the descending aorta, for gaseous exchange. In healthy infants the ductus arteriosus closes in the first 48hrs after birth (Drayton & Skidmore, 1987), and prematurity is a major factor in determining patency postnatally (Gersony, 1986). In the neonate, patency of the ductus arteriosus increases pulmonary blood flow and increases the load to the left ventricle. Pérez Fontán, Clyman, Mauray, Heymann & Roman (1987) showed that the effect of re-opening of the ductus arteriosus on FRC and dynamic lung compliance in newborn lambs was very small compared to the effects on pulmonary blood flow, pulmonary arterial pressure and left ventricular diastolic pressure. Furthermore, gas exchange was more efficient in lambs with a patent ductus compared to lambs with a closed ductus. PaO<sub>2</sub> tended to be higher and arterial-alveolar P<sub>o2</sub> difference was less, presumably due to left to right shunting and recirculation of the blood when the ductus was patent. Thus in the instance of right to left shunting, the amplitude of the alternating stimulus in the blood will be reduced as blood is diverted away from the pulmonary circulation. However in the instance of left to right shunting, the oscillations in arterial P<sub>o2</sub> may be enhanced due to the increase in pulmonary blood flow and arterial pressure. The infants in this study were healthy and born at term, and there is no reason to suspect that a patent ductus was a conflicting factor in older infants.

### **3.4.2.b Changes in pulmonary mechanics with postnatal age**

There are considerable changes in pulmonary mechanics of the newborn baby over the first year of life (see section 4.4.3.f for full review). At birth, the newborn has a highly compliant chest wall whilst the lung is much less compliant (Papastamelos, Panitch, England & Allen, 1995) and over the first postnatal year specific lung compliance, and total respiratory compliance increases (Rabbette, Fletcher, Dezateux, Soriano-Brucher & Stocks, 1994). In part to overcome a non-compliant lung, the newborn breathes at a higher EELV which may help in maintaining an inflated lung (Fisher et al, 1982; Kosch & Stark, 1984). This could provide an additional dilution factor if an infant was breathing at a high EELV, however this would suggest smaller chemoreflex responses in younger infants compared to older infants. I have no information on EELV in these infants, but my results do not support the idea that a high EELV reduced chemoreflex responses in younger infants.

### **3.4.2.c Effect of sleep state on the chemoreflex respiratory responses**

The newborn shows oscillations in respiratory variables during sleep (Hathorn, 1978). The respiratory pattern is more regular during NREM sleep, although these oscillations persist. Respiratory frequency is typically slower during NREM compared to REM sleep, and  $V_t$  is typically larger. During NREM sleep oscillations in  $V_t$  and  $f$  occur out of phase such that  $V_E$  remains stable. During REM sleep, the amplitude of these oscillations is higher, accounting in part for the variability  $V_E$ . Furthermore, oscillations in  $V_t$  and  $f$  occur in phase so as to produce an oscillation in  $V_E$ .

The chemoreflex response to an imposed stimulus is influenced by sleep state, but reports in the literature are conflicting. Bolton & Herman (1974) reported that the fall in  $V_E$  during hyperoxia was similar during REM and NREM in newborn babies. In newborn lambs, puppies and calves, the response to progressive hypoxia was less or absent during REM compared to NREM sleep (Henderson-Smart & Read, 1979; Jeffery & Read, 1980). In lambs, this response was explicable in terms of ribcage collapse during inspiration. Haddad, Gandhi & Mellins (1982) investigated the maturation of the ventilatory response to hypoxia in newborn puppies. During REM sleep puppies aged 14-29d increased  $V_E$  during hypoxia. However for NREM sleep, the increase in  $V_E$  during hypoxia was depressed in puppies at 14d due to an increase in  $t_E$ , whilst older puppies increased  $V_E$ . Arousal responses to hypoxia also occur at a lower oxygen saturation during REM compared to NREM sleep (Henderson-Smart & Read, 1979; Jeffery & Read, 1980).

Observations of the chemoreflex response to CO<sub>2</sub> are no more consistent than that to hypoxia. Andersson, Gennser & Johnson (1986) found in newborn infants aged 2-5d (n=16) that V<sub>E</sub> increased during 5% CO<sub>2</sub> breathing for both REM and NREM sleep. Davi, Sankaran, MaCallum, Cates & Rigatto (1979) found in term infants aged 3d and preterm infants aged 21d that V<sub>E</sub> was increased during both REM and NREM sleep to 3% CO<sub>2</sub> breathing. Similarly in newborn monkeys up to 7d postnatally, there was no difference in the ventilatory response to CO<sub>2</sub> between active and quiet sleep. Guthrie, Standaert, Hodson & Woodrum (1980, 1981) found that the ventilatory response to 2-5% CO<sub>2</sub> was no different between REM and NREM sleep at 7d, but was less during REM sleep at 21d. Contrastingly in infants studied over the first 4mths of life, Haddad, Leistner, Epstein, Epstein, Grodin & Mellins (1980) found that the increase in V<sub>E</sub> to 2% CO<sub>2</sub> breathing was no different between REM and NREM sleep. In agreement with observations in animals, Moriette, van Reempts, Moore, Cates & Rigatto (1985) found that for both preterm (10d; n=11) and term infants (3d; n=14) the increase in V<sub>E</sub> during 5% CO<sub>2</sub> breathing was less during active sleep compared to quiet sleep. In summary, the literature suggests in some species a depression of CO<sub>2</sub> sensitivity during REM sleep that develops postnatally and is not present in very young neonates.

I judged sleep state on the basis of behavioural observations and the criteria of Prechtl (1974). I characterized quiet sleep (NREM) by a regular breathing pattern, a lack of eye movement, lack of body movement except in the case of startles and lack of 'sucking' behaviour, except in the case of short bursts of sucking of *ca.* 1-2sec duration. These criteria are used conventionally and accepted as a procedure to categorize sleep state in the absence of electroencephalogram recordings. I have attempted to perform all measurements during quiet sleep on the basis of these observations.

### **3.4.3 The alternate breath test as a technique to assess peripheral chemoreceptor sensitivity to hypoxia**

The alternate breath test has been used in kittens, lambs and newborn infants to measure the respiratory chemoreflex to hypoxia (Hanson et al, 1989; Williams et al, 1990; Williams et al, 1991; Calder et al, 1994a). These studies have shown a postnatal increase in the response to the test, and that carotid sinus nerve section virtually abolishes the response. Furthermore, the test has been used to show that respiratory chemoreflex responses to hypoxia are poor in chronically hypoxic kittens (Hanson et al, 1989) and infants who have suffered chronic lung disease (Calder et al, 1994b). The rapid nature of the alternating hypoxic stimulus, and the blunted chemoreflex response

in carotid-denervated lambs and chronically hypoxic kittens have all been taken as evidence that the respiratory response is mediated by the arterial chemoreceptors.

Other methods have been used to assess peripheral chemoreflexes (see section 3.4.4), however the alternate breath test offers some advantages over other techniques. Perhaps most important is that the alternate breath test can be repeated over a large number of breaths (up to 100). As the inspired stimulus is alternated on a single breath basis, up to 98 (excluding the first and last breath) paired data comparisons can be made because each breath is compared to the immediately preceding breath. These paired comparisons can then be averaged, and I have used linear regression to describe the cumulative chemoreflex response as performed previously (Williams 1990; Williams et al, 1991). The slope of the line describes the mean breath-by-breath alternation. Linear regression has the advantage that it provides a more accurate measure of the breath to breath alternation compared to measuring the overall mean (sum of all differences / total number of breaths), and also gives a correlation coefficient to indicate how well the chemoreflex response is described by a straight line. In other words, the correlation coefficient gives a measure of how regularly the reflex alternation in the respiratory variable occurs.

The alternate breath test compares the magnitude of the breath-by-breath percentage alternation measured during test runs and control runs. The purpose of the control run is to determine on a breath to breath basis how great an alternation one might expect from random fluctuations in  $PAO_2$  and  $PACO_2$ , the experimental method and the method of analysis. It has been shown that  $PAO_2$  and  $PACO_2$  oscillate at respiratory frequency (Haldane & Priestly, 1935; Chilton & Stacey, 1952; DuBois, Britt & Fenn, 1952; Yokota & Kreuzer, 1973) and that respiratory variables fluctuate on a breath-to-breath basis during quiet sleep (Hathorn, 1978). This alone suggests that during control periods, the cumulative summation of breath to breath fluctuations in respiratory variables will result in some degree of alternation. Furthermore, auditory stimulation may be provided when the solenoids switch the flow of inspired gas, or the switching of gas *per se* may have an effect on breathing via trigeminal stimulation (Trippenbach, 1981). Care was taken to balance the flow of inspired gas in both delivery lines which should have minimized any response of upper airway receptors to changes in airflow (Sant'Ambrogio et al, 1983). To take into account an alternation that may be measured from random fluctuations in respiratory variables, it is imperative that chemoreflex responses to changes in  $F_{iO_2}$  are compared to control runs when  $F_{iO_2}$  does not alternate but the same methodology is applied.

This method of comparison for the purpose of data analysis contrasts to studies performed in the adult. Metias et al. (1981) measured the chemoreflex response to single breath alternations in  $P_{ACO_2}$ . The breath-by-breath alternation was summed cumulatively and the mean alternation calculated (linear regression was not used), and compared to zero by *t*-test. This is problematic as the assumption is made that in a control situation the breath to breath alternation is zero, and there is evidence from my results and others that this is not the case (Hanson et al, 1989; Williams & Hanson, 1990; Williams et al, 1991). Most importantly, it does not assess the alternation one might expect from the methodology alone.

I have also developed a new method for detecting responders from non-responders. This differed from Williams' (1990) speculation on a possible method to detect responders: *"The correlation coefficients were usually high during test runs with a large proportion of correlation coefficients being over 0.8. However, during control runs similar proportions of low and high correlation coefficients were observed. It is possible that in future studies these values in conjunction with slope values may prove useful in identifying non-responders from responders"*. I observed that control runs frequently had high correlation coefficients when the slope of the linear regression was low, and felt that it was difficult to incorporate this measure into the analysis. Instead I adopted a different approach.

Infants tended to differ in the degree of reflex alternation shown during control runs (see figure 3.3.2.i-ix). It was clear that for an individual infant, the chemoreflex respiratory response was the increase in the reflex alternation from control during test runs. However, the size of the alternation tended to vary between control and test runs. Thus, when data were averaged for each infant some of the information was lost, and there did not appear to be a simple method to compare between the average test and control responses to detect responders. I chose to use all responses obtained from all infants to generate a distribution of responses. I have made the assumption that given a large enough sample from the population, the distribution of control response would be normal. Statistically, a sample of  $n > 30$  for a randomly fluctuating variable is assumed to be normal for the purpose of using parametric statistics. With 13 infants in this study, and either 2 or 3 responses for infants, I had a sample of 33 responses, and a normal distribution of control response was developed for each respiratory variable.

Thus, I decided on two criteria that a test response must fulfil to be deemed significant. From the control distribution I calculated the critical value, which was the 95% percentile:  $\text{critical value} = \text{mean} + (1.645 \cdot \text{standard deviation})$ . The first criterion was that test responses must be greater than this critical value, and so would occur in the top

5% of the control distribution. I also wanted to take into account the degree of alternation measured during control runs for each infant. For instance if a test alternation was greater than the critical value calculated from the control distribution, but was no different from that individual's control alternation, then there was no chemoreflex respiratory response. So the second criterion that test responses had to fulfil was that the magnitude of the test alternation was at least twice as great as the largest alternation obtained during control runs for that infant. Only if both the conditions were met was the chemoreflex alternation for that respiratory variable deemed significant. A responder was defined by showing a significant alternation in at least one respiratory variable.

Although not conventional definitions for chemoreflex respiratory responses, these were in fact stringent criteria for determining a significant alternation. A significant alternation may have occurred when test responses were less than twice the greatest control alternation in an infant, for instance if the control response was of poor quality and showed a large breath-by-breath alternation. Alternatively, I could have used the averaged control response to compare with test alternations. However, it was necessary to draw guidelines for what might be determined as 'clinically significant' and to use statistics to define those guidelines. I have made the assumption that the sample is from a normally distributed population to determine a critical value for significant alternations. I have not ascribed a level of significance (P value) to this method of analysis. Therefore I reason that in the instance that the population is not normally distributed, the implications for my method of analysis are minimal. If I were to calculate specific P values for each alternation then the assumption of normality would have to be proven. However as I have not done this, I am confident that this new analysis is a useful method to determine respiratory variables that show a chemoreflex.

### **3.4.4 Other tests of peripheral chemosensitivity**

#### **3.4.4.a Dejours' method: Single breath of oxygen**

The single breath of oxygen has the advantage that it is easy and safe to use on newborn babies. It has been successfully used to demonstrate a postnatal increase in hypoxia chemosensitivity (Girard et al, 1960; Crance et al, 1971; Hagan & Gulston, 1981; Williams 1990), however it does possess some drawbacks.

The response to transient hyperoxia is variable. The rise in PaO<sub>2</sub> will be largely determined by the volume of the inspired breath during oxygen breathing. These

measurements are frequently performed during quiet sleep which will minimize the breath to breath variation in  $V_{Ti}$  (compared to REM sleep), however breath to breath changes still occur during quiet sleep (Hathorn, 1978). Obviously the differences in  $V_{Ti}$  between infants will be greater than within infants, and will also influence the rise in  $PaO_2$ . Furthermore, it is difficult to measure the strength of the stimulus to such a transient rise in  $PaO_2$ . Oxygen saturation ( $SaO_2$ ) would not provide a good measure of the stimulus as the upper portion of the oxygen saturation/haemoglobin dissociation curve is relatively flat, and so a rise in  $PaO_2$  will occur with very little change in  $SaO_2$ . Mass spectrometry should give a reliable measure for  $etO_2$  concentration, however many of the earlier studies were without this information.

More complicated however is identifying the breath which responds to the hyperoxic stimulus. Different investigators have employed different techniques to assess the fall in  $V_E$  to a transient rise in  $PaO_2$ . Girard et al. (1960) measured the fall in  $V_E$  for the first 6sec after  $O_2$  breathing. Hagan & Gulston (1981) measured the percentage change from control on the second and third breaths during  $O_2$  breathing. Aizad et al. (1984) measured the fall in  $V_E$  to 5 breaths of  $O_2$  in 15sec epochs over the first minute of air breathing. Hertzberg & Lagercrantz (1987) compared 30sec of  $O_2$  breathing to 30sec of control to detect a fall in  $V_E$ . It is not possible to choose only one breath to analyse as the hyperoxic stimulus is likely to affect more than one breath, however analysis of chemoreflex response over longer periods will underestimate the fall in ventilation. Frequently, investigators have measured the fall in  $V_E$  when switching between mild hypoxia and transient hyperoxia to enhance the rise in  $PaO_2$  as this will augment the response. The array of analysis techniques used to measure the chemoreflex makes the comparison between studies less valid.

#### **3.4.4.b The respiratory response to acute hypoxia**

Phase I of the BVR is mediated by the peripheral chemoreceptors, and it has been suggested that phase II is due to the depressant effects of hypoxia centrally (Gluckman & Johnston, 1987; Martin-Body, 1988; Martin-Body & Johnston, 1988; Williams & Hanson, 1989). It is possible to assess hypoxic chemosensitivity by the measuring the increase in  $V_E$  to hypoxia during the first phase of the BVR. It has been demonstrated in newborn infants (Miller & Smull, 1955), kittens (McCooke & Hanson, 1987), lambs (Bureau et al, 1987) and monkeys (Woodrum et al, 1981) that the magnitude of the ventilatory increase during phase I is greater in older animals compared to younger animals.

Another strategy to investigate the postnatal development of respiratory chemoreflexes has been to study the time course over which the neonate develops a sustained ventilatory response to hypoxia, or the time at which phase II disappears. This has been assumed to reflect the postnatal increase in chemoreceptor sensitivity, ventilation being sustained when the increase in peripheral chemoreceptor activity counteracts any depressant effect of hypoxia centrally. However, the development of a sustained increase in  $\dot{V}_E$  could also be due to a decline in the depressant or inhibitory effects exerted on breathing by hypoxia and in turn maturation of central control of breathing. Nevertheless, several investigators have used this method to conclude a postnatal maturation of hypoxia chemosensitivity (Dawes & Mott, 1959; Brady & Ceruti, 1966; Brady & Dunn, 1970; Eden & Hanson, 1985, 1987a).

Much of the early work investigating hypoxia chemosensitivity in newborn infants measured the ventilatory response to 15% (Cross & Warner, 1951; Cross & Oppé, 1952; Rigatto et al, 1975a) or 12% oxygen (Miller & Smull, 1955; Brady & Ceruti, 1966). It is not currently acceptable by Ethics Committees to use this severity of hypoxia for a prolonged period in newborn babies. In addition, parents are increasingly aware of the care of their babies and are concerned about the risks associated with breathing low oxygen. Perhaps for this reason, if for nothing more academic, it is not acceptable for research purposes to use acute hypoxia as a stimulus to assess peripheral chemoreceptor function newborn babies.

#### **3.4.4.c The compensatory response to a sigh**

Fleming, Gonclaves, Levine & Woollard (1984) used the response to a naturally occurring sigh as a test of peripheral chemosensitivity. They defined a sigh as "*a breath which was at least twice as large as the other breaths in the sequence and which was followed by a transient fall in ventilation or an apnoeic pause which was itself at least twice as long as the average breath duration*", and measured respiration 1min before and up to 2min after a spontaneous sigh. Using equations to fit the compensatory respiratory response to a sigh, they found a maturation of the response over the first 7mths of life. Infants aged less than 4d showed a response that was "*highly stable but sluggish*", infants up to 4mths showed a less stable response and  $\dot{V}_E$  continued to oscillate for a longer period following the sigh, and infants 4-8mths showed a mature response and  $\dot{V}_E$  recovered rapidly.

The main problem with this technique is that a sigh is completely unpredictable. Furthermore, the frequency of naturally occurring sighs decreases with increasing postnatal age. It is not possible to standardize the stimulus between infants, and indeed

even between consecutive events in the same infant. The response to a sigh will be determined by changes in both  $\text{PaO}_2$  and  $\text{PaCO}_2$ , and it is not possible to separate the two stimuli to study in isolation. A fall in  $\text{PaCO}_2$  will reduce respiratory output from the central chemoreceptors, and if the compensatory response is measured over 2min then there is likely to be some central component. The technique does not favour making a reproducible measure of peripheral chemosensitivity.

### **3.4.5 Maturation of respiratory chemoreflexes**

The purpose of these experiments was to assess the maturation in hypoxia chemosensitivity beyond the first week of life. I chose to study newborn infants on *ca.* postnatal day 2 ( $42.5 \pm 6.8$ hrs; mean postnatal age  $\pm$  S.E.M.) as the observations of Williams et al. (1991) had indicated that a chemoreflex response was shown at this age. Ideally it would have been preferable to study infants towards the end of the first postnatal week, perhaps at a similar age to the oldest age group in the study of Williams et al. (1991). However, I found that it was not possible to study infants at this age on the postnatal wards because mothers were frequently discharged on postnatal day 2, and as a consequence only 3 of the infants in my study were more than 72hrs old.

The observations of Williams et al. (1991) suggest maturation of the respiratory chemoreflex between 24-48hrs and 5-8d. At the earlier age significant alternations were seen for  $V_{\text{ti}}$ ,  $t_{\text{I}}$ ,  $V_{\text{ti}}/t_{\text{I}}$  and  $t_{\text{I}}/T_{\text{tot}}$  with a mean breath-by-breath alternation of *ca.* 4% for test and 2% for control runs, and at the older age significant alternations were seen additionally in  $t_{\text{E}}$ ,  $f$  and  $V_{\text{E}}$ , with a mean breath-by-breath alternation of *ca.* 5-8% for test and 2% for control runs. This was a cross sectional study and hence it is difficult to extrapolate to my findings. For study 1, I observed a median alternation of *ca.* 3-5% for test and 2% for control runs with significant alternations in  $t_{\text{I}}$ ,  $t_{\text{E}}$ ,  $f$ ,  $V_{\text{ti}}/t_{\text{I}}$  and  $V_{\text{E}}$ . However I was unable to measure these responses later during the first postnatal week, so I do not know if these infants similarly would have showed an alternation of 5-8% during test runs.

I measured chemoreflex responses again at  $46.6 \pm 3.4$ d (mean postnatal age  $\pm$  S.E.M.), and found no significant increase in the magnitude of the test response (median alternation 4-5%), however a greater number of respiratory variables showed test responses significantly greater than control (additional variables showing significant alternations were  $V_{\text{ti}}$ ,  $V_{\text{te}}$  and  $V_{\text{te}}/t_{\text{E}}$ , but not  $t_{\text{I}}$ ). Chemoreflex responses for some infants showed an increase, and for other infants a decrease, in the average test alternation (figures 3.3.2.i-ix). When I analysed the number of respiratory variables that

showed a significant alternation for each infant on each study occasion, six infants increased, six infants decreased and one infant showed no change in the number of variables with a significant alternation (table 3.3.4.ii). Therefore as a group, I found no further increase in the peripheral chemoreflex after *ca.* the second postnatal day. I therefore conclude that the predominant increase in hypoxia sensitivity of the peripheral chemoreceptors occurs over the first two postnatal days.

It is clear from nerve recording studies in animals both *in vivo* (Hanson et al, 1987; Kumar & Hanson, 1989; Marchal et al, 1992; Carroll et al, 1993) and *in vitro* (Kholwadwala & Donnelly, 1992; Pepper et al, 1995) that the chemoreceptor response to hypoxia continues to increase beyond the second postnatal day. In newborn infants it is only possible to measure hypoxia chemosensitivity non-invasively, and some of the foregoing studies report an increase in hypoxia chemosensitivity between a few days of age and up to the second postnatal week. Aizad et al. (1984) showed a greater fall in  $\dot{V}_E$  in preterm infants aged 9d compared to term infants aged 3d. Ventilatory studies in newborn lambs have found that the fall in  $\dot{V}_E$  to 5 or 10sec O<sub>2</sub> breathing was greater at 10d than at 2d (Bureau & Bégin, 1982). Therefore, the literature suggests that a greater chemoreflex response may have been present to the alternate breath test if infants were studied at 1-2wks of age. I was not able to study infants at this age as I was restricted to performing the study when the mothers and infants were on the postnatal wards, or when they returned to the hospital for their postnatal check-up. Although not ideal, I have shown that a significant alternation in at least one respiratory variable was present in all infants at the time of study 1.

Most of the literature (reviewed in section 3.1) assessing the development of respiratory chemoreflexes and hypoxia chemosensitivity has concentrated on the first two weeks of postnatal life. One other study has investigated the development of peripheral chemoreflexes beyond the first postnatal month. Parks, Beardsmore, MacFayden, Pallot, Goodenough, Carpenter & Simpson (1991) found that the response of infants aged 1, 2 and 3mths to a single breath of 100% oxygen was not significantly different with age when the fall in  $\dot{V}_E$  was expressed as a percentage change, or corrected for weight or body length. This indicates that chemoreceptor resetting to hypoxia does not continue after the end of the first month and my results confirm this.

### **3.4.6 Variability between infants**

It is clear from the average chemoreflex responses in figures 3.3.2.i-ix that there is a large variation in response between infants. Three infants ("Mac", "Con" and "Lon") showed

very large chemoreflex responses at the time of study 1, but not for study 2. These infants continued to show significant alternations for study 2, however the magnitude of the breath-by-breath alternation was less. There are several possibilities which may explain this response.

First, these three infants showed a fall in the mean breath-by-breath percentage alternation during test runs, but they showed a similar fall (albeit not as dramatic) during control runs. Hence, they continued to show significant alternations because the change from control was still significant. This reflects a maturation of the ventilatory pattern as respiration becomes more stable with increasing postnatal age.

Secondly, it is possible that the stimulus was inadequate in these infants for study 2 as discussed in section 3.4.2.a. It appears from the very large responses for study 1 that the stimulus was indeed greater. However, I was unable to sample end-tidal gas concentrations so have no information on the stimulus size between the two studies. Clearly, the stimulus was not inadequate for all infants as the breath-by-breath alternation during test runs increased for other infants.

Thirdly, it is possible that a reduction in hypoxia chemosensitivity was the cause for a smaller alternation during test runs at the time of study 2. There has been some speculation that SIDS infants suffer a reduction in chemoreceptor sensitivity to hypoxia and hypercapnia at 3-5mths when the incidence of SIDS is greater. On the whole, this theory has not been substantiated in animals. However there is some preliminary evidence in newborn rabbits (Bee, Wright & Pallot, 1993) that hypoxia sensitivity of the peripheral chemoreceptors falls at around 5wks of age, which is after resetting has occurred. It seems unlikely that the infants in my study suffered such a transient loss of hypoxia sensitivity and there was no reason to assume they were of ill health. These infants maintained a chemoreflex response, but the magnitude of the breath-by-breath alternation was not as great compared to study 1. Alternatively, it is possible that hypoxia sensitivity was reduced due to an effect of sleep state on the chemoreflex response (see section 3.4.2.c). Sleep state was judged behaviourally, and if the measurements were performed whilst these 3 infants were in REM instead of NREM sleep, they may have experienced a fall in hypoxia sensitivity. Based on the criteria described by Precht (1974) (see also section 3.4.2.c), I judged that all recordings were performed during quiet sleep (NREM).

Due to the conditions under which these experiments were performed, it was not possible to perform repeat studies to assess the variability of the chemoreflex response within infants. The duration of the study period was frequently 2-3hrs, allowing for

feeding time and for the infant to fall asleep, although the data collection period may have been only 20-30min. In a lot of instances this made the mothers slightly anxious, and compounded by the short duration of their stay in hospital, it was not feasible to repeat the study on the same or following day. Particularly as I was already recruiting infants whose mothers were prepared to return for the second study at *ca.* 4-6wks, it was not acceptable to repeat the study a third time. I also encountered competition from other researchers in the hospital who were interested in recruiting healthy mothers and babies. It is important to assess the variability of the chemoreflex response within infants, however this was not an appropriate sample of subjects to address that particular issue. To answer this question in part, I have measured chemoreflex responses in 3 adults on 3 separate occasions in Chapter 5. This does not help to answer the question of variability in infants, but to some extent provides an indication of the reproducibility of the response.

### **3.4.7 Clinical Importance**

Poor respiratory control is postulated to be a cause of SIDS because in the face of rising hypoxia and hypercapnia there is no arousal response to re-establish ventilation and prevent death. Thus, victims of SIDS must suffer from either an absent or inadequate afferent input from the peripheral chemoreceptors, or they are unable to produce an arousal response to increased chemoreceptor discharge during hypoxia.

The role of chemoreceptor function in controlling breathing has been considered in relation to SIDS. It is possible that delayed resetting of peripheral hypoxia chemosensitivity may prevent an adequate response to acute hypoxia, especially if the  $P_{O_2}$  at birth is crucial in determining the time course for resetting. Evidence for the  $P_{O_2}$  at birth determining chemoreceptor hypoxia sensitivity in man, comes from the response to hypoxia in natives of high altitude (Lahiri & Edelman, 1969) who demonstrated minimal decline in ventilation to 2 breaths of  $O_2$  at altitude in comparison to acclimatized lowlanders. At sea level, acclimatized highlanders exhibited a much less pronounced increase in ventilation to 3 or 5 breaths of  $N_2$  than did lowlanders. Hence, both the peripheral chemosensitivity to hyperoxia and hypoxia is blunted in natives of high altitude. In animals, chronic hypoxia reduces respiratory chemoreflexes in kittens (Hanson et al, 1989). In newborn infants who have suffered BPD and are believed to be chronically hypoxic, respiratory chemoreflexes also appear to be reduced (Calder et al, 1994b; Katz-Solomon & Lagercrantz, 1994). This reduction in hypoxia chemosensitivity, popularly referred to as 'blunting', is believed to be intricately linked to the oxygen level when chemoreceptor resetting occurs.

Weak peripheral chemoreflexes may also result after resetting of hypoxia chemosensitivity if other factors interact at a later stage to reduce the gain of respiratory chemoreflexes. An example is the effect of high ambient temperature, as poor thermoregulation has also been implicated in the aetiology of SIDS. Watanabe, Kumar & Hanson (1993a) used the alternate breath test in kittens at different ambient temperatures. They found in kittens aged 2-7d and 28-39d that the gain of respiratory chemoreflexes to both hypoxia and CO<sub>2</sub> was less at 30°C compared to 25°C. This was accompanied by a fall in oxygen consumption at 30°C. Poor thermoregulation may have a fatal effect if it reduces peripheral chemoreflexes in newborn infants.

My observations are important in the context of normal maturation of peripheral chemoreflexes and respiratory control in the newborn. I found that hypoxia chemosensitivity did not increase with age, which suggests that a substantial portion of the resetting process had already taken place. If there is a link between SIDS and peripheral chemoreflexes, it is possible that the gain of respiratory chemoreflexes may be reduced after resetting by some pathology suffered by the infant. Clearly, interrupting the normal process of resetting influences respiratory chemoreflexes in later life, however other factors may interact to reduce chemoreflex gain in an otherwise healthy infant.

### **3.4.8 Summary**

My results show that the chemoreflex respiratory response of the newborn infant to alternate breaths of air and a hypoxic mixture remains unchanged from *ca.* 48hrs to *ca.* 6wks of age. Thus, it appears that there is no additional maturation of respiratory chemoreflexes evident between the first week and the second month of postnatal life, and that chemoreceptor resetting to hypoxia is virtually complete within the first 48hrs after birth. This is more rapid than has been reported previously in other species.

To investigate further the changes in hypoxia sensitivity that may occur after the first month of life, I have performed experiments in adults in Chapter 5. This facilitates the comparison of chemoreflex responses to the alternate breath test between infants and adults. Previously there were no reports of the degree of reflex alternation to an alternating hypoxic stimulus in the adult.

## **4.0 INTERACTION BETWEEN RESPIRATORY CHEMOREFLEXES AND MECHANOREFLEXES DURING QUIET BREATHING IN THE NEWBORN INFANT**

### **4.1 Introduction**

As in the adult, the newborn infant matches ventilation to metabolism by using inputs from the peripheral arterial chemoreceptors and mechanoreceptors in the lung and upper airway, however the relative contribution from these receptors may differ between the neonate and adult.

Respiratory chemoreflex responses to hypoxia develop postnatally in the newborn baby as discussed in Chapter 1 (for review see Blanco, 1994). The postnatal maturation of the respiratory chemoreflex response to hypoxia has been reported using the alternate breath test (Williams et al, 1991), 30sec of 100% O<sub>2</sub> breathing (Hertzberg & Lagercrantz, 1987), and a single breath (Wilkie et al, 1987; Hagan & Gulston, 1981; 1987) two breaths (Girard et al, 1960), five breaths (Crance et al, 1971) and five minutes of 100% O<sub>2</sub> (Cross & Warner, 1951). The maturation of hypoxia chemosensitivity has been shown to occur over the first two weeks of life using all these non-invasive methods.

The Hering-Breuer inflation reflex is mediated by the vagus and is a response to stimulation of the slowly adapting stretch receptors (Breuer, 1868; Hering, 1868; Adrian, 1933). In the newborn, mechanoreceptor activity from vagal afferents in the lung is important for the maintenance of lung volume and lung inflation. The vagal afferents appear to play a crucial role in the maintenance of normal ventilation as vagotomy causes a decrease in ventilation in newborn rats (Fedorko et al, 1988). Similarly in young lambs, Johnson (1986) reported that the mechanism responsible for the braking of expiratory flow was essential for the rhythmic nature of spontaneous breathing during slow wave sleep.

Cross et al. (1960) first reported the presence of the HBIR in sleeping newborn infants. They found that the apnoeic response to lung inflation was present at birth but declined over the first 2 postnatal days. Fisher et al. (1982) also reported a decline in the strength of the HBIR in term infants over the first few postnatal hours using the end-expiratory occlusion method. However, Kirkpatrick, Olinsky, Bryan & Bryan (1976) measured the HBIR in term infants within the first 24hr and again at 4-6days and found no change in the strength of the response. Rabbette, Costeloe & Stocks (1991) also

showed that the strength of the HBIR did not decline between the first few postnatal days and 6 weeks of age.

The reflex mechanism can still be evoked in children up to 4 years of age using both the end-inspiratory (Rabbette et al, 1994; Marchal & Crance, 1987) and end-expiratory occlusion (Witte & Carlo, 1987) methods, however it is weaker during infancy compared to the neonatal period (Rabbette et al, 1994). Moreover, Rabbette et al. (1994) could not attribute the decline in strength of the HBIR in older infants to an increase in total respiratory system compliance. In contrast, in anaesthetised adult man, Widdicombe (1961) showed that the response to lung inflation was relatively weak. And by using CO<sub>2</sub> rebreathing in unanaesthetised human subjects, Clark & von Euler (1972) concluded that the inflation reflex could not be elicited within the normal tidal volume range. Neither does adult man show a HBIR to end-expiratory nasal occlusion (Issa & Sullivan, 1983). The exact time at which a decline in the relative contribution the HBIR makes to quiet breathing remains controversial.

Previous studies in adults suggest an interaction between chemo- and mechanoreflexes. Adult dogs show a HBIR which is diminished by hypoxia or hypercapnia (Bouverot, Crance & Dejours, 1970), so it appears that increased chemical drive attenuates the inhibitory reflex. Conversely, it is well established that the ventilatory response to hypercapnia or hypoxia is influenced by vagal mechanoreflexes, as in the adult rabbit vagotomy prevents the rise in respiratory frequency during hypercapnia (Richardson & Widdicombe, 1969). Frantz & Milic-Emili (1975) measured in the newborn infant the pressure generated at the mouth to end-expiratory occlusions, and showed an increase in inspiratory pressure over successive inspirations, and a response that was greater in older compared to younger infants. More recently Matsuoka & Mortola (1995) have shown in newborn rat pups that hypoxia or hypercapnia shorten the apnoea in response to lung inflation at 8 days but not at 2 days. Presumably both of these observations in the neonate are due to postnatal resetting of chemosensitivity (Williams et al, 1991; Williams et al, 1990; Hanson et al, 1989), as the younger pups or infants were relatively insensitive to hypoxia or hypercapnia compared to the older subjects. Such studies suggest a possible role for interaction between chemo- and mechanoreflexes, but this has not been directly investigated in the human neonate.

From the observations in Chapter 2 and from previous studies using the alternate breath test in healthy term infants (Williams et al, 1991; Calder et al, 1994a), it was apparent that the pattern of the respiratory chemoreflex response differed between infants. In response to the alternations in Fio<sub>2</sub>, most infants showed a slight lengthening of t<sub>I</sub> when V<sub>ti</sub> increased. However, the effects on expiratory time t<sub>E</sub> were different between

infants. Some infants showed large increases in  $t_E$  when  $V_{Ti}$  increased and so the  $t_E$  component of their chemoreflex was large. Other infants showed chemoreflex responses where  $t_E$  was tightly regulated, and only small increases in  $t_E$  were associated with an increase in  $V_{Ti}$ , hence the  $t_E$  component of their chemoreflex was small.

From these observations a question began to emerge, that being, could the variation in the  $t_E$  component of the chemoreflex respiratory response be attributed to mechanoreflex control of breathing in some infants? It appeared that there were two possibilities, first that the variation in  $t_E$  during alternations in  $F_{iO_2}$  was a chemoreflex whereby the hypoxic stimulus prolonged the expiratory component of the breath cycle, and secondly that the prolongation in  $t_E$  was actually a mechanoreflex response to the increases in  $V_{Ti}$ . I hypothesised that a large  $t_E$  chemoreflex response was in part due to a strong mechanoreflex control on breathing. I tested this hypothesis using both the alternate breath test and the end-inspiratory occlusion technique (Rabbette et al, 1991; Rabbette et al, 1994) to determine if there was a relationship between chemoreflex and mechanoreflex control in newborn infants.

## 4.2 Methods

### 4.2.1 Subject information

Subject selection is outlined in section 2.2.1. Infant data for the 17 successfully completed trials are given in table 4.2.1.i (gestation  $39 \pm 0.4$  wk, mean  $\pm$  S.E.M; postnatal age  $57 \pm 6$  hr). The order of the chemoreflex and mechanoreflex components of the study was randomised, and the order in which they were performed tabulated with total duration of time to complete both measurements.

Infant	Sex	Delivery	First test	Duration of study (hrs)	Gestation (wk)	Postnatal age (hr)	Birth weight (g)	Crown heel length (cm)
1	M	Vent	Alt	3.75	38	42	3108	50.0
2	F	EMCX	Alt	1.25	43	98	3420	54.0
3	F	ELCS	Alt	2.25	38	72	2730	47.0
4	M	EMCX	Alt	3.58	38	69	3420	52.0
5	F	ELCS	Occ	2.00	41	88	3800	54.0
6	F	Fd	Alt	3.67	41	36	3140	52.0
7	F	ELCS	Alt	3.67	37	26	2860	48.0
8	F	SVD	Occ	1.25	39	26	3680	54.5
9	F	EMCX	Occ	3.00	41	90	3740	52.0
10	F	ELCS	Alt	1.50	38	52	2520	50.0
11	F	ELCS	Alt	1.50	39	49	3240	52.0
12	M	Vent	Alt	2.00	37	38	2420	46.0
13	M	ELCS	Occ	2.00	38	73	2940	50.0
14	F	EMCX	Occ	2.25	41	68	3120	52.0
15	F	ELCS	Alt	2.00	38	77	4080	57.0
16	M	Fd	Occ	2.00	40	46	3340	51.0
17	F	SVD	Occ	1.50	41	19	3860	58.0
Mean $\pm$ S.E.M				2.30 $\pm$ 0.21	39 $\pm$ 0.4	57 $\pm$ 6	3260 $\pm$ 116	52.0 $\pm$ 0.8

Table 4.2.1.i Subject information. Delivery type Vent=ventouse, EMCX=emergency caesarean section, ELCS=elective caesarean section, SVD=standard vaginal delivery, Fd=forceps. Alt=alternate breath test performed first, Occ=end-inspiratory occlusion test performed first.

### 4.2.2 Experimental Conditions

#### *Alternate breath test*

The experimental set-up for the alternate breath test is described in section 2.1.2 and illustrated in Figure 2.1.2.i.

*Airway occlusion*

The experimental set-up for the airway occlusion technique is shown in figure 4.2.2.i. The airway occlusion technique (Rabbette et al, 1991; Rabbette et al, 1994) was used to measure mechanoreflexes. A small face-mask (Rendell-Baker Soucek, U.K., size 0) attached to a pneumotachometer (PK Morgan, UK; max flow rate=30 l/min, dead space=4-7ml) and with a port to a pressure transducer (Furness Controls Limited, U.K.,  $\pm 5$ kPa), was sealed around the infant's nose and mouth with silicone putty. Changes in air flow were electronically integrated to derive tidal volume, and recorded on a 2 channel chart recorder (Gould, U.K.) with pressure at the airway opening.

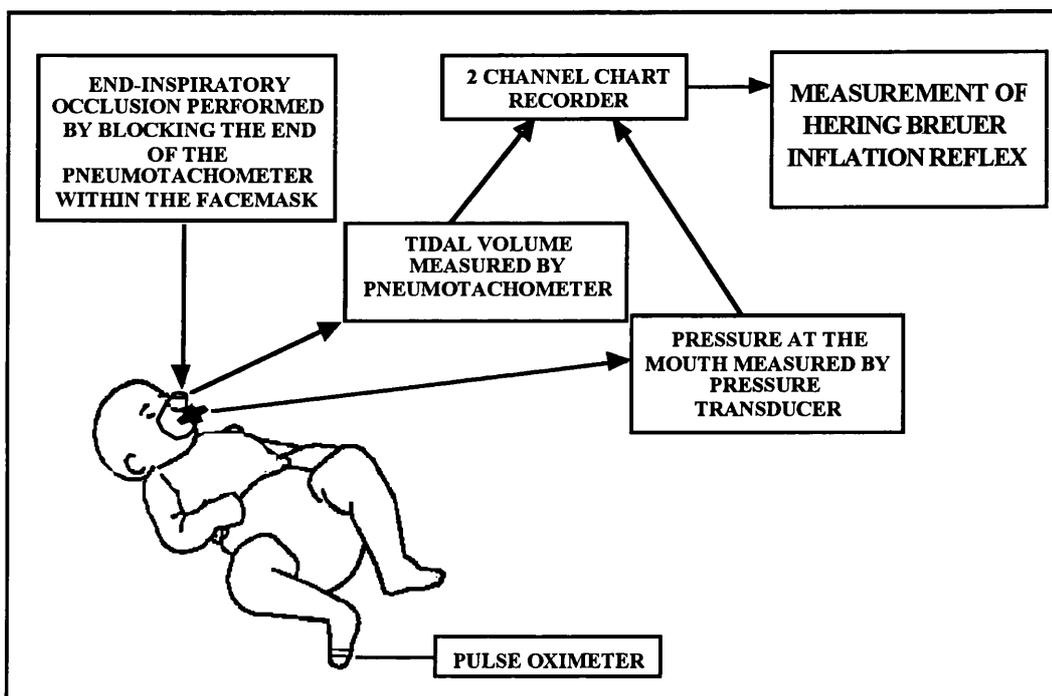


Figure 4.2.2.i Diagram of experimental set-up for end inspiratory occlusions.

### 4.2.3 Experimental protocol

The order of the alternate breath test and end-inspiratory occlusion test was randomised between infants as described in table 4.2.1.i. For the alternate breath test, a minimum of two test and two control runs were performed in each infant of a maximum duration each of 100 breaths or 3min. The order of control and test runs was also randomised.

For end-inspiratory occlusions the infants were usually supine. After the infant was breathing quietly through the facemask, a period of at least 10 regular breaths were recorded prior to end-inspiratory occlusion. Occlusion was achieved by the operator placing a thumb over the end of the pneumotachometer head (*ca.* 2 sec), thus preventing

expiration and maintaining an inflated lung. A minimum of 3 end-inspiratory occlusions, at least 1 min apart, were performed in each infant.

#### 4.2.4 Data analysis

Figure 4.2.4.i shows an example of an end-inspiratory occlusion in one infant. Mechanoreflex responses were accepted if the pressure signal indicated a complete seal during end-inspiratory occlusion, and that the onset of the occlusion occurred no later than 20% after the beginning of expiration. The mechanoreflex response was measured as percentage increase in  $t_E$  for the occluded breath as a percentage of control:

$$\text{Percentage increase } t_E = 100 \times (\text{occluded } t_E - \text{control } t_E) / \text{control } t_E.$$

Occluded breaths were compared to the mean of the 10 breaths prior to occlusion using Wilcoxon matched pairs test. It was not possible to obtain 3 satisfactory occlusions in all infants, therefore for two infants only 2 occlusions each were used for analysis, and for one other infant only one satisfactory occlusion was used for analysis.

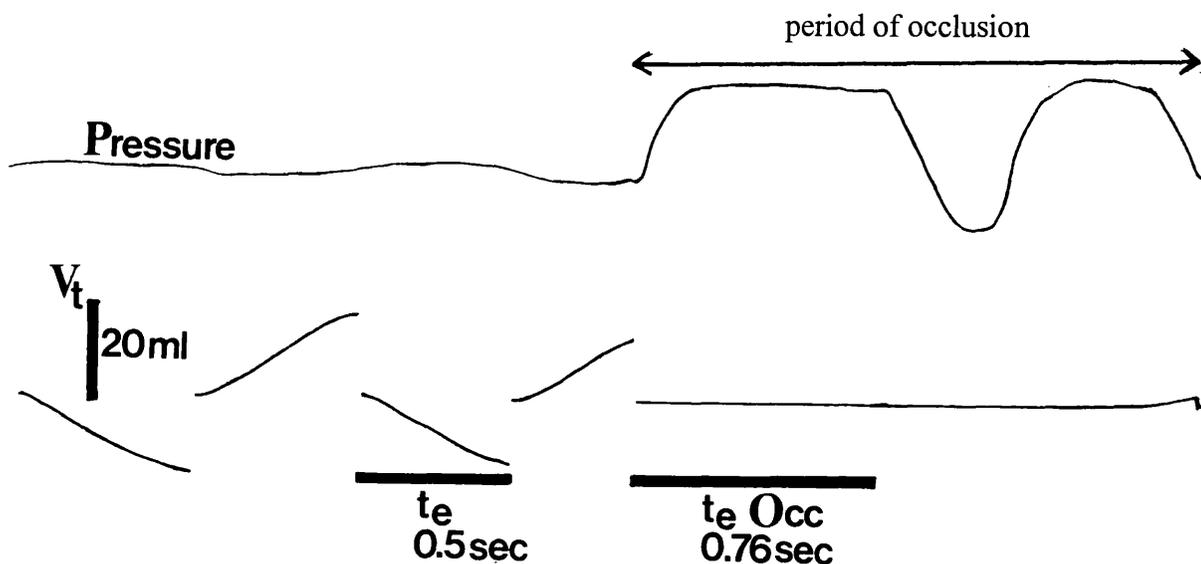


Figure 4.2.4.i An example of an end-inspiratory occlusion in one infant;  $V_t$  (mls),  $t_E$  (sec),  $t_E$  Occ (sec) and pressure at the nose (uncalibrated).

## **4.3 Results**

### **4.3.1 Phase relationships between alternating respiratory variables of the chemoreflex respiratory response**

An example of the chemoreflex responses for two infants is shown in figure 4.3.1.i. Infant A shows an "in phase" chemoreflex response between  $V_{ti}$  and  $t_E$ , such that increases in  $V_{ti}$  are accompanied by increases in  $t_E$ , whilst infant B shows an "out of phase" chemoreflex response between  $V_{ti}$  and  $t_E$  and so increases in  $V_{ti}$  are accompanied by decreases in  $t_E$ .

Linear regression fitted to each of two chemoreflex responses per infant was used to describe the phase relationship between  $V_{ti}$  and  $t_E$  (see section 2.2.3.a). To consider the validity of this type of comparison, the slope and correlation coefficient of the regression line fitted to the alternation was tabulated for  $V_{ti}$  and  $t_E$  responses in table 4.3.1.i. Overall the correlation coefficients of many of alternations were low, therefore using the regression line to describe the pattern of alternation between respiratory variables to any degree of precision was difficult. However using this method for analysis, 3 infants showed "in phase" alternations between  $V_{ti}$  and  $t_E$ , 5 infants showed "out of phase" alternations between  $V_{ti}$  and  $t_E$  and 9 infants showed "mixed" responses (i.e. one of each "in phase" and "out of phase"). These nine infants show that there did not appear to be any consistency in the pattern of chemoreflex response shown between infants, because for the two test responses used to describe their chemoreflex response, each showed a different pattern of alternation between  $V_{ti}$  and  $t_E$ .

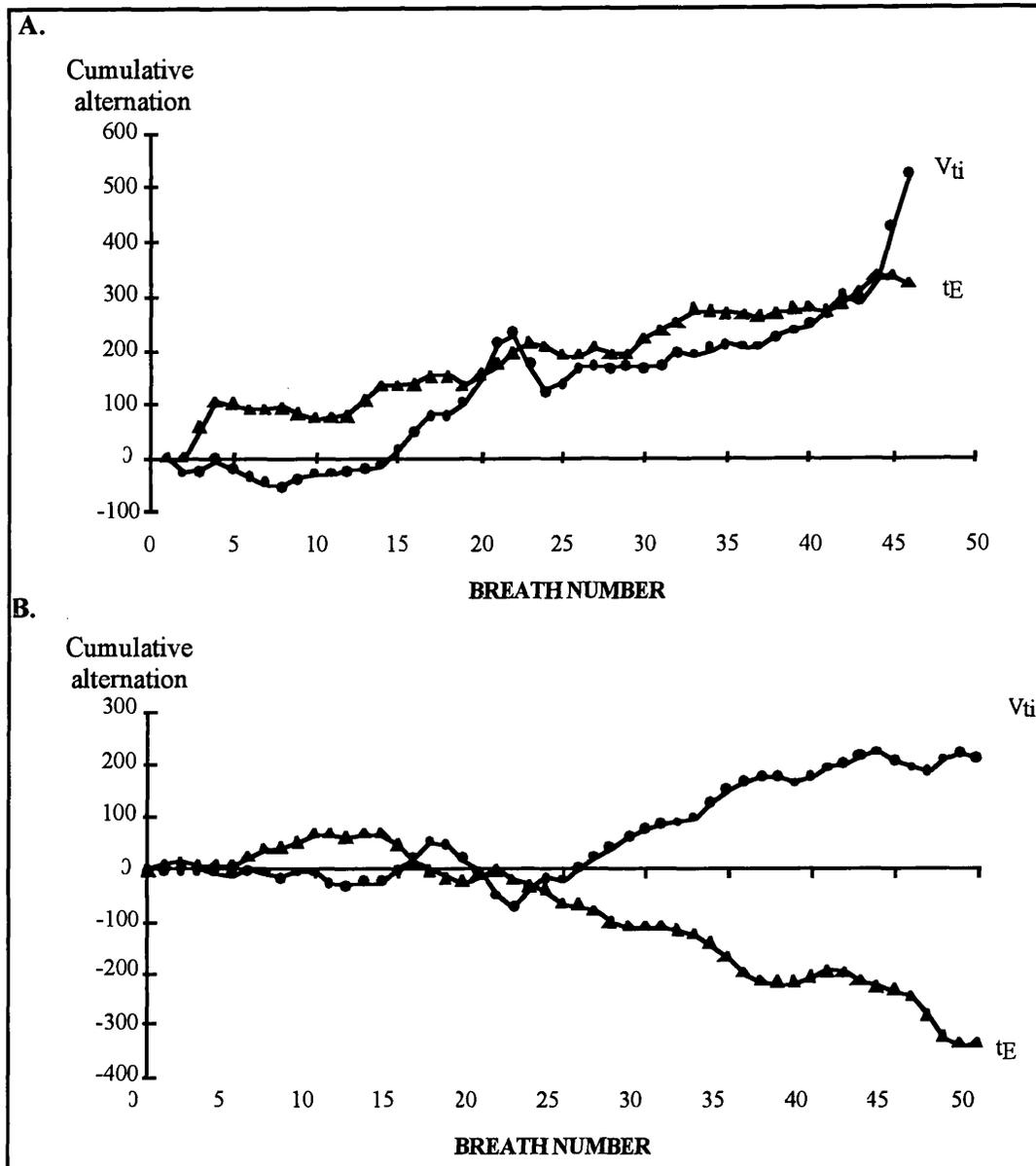


Figure 4.3.1.i The chemoreflex respiratory response to alternate breaths of  $F_{iO_2}$  0.21 and 0.16 for one infant showing an "in phase" (A) and another showing an "out of phase" (B) relationship between alternating  $V_{ti}$  and  $t_E$ . Mean percentage breath-by-breath alternation is plotted cumulatively with respect to breath number. Closed circles,  $V_{ti}$ ; closed triangles,  $t_E$ .

Phase relationship between $V_{ti}$ and $t_E$	$V_{ti}$		$t_E$	
	mean breath-by- breath % alt.	r	mean breath-by- breath % alt.	r
IN	1.93	0.51	1.70	0.45
IN	12.45	0.96	6.42	0.45
IN	27.88	0.96	6.01	0.89
IN	5.41	0.66	4.51	0.98
MIXED OUT	6.61	0.91	0.80	0.50
IN	3.34	0.78	2.92	0.90
IN	1.34	0.40	5.65	0.96
IN	3.01	0.70	6.26	0.72
OUT	13.78	0.90	5.49	0.94
OUT	14.13	0.93	3.47	0.64
MIXED OUT	7.65	0.63	7.68	0.94
IN	10.78	0.89	11.76	0.94
MIXED OUT	6.91	0.80	3.40	0.79
IN	17.43	0.92	3.86	0.83
OUT	3.95	0.86	10.69	0.92
OUT	6.40	0.91	4.34	0.74
MIXED OUT	9.91	0.95	1.46	0.44
IN	3.36	0.57	11.18	0.92
OUT	18.53	0.94	13.53	0.97
OUT	13.08	0.90	3.26	0.60
MIXED OUT	8.31	0.79	0.72	0.42
IN	9.25	0.92	3.92	0.88
OUT	6.00	0.90	2.62	0.82
OUT	7.40	0.95	1.65	0.56
MIXED OUT	18.36	0.94	0.60	0.08
IN	7.08	0.65	16.04	0.87
MIXED IN	2.00	0.44	8.36	0.99
OUT	2.05	0.55	7.61	0.98
MIXED IN	4.70	0.96	0.02	0.01
OUT	4.50	0.93	1.86	0.82
MIXED OUT	5.88	0.70	9.61	0.94
IN	8.45	0.88	7.51	0.95
OUT	9.74	0.88	9.02	0.92
OUT	9.37	0.91	13.41	0.92

Table 4.3.1.i Summary of regression analysis fitted to  $V_{ti}$  and  $t_E$  alternation responses. Phase relationship between  $V_{ti}$  and  $t_E$ , slopes and correlation coefficients (r).

### 4.3.2 Chemoreflex respiratory responses for all infants

The mean breath-by-breath alternations for control and test runs are given in table 4.3.2.i. Test responses were compared to control for each respiratory variable (see section 2.2.3.b). For all infants grouped together, mean test chemoreflex respiratory responses were significantly greater than mean control responses for  $V_{ti}$ ,  $t_E$ ,  $f$ ,  $V_{ti}/t_I$ ,  $t_I/t_{Tot}$  and  $V_E$  (Wilcoxon matched pairs,  $P < 0.05$ ).

		$V_{ti}$	$V_{te}$	$t_I$	$t_E$	$f$	$V_{ti}/t_I$	$V_{te}/t_E$	$t_I/t_{Tot}$	$V_E$
CONTROL	median	3.18	4.89	1.58	2.87	2.42	1.98	6.31	2.15	3.00
n=17	95% confidence limits	1.52-4.04	2.66-5.55	1.38-3.25	1.92-3.96	1.42-3.06	1.15-3.67	4.21-7.91	1.42-2.98	1.50-3.64
TEST	median	7.19	5.84	2.42	5.96	4.19	4.29	7.13	3.20	4.69
n=17	95% confidence limits	5.18-12.20	3.99-6.72	2.07-5.42	3.63-8.62	2.58-5.47	2.41-8.08	4.83-9.31	2.06-5.71	2.82-7.89
p VALUE		0.01	NS	NS	0.01	0.01	0.05	NS	0.01	0.05

Table 4.3.2.i. Summary of mean control and test chemoreflex respiratory responses (median, 95% confidence limits) to alternate breaths of  $F_{iO_2}$  0.21 (control) and 0.21 or 0.16 (test).

### 4.3.3 Assessment of significant chemoreflex responses for each infant

To assess the number of infants showing a significant chemoreflex respiratory response, test responses were compared to control responses for each infant (see section 2.2.3.c). Fourteen of 17 infants showed a significant chemoreflex respiratory response, that is at least one respiratory variable of the chemoreflex showed a significant response. The three infants who did not show a significant chemoreflex response were aged 49, 72 and 77 hrs postnatally, and were born at 39, 38 and 38 wks gestation respectively. Details of the total number of infants showing significant chemoreflex responses for each respiratory variable are given in table 4.3.3.i. Chemoreflex respiratory responses were plotted for each infant as the difference between mean test and control for each respiratory variable in figures 4.3.3.i-ix. Significant chemoreflex responses are marked by an asterisk for each infant.

Respiratory variable	$V_{ti}$	$V_{te}$	$t_I$	$t_E$	$f$	$V_{ti}/t_I$	$V_{te}/t_E$	$t_I/T_{Tot}$	$V_E$
Number of infants showing significant responses (total=17)	9	4	6	7	7	8	3	7	8

Table 4.3.3.i Number of infants showing significant chemoreflex respiratory responses to breath-by-breath alternations in  $F_{iO_2}$  between 0.21 and 0.16 for each respiratory variable.

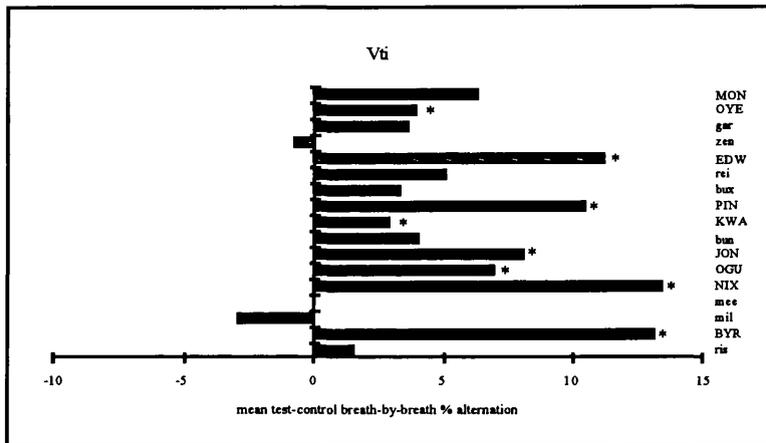


Figure 4.3.3.i  $V_{ti}$  chemoreflex respiratory responses (mean test - control breath-by-breath % alternation) to alternate breaths of  $F_{iO_2}$  0.21 and 0.16. Significant  $V_{ti}$  alternations are denoted by asterisk (\*).

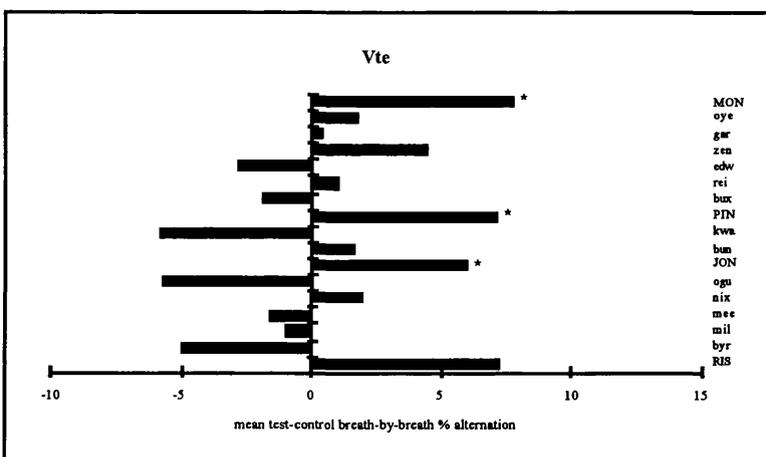


Figure 4.3.3.ii  $V_{te}$  chemoreflex respiratory responses (mean test - control breath-by-breath % alternation) to alternate breaths of  $F_{iO_2}$  0.21 and 0.16. Significant  $V_{te}$  alternations are denoted by asterisk(\*).

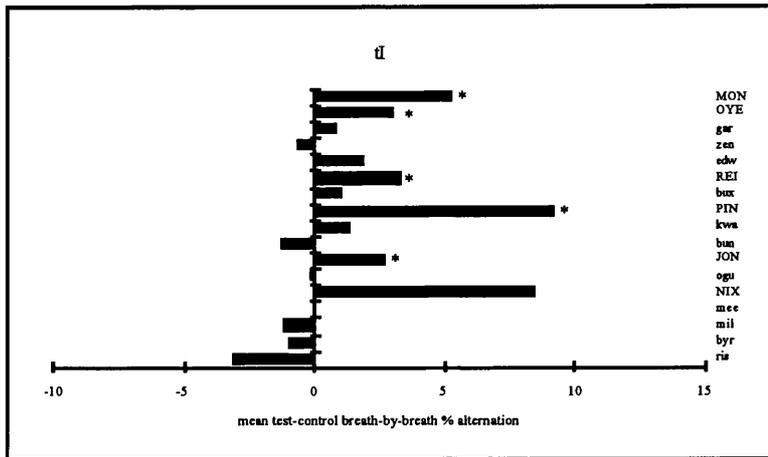


Figure 4.3.3.iii t<sub>I</sub> chemoreflex respiratory responses (mean test - control breath-by-breath % alternation) to alternate breaths of Fio<sub>2</sub> 0.21 and 0.16. Significant t<sub>I</sub> alternations are denoted by asterisk (\*).

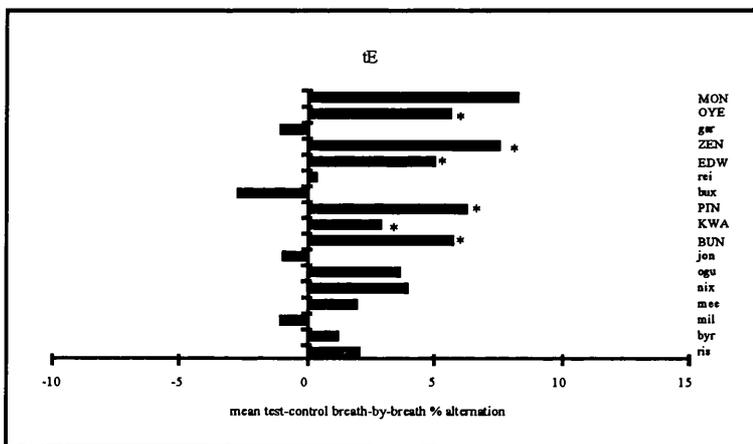


Figure 4.3.3.iv t<sub>E</sub> chemoreflex respiratory responses (mean test - control breath-by-breath % alternation) to alternate breaths of Fio<sub>2</sub> 0.21 and 0.16. Significant t<sub>E</sub> alternations are denoted by asterisk (\*).

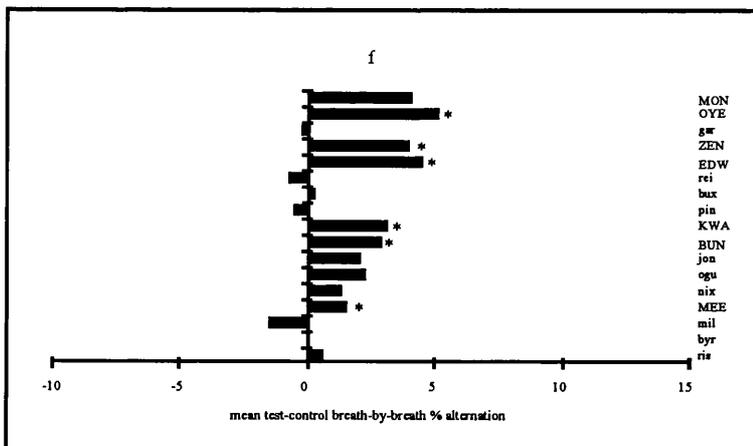


Figure 4.3.3.v f chemoreflex respiratory responses (mean test - control breath-by-breath % alternation) to alternate breaths of Fio<sub>2</sub> 0.21 and 0.16. Significant f alternations are denoted by asterisk (\*).

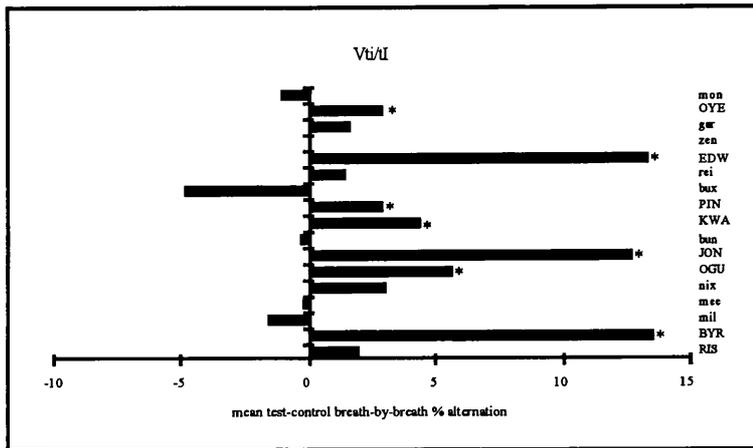


Figure 4.3.3.vi  $V_{ti}/t_I$  chemoreflex respiratory responses (mean test - control breath-by-breath % alternation) to alternate breaths of  $F_{iO_2}$  0.21 and 0.16. Significant  $V_{ti}/t_I$  alternations are denoted by asterisk (\*).

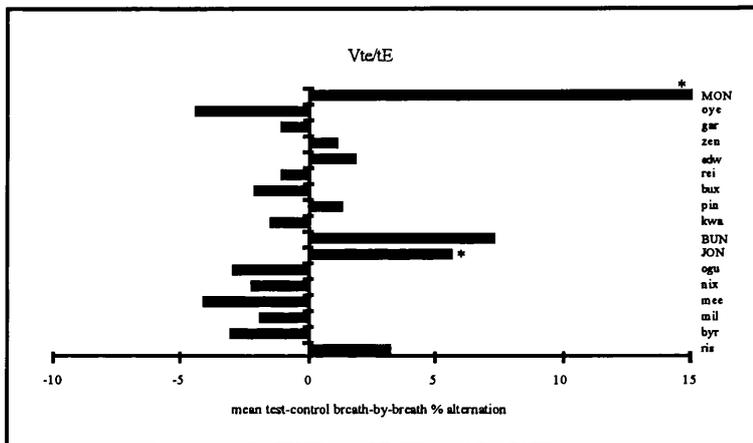


Figure 4.3.3.vii  $V_{te}/t_E$  chemoreflex respiratory responses (mean test - control breath-by-breath % alternation) to alternate breaths of  $F_{iO_2}$  0.21 and 0.16. Significant  $V_{te}/t_E$  alternations are denoted by asterisk (\*).

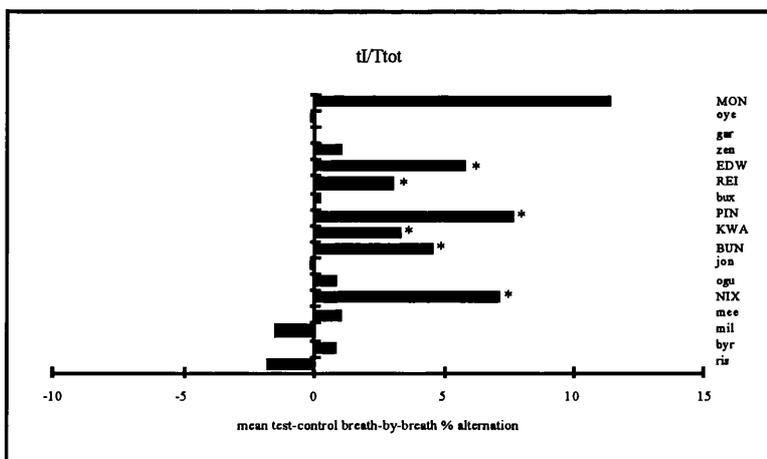


Figure 4.3.3.viii  $t_I/T_{tot}$  chemoreflex respiratory responses (mean test - control breath-by-breath % alternation) to alternate breaths of  $F_{iO_2}$  0.21 and 0.16. Significant  $t_I/T_{tot}$  alternations are denoted by asterisk (\*).

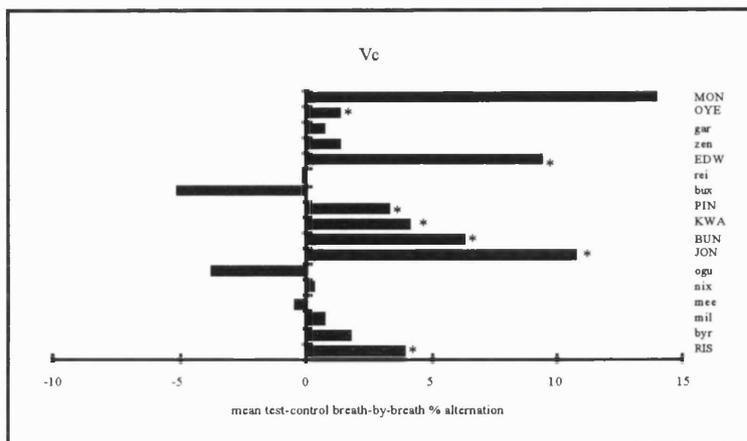


Figure 4.3.3.ix VE chemoreflex respiratory responses (mean test - control breath-by-breath % alternation) to alternate breaths of Fio<sub>2</sub> 0.21 and 0.16. Significant VE alternations are denoted by asterisk (\*).

The three infants who did not show a significant chemoreflex respiratory response are represented in figures 4.3.3.x-xii. Each control response and each test response is shown for all respiratory variables analysed. The infant in figure 4.3.3.x shows very small test responses to the alternations in Fio<sub>2</sub> between 0.21 and 0.16, and so the absence of a test response greater than the critical value (see table 2.2.3.c.i.) means that this infant is a non-responder. In addition, the infants in figures 4.3.3.xi-xii not only show small test responses for some respiratory variables, but also show large control responses for other respiratory variables. That is, during control runs when Fio<sub>2</sub> alternated between Fio<sub>2</sub> 0.21 and 0.21, the breath-by-breath % alternation was large. So although the test response may show a greater breath-by-breath % alternation than the critical value (see table 2.2.3.c.i.), if the test response was not at least twice as great as the individual's control response, the alternation was not significant. This is the possibility of a type 2 error, which is discussed in greater detail in section 5.4.4.e, in relation to respiratory chemoreflex testing in the adult.

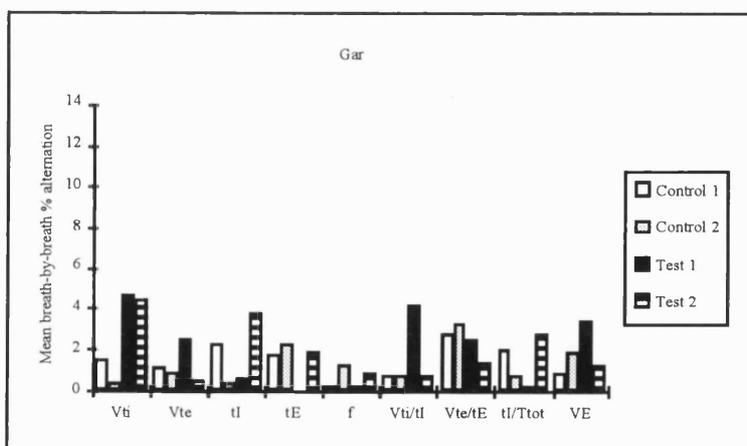


Figure 4.3.3.x Control (alternations between Fio<sub>2</sub> 0.21 and 0.21) and test (alternations between Fio<sub>2</sub> 0.21 and 0.16) responses (mean breath-by-breath % alternation) for non-responder Gar.

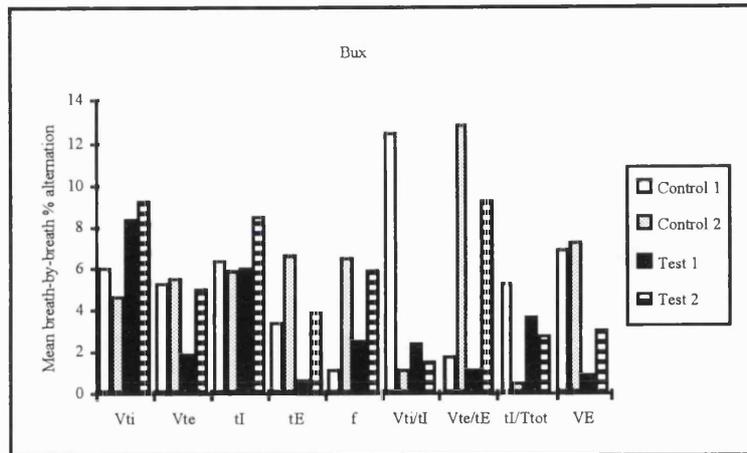


Figure 4.3.3.xi Control (alternations between  $F_{iO_2}$  0.21 and 0.21) and test (alternations between  $F_{iO_2}$  0.21 and 0.16) responses (mean breath-by-breath % alternation) for non-responder Bux.

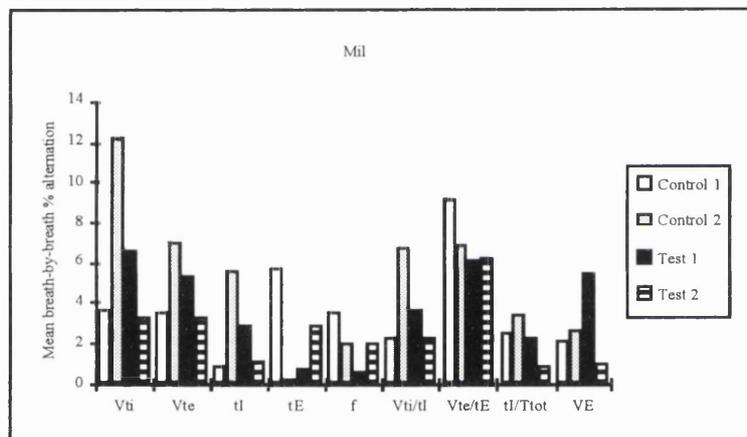


Figure 4.3.3.xii Control (alternations between  $F_{iO_2}$  0.21 and 0.21) and test (alternations between  $F_{iO_2}$  0.21 and 0.16) responses (mean breath-by-breath % alternation) for non-responder Mil.

#### 4.3.4 Variation in the pattern of the chemoreflex response: Effect of breath-by-breath alternations in $F_{iO_2}$ between 0.21 and 0.16 on $V_{ti}$ , $t_i$ and $t_E$

In 6 of 17 infants the volume component of their chemoreflex responses was calibrated in mls. This meant that the effect of a chemical stimulus on breathing could be observed in terms of volume and timing. Chemoreflex responses to the alternate breaths of  $F_{iO_2}$  0.21 and 0.16 are shown in figures 4.3.4.i-vi in different infants. The most notable difference between these graphs are the chemoreflex responses for infants in figures 4.3.4.iv and v. These 2 infants show greater variation in  $t_E$  in response to the breath-by-breath alternations in  $F_{iO_2}$  than the other infants. The infant in figure 4.3.4.iv

showed a large breath-by-breath alternation in the  $t_E$  (11.76%,  $r=0.94$ ) component of their chemoreflex response, which would tend to suggest that the variation in  $t_E$  was under chemoreflex control. That is, the variation in  $t_E$  occurred in a regular manner such that it produced a highly linear response, presumably in response to the breath-by-breath changes in  $F_{iO_2}$ . In contrast, the infant in figure 4.3.4.v showed small breath-by-breath alternations in both the  $V_{Ti}$  (1.93%,  $r=0.51$ ) and  $t_E$  (1.70%,  $r=0.45$ ) components of the chemoreflex response. So although there was large variation in  $V_{Ti}$  and  $t_E$  between breaths, it did not occur in a regular manner, and so the measured chemoreflex response was small. To determine if this pattern of breathing was under mechanoreflex control, chemoreflex responses were compared to mechanoreflex responses in section 4.3.7.

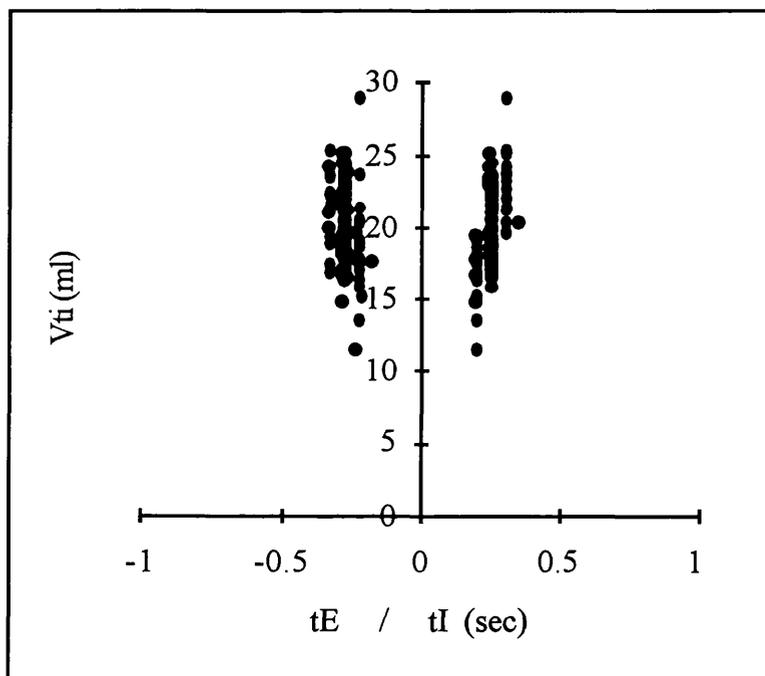


Figure 4.3.4.i Chemoreflex response to breath-by-breath alternations in  $F_{iO_2}$  between 0.21 and 0.16 for one infant. Mean  $V_{Ti}$  breath-by-breath alternation = 1.37 % ( $r=0.84$ ), mean  $t_E$  breath-by-breath alternation = 4.21 % ( $r=0.92$ ).

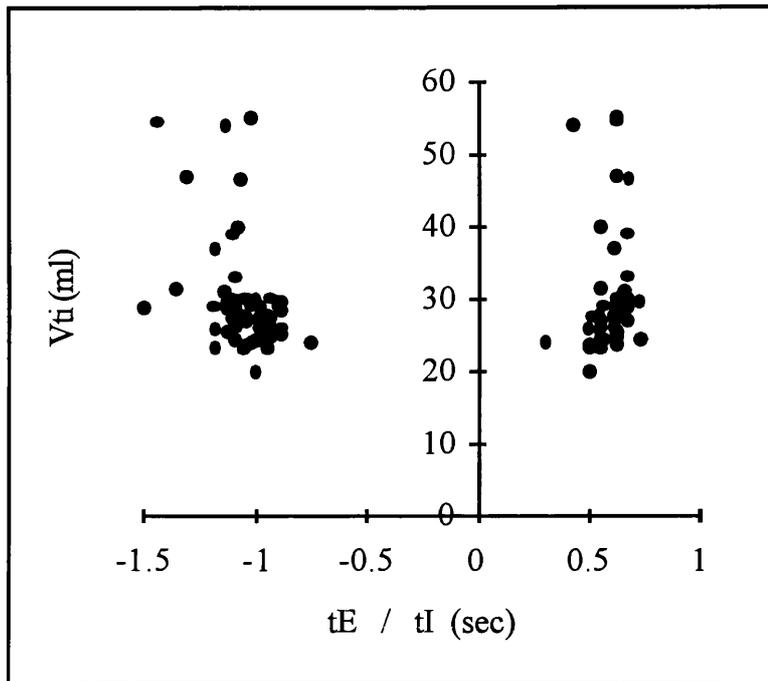


Figure 4.3.4.ii Chemoreflex response to breath-by-breath alternations in  $F_{iO_2}$  between 0.21 and 0.16 for one infant. Mean  $V_{Ti}$  breath-by-breath alternation = 1.34 % ( $r=0.40$ ), mean  $t_E$  breath-by-breath alternation = 5.65 % ( $r=0.96$ ).

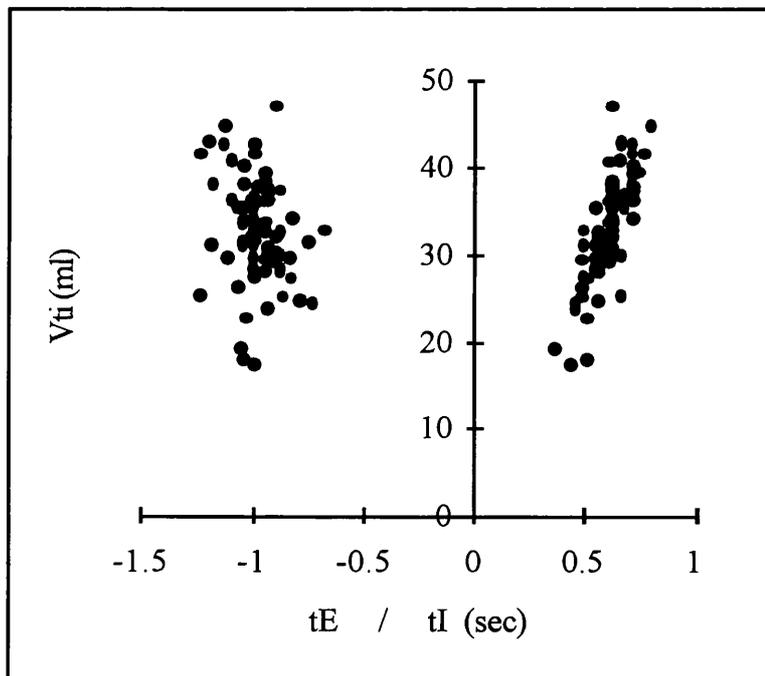


Figure 4.3.4.iii Chemoreflex response to breath-by-breath alternations in  $F_{iO_2}$  between 0.21 and 0.16 for one infant. Mean  $V_{Ti}$  breath-by-breath alternation = 3.34 % ( $r=0.78$ ), mean  $t_E$  breath-by-breath alternation = 2.92 % ( $r=0.90$ ).

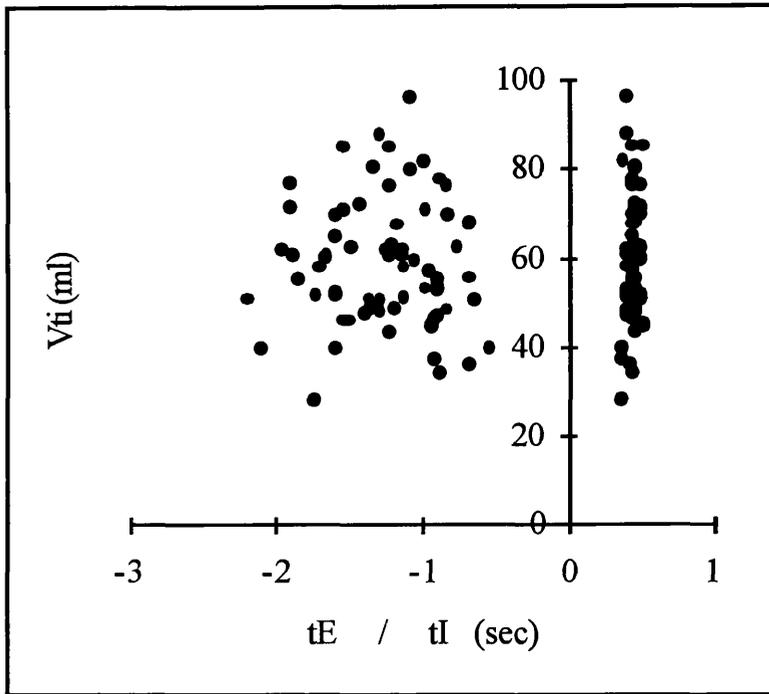


Figure 4.3.4.iv Chemoreflex response to breath-by-breath alternations in  $F_{iO_2}$  between 0.21 and 0.16 for one infant. Mean  $V_{Ti}$  breath-by-breath alternation = 10.78 % ( $r=0.89$ ), mean  $t_E$  breath-by-breath alternation = 11.76 % ( $r=0.94$ ).

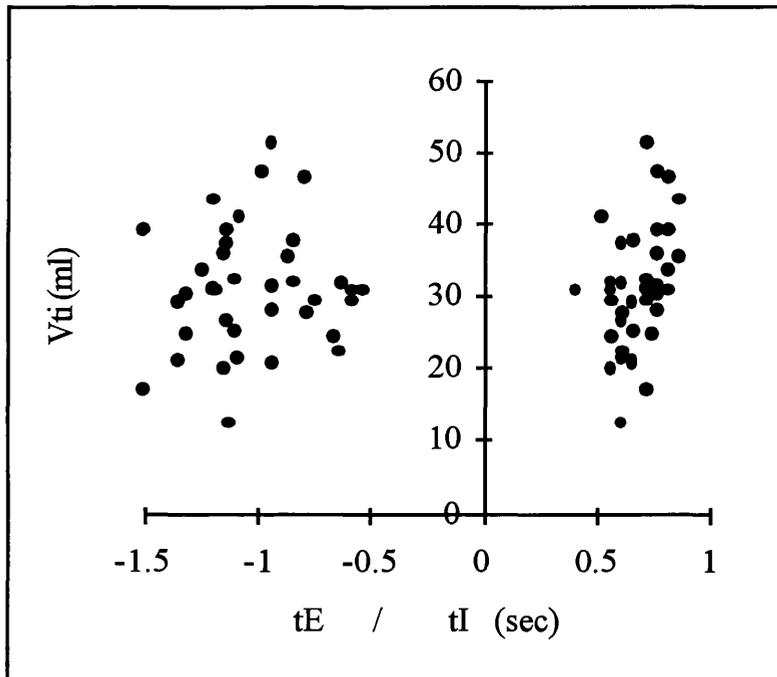


Figure 4.3.4.v Chemoreflex response to breath-by-breath alternations in  $F_{iO_2}$  between 0.21 and 0.16 for one infant. Mean  $V_{Ti}$  breath-by-breath alternation = 1.93 % ( $r=0.51$ ), mean  $t_E$  breath-by-breath alternation = 1.70 % ( $r=0.45$ ).

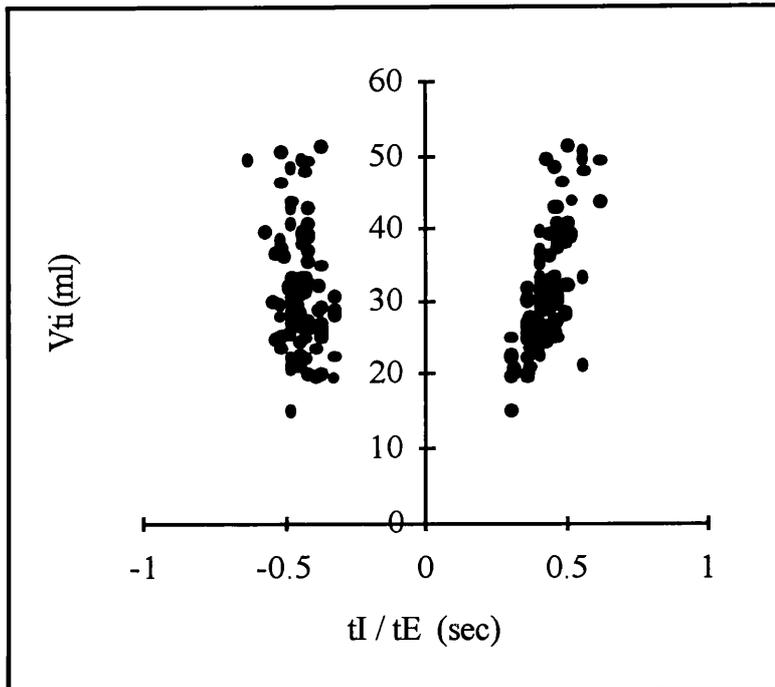


Figure 4.3.4.vi Chemoreflex response to breath-by-breath alternations in  $F_{iO_2}$  between 0.21 and 0.16 for one infant. Mean  $V_{ti}$  breath-by-breath alternation = 13.78 % ( $r=0.90$ ), mean  $t_E$  breath-by-breath alternation = 5.49 % ( $r=0.94$ ).

### 4.3.5 Effect of postnatal age on chemoreflex respiratory responses

Chemoreflex responses were plotted against postnatal age in figures 4.3.5.i-ix. Infants were separated into those showing significant and non-significant alternations for each respiratory variable. When the strength of the chemoreflex was related to postnatal age for all infants as a group, there was no correlation found between a greater chemoreflex response and an increase in postnatal age. Correlation coefficients were  $r=0.28$  for  $V_{ti}$ ,  $r=-0.50$  for  $V_{te}$ ,  $r=-0.06$  for  $t_I$ ,  $r=-0.14$  for  $t_E$ ,  $r=-0.24$  for  $f$ ,  $r=0.27$  for  $V_{ti}/t_I$ ,  $r=-0.61$  for  $V_{te}/t_E$ ,  $r=-0.19$  for  $t_I/T_{tot}$  and  $r=-0.52$  for  $V_E$ .

There was a tendency for infants with a significant alternation in  $V_{ti}$  to show a greater chemoreflex response for an increase in postnatal age, however this was not seen in any other respiratory variable, and so does not provide evidence for a direct link between postnatal age and chemoreflex responses in these infants.

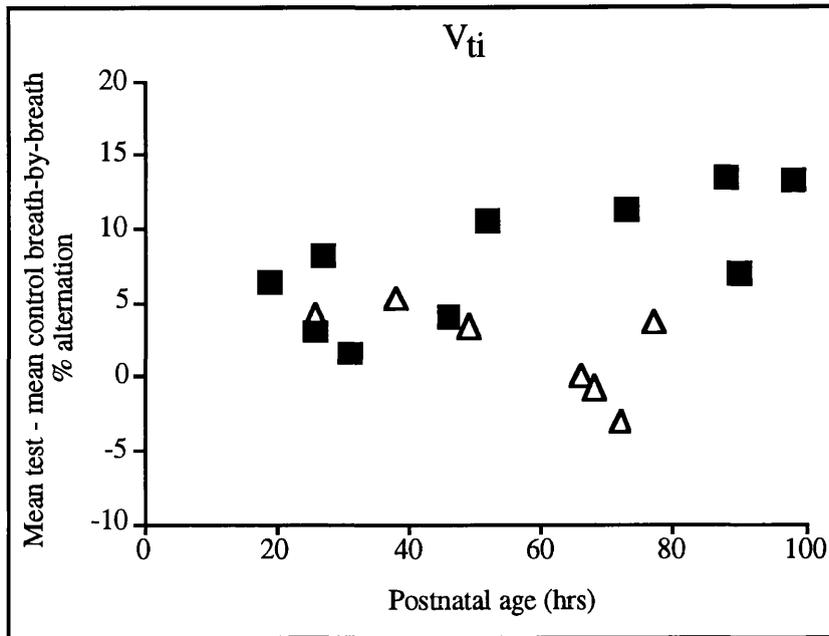


Figure 4.3.5.i  $V_{ti}$  chemoreflex response (mean test - control breath-by-breath % alternation) plotted against postnatal age (hrs). Filled squares are infants showing a significant alternation, and open triangles are infants showing a non-significant alternation.

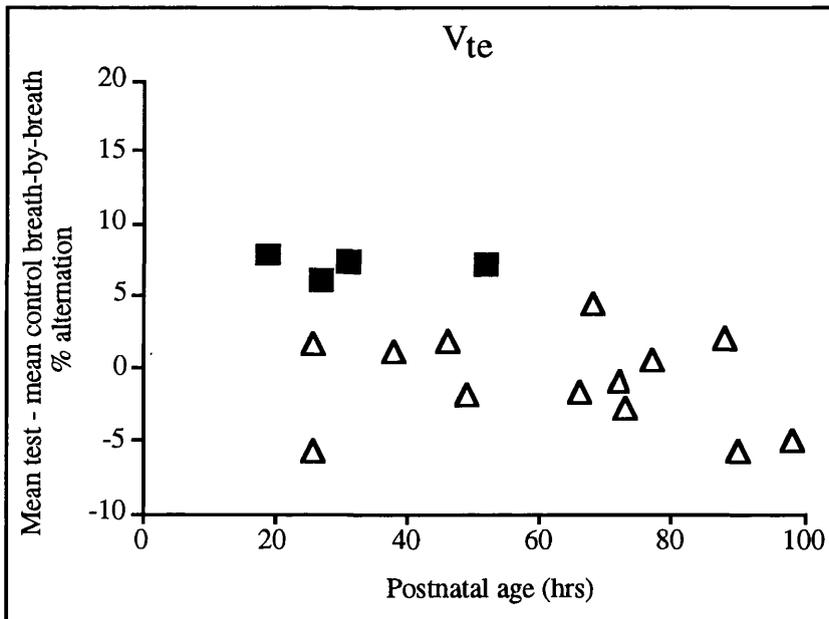


Figure 4.3.5.ii  $V_{te}$  chemoreflex response (mean test - control breath-by-breath % alternation) plotted against postnatal age (hrs). Filled squares are infants showing a significant alternation, and open triangles are infants showing a non-significant alternation.

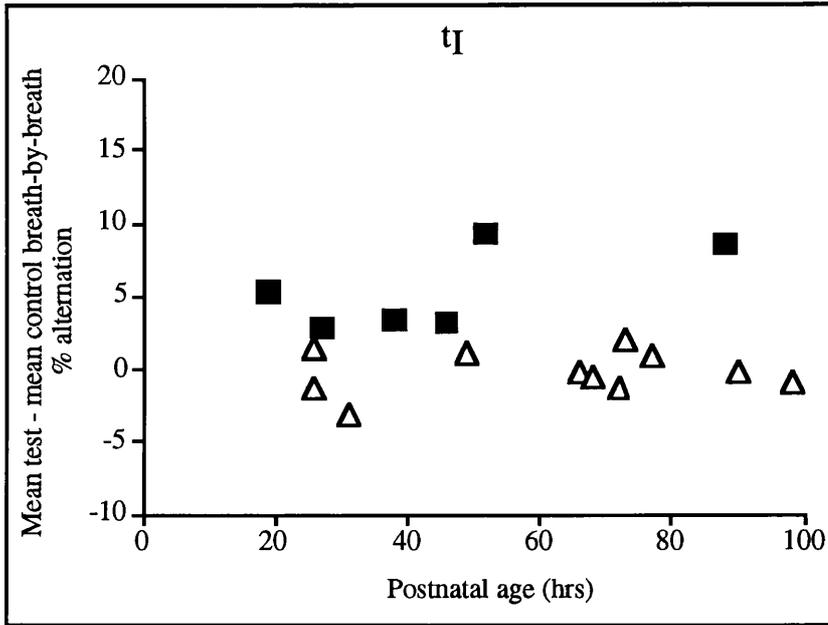


Figure 4.3.5.iii t<sub>I</sub> chemoreflex response (mean test - control breath-by-breath % alternation) plotted against postnatal age (hrs). Filled squares are infants showing a significant alternation, and open triangles are infants showing a non-significant alternation.

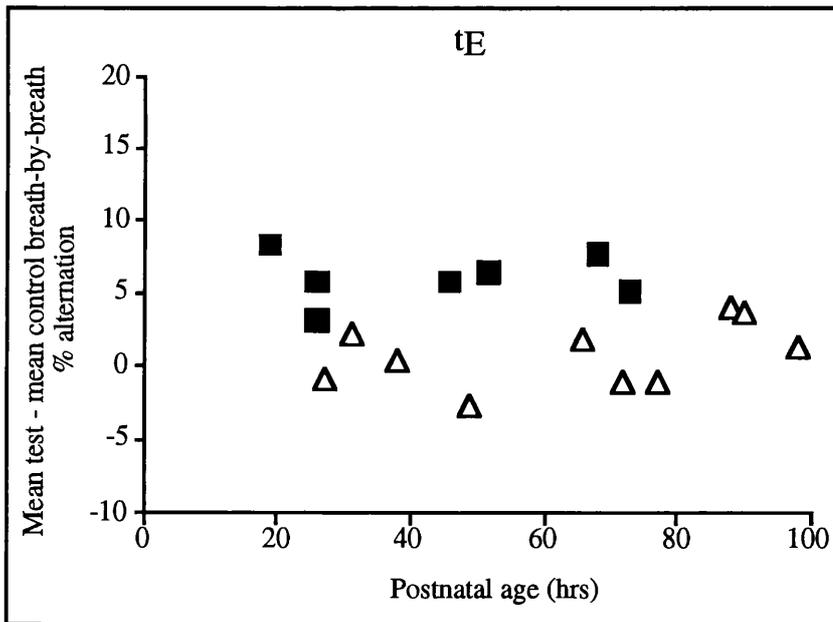


Figure 4.3.5.iv t<sub>E</sub> chemoreflex response (mean test - control breath-by-breath % alternation) plotted against postnatal age (hrs). Filled squares are infants showing a significant alternation, and open triangles are infants showing a non-significant alternation.

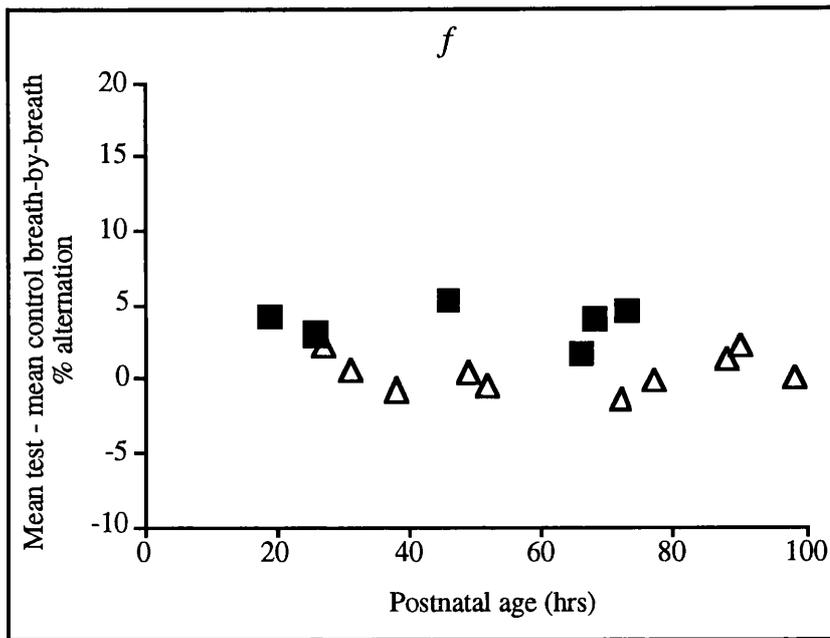


Figure 4.3.5.v  $f$  chemoreflex response (mean test - control breath-by-breath % alternation) plotted against postnatal age (hrs). Filled squares are infants showing a significant alternation, and open triangles are infants showing a non-significant alternation.

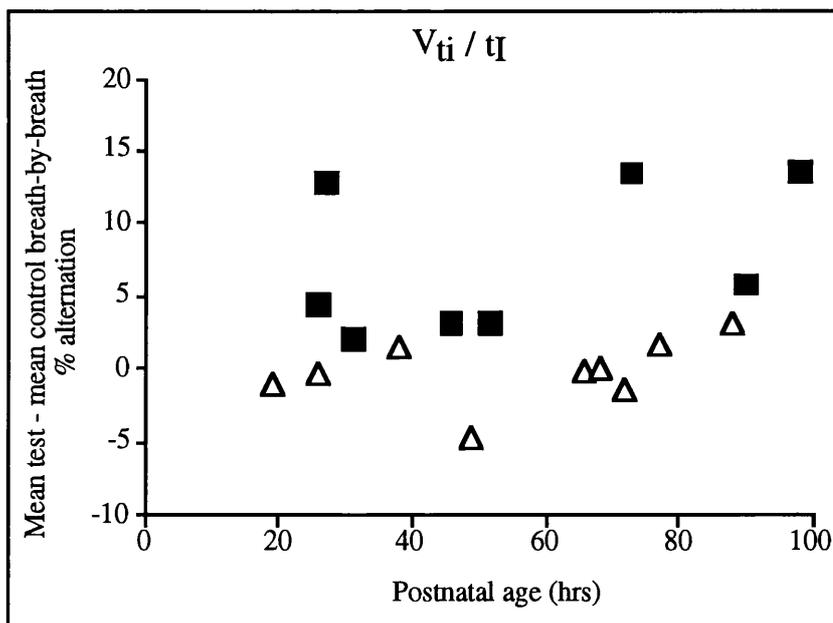


Figure 4.3.5.vi  $V_{ti}/t_I$  chemoreflex response (mean test - control breath-by-breath % alternation) plotted against postnatal age (hrs). Filled squares are infants showing a significant alternation, and open triangles are infants showing a non-significant alternation.

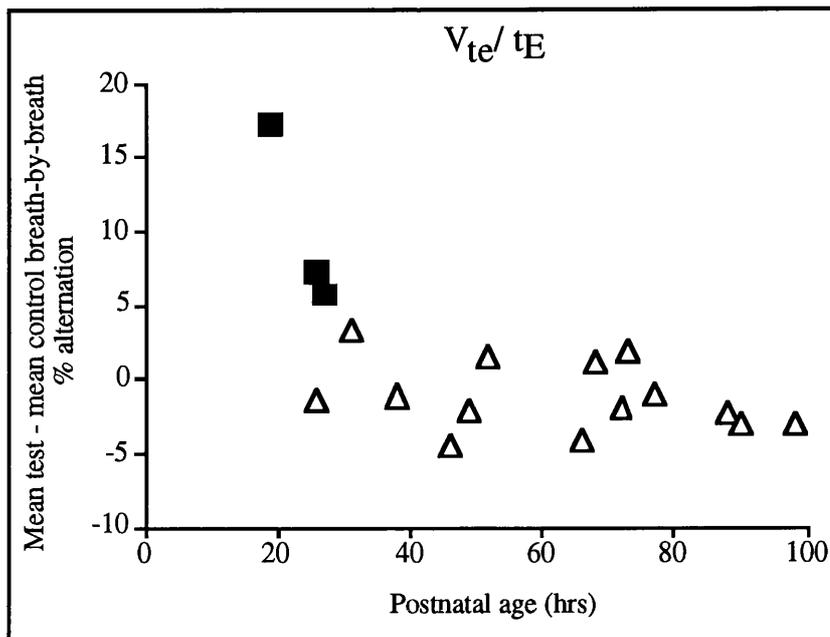


Figure 4.3.5.vii  $V_{te}/t_E$  chemoreflex response (mean test - control breath-by-breath % alternation) plotted against postnatal age (hrs). Filled squares are infants showing a significant alternation, and open triangles are infants showing a non-significant alternation.

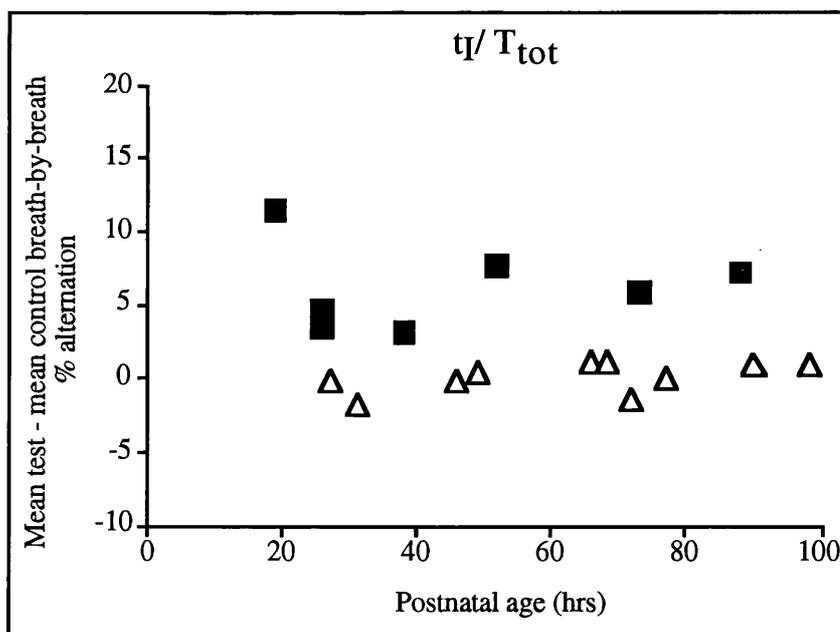


Figure 4.3.5.viii  $t_I/T_{tot}$  chemoreflex response (mean test - control breath-by-breath % alternation) plotted against postnatal age (hrs). Filled squares are infants showing a significant alternation, and open triangles are infants showing a non-significant alternation.

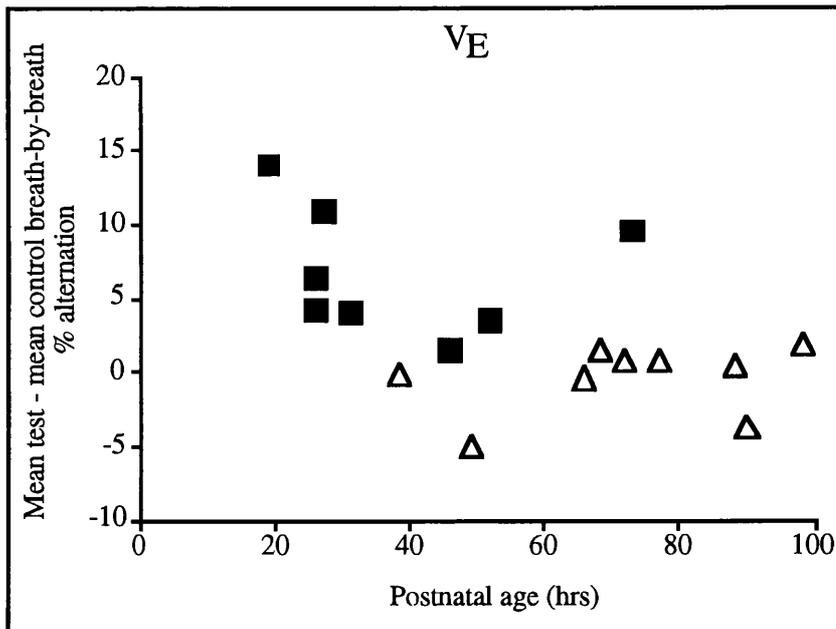


Figure 4.3.5.ix  $V_E$  chemoreflex response (mean test - control breath-by-breath % alternation) plotted against postnatal age (hrs). Filled squares are infants showing a significant alternation, and open triangles are infants showing a non-significant alternation.

#### 4.3.6 Mechanoreflex responses for all infants

The mechanoreflex response to end-inspiratory occlusion was calculated as the percentage prolongation in  $t_E$ . The median prolongation in  $t_E$  for all infants was 115% (95% confidence limits 84-162%;  $p < 0.01$  Wilcoxon matched pairs test). The range of mechanoreflex responses plotted as the percentage prolongation in  $t_E$  is shown in figure 4.3.6.i. There was considerable variation in the magnitude of the response both within (particularly infant 11) and between infants. The mean mechanoreflex response, expressed in seconds, is shown in figure 4.3.6.ii. for each infant. All infants showed a prolongation in  $t_E$  in response to the occlusion. Mean mechanoreflex responses were related to postnatal age, gestation, birthweight and crown rump length by multiple linear regression but no significant relationship was found.

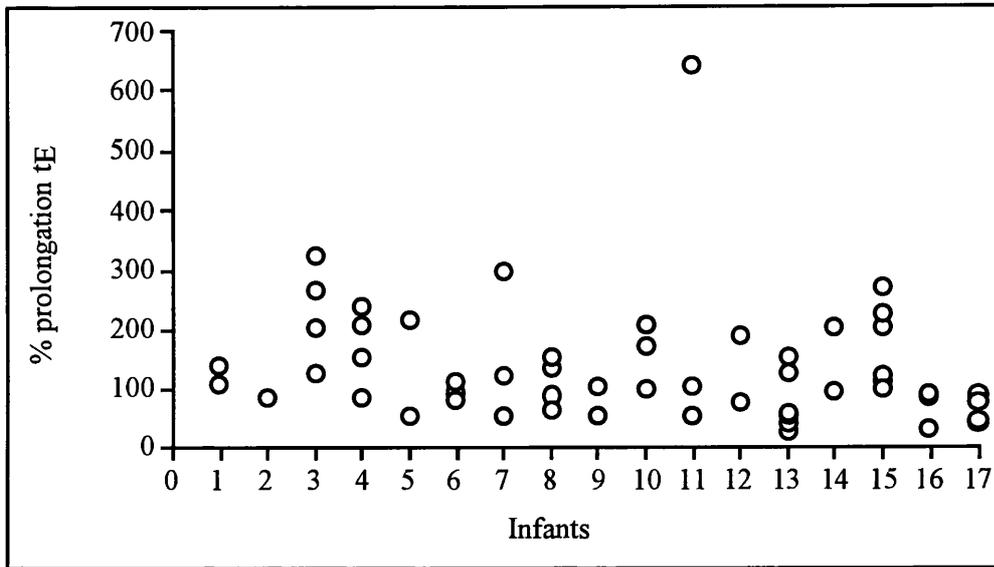


Figure 4.3.6.i Range of mechanoreflex responses (% prolongation in  $t_E$ ) for all infants in response to end-inspiratory occlusion.

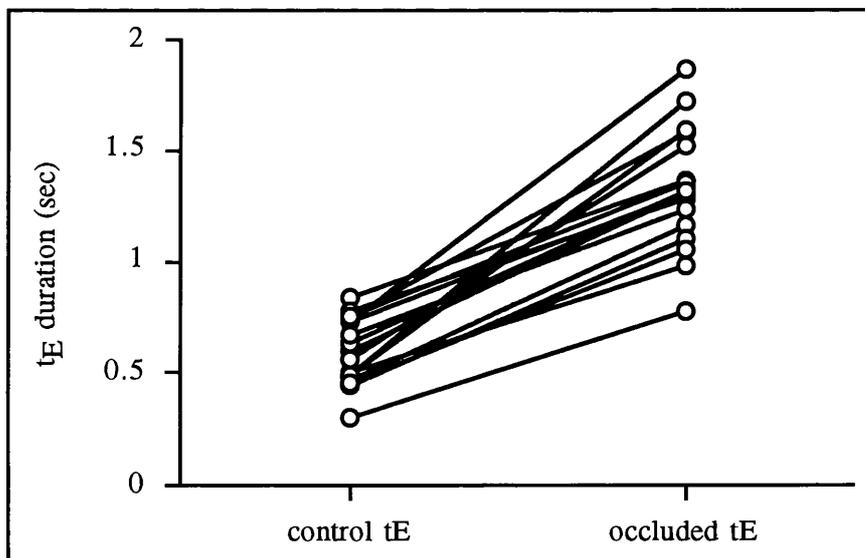


Figure 4.3.6.ii Mean control and occluded  $t_E$  (sec) for each infant in response to end-inspiratory occlusion.

### 4.3.7 Measurement of peak inspiratory flow and relationship with chemoreflex responses

It was possible to measure peak inspiratory flow during control breaths prior to end-inspiratory occlusion from the pneumotachometer volume signal. This data was used as an estimate of peak inspiratory flow for an infant during the alternate breath test as it was not possible to obtain information on flow rates when respiration was measured by RespiTrace. Mean peak inspiratory flow for each infant (aged 19-98hr) is plotted against birthweight in figure 4.3.7.i. There was no correlation between peak inspiratory flow and birthweight. These measurements of peak inspiratory flow from the pneumotachometer may underestimate flow when the infant is breathing via a nasal catheter. However using these peak inspiratory flow rates as a guide, there was no indication that the size of the infant was related to peak inspiratory flow.

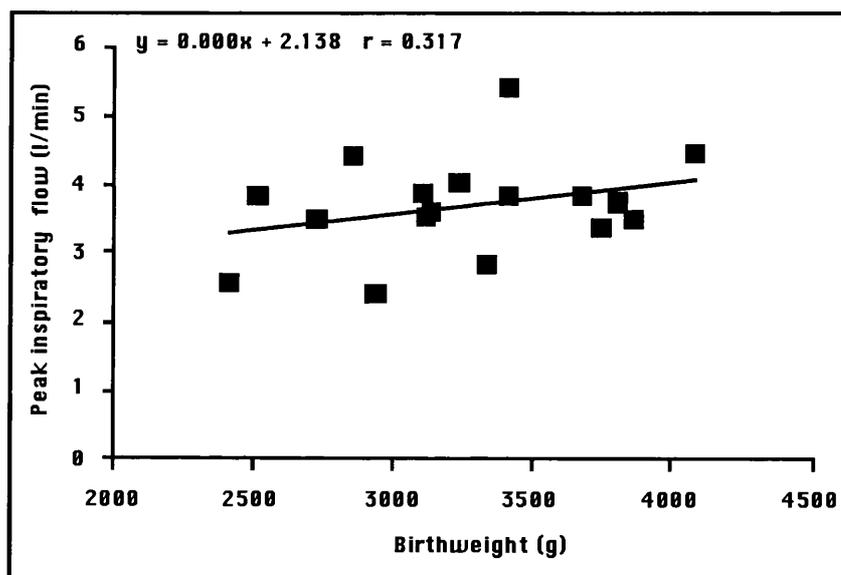


Figure 4.3.7.i Relationship between peak inspiratory flow (l/min) measured from the pneumotachometer signal and birthweight (g).

Given that there was an inherent variation in peak inspiratory flow between infants, it was necessary to investigate the relationship between the magnitude of the chemoreflex response and peak inspiratory flow. Chemoreflex responses were plotted against peak inspiratory flow rates in figures 4.3.7.ii-x but there was no consistent relationship between the two variables in each case. Thus, the smaller chemoreflex responses in some infants were not explicable in terms of an inadequate flow of the inspired gas stimulus.

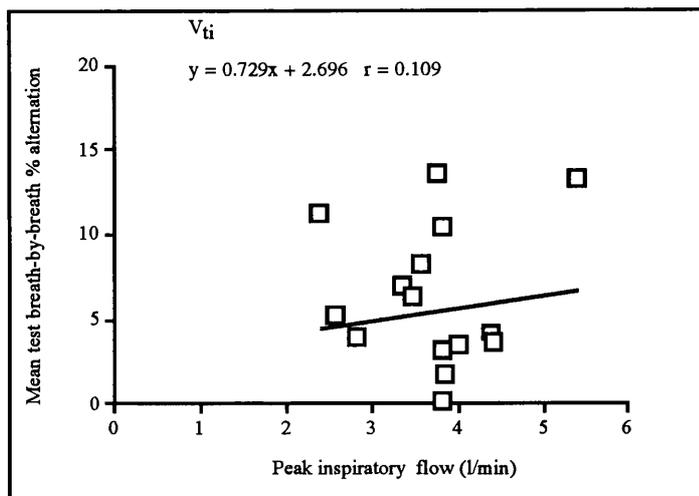


Figure 4.3.7.ii Relationship between  $V_{ti}$  chemoreflex response (mean test breath-by-breath % alternation) and peak inspiratory flow (l/min).

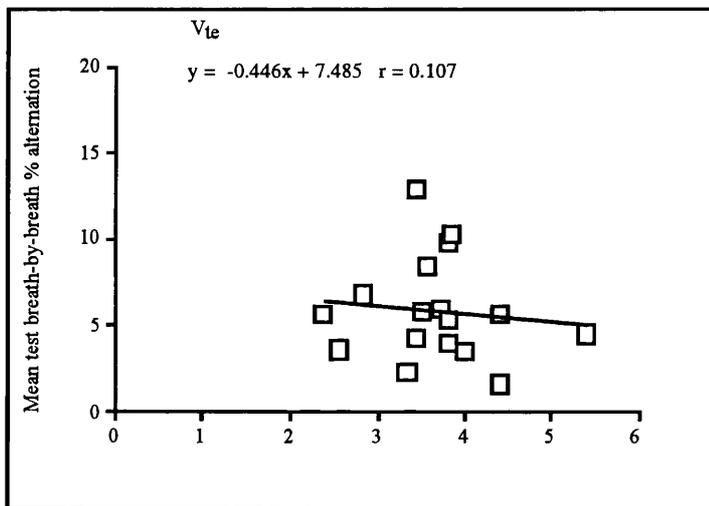


Figure 4.3.7.iii Relationship between  $V_{te}$  chemoreflex response (mean test breath-by-breath % alternation) and peak inspiratory flow (l/min).

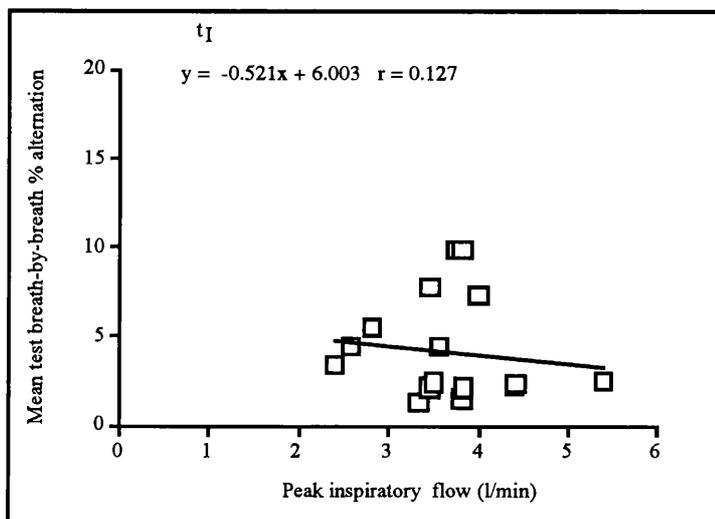


Figure 4.3.7.iv Relationship between  $t_I$  chemoreflex response (mean test breath-by-breath % alternation) and peak inspiratory flow (l/min).

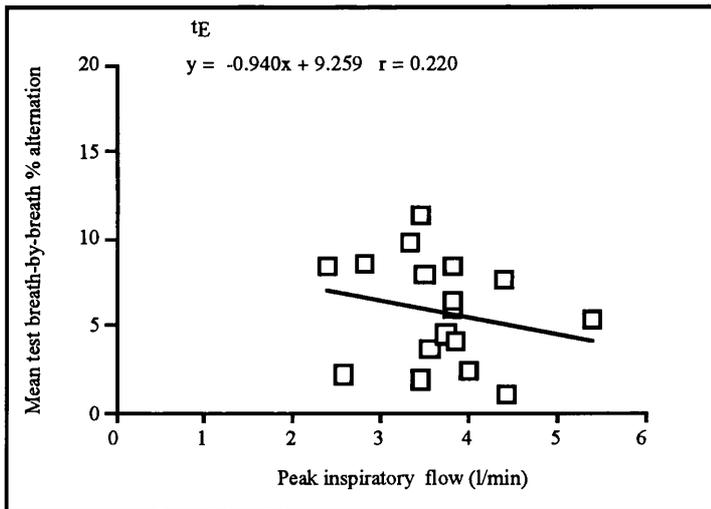


Figure 4.3.7.v Relationship between  $t_E$  chemoreflex response (mean test breath-by-breath % alternation) and peak inspiratory flow (l/min).

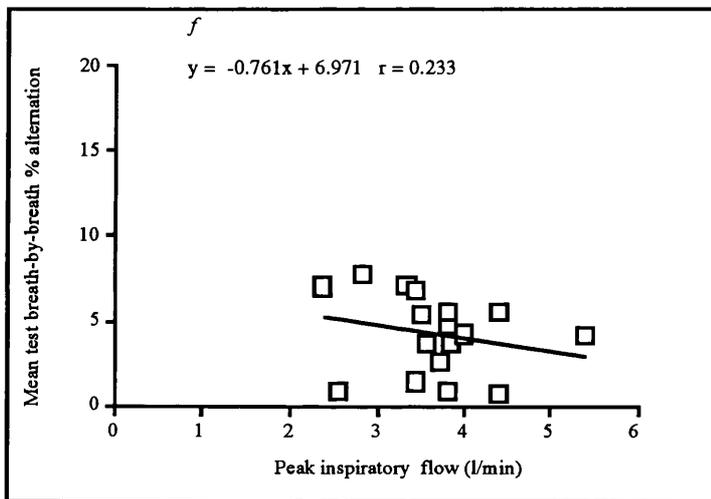


Figure 4.3.7.vi Relationship between  $f$  chemoreflex response (mean test breath-by-breath % alternation) and peak inspiratory flow (l/min).

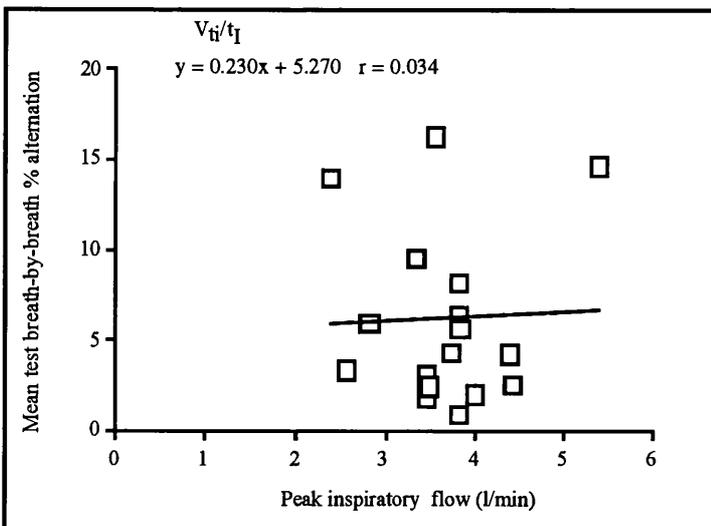


Figure 4.3.7.vii Relationship between  $V_{ti}/t_I$  chemoreflex response (mean test breath-by-breath % alternation) and peak inspiratory flow (l/min).

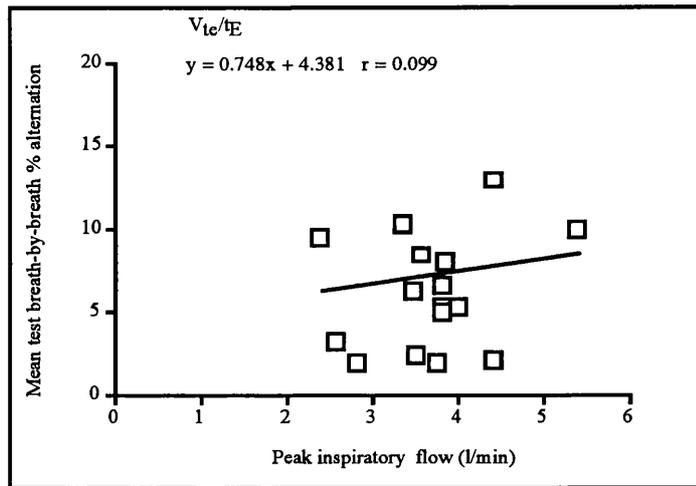


Figure 4.3.7.viii Relationship between  $V_{te}/V_E$  chemoreflex response (mean test breath-by-breath % alternation) and peak inspiratory flow (l/min).

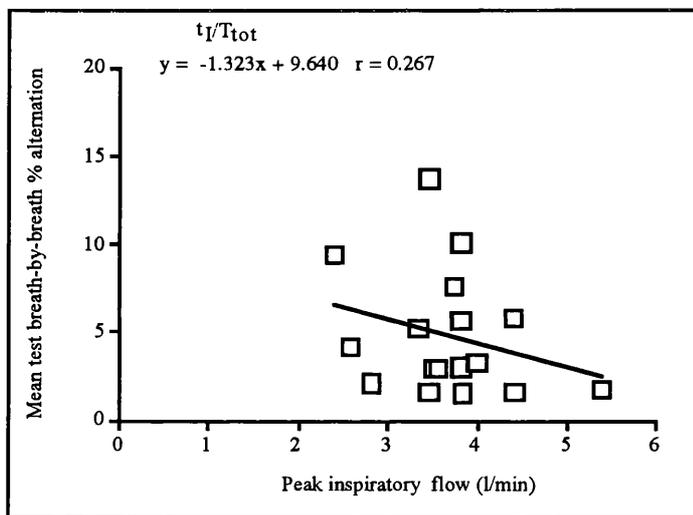


Figure 4.3.7.ix Relationship between  $t_I/T_{tot}$  chemoreflex response (mean test breath-by-breath % alternation) and peak inspiratory flow (l/min).

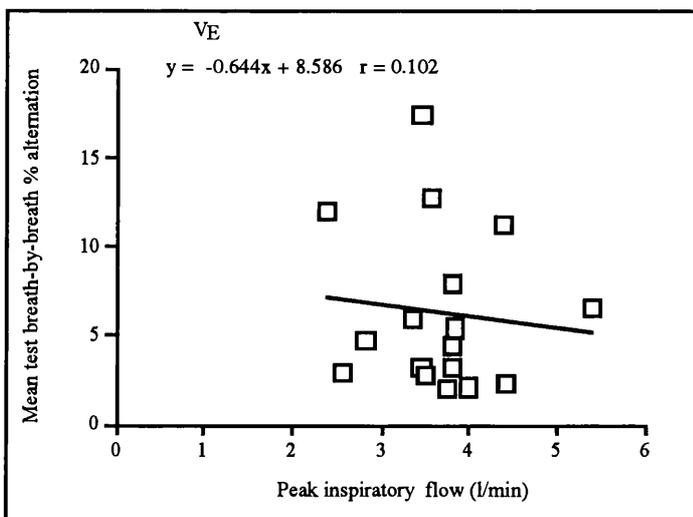


Figure 4.3.7.x Relationship between  $V_E$  chemoreflex response (mean test breath-by-breath % alternation) and peak inspiratory flow (l/min).

### 4.3.8 Relationship between chemo- and mechanoreflexes

To investigate the relationship between mechanoreflexes and the components of the chemoreflex response, the magnitude of the mechanoreflex response was plotted against the magnitude of the chemoreflex response. For each respiratory variable, the average control % breath-by-breath alternation was subtracted from the average test % breath-by-breath alternation in every infant. Each infant was then represented by a single point. Responses are shown in figures 4.3.8.i-ix. Regression analysis was used to describe the relationship and the correlation coefficients calculated were  $V_{ti}$  ( $r=-0.55$ ,  $P<0.03$ ),  $V_{te}$  ( $r=-0.05$ , NS),  $t_I$  ( $r=-0.23$ , NS),  $t_E$  ( $r=-0.49$ ,  $P<0.05$ ),  $f$  ( $r=-0.53$ ,  $P<0.03$ ),  $V_{ti}/t_I$  ( $r=-0.64$ ,  $P<0.01$ ),  $V_{te}/t_E$  ( $r=-0.26$ , NS),  $t_I/T_{tot}$  ( $r=-0.35$ , NS) and  $V_E$  ( $r=-0.51$ ,  $P<0.05$ ) when all infants were grouped together irrespective of which showed a significant alternation. In summary, infants tended to show a weaker mechanoreflex response to airway occlusion for a stronger chemoreflex response to the breath-by-breath alternations in  $F_{iO_2}$ . Infants showing a significant alternation in a respiratory variable always showed a smaller response to end-inspiratory occlusion compared to infants with non-significant alternations.

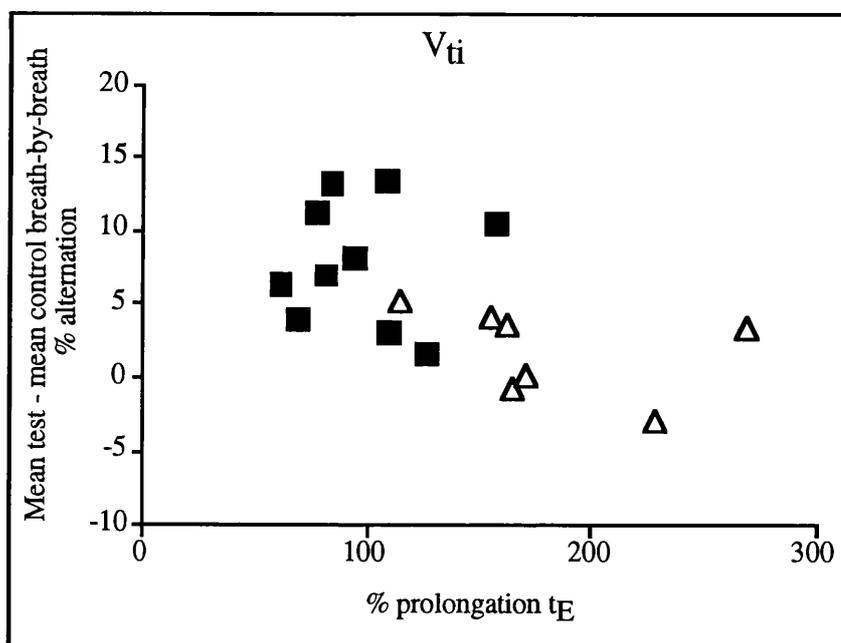


Figure 4.3.8.i Relationship between  $V_{ti}$  chemoreflex response (mean test - control breath-by-breath % alternation) and mechanoreflex response (% prolongation  $t_E$ ). Filled squares are infants showing a significant alternation, and open triangles are infants showing a non-significant alternation.

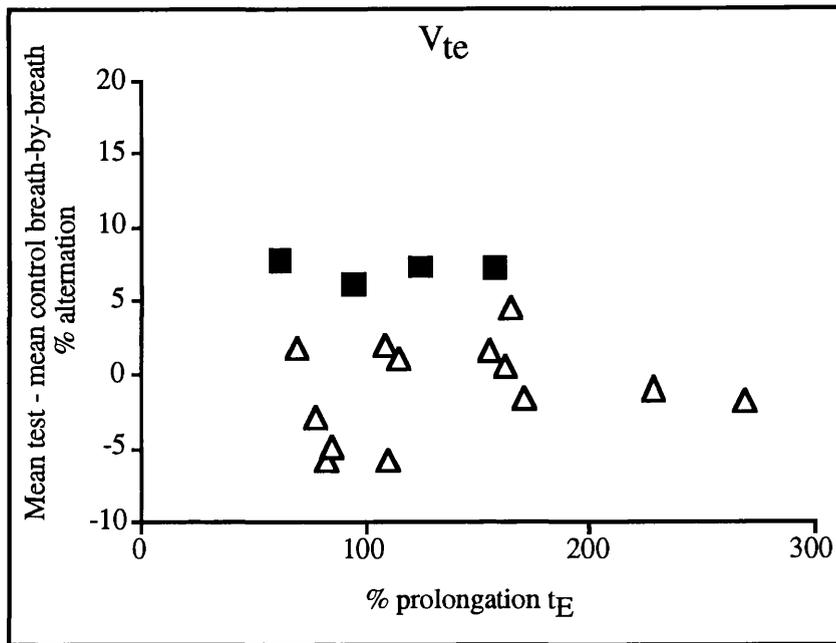


Figure 4.3.8.ii Relationship between  $V_{te}$  chemoreflex response (mean test - control breath-by-breath % alternation) and mechanoreflex response (% prolongation  $t_E$ ). Filled squares are infants showing a significant alternation, and open triangles are infants showing a non-significant alternation.

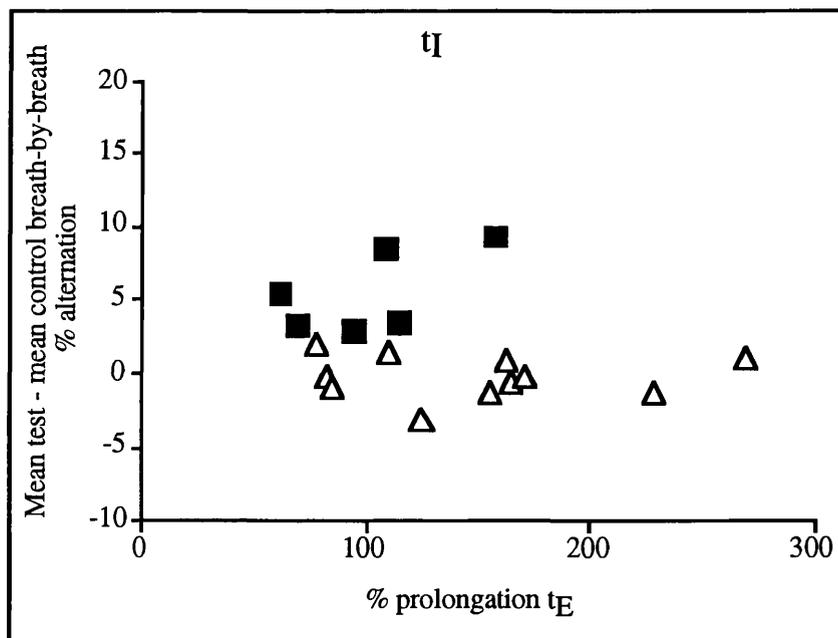


Figure 4.3.8.iii Relationship between  $t_I$  chemoreflex response (mean test - control breath-by-breath % alternation) and mechanoreflex response (% prolongation  $t_E$ ). Filled squares are infants showing a significant alternation, and open triangles are infants showing a non-significant alternation.

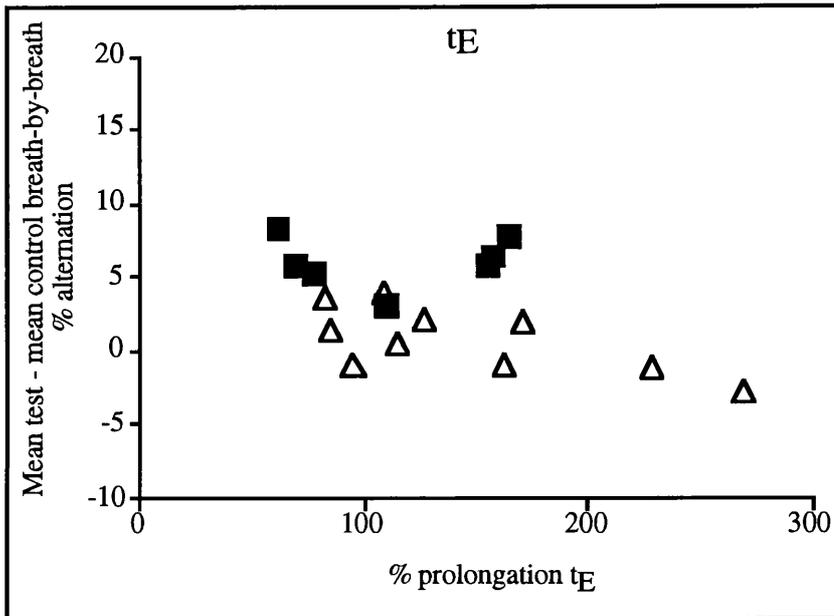


Figure 4.3.8.iv Relationship between  $t_E$  chemoreflex response (mean test - control breath-by-breath % alternation) and mechanoreflex response (% prolongation  $t_E$ ). Filled squares are infants showing a significant alternation, and open triangles are infants showing a non-significant alternation.

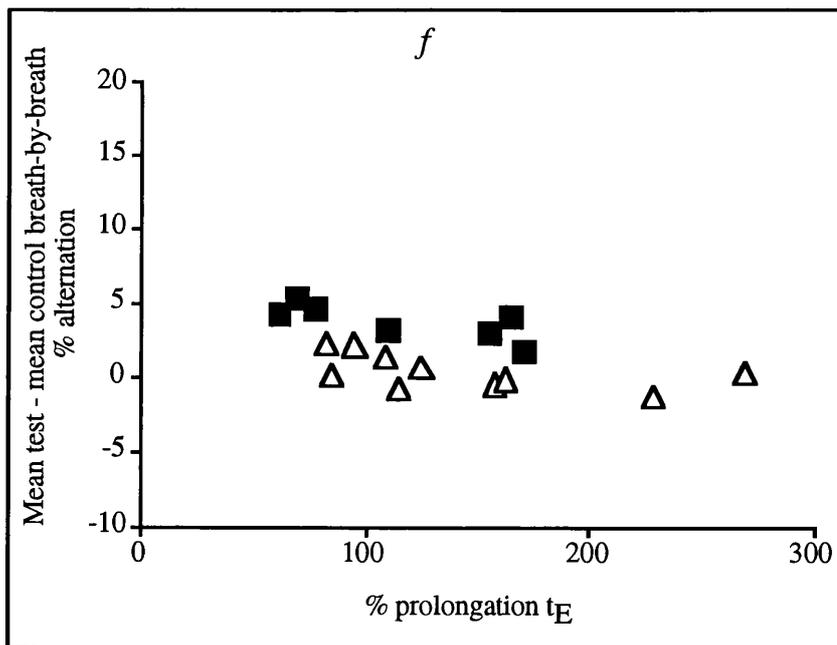


Figure 4.3.8.v Relationship between  $f$  chemoreflex response (mean test - control breath-by-breath % alternation) and mechanoreflex response (% prolongation  $t_E$ ). Filled squares are infants showing a significant alternation, and open triangles are infants showing a non-significant alternation.

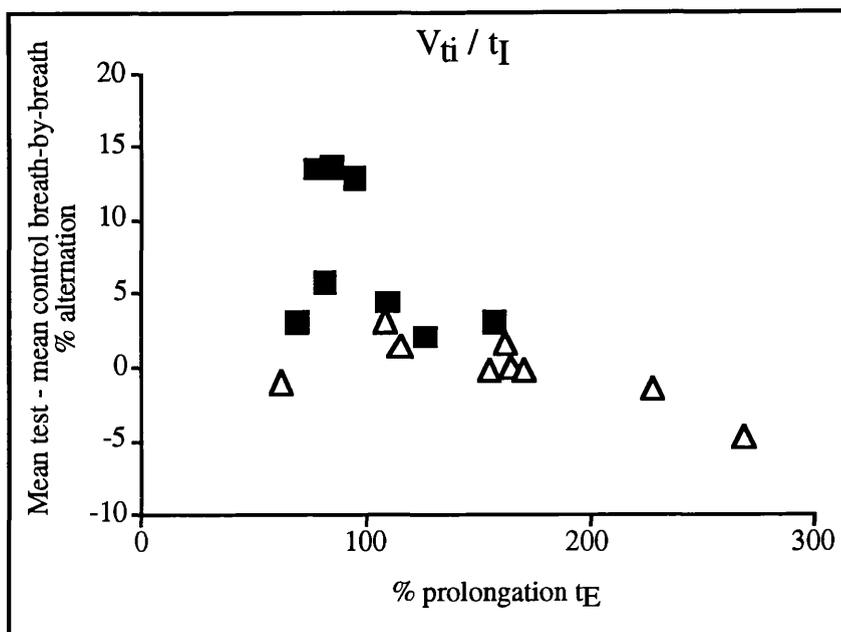


Figure 4.3.8.vi Relationship between  $V_{ti}/t_I$  chemoreflex response (mean test - control breath-by-breath % alternation) and mechanoreflex response (% prolongation  $t_E$ ). Filled squares are infants showing a significant alternation, and open triangles are infants showing a non-significant alternation.

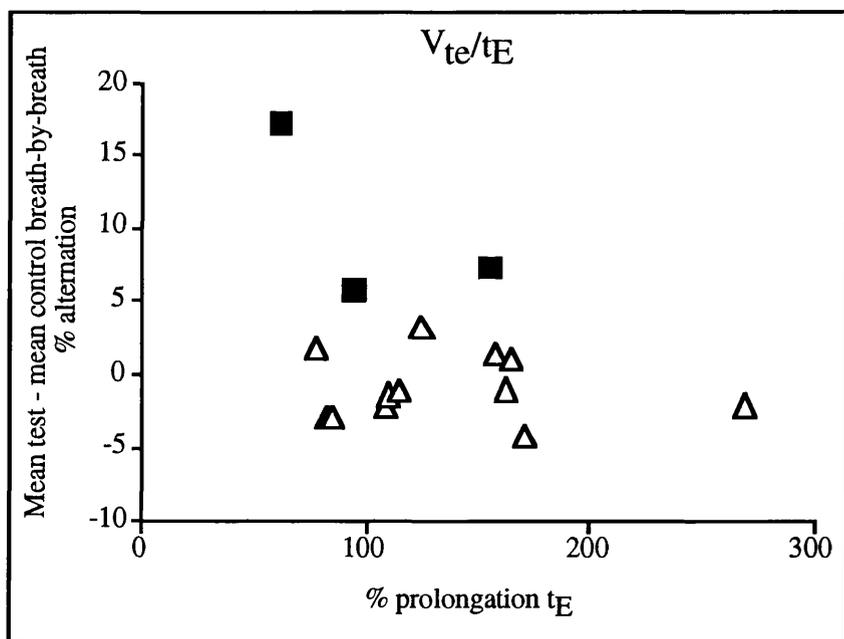


Figure 4.3.8.vii Relationship between  $V_{te}/t_E$  chemoreflex response (mean test - control breath-by-breath % alternation) and mechanoreflex response (% prolongation  $t_E$ ). Filled squares are infants showing a significant alternation, and open triangles are infants showing a non-significant alternation.

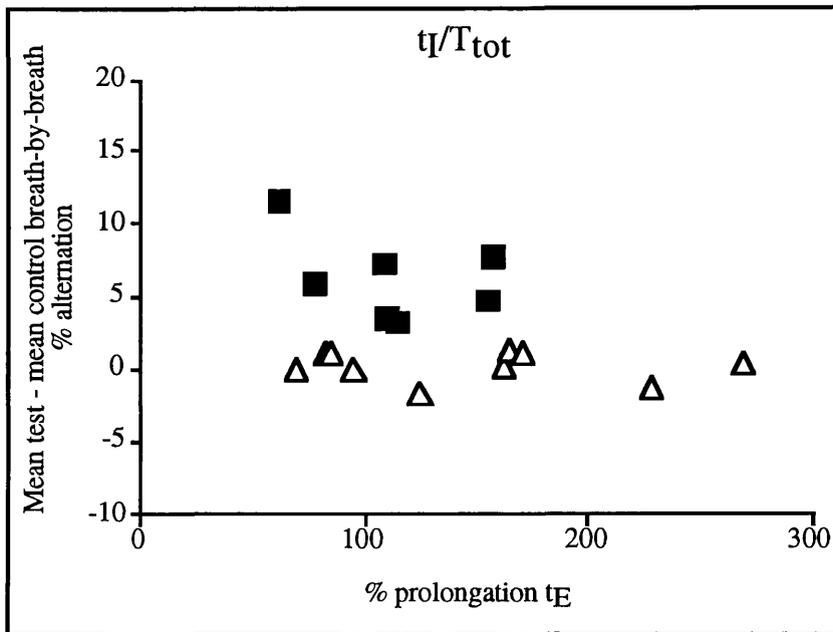


Figure 4.3.8.viii Relationship between  $t_I/T_{tot}$  chemoreflex response (mean test - control breath-by-breath % alternation) and mechanoreflex response (% prolongation  $t_E$ ). Filled squares are infants showing a significant alternation, and open triangles are infants showing a non-significant alternation.

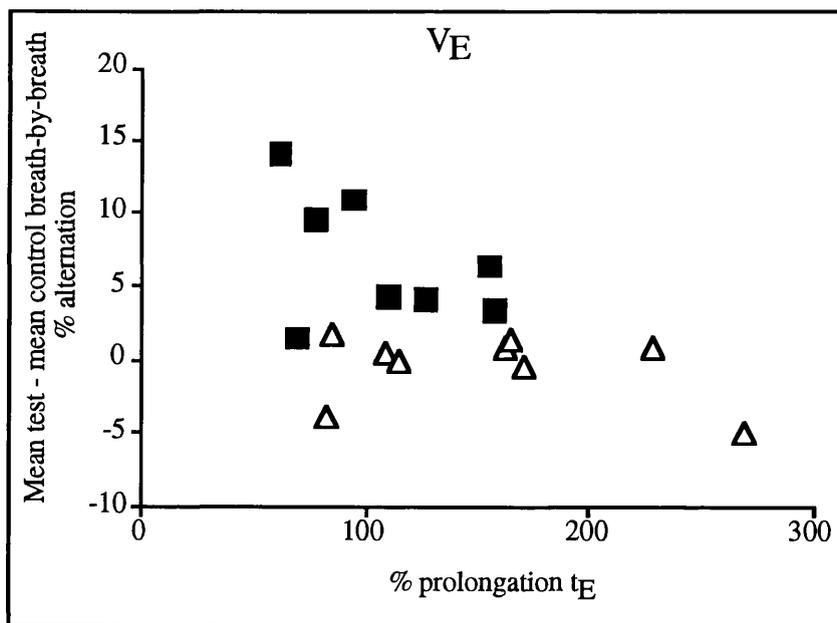


Figure 4.3.8.ix Relationship between  $V_E$  chemoreflex response (mean test - control breath-by-breath % alternation) and mechanoreflex response (% prolongation  $t_E$ ). Filled squares are infants showing a significant alternation, and open triangles are infants showing a non-significant alternation.

## 4.4 Discussion

### 4.4.1 Overview

I have used the alternate breath test to measure respiratory chemoreflexes to hypoxia, and the end-inspiratory occlusion technique to measure HBIR in newborn infants. I found a significant chemoreflex response in 14 of the 17 infants, that is at least one respiratory variable of their chemoreflex response showed a significant alternation. The three infants who did not show a significant chemoreflex response were not different in terms of gestation or postnatal age (see section 4.3.3). One infant showed small test responses (see figure 4.3.3.x), whilst the other two infants showed large breath-by-breath % alternations for control responses, hence their chemoreflex responses were classified as non-significant. All 17 infants showed a significant prolongation in  $t_E$  in response to end-inspiratory occlusion.

It had been observed previously, in studies using the alternate breath test to measure chemoreflex responses in newborn infants up to 2 months of age, that some infants showed large increases in  $t_E$  when  $V_{ti}$  increased and so the  $t_E$  component of their chemoreflex was large (see infant in figure 4.3.4.iv). In contrast, other infants showed a much smaller chemoreflex-mediated alternation in  $t_E$  when there was large variation in  $t_E$  (see infant in figure 4.3.4.v), or more frequently small increases in  $t_E$  associated with an increase in  $V_{ti}$  (see infant in figure 4.3.4.i-iii). If the large breath-by-breath alternation in  $t_E$  was a mechanoreflex response to increases in  $V_{ti}$ , then it would be expected that those infants would also show a strong response to the end-inspiratory occlusion test. My hypothesis predicted a positive correlation between the  $t_E$  component of the chemoreflex and the mechanoreflex response to end-inspiratory occlusion. However, I have found a negative interaction between chemo- and mechanoreflexes in these infants, such that mechanoreflex responses were inversely correlated ( $P < 0.05$ ) with the  $V_{ti}$ ,  $t_E$ ,  $f$ ,  $V_{ti}/t_I$ , and  $V_E$  components of the chemoreflex response (see figure 4.3.8.i-ix). These results suggest that the variation in the  $t_E$  component of the chemoreflex respiratory responses between infants is not due to a difference in mechanoreflexes. However, the data raises the interesting possibility that mechanoreflex control of breathing is more important in some infants who show poor chemoreflexes.

## **4.4.2 Respiratory chemoreflex response to the alternate breath test: possible causes for poor respiratory chemoreflexes**

### **4.4.2.a Postnatal age**

Poor respiratory chemoreflexes are observed in infants on the day of birth when measured by the alternate breath test (Williams et al, 1991) presumably because chemoreceptor hypoxia sensitivity has not reset from the fetal range at this early stage (Carroll et al, 1993; Marchal et al, 1992a; Kumar & Hanson, 1989; Blanco et al, 1984a). In Chapter 3, I measured the respiratory response to alternating breaths of Fio<sub>2</sub> 0.21 and 0.16 in infants during the first week of life and again at week 6, and found that the chemoreflex response did not increase significantly with age over this period. In the present study, infants were recruited postnatally to examine the interaction between chemo- and mechanoreflexes, such that they would show a chemoreflex response (postnatal age  $57 \pm 6$  hr, mean  $\pm$  S.E.M). In fact, once again there was no correlation between the strength of the chemoreflex response and postnatal age for these infants, as illustrated in figures 4.3.4.i-ix. That is, those infants showing the smallest chemoreflex respiratory responses were not the youngest in the group. Therefore it seems unlikely that the observed negative interaction between chemo- and mechanoreflexes can be attributed to postnatal age.

### **4.4.2.b Respiratory disease**

Reduced respiratory chemoreflexes have been implicated in infants who have suffered bronchopulmonary dysplasia (BPD) (Calder et al, 1994b; Katz-Salamon & Lagercrantz, 1994). It has been suggested that infants who have suffered BPD are also chronically hypoxaemic (Uyboco, Kwiatkowski, Cates, Kavanagh & Rigatto, 1989). Kittens reared in chronic hypoxia show reduced hypoxia chemosensitivity compared to normoxic kittens (Hanson, Kumar & Williams, 1989). Similarly, chronically hypoxic newborn rats fail to show a typical biphasic ventilatory response to hypoxia compared to normoxic rat pups, that is an increase in ventilation during phase I due to peripheral chemoreceptor stimulation (Eden & Hanson, 1987b; Elnazir, Pepper & Kumar, 1993). Poor respiratory chemoreflexes to hypoxia could be explained if infants were chronically hypoxic from birth: however the infants in this study were all healthy and had no history of respiratory distress syndrome.

#### **4.4.2.c Inadequate stimulus**

Another source of variation in the chemoreflex response could be due to a difference in stimulus intensity between infants. All infants received the same inspired flow of 2.5l/min which is in excess of minute ventilation, but if peak inspiratory flow was greater than 2.5l/min in an infant there would be some transient dilution of the inspire with air. Infants received the inspired flow via a nasal catheter, and breathing was measured by RespiTrace throughout the alternate breath test. Therefore, it was not possible to measure peak inspiratory flow in these infants during the alternate breath test. However, as a guide to values for peak inspiratory flows in individual infants, calculations were made from the volume signal of the pneumotachometer during end-inspiratory occlusions. Peak inspiratory flow was measured from the control breaths prior to end-inspiratory occlusion and plotted against body weight (figure 4.3.7.i.) and chemoreflex responses (figures 4.3.7.ii-x.). There was no correlation between peak inspiratory flow and body weight, nor was there any correlation with chemoreflex responses. Some respiratory variables showed a very weak positive relationship with peak inspiratory flow ( $V_{ti}$  and  $V_{te}/t_E$ ), whilst other respiratory variables showed a weak negative relationship with peak inspiratory flow ( $V_{te}$ ,  $t_I$ ,  $t_E$ ,  $f$ ,  $t_I/T_{tot}$  and  $V_E$ ). A strong negative relationship between chemoreflex responses and peak inspiratory flow would suggest that the flow of inspired gas was insufficient to meet peak flow in some infants, however the results do not support this.

If the resistance to breathing was increased when the infant was wearing the facemask attached to the pneumotachometer, then these measurements of peak inspiratory flow may underestimate peak flow achieved when the infant was breathing via the nasal catheter. However the small increase in resistance associated with wearing a facemask is said to affect respiratory pattern less than the stimulation of receptors in the trigeminal area (Fleming et al, 1982; Dolfin et al, 1983), so the approximation of peak inspiratory flow rates from the pneumotachometer tidal volume signal may be adequate to determine any relationship with the chemoreflex response.

It was not possible to sample end-expiratory gas concentrations during the alternations in  $F_{iO_2}$ , so I could not measure the level of hypoxia reached in an infant. To eradicate the problem of standardising stimulus intensity between infants there are two possibilities, first, to increase the flow of inspired gas, and secondly to increase the severity of the hypoxia. I found that increasing the flow of inspired gas frequently caused arousal, so it was preferable to use a lower flow and maintain a state of quiet sleep than attempt to meet peak inspiratory flow with a greater inspired flow rate. Nor

did I want to use a more severe hypoxic stimulus as it was doubtful whether approval would be given for it by the Ethical Committee at University College Hospital.

#### **4.4.3 Mechanoreflex response to end-inspiratory occlusions: possible reasons for variation in the HBIR**

It is possible that the greater responses to end-inspiratory occlusion occurred in less mature infants, as a correlation between HBIR with both gestation and postnatal age has been reported (see below).

##### **4.4.3.a Gestational age**

The literature comparing the strength of the HBIR between term and preterm infants is conflicting. Preterm infants have been shown to exhibit a stronger HBIR to end-expiratory occlusion compared to term infants (Kirkpatrick et al, 1976; Olinsky, Bryan & Bryan, 1974). Bodegard, Schweiler, Skoglund & Zetterstom (1969) used end-inspiratory occlusion in term and preterm infants and showed that the strength of the HBIR increased in preterm infants up to 37 weeks (postconceptional age), and then declined over the subsequent weeks to 43 postconceptional weeks. Both Fox, Kosch, Feldman & Stark (1988) and Thach, Frantz, Adler & Taeusch (1978) report similar strength of the HBIR in term and premature infants. In my study all infants were  $\geq$  37wks gestation and I found no relationship between the strength of the HBIR with gestation by multiple linear regression, so it seems unlikely that larger mechanoreflex responses were due to prematurity.

##### **4.4.3.b Postnatal age**

Numerous studies have investigated the postnatal decline in the strength of the HBIR (see section 4.1), however there is now reasonable evidence to suggest that the strength of the reflex is not significantly changed over the first two months of age (Rabbette et al, 1991). Infants ranged between 1-4 days old when the measurements were made and again I found no relationship with the strength of the HBIR by multiple linear regression. Hence, the small mechanoreflex responses did not occur simply in older infants.

#### **4.4.3.c Variation in mechanoreflex response within infants**

Most infants had three end-inspiratory occlusion tests which were satisfactory for analysis, but in two infants only two occlusions each were used for analysis, and for one other infant only one satisfactory occlusion was used for analysis. Clearly, there was variation in the response to occlusion within an infant as shown in figure 4.3.6.i. This variation has also been reported by Rabbette et al. (1991). Occlusion responses were averaged to minimize the variability, however without a very large number of responses for each infant it is not possible to eliminate this error.

#### **4.4.3.d Other receptors which may be stimulated by end-inspiratory occlusion: Pressure receptors in the upper airway**

Evidence for the role of mechanoreceptors in the upper airway exerting an effect on respiratory frequency, and the reflex response to occlusion, is provided by the greater response to nasal occlusion compared to tracheal occlusion in the lamb (Igras & Fewell, 1991; Webb, Hutchison & Davenport, 1994) and piglet (Barrington & Allen, 1992). A stronger HBIR in some infants could be due to the stimulation of pressure receptors in the upper airway. Pressure receptors and other receptors sensitive to flow, "respiratory drive" and temperature have been characterised in the larynx, with pressure receptors being the most abundant (Sant'Ambrogio et al, 1983; Ukabam et al, 1992). Stimulation of pressure receptors would prolong the HBIR response to occlusion, however the effect of positive pressure in the upper airway on respiratory frequency is inconsistent and weak compared to that of negative pressure (Mathew, Abu-Osba & Thach, 1982c). A positive pressure plateau is produced by end-inspiratory occlusion, thus any prolongation of  $t_E$  by stimulation of pressure receptors would be small. However, without measuring the pressure achieved during occlusion I cannot assess the contribution of this effect in individual infants.

#### **4.4.3.e Other receptors which may be stimulated by end-inspiratory occlusion: Chest wall receptors**

The mechanoreflex response to airway occlusion involves not only the pulmonary stretch receptors and mechanoreceptors in the upper airway, but could also stimulate receptors in the chest wall. Knill & Bryan (1976) observed an inhibitory effect on inspiration when the chest wall was rapidly compressed in the newborn baby, thus stimulation of afferents from the chest wall can terminate inspiration prematurely. This inhibitory reflex may be evoked if occlusion occurs during inspiration, however all occlusions in this study were performed at end-inspiration. In fact, all infants

lengthened  $t_E$  in response to occlusion (see figure 4.3.5.ii), and I saw no evidence of the inhibitory reflex. Obviously, this reflex is of greater importance when the chest wall is highly compliant in the newborn period (Gerhardt & Bancalari, 1980; Davis, Coates, Papageorgiou & Bureau, 1988; Colin, Wohl, Mead, Ratjen, Glass & Stark, 1989; Papastamelos, Panitch, England & Allen, 1995).

#### **4.4.3.f Effect of end-expiratory lung volume (EELV) on HBIR**

Due to the highly compliant chest wall in the newborn (Gerhardt & Bancalari, 1980; Davis et al, 1988; Colin et al, 1989; Papastamelos et al, 1995), the FRC is 15-20% of TLC compared to 35-40% in the adult (Avery & Cook, 1961; Agostoni & Mead, 1965). If the newborn breathed from an FRC that was close to residual volume, gas exchange would be inefficient. In fact, the newborn actively maintains EELV above passive FRC to compensate for this. Olinsky et al. (1974) suggested that the newborn's rapid respiratory rate prevents passive expiration to FRC and, in the absence of expiratory muscle activity, EELV would be higher than passive FRC. Only during periodic breathing and apnoeic periods did Olinsky et al. (1974) find that there was sufficient time to reach passive FRC. Fisher et al. (1982) and Kosch & Stark (1984) also found that the newborn at a few days of age maintained EELV above passive FRC. Kosch & Stark (1984) agree with Olinsky et al. (1974), that respiratory timing is the most important factor in determining EELV. However they dispute the conclusions of Griffiths, Nowaraj & Mortola (1983) who suggest that an elevated EELV is produced by expiratory braking mechanisms (such as the persistence of inspiratory muscle activity) because the later portion of expiration is predominantly passive. Kosch & Stark (1984) argue that expiratory braking plays a role in the maintenance of EELV during early expiration by decreasing the rate of lung deflation and prolonging expiratory time. However, once braking mechanisms terminate, as in later expiration, EELV is determined by  $t_E$  and the slope of the expiratory flow volume curve (expiratory time constant). In the context of the regulation of  $t_E$  to elevate EELV above passive FRC as a ventilatory strategy, we can see that the HBIR may be playing an important physiological role.

It is possible that those infants showing a strong HBIR in my study were breathing at an elevated (EELV). Raising EELV causes an increase in  $t_E$  in sleeping newborn infants and anaesthetised dogs (Bartoli, Bystrzycka, Guz, Jain, Noble & Trenchard, 1973; Stark & Frantz, 1979), although not in adult man (Hamilton, Horner, Winning & Guz, 1990). Stark & Frantz (1979) produced a mean increase in EELV of 17.5ml by application of continuous negative pressure (CNEG) in 15 term infants. They estimated the increase in EELV to be *ca.* 20% of FRC, and the mean duration of expiration increased from 0.62

to 0.84sec. They found no significant effect on  $V_t$  when EELV was made to increase. Bartoli et al. (1973) increased EELV by 20-100ml in anaesthetized dogs (tidal volume ranged between 120-310ml) which produced a significant lengthening of  $t_E$ . In contrast, Hamilton et al. (1990) increased EELV by up to 350ml ( $V_{ti}$  341-563ml; FRC 2.5-5.3l) by the application of expiratory loads in 5 sleeping laryngectomized men without effect on  $t_E$  or  $t_I$ . In all three instances, the increase in EELV is roughly similar to  $V_{ti}$ . This indicates that vagal afferent information from the lung exerts an influence over respiratory rhythm in the newborn baby and dog who show a HBIR, but not in man who does not during unstimulated tidal breathing.

We have no information on EELV in these infants, but an elevated EELV could explain why HBIR responses were greater in some infants. From the observations of Stark & Frantz (1979), it is possible to calculate the dilution of an inspired stimulus if EELV is increased. If we assume a FRC of 100ml and  $V_t$  of 30ml in the newborn baby, then a 15-20ml increase in EELV (that which had a significant effect on  $t_E$ ) will increase end-inspiratory lung volume by 10-15%. The dilution of an inspired stimulus will depend on the volume of air in the lung at end inspiration. The ratio of  $V_t$  to end-inspiratory lung volume is affected very little from breathing at FRC (0.23) compared to an elevated EELV of 15-20ml (0.20). I would not expect the inspired stimulus to be greatly reduced, based on these calculations. Thus, an elevated EELV may account for a greater HBIR, but it does not explain a reduced respiratory chemoreflex to hypoxia by means of a diminished stimulus.

#### **4.4.4 Assessment of the end-inspiratory occlusion technique to measure HBIR**

Breuer (1868) and Hering (1868) first reported the presence of HBIR in dogs using lung inflation technique. Cross et al. (1960) used the same technique to report the presence of the HBIR in the newborn baby. Clark & von Euler (1972) investigated the role of the HBIR in conscious humans using CO<sub>2</sub> rebreathing. The problem associated with using these techniques is that a difference in CO<sub>2</sub> sensitivity between subjects influences the interpretation of the apnoeic response. Younes, Vaillancourt & Milic-Emili (1974) found in cats that the duration of the apnoeic response to lung inflation suggested an interaction between the declining vagal inspiratory inhibitory activity and the increasing excitatory influences from rising arterial CO<sub>2</sub> and falling arterial O<sub>2</sub>. Thus, during prolonged lung inflation the apnoeic response may be related more to afferent chemoreceptor input than to stimulation of pulmonary stretch receptors.

To avoid the problem of increased chemical drive to breathe during the inhibitory response, the end-inspiratory (Bodegard et al. 1969; Marchal & Crance, 1987; Rabbette et al, 1991; Rabbette et al, 1994) and end-expiratory occlusion (Olinsky et al, 1974; Kirkpatrick et al. 1976; Fisher et al, 1982; Witte & Carlo, 1987) technique have been used to measure HBIR in newborn infants. Due to the brevity of the occlusion, there is unlikely to be much change in arterial blood gases. One disadvantage of the occlusion technique is that it may elicit the intercostal-phrenic inhibitory reflex if occlusion is performed during inspiration (see section 4.4.3.e), however this can be avoided by performing occlusions at end-inspiration/early expiration.

Elastic and resistive loads have also been used to measure HBIR in term and preterm infants (Kosch, Davenport, Wozniak & Stark, 1985; Kosch, Davenport, Wozniak & Stark, 1986; Fox et al, 1988). The interpretation of the strength of the HBIR can be difficult using these methods, and neural timing from diaphragm electromyogram (EMG) provides a better indication of the reflex than mechanical timing from the volume trace.

Rabbette et al. (1991) used both the end-inspiratory and end-expiratory occlusion to assess the variability of the technique. They found that whilst both occlusions produced a significant prolongation of  $t_I$  or  $t_E$ , occlusions at end-expiration more frequently aroused the infant from quiet sleep and pressure recordings were more likely to be disturbed by glottis closure. In addition, the end-expiratory occlusion necessitated several repeat occlusions at the same lung volume to reduce the variability in response. Hence, I chose to use end-inspiratory occlusions to measure the HBIR. As discussed in section 4.4.3.d, the effects of negative pressure in the upper airway are greater on respiratory pattern than positive pressure, thus further supporting the use of occlusions at end-inspiration in preference to end-expiration.

#### **4.4.5 Assessment of chemo- and mechanoreflex interaction**

##### **4.4.5.a Analysis of phase relationships between alternating respiratory variables**

In section 4.3.1, I attempted to describe the chemoreflex respiratory response for alternating respiratory variables  $V_{tI}$  and  $t_E$  as "in phase" and "out of phase". This method was hindered by chemoreflex responses which showed poor correlation coefficients fitted to the cumulative alternation by linear regression (see table 4.3.1.i). If the response was not well described by linear regression, then it is difficult and perhaps meaningless to use the slope of that line to determine the pattern of alternation between  $V_{tI}$  and  $t_E$ . Unfortunately due to infants showing both types of chemoreflex patterns, I

had also to classify infants as belonging to a third group, that was "mixed" (showing one each of "in phase" and "out of phase"). More than half of the infants were classified as "mixed" for the pattern of alternation between  $V_{ti}$  and  $t_E$ . Clearly if some infants demonstrated both "in phase" and "out of phase" chemoreflex response patterns, then it no longer seemed valid to correlate the strength of the mechanoreflex response with this classification of chemoreflex responses. So, although these observations had led rise to my hypothesis of an influence of the HBIR on chemoreflex respiratory responses, this method of analysis did not prove successful in addressing the interaction between chemo- and mechanoreflexes.

#### **4.4.5.b Effect of breath-by-breath alternations in $F_{iO_2}$ between 0.21 and 0.16 on $V_{ti}$ , $t_I$ and $t_E$**

In section 4.3.4 I showed the relationship between  $V_{ti}$ ,  $t_I$  and  $t_E$  in six of 17 infants whose tidal volume signals were calibrated in mls. This method for presentation of the relationship between  $V_{ti}$ ,  $t_I$  and  $t_E$  has been used previously by Clark & von Euler (1972), and Cunningham & Gardner (1972). They both described changes in tidal volume independent of changes in inspiratory time within the tidal breathing range, and associated shortening of expiratory time with increases in  $V_{ti}$ . Clark & von Euler (1972) also found that a shortening of  $t_I$  for an increase in  $V_{ti}$  could not be elicited until the subject was breathing at 1.5-2.0 x eupnoeic values, hence the conclusion that in adult man the HBIR could not be evoked within the normal tidal volume range. In this respect, the relation between  $V_{ti}$ ,  $t_I$  and  $t_E$  in infants figures 4.3.4.i-vi appear very similar to those described in the adult.

I examined the relation between  $V_{ti}$ ,  $t_I$  and  $t_E$  when the infants received the alternating hypoxic stimulus. This would provide a mild stimulus for the augmentation of  $V_{ti}$ , however it is not directly comparable to the much larger changes in  $V_{ti}$  evoked by  $CO_2$  rebreathing in the adult. I had hypothesised that small changes in  $V_{ti}$  would produce a reflex effect on respiratory timing as the newborn shows a HBIR to end-inspiratory occlusion. I found in all six infants that  $t_I$  was closely regulated for increases in tidal volume. On average, the breath-by-breath alternation in  $V_{ti}$  in response to the alternating hypoxic stimulus was 10%. This in itself may have been too small to produce a reflex effect on respiratory timing. Also, the increase in  $V_{ti}$  was not necessarily induced rapidly in early inspiration, so perhaps it would not be expected to shorten that inspiration. In fact, it was evident that in some infants (see figure 4.3.4.iii and 4.3.4.vi) changes in  $V_{ti}$  evoked prolongation of  $t_I$  such that respiratory drive ( $V_{ti}/t_I$ ) was maintained. Nor did I observe a reflex shortening of  $t_E$  for increases in  $V_{ti}$ . Once

again this may be due to the small increases in  $V_{ti}$ , or the speed at which they were induced.

Thus, I was not able to comment on the presence of the HBIR during quiet breathing using this method of analysis. Either there was no reflex control on respiratory timing for increases in tidal volume, or the changes in  $V_{ti}$  were insufficiently large to induce the reflex effect. The method of Clark & von Euler (1972) to display the relationship between volume and timing has not previously been used in the neonate. The evidence from performing end-inspiratory, or expiratory occlusions, and from the early observations employing the technique of lung inflation (Cross et al, 1960), do support the presence of a strong HBIR in the newborn. This leads me to suggest that the stimulus I used did not produce a large enough change in  $V_t$ . If I had used a more severe hypoxic stimulus to evoke larger breath-by-breath changes in  $V_{ti}$ , then I may have seen the reflex effect on  $t_I$  and  $t_E$ . If however larger volumes were necessary to produce this reflex response, then the volumes involved would begin to approach the 1.5 times eupnoeic threshold described by Clark & von Euler (1972). Thus, the newborn would not be dissimilar to the adult in that the reflex shortening of  $t_I$  was not observed in 'range 1'. Rather,  $t_I$  was independent of changes in  $V_t$  for range 1. Clearly, it is not questionable that the newborn shows a HBIR, but perhaps the terminology used to describe its presence is incorrect. To suggest that the reflex is present during quiet breathing implies that all breaths are under this reflex control. It is clear that the reflex is evoked in response to some challenge, however without using a greater stimulus to produce larger changes in  $V_t$  (perhaps by CO<sub>2</sub> re-breathing), it is not possible to say that tidal breathing is under HBIR control. Perhaps it is only in the instance when  $V_t$  deviates from a tidal range that the reflex is responsible for controlling respiratory pattern. In summary, this method of graphical presentation was not helpful to compare chemo- and mechanoreflex interaction in the individual infant.

#### **4.4.5.c Comparison of percentage prolongation in $t_E$ to occlusion with mean breath-by-breath percentage alternation to the alternating hypoxic stimulus**

My next strategy to investigate the interaction between chemo- and mechanoreflexes was to correlate the strength of the HBIR with the individual components of the chemoreflex respiratory response. As no one respiratory variable gave any better indication of the chemoreflex response than another, all nine respiratory variables were plotted separately against the percentage prolongation in  $t_E$  to occlusion in figures 4.3.8.i-ix. I found a negative interaction between the chemoreflex response to breath-by-breath alternations in  $F_{iO_2}$  and the HBIR response to end-inspiratory occlusion for  $V_{ti}$ ,  $t_E$ ,  $f$ ,  $V_{ti}/t_I$ , and  $V_E$  ( $P < 0.05$ ).

I am not aware of any other workers who have attempted to relate chemo- and mechanoreflex control in this manner in the adult or in the neonate. Hypercapnia and hypoxia have been shown to attenuate the HBIR in the adult (Bouverot et al, 1970) and increasing chemical drive is thought to determine the duration of the HBIR apnoeic response (Younes et al, 1974). In the neonate (Frantz & Milic-Emili, 1975; Matsuoka & Mortola, 1995) relative insensitivity to hypoxia prolongs the HBIR response shortly after birth, and this effect is less as postnatal resetting of hypoxia chemosensitivity occurs. However, the relative strength of chemo- and mechanoreflexes for individual infants and their interaction to control respiration has not been examined. In particular, longitudinal measurements of the development of the chemoreflex and the decline in strength of the HBIR have not been made. My results have raised the interesting suggestion that the two control mechanisms may interact in the neonatal period. A possible physiological role for this is discussed.

#### **4.4.6 Possible physiological role for chemo- and mechanoreflex interaction in the newborn period**

Both intact peripheral chemoreceptor function (Hofer, 1984; Bureau et al, 1985b; Donnelly & Haddad, 1990) and vagally mediated afferent information from the lung and upper airways (Johnson, 1986; Fedorko et al, 1988) are necessary for the newborn to maintain a regular respiratory pattern, adequate ventilation and indeed sustain life. Precisely how great the individual contributions of these control mechanisms are in the newborn is not well understood.

A possible physiological role for the HBIR has been described in relation to newborn infants maintaining an elevated EELV by the regulation of respiratory timing i.e. a slowing of expiratory time (Kosch & Stark, 1984). Breathing at an elevated EELV may reduce the risk of airway closure and atelectasis. The chest wall is highly compliant during the period when the HBIR is strong, but the postnatal increase in total respiratory system compliance appears to be unrelated to the decline in HBIR strength (Rabbette et al, 1994). It appears that the HBIR is most important in the control of lung volume to preserve FRC and possibly maintain an elevated EELV, as at rapid respiratory frequencies deflation of the lung to FRC is prevented (Olinsky et al, 1974; the importance of maintaining an FRC in relation to inflation pressure is discussed in section 1.6.1).

For the first few weeks of life, the neonatal ventilatory response to sustained hypoxia is biphasic (Cross & Warner, 1951; Cross & Oppé, 1952). That is, the initial increase in

ventilation is followed by a fall in ventilation to, or below, pre-hypoxic levels. A biphasic ventilatory response to sustained acute hypoxia has been reported in several neonatal species (Cross & Oppé, 1952; Schweiler, 1968; Woodrum et al, 1981; LaFramboise et al, 1981; Grunstein et al, 1981; Bureau et al, 1984; Blanco et al, 1984b; McCooke & Hanson, 1986; Eden & Hanson, 1987; Martin-Body & Johnston, 1988). The reduction of ventilation is believed to be due to the action of hypoxia on the brainstem (Dawes, Gardner, Johnston & Walker, 1983; Gluckman & Johnston, 1987; Martin-Body, 1988; Martin-Body & Johnston, 1988; Williams & Hanson, 1989) and is concurrent with a fall in oxygen consumption (Mortola & Rezzonico, 1988; Mortola, Rezzonico & Lanthier, 1989; Suguihara, Bancalari, Hehre, Duara & Gerhardt, 1994). With increasing postnatal age, the neonate is able to show a sustained increase in  $V_E$  to hypoxia (Bureau et al, 1984; Eden & Hanson, 1987a).

If chemo- and mechanoreflex control of breathing do interact in the newborn period so as to play a physiological role, it seems unlikely that the interaction would occur at the level of the carotid body because the carotid body does not receive efferent information from mechanoreceptors in the lung. Both chemoreceptors (Kalia & Richter, 1985; Davies, Kubin & Pack, 1987) and pulmonary stretch receptors (Donoghue, Felder, Jordan & Spyer, 1984; Donoghue, Felder, Gilbey, Jordan & Spyer, 1985) send projections to the NTS, so it seems likely that any interaction between chemo- and mechanoreflexes would occur centrally.

During the period when hypoxia causes a reduction in  $V_E$ , it is important that the newborn maintains FRC and keeps the lung inflated. The HBIR helps to maintain an elevated EELV, and in conjunction with expiratory braking, will allow for a greater volume of air in the lungs and promote gas exchange. The interaction of chemo- and mechanoreflexes may be clinically important during the period when the newborn still shows a biphasic ventilatory response to hypoxia. So, when chemoreceptor stimulation is insufficient to sustain an increase in  $V_E$ , mechanoreflexes could operate to maintain FRC. This interaction could only occur centrally, and I can only speculate that vagally-mediated volume related feedback may prevent slowing of respiratory frequency to such an extent that EELV could be reduced below FRC during phase II of the BVR.

There have been no previous reports for chemo- and mechanoreflex interaction playing a physiological role in the newborn. My observations suggest that mechanoreflexes are strong during a period when chemoreflexes are not fully mature. Alternatively, it may be that when chemoreceptor stimulation during hypoxia is insufficient to sustain an increase in  $V_E$ , mechanoreflexes may interact to slow respiratory frequency and allow maximal gaseous exchange.

#### **4.4.7 Summary**

In summary, I found that in infants who showed the largest mechanoreflex responses, chemoreflex responses were small, and this raises that possibility that the newborn maintains a powerful HBIR until resetting of chemoreceptor activity has occurred. To address this question fully, it would be necessary to perform longitudinal measurements in those infants who showed a strong HBIR and weak respiratory chemoreflex. If the HBIR became weaker at a time when the respiratory chemoreflex appeared, then it would suggest a direct link between the two reflexes which facilitate ventilatory control in the neonate. My preliminary observations suggest that mechanoreflex control of breathing may be stronger in some infants who have weak chemoreflexes, presumably until hypoxia chemosensitivity has reset from the fetal range. Further work is needed to determine the exact time course for the development of these reflexes from birth in individual infants.

## 5.0 ASSESSMENT OF CHEMOREFLEX RESPIRATORY RESPONSE TO A BREATH-BY-BREATH ALTERNATION IN $F_{iO_2}$ IN ADULT MAN

### 5.1 Introduction

The use of alternate breath techniques to measure chemoreflex responses in adult man has on the whole been restricted to producing  $CO_2$  alternations. Steady state hypoxia has been used to augment the respiratory response to breath-by-breath  $CO_2$  alternations (Ward & Cunningham, 1977a, 1977b; Ward et al, 1979; Metias et al, 1981; Cunningham et al, 1986) however the effect on respiration of  $O_2$  alternations has been virtually neglected. In newborns, assessment of the chemoreflex respiratory response to  $O_2$  alternations provides information on the postnatal resetting of hypoxia sensitivity of the carotid body. In the adult, measurement of chemoreflex respiratory responses may have been focused on  $CO_2$  as a stimulus because it gives an indication of respiratory drive.

The alternate breath test has been used in several different newborn species (see section 3.1.3.d for review), but there are no previous reports of the degree of reflex alternation induced by  $F_{iO_2}$  oscillations in the adult. Ward et al. (1986) produced oscillations in  $PET_{O_2}$  between 80 and 45 torr against a background of  $PET_{CO_2}$  10 torr. However, the purpose of this study was to relate the latency between the stimulus and the onset of the response, and to detect if the inspiratory or expiratory phase of the breath was affected. Therefore it was not possible to draw any comparisons between this study in the adult and the degree of reflex alternation I measured in infants (see Chapter 3). I have measured the chemoreflex response to single breath alternations between  $F_{iO_2}$  0.21-0.16 in infants at *ca.* 6wks of age, and my results suggest that the respiratory response was mature. However without using the method in adults, I had no indication of the mean breath-by-breath percentage alternation I would expect in respiratory variables. Thus, it was important to establish the degree of alternation I would expect to see in an infant showing a mature chemoreflex respiratory response, based on observations in the adult. Some of these results have been published in brief (Calder, Waites, Wong & Hanson, 1995b).

## 5.2 Methods

### 5.2.1 Subject Information

Subjects were required to complete a questionnaire (see Appendix 6). The results of the questionnaire for the 14 subjects that were used for analysis are shown in table 5.2.1.i.

AGE	20-29 years	n=12		
	30-39 years	n=2		
	40-49 years	n=0		
SEX	Male	n=8	Female	n=6
HEIGHT	5"3' - 6"7'			
WEIGHT	40-50kg	n=0	71-80kg	n=5
	51-60kg	n=6	81+kg	n=0
	61-70kg	n=3		
DO YOU SMOKE?	NO	n=7		
	YES	n=7		
If NO, have you smoked in the past?		n=1		
Were you a	light smoker?			
	moderate smoker?	n=1		
	heavy smoker?			
If YES, do you smoke	less than 5 per day	n=6		
	6-10 per day	n=0		
	11-15 per day	n=1		
	16-20 per day	n=0		
	21+ per day	n=0		
HOW OFTEN DO YOU EXERCISE?				
	less than once per week	n=4		
	1-2 times per week	n=3		
	3-4 times per week	n=4		
	more than 5 times per week	n=3		
GENERAL HEALTH				
How would you rate your general health?	good - average			
Do you have any specific history of cardiorespiratory disease or allergy?	hay fever	n=2		

Table 5.2.1.i Summary of subject information

### 5.2.2 Experimental conditions

The experimental set-up for the alternate breath test is described in section 2.3.2 and illustrated in figure 2.3.2.i. The experimental protocol and data analysis are described in sections 2.3.3 and 2.3.4 respectively.

### 5.3 Results

#### 5.3.1 Effect of wearing a nose mask on baseline respiratory pattern

To observe the effect of wearing a nose mask on respiration,  $V_{ti}$ ,  $t_I$ ,  $t_E$  and  $f$  were calculated in 14 subjects during control runs with no nose mask and control runs wearing a nose mask. There was a significant effect of wearing a nose mask on  $V_{ti}$ ,  $t_I$ , and  $t_E$  (Wilcoxon matched pairs; table 5.3.1.i). The effect of wearing a nose mask on raw respiratory variables for each individual is shown in figures 5.3.1.i - iv.

	% change $V_{ti}$	% change $t_I$	% change $t_E$	% change $f$
Mean (n=14)	16.76	13.54	-8.26	-0.87
S.E.M	5.44	3.90	2.55	3.16
p value	0.02	0.01	0.01	0.50

Table 5.3.1.i Percentage change from baseline in respiratory variables on wearing a nose mask. Mean  $\pm$  S.E.M

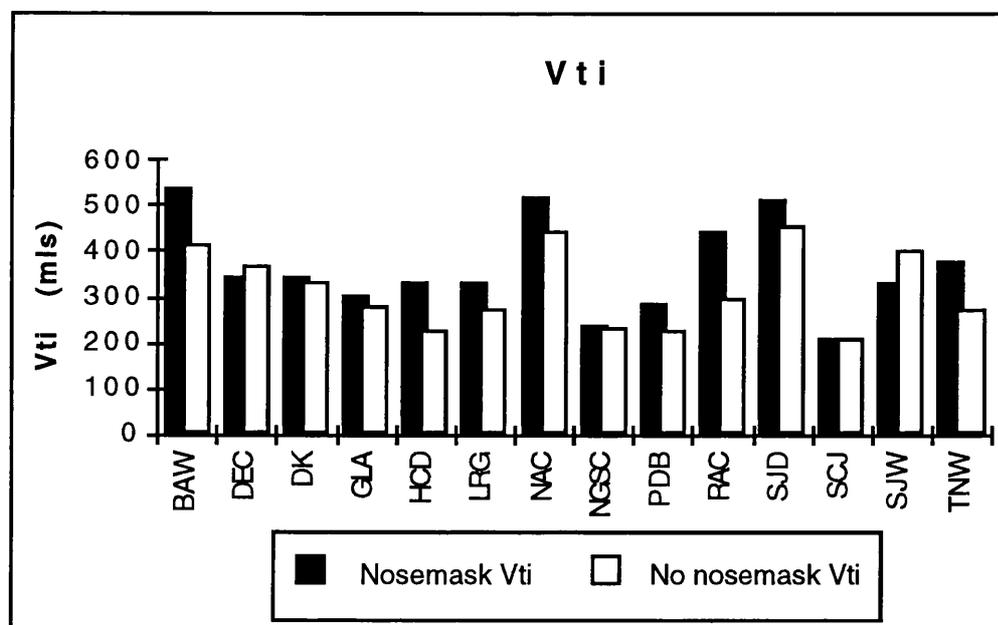


Figure 5.3.1.i The effect of wearing a nose mask on  $V_{ti}$ .

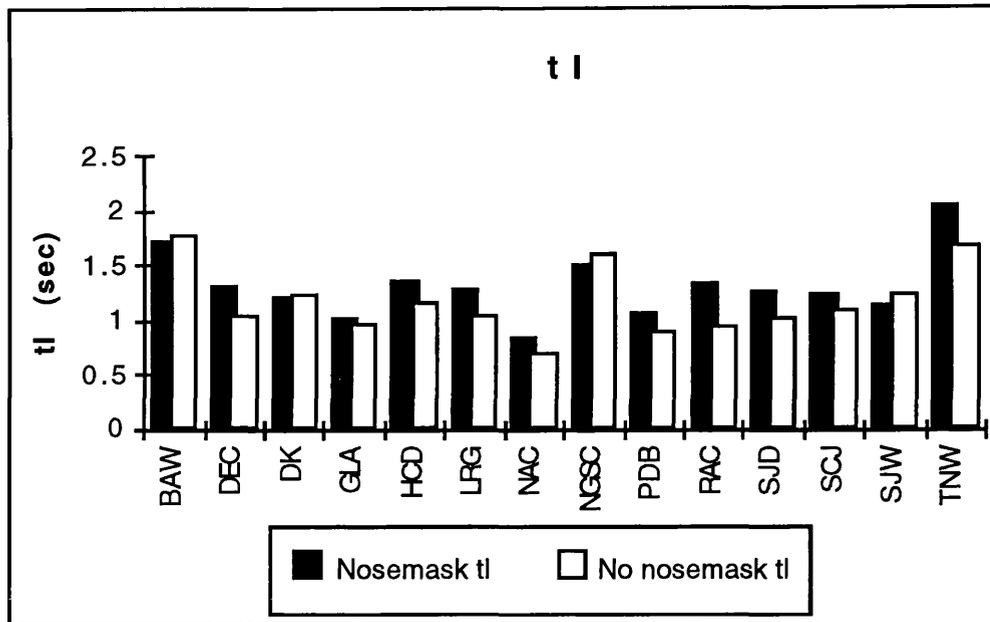


Figure 5.3.1.ii The effect of wearing a nose mask on t<sub>I</sub>.

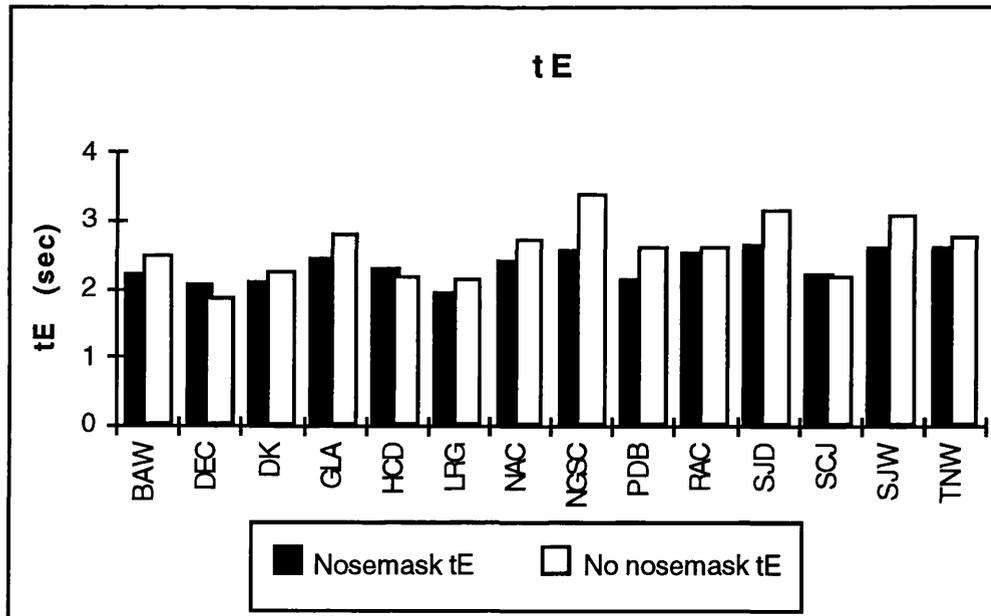


Figure 5.3.1.iii The effect of wearing a nose mask on t<sub>E</sub>.

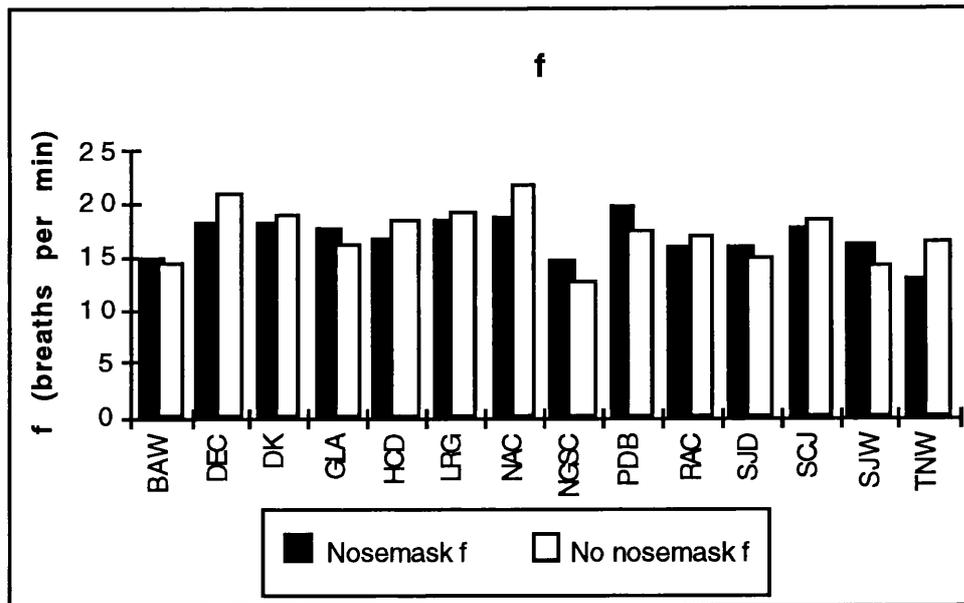


Figure 5.3.1.iv The effect of wearing a nose mask on *f*.

(n=14)		$V_{ti}$	$V_{te}$	$t_I$	$t_E$
Control no nose mask	median	4.77	7.05	4.70	5.52
	25th-75th percentile	2.31-9.34	3.34-8.20	3.49-6.85	3.87-7.30
Control with NOSEMASK	median	3.58	3.44	3.81	3.87
	25th-75th percentile	2.77-4.51	2.26-3.86	3.14-4.83	2.81-4.50
	p value	0.04	0.02	0.06	0.06

	<i>f</i>	$V_{ti}/t_I$	$V_{te}/t_E$	$t_I/T_{tot}$	$V_E$
median	4.73	4.10	6.15	4.91	4.44
25th-75th percentile	2.75-6.46	3.38-4.91	4.54-9.43	2.69-6.90	3.08-5.34
median	3.02	2.46	4.81	3.74	2.82
25th-75th percentile	2.51-4.38	2.19-3.47	3.31-6.46	3.44-4.73	2.31-3.96
p value	0.05	0.03	0.08	0.51	0.04

Table 5.3.1.ii The effect of wearing a nose mask on breath-by-breath percentage alternation for control runs (n=14).

The effect of wearing a nose mask on the mean percentage breath-by-breath % alternation for each respiratory variable was also compared in table 5.3.1.ii. There was significantly less breath-by-breath % alternation for  $V_{ti}$ ,  $V_{te}$ , *f*,  $V_{ti}/t_I$  and  $V_E$  (Wilcoxon matched pairs  $p < 0.05$ ). Thus it appeared that wearing a nose mask had a regularizing effect on respiratory pattern.

### 5.3.2 Chemoreflex respiratory responses to breath-by-breath alternations in $F_{iO_2}$ between 0.21 and 0.16

Figure 5.3.2.i shows an example of the chemoreflex respiratory response for one subject to single breath alternations in  $F_{iO_2}$  between 0.21 and 0.16. There is an obvious breath-by-breath oscillation in  $e_tO_2$  in response to the alternations in  $F_{iO_2}$ . A small breath-by-breath alternation in tidal volume can be seen between some breaths. 9 of 14 subjects showed a significant chemoreflex respiratory response. This was defined by a significantly greater breath-by-breath percentage alternation for test compared to control runs (wearing a nose mask) for at least one respiratory variable (for criteria defining a significant response see Methods section 2.3.4). The number of subjects showing significant chemoreflex respiratory responses for each respiratory variable is shown in table 5.3.2.i. The range of chemoreflex responses to test alternations and group means  $\pm$  S.E.M are shown table 5.3.2.ii. The greatest mean  $\pm$  S.E.M alternations were observed for  $V_{te}/t_E$  ( $6.76 \pm 1.11\%$ ),  $t_I$  ( $5.21 \pm 0.97\%$ ),  $V_{te}$  ( $5.15 \pm 0.71\%$ ), and  $t_I/T_{tot}$  ( $5.06 \pm 0.77\%$ ). Figure 5.3.2.ii shows an example of a subject who showed a significant chemoreflex respiratory response to the breath-by-breath alternations in  $F_{iO_2}$ , and an example of a subject who did not show a response is shown by Figure 5.3.2.iii. For the non-responder, breath-to-breath percentage alternations were similar for test and control responses. For the responder, significant alternations were seen in  $t_E$ ,  $f$ , MEF ( $V_{te}/t_E$ ) and  $V_E$ .

Chemoreflex responses were analysed by subtracting the average control response (wearing a nose mask) from the average test responses for each variable. This was necessary because control responses showed variation between subjects, and is outlined in section 2.3.4.c. A summary of the chemoreflex respiratory response for all subjects are shown in figures 5.3.2.iv-vii.

Respiratory variable	$V_{ti}$	$V_{te}$	$t_I$	$t_E$	$f$	$V_{ti}/t_I$	$V_{te}/t_E$	$t_I/T_{Tot}$	$V_E$
Number of subjects showing significant responses (n=14)	3	4	3	4	3	4	4	3	3

Table 5.3.2.i Number of subjects showing significant chemoreflex respiratory responses to breath-by-breath alternations in  $F_{iO_2}$  between 0.21 and 0.16 for each respiratory variable.

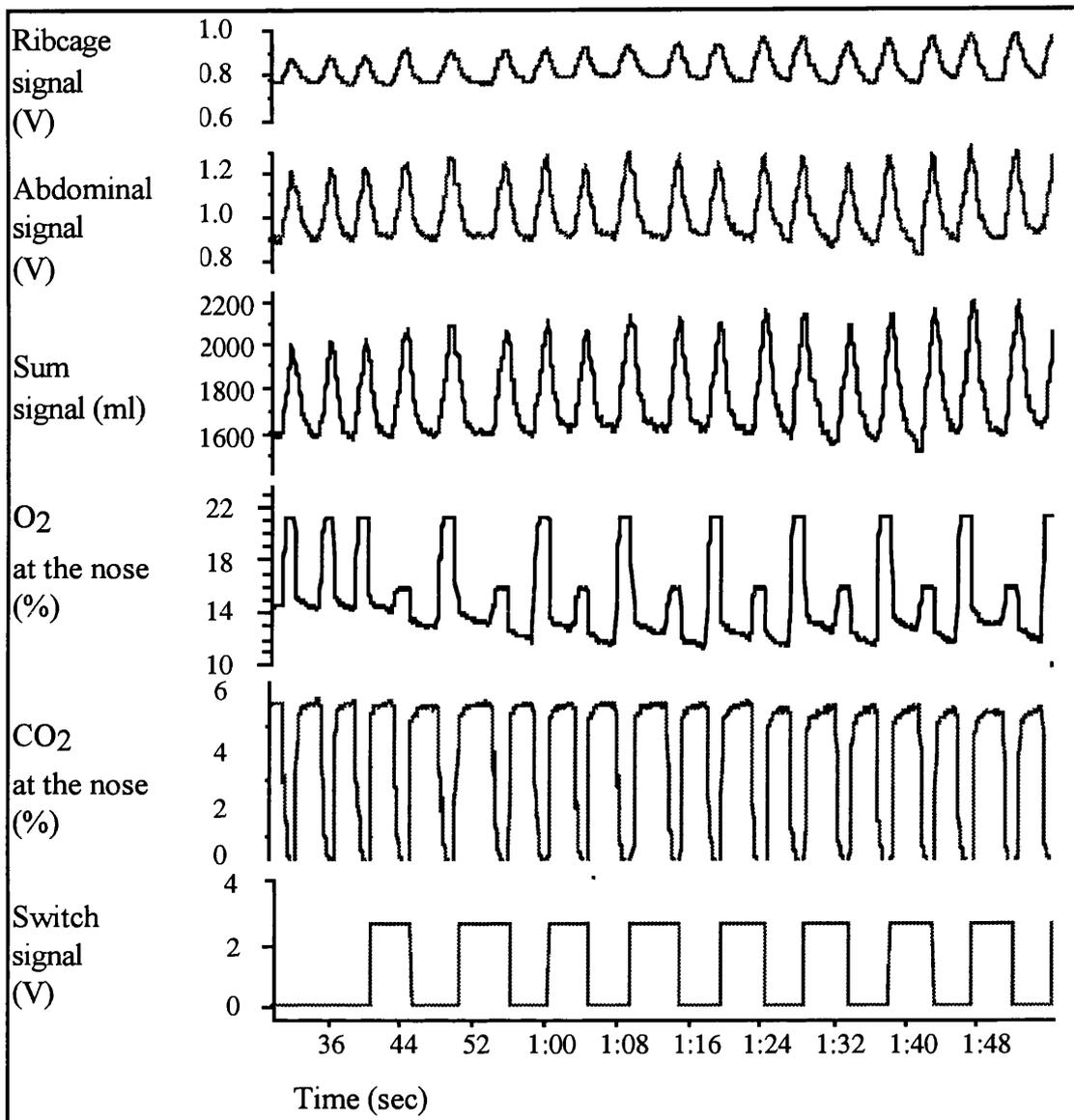


Figure 5.3.2.i The chemoreflex respiratory response to 1 breath alternations in  $F_{iO_2}$  between 0.21 and 0.16. Ribcage (V), abdominal (V) and sum (ml) Respiration signals, O<sub>2</sub> at the nose (%), CO<sub>2</sub> at the nose (%) and the switch signal (V).

	Vti	Vte	iI	tE	f	Vti/tI	Vte/tE	Tm	VE
BAW	4.89	0.5	0.8	5.56	2.72	4.1	5.06	3.59	5.32
	0.41	8.05	4.28	<i>10.29</i>	4.14	4.75	<i>18.25</i>	8.61	7.24
	3.15	3.52	2.53	4.76	3.85	5.7	1.23	1.27	4.13
DEC	1.28	7.38	4.47	3.28	0.06	5.92	3.75	4.85	3.17
	6.09	1.62	<i>10.52</i>	1.01	2.75	4.51	2.78	7.81	2.59
	0.98	4.34	0.24	9	6.17	0.73	<i>12.87</i>	5.67	6.45
DK	0.74	2.66	3.53	3.53	0.6	2.8	6.17	4.11	2.1
	0.51	1.93	1.63	0.92	0.1	1.12	2.85	1.49	0.93
	0.01	2.31	3.91	1.24	0.7	3.93	3.52	3.11	0.16
GLA	3.88	4	4.13	2.7	0.44	0.29	6.8	4.67	4.49
	2.33	11.63	7.39	<i>18.02</i>	<i>14.64</i>	9.78	29.9	8.14	<i>19.54</i>
	0.57	8.25	1.27	10.79	5.92	1.75	2.77	7.11	3.6
HCD	2.7	<i>9.52</i>	0.18	1.91	1.32	2.57	11.3	1.49	5.31
	2.79	6.84	2.09	3.04	2.71	0.69	3.78	0.58	1.58
	0.81	2.96	3.48	5.58	4.88	4.29	<i>8.52</i>	1.4	6.49
LRG	0.81	1.35	2.6	5.83	4.43	1.78	7.16	1.97	4.45
	0.63	1.38	0.83	3.52	1.73	1.46	4.88	2.49	1.98
	1.92	0.81	2.68	2.96	2.6	0.78	2.08	0.03	1.08
NAC	5.34	13.03	6.6	6.6	10.98	<i>6.76</i>	1.26	<i>23.32</i>	<i>13.59</i>
	11.4	18.45	19.84	6.27	10.05	<i>8.21</i>	<i>23.6</i>	<i>13.44</i>	4.3
	<i>31.84</i>	3.73	<i>36.37</i>	<i>13.45</i>	<i>18.44</i>	4.32	15.83	<i>18.49</i>	2.09
NGSC	0.14	7.67	4.5	4.5	9.83	5.06	4.68	2.14	9.68
	1.1	4.36	3.6	8.54	4.78	2.56	4.11	8.55	4.21
	4.56	1.03	4.9	4.08	1.01	0.38	5.01	5.99	2.09
PDB	1.92	2.16	2.61	2.02	2.04	0.67	4	0.52	0.14
	1.17	1.37	0.55	0.55	0.86	0.93	0.61	0.34	0.03
	6	5.34	6.76	8.02	7.82	0.74	13.14	0.81	5.01
RAC	0.21	1.28	2.94	1.37	0.2	2.77	0.18	2.77	0.51
	5.54	0.31	4.28	1.28	2.38	1.28	0.93	1.98	1.61
	3.97	0.47	2.89	0.66	0.54	1.09	0.21	2.38	1.87
SCJ	4.26	1.09	4.23	1.33	2.21	0.02	2.45	1.94	0.11
	5.43	4.82	3.19	3.19	<i>9.44</i>	<i>7.67</i>	2.25	4.58	4.45
	4.98	7.36	6.03	4.63	5.04	1.05	2.7	0.7	0.83
SJD	5.81	<i>11.28</i>	1.75	<i>15.71</i>	12.99	4.34	<i>26.18</i>	<i>12.32</i>	<i>11.38</i>
	6.64	1.09	11.42	4.4	0.84	4.8	3.19	<i>11.11</i>	2.99
	3.8	3.66	7.03	2.08	0.64	3.29	5.58	6.71	4.36
SJW	7.76	13.24	3.09	2.91	1.85	4.62	16.17	4.56	11.47
	6.15	7.22	4.82	0.8	2.34	1.79	6.1	2.99	3.95
	<i>10.45</i>	<i>17.17</i>	3.08	7.37	3.5	<i>13.78</i>	9.95	6.56	10.04
TNW	5.99	5.37	2.78	6.34	4.37	8.68	1.08	1.84	4.93
	<i>17.59</i>	0.16	<i>16.49</i>	0.2	7.43	1.03	0.07	9.26	2.16
	<i>11.21</i>	5.63	2.69	2.69	1.58	0.21	0.95	0.63	0.76
Mean ±	4.71	5.15	5.21	4.83	4.31	3.40	6.76	5.06	4.36
S.E.M.	± 0.87	± 0.71	± 0.97	± 0.64	± 0.66	± 0.47	± 1.11	± 0.77	± 0.63

Table 5.3.2.ii Chemoreflex responses (mean breath-by-breath % alternation) to test alternations between Fio<sub>2</sub> 0.21-0.16 for all subjects. Italics denote significant alternations. Grouped means ± S.E.M. for all variables.

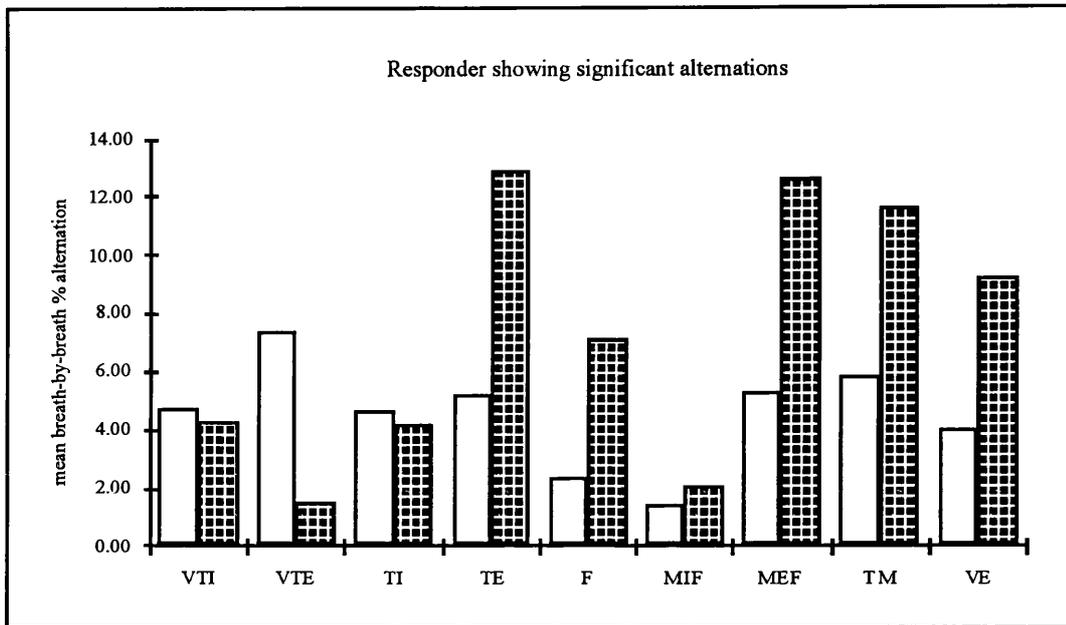


Figure 5.3.2.ii Subject showing a significant chemoreflex response with significant alternations in  $t_{E,f}$ , MEF ( $V_{tE}/t_E$ ) and  $V_E$ . Open bars are control responses, hatched bars are test responses (alternations between  $F_{iO_2}$  0.21 and 0.16).

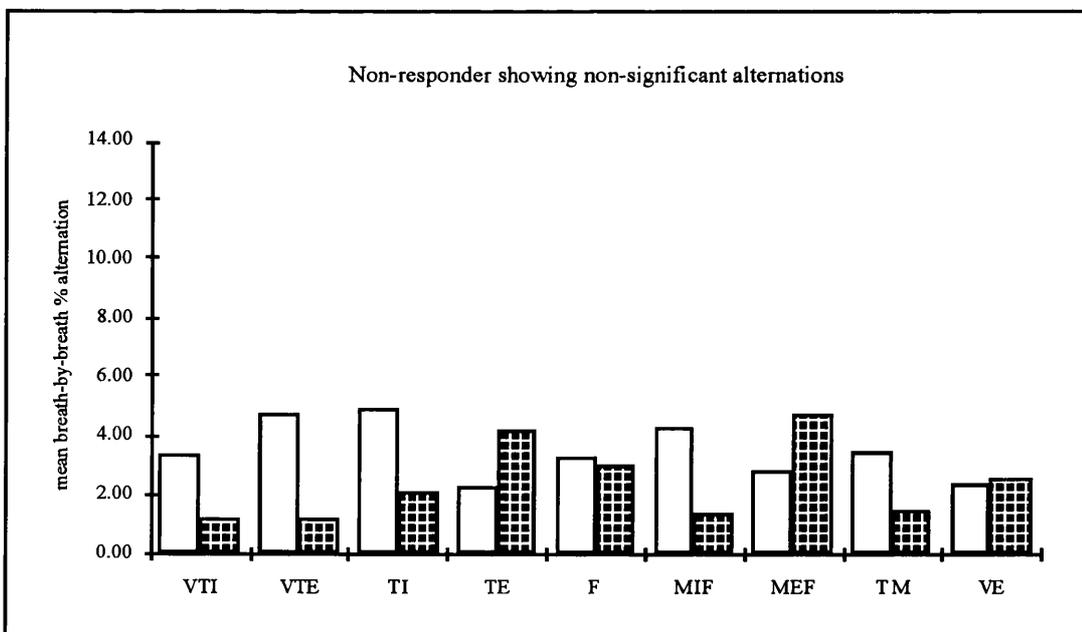


Figure 5.3.2.iii Subject showing no significant chemoreflex response. Open bars are control responses, hatched bars are test responses (alternations between  $F_{iO_2}$  0.21 and 0.16).

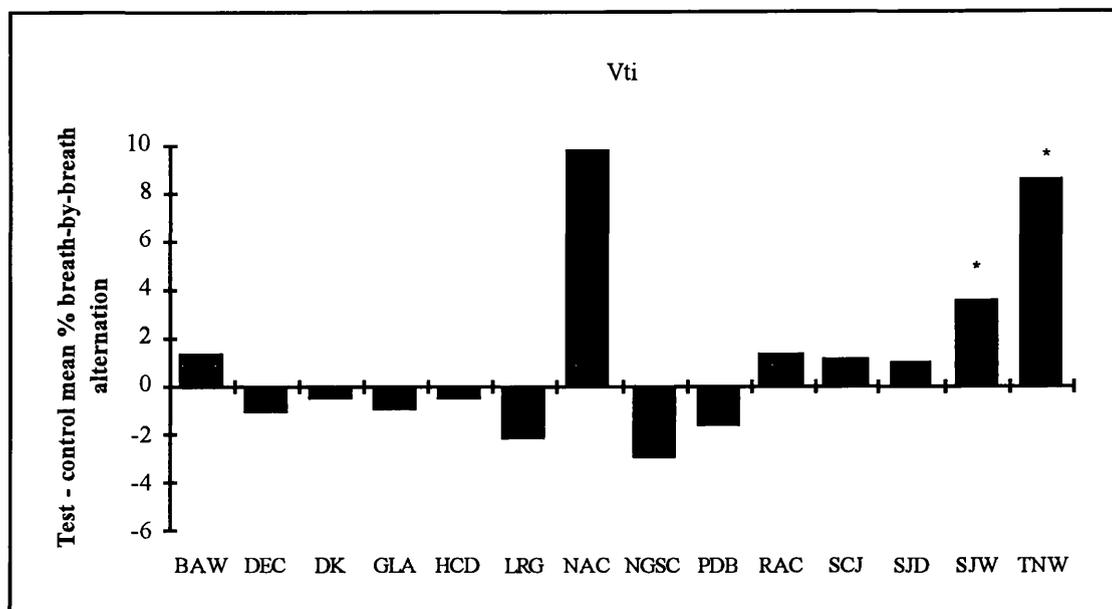


Figure 5.3.2.iv  $V_{ti}$  chemoreflex response to alternations in  $F_{iO_2}$  between 0.21 and 0.16. Average test - control mean % breath-by-breath alternation. Asterisks denote subjects that showed significant alternations in that respiratory variable.

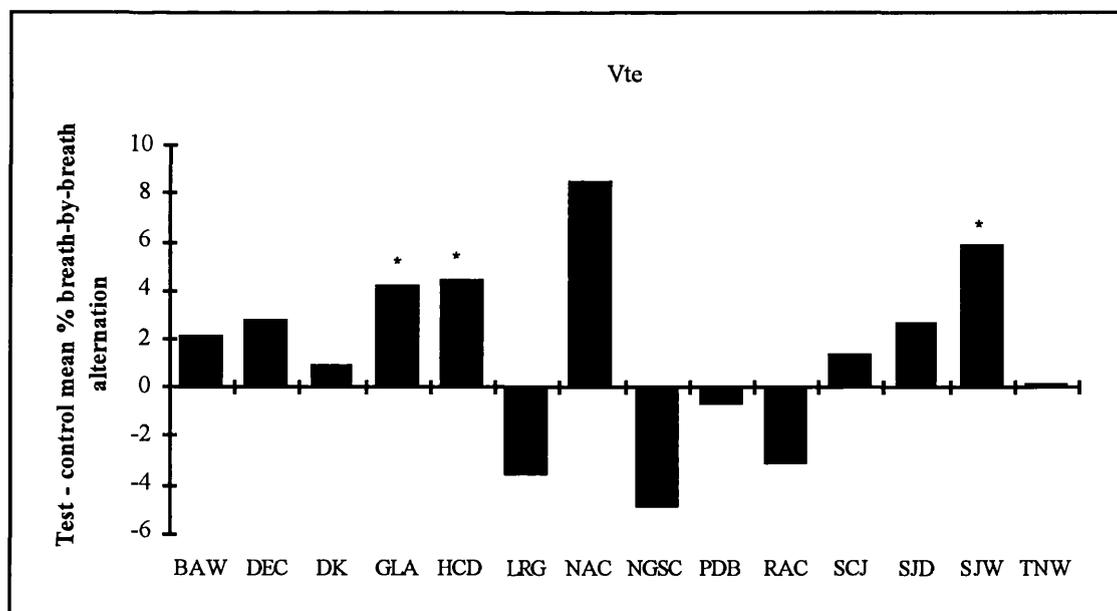


Figure 5.3.2.v  $V_{te}$  chemoreflex response to alternations in  $F_{iO_2}$  between 0.21 and 0.16. Average test - control mean % breath-by-breath alternation. Asterisks denote subjects that showed significant alternations in that respiratory variable.

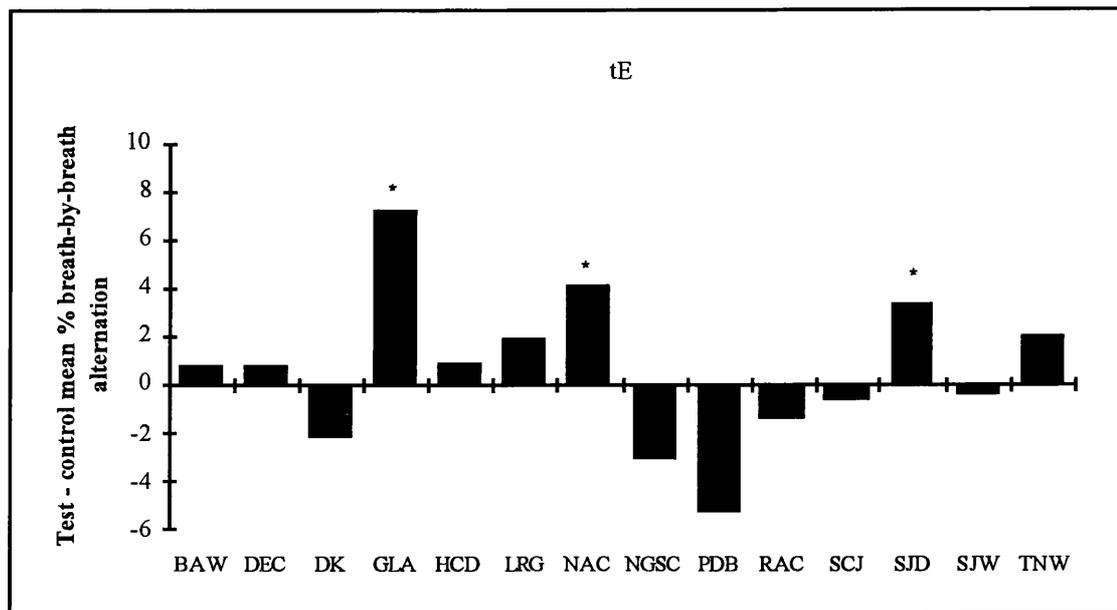


Figure 5.3.2.vi t<sub>E</sub> chemoreflex response to alternations in Fio<sub>2</sub> between 0.21 and 0.16. Average test - control mean % breath-by-breath alternation. Asterisks denote subjects that showed significant alternations in that respiratory variable.

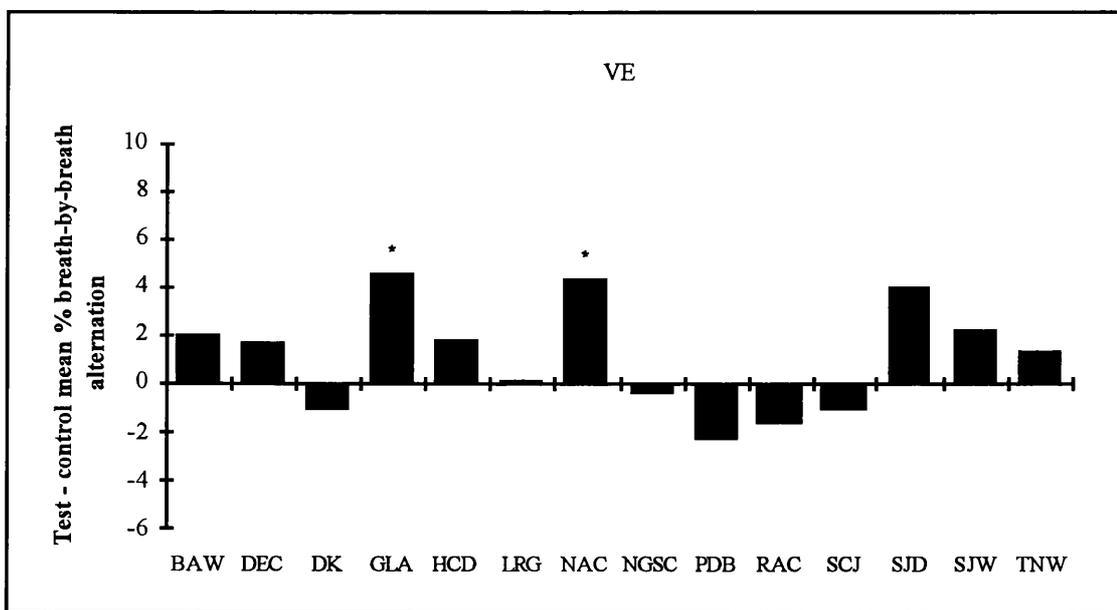


Figure 5.3.2.vii V<sub>E</sub> chemoreflex response to alternations in Fio<sub>2</sub> between 0.21 and 0.16. Average test - control mean % breath-by-breath alternation. Asterisks denote subjects that showed significant alternations in that respiratory variable.

### 5.3.3 Chemoreflex respiratory responses to breath-by-breath alternations in $F_{iO_2}$ between 0.26 and 0.16

The chemoreflex response to breath-by-breath alternations in  $F_{iO_2}$  between 0.26 and 0.16 was assessed in 12 of the 14 subjects. Only 4 of 12 subjects showed a significant chemoreflex response (a significant alternation in at least one respiratory variable) to alternations in  $F_{iO_2}$  between 0.26 and 0.16, compared to 9 of 14 subjects who showed a significant chemoreflex response to alternations in  $F_{iO_2}$  between 0.21 and 0.16. The group mean  $\pm$  S.E.M. test alternations were  $3.89 \pm 0.61\%$  for  $V_{ti}$ ,  $4.00 \pm 0.69\%$  for  $V_{te}$ ,  $3.94 \pm 0.55\%$  for  $t_I$ ,  $3.48 \pm 0.56\%$  for  $t_E$ ,  $2.51 \pm 0.39\%$  for  $f$ ,  $3.07 \pm 0.46\%$  for  $V_{ti}/t_I$ ,  $5.37 \pm 0.9\%$  for  $V_{te}/t_E$ ,  $3.65 \pm 0.62\%$  for  $t_I/T_{Tot}$  and  $3.25 \pm 0.45\%$  for  $V_E$ . The number of subjects who showed a significant chemoreflex response for each variable is shown in table 5.3.3.i. A summary of the responders and non-responders for the 12 subjects measured in both test conditions is given in table 5.3.3.ii. Chemoreflex responses to the alternations in  $F_{iO_2}$  0.26-0.16 are shown in figure 5.3.3.i-iv. There was a general trend for chemoreflex respiratory responses to be less for the  $F_{iO_2}$  0.26-0.16 stimulus. Thus both the number of respiratory variables showing significant alternations and the size of the breath-by-breath alternation is less for  $F_{iO_2}$  alternations between 0.26 and 0.16, compared to alternations between 0.21 and 0.16.

Respiratory variable	$V_{ti}$	$V_{te}$	$t_I$	$t_E$	$f$	$V_{ti}/t_I$	$V_{te}/t_E$	$t_I/T_{Tot}$	$V_E$
Number of subjects showing significant responses (n=12)	3	2	1	1	0	2	1	0	0

Table 5.3.3.i Number of subjects showing significant chemoreflex respiratory responses to breath-by-breath alternations in  $F_{iO_2}$  between 0.26 and 0.16 for each respiratory variable.

		Test $F_{iO_2}$ 0.26-0.16		
		Responders	Non-responders	Total
Test $F_{iO_2}$ 0.21-0.16	Responders	3	4	7
	Non-responders	1	4	5
		4	8	12

Table 5.3.3.ii Summary of responders and non-responders to both test protocols.

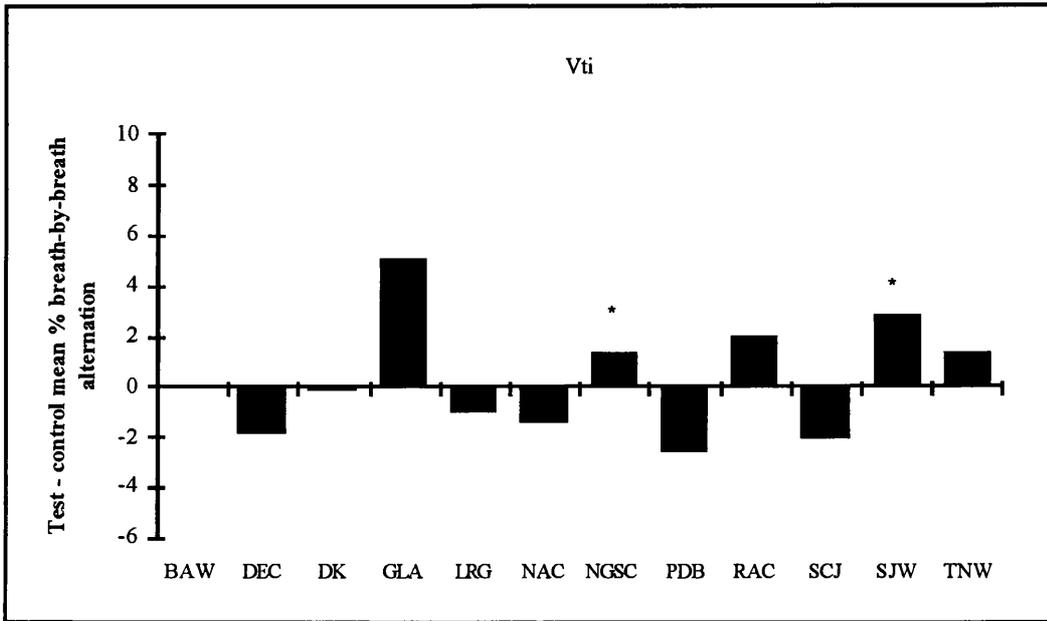


Figure 5.3.3.i V<sub>ti</sub> chemoreflex response to alternations in F<sub>io2</sub> between 0.26 and 0.16. Average test - control mean % breath-by-breath alternation. Asterisks denote subjects that showed significant alternations in that respiratory variable.

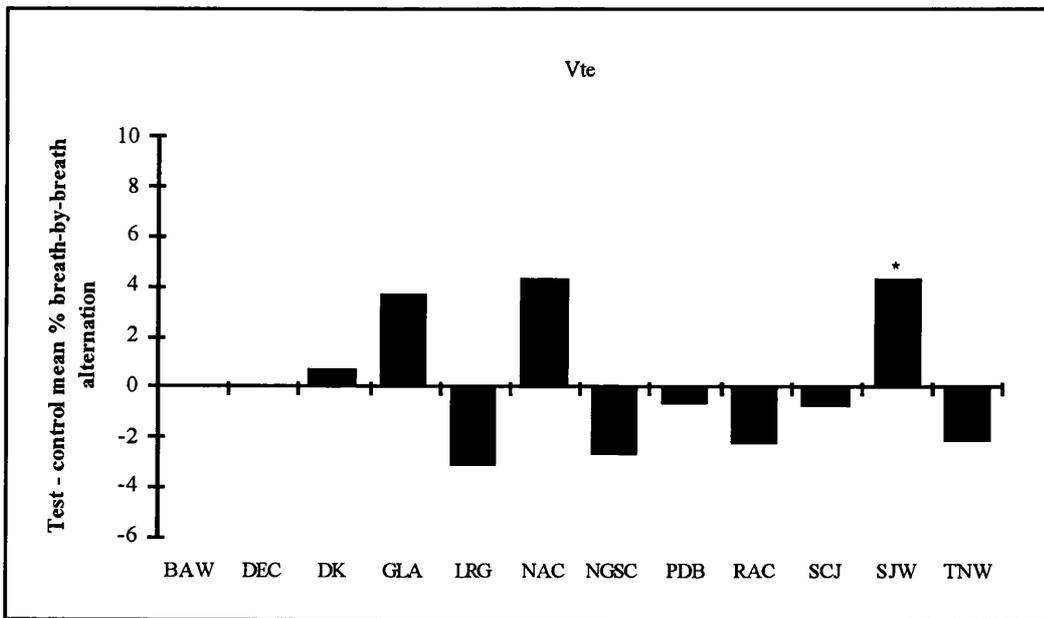


Figure 5.3.3.ii V<sub>te</sub> chemoreflex response to alternations in F<sub>io2</sub> between 0.26 and 0.16. Average test - control mean % breath-by-breath alternation. Asterisks denote subjects that showed significant alternations in that respiratory variable.

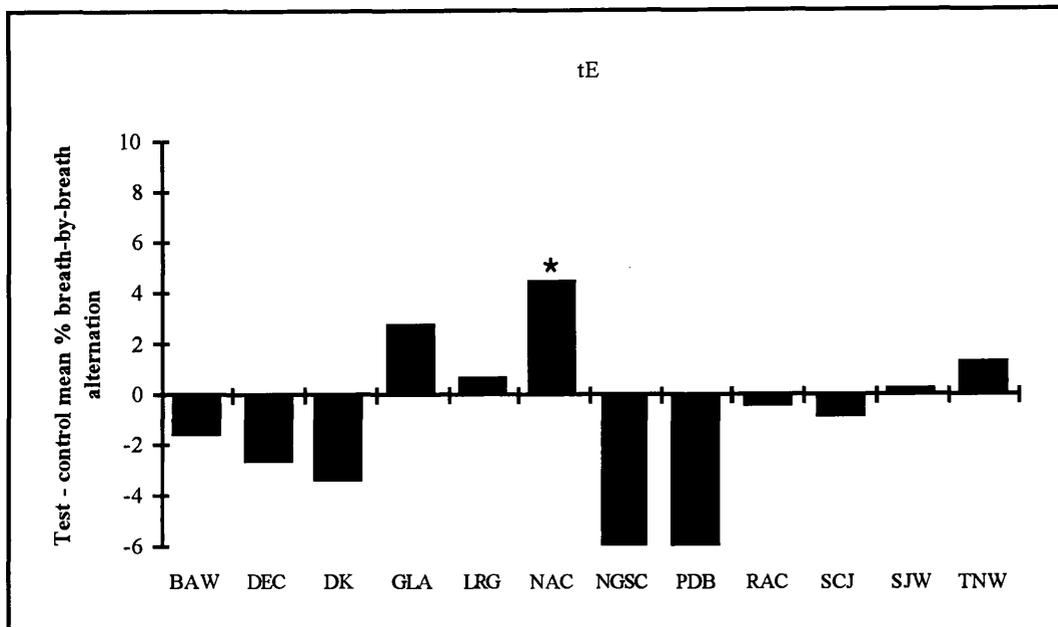


Figure 5.3.3.iii tE chemoreflex response to alternations in Fio<sub>2</sub> between 0.26 and 0.16. Average test - control mean % breath-by-breath alternation. Asterisks denote subjects that showed significant alternations in that respiratory variable.

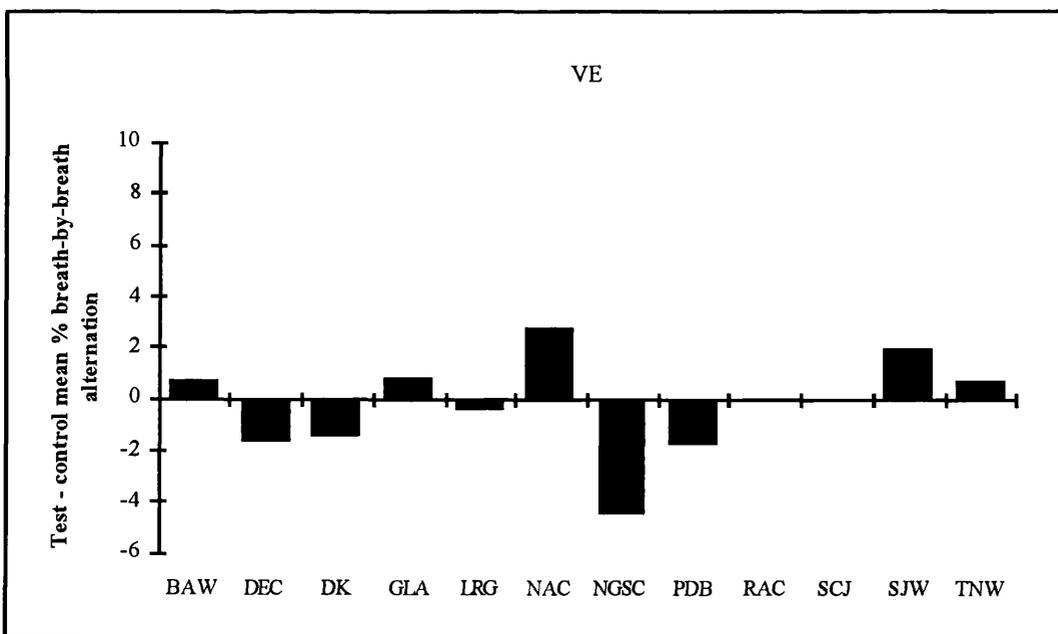


Figure 5.3.3.iv VE chemoreflex response to alternations in Fio<sub>2</sub> between 0.26 and 0.16. Average test - control mean % breath-by-breath alternation. No subjects showed significant alternations in this respiratory variable.

### **5.3.4 Within subject variation**

To investigate the variability of the chemoreflex response for a given subject, 3 subjects were tested repeatedly on 3 separate days. The chemoreflex responses to alternations in  $F_{iO_2}$  0.21-0.16 were compared to control (wearing a nose mask) responses for the group and were tested for significance by the criteria outlined in section 2.3.4. Control responses, no nose mask and wearing a nose mask, and test responses are shown in figure 5.3.4.i-iii for each subject on each experimental day. In general, control runs without a nose mask were highly variable in all three subjects. In contrast, control runs wearing a nose mask were similar on all three occasions for subjects BAW and NAC, but more variable for SJW. Test runs alternating between  $F_{iO_2}$  0.21-0.16 were highly variable in all subjects with respect to magnitude. SJW showed a tendency for significant alternations in  $V_{ti}$  ( $n=3$ ) and  $V_{te}$  ( $n=2$ ) on all occasions. There was less of a tendency for NAC and BAW to show chemoreflex responses in the same respiratory variables, however test responses for NAC suggest that significant alternations tended to occur in respiratory variables MEF ( $n=2$ ),  $T_m$  ( $n=2$ ) and  $V_E$  ( $n=2$ ). Test runs were compared to control runs measured on the same day for statistical comparison.

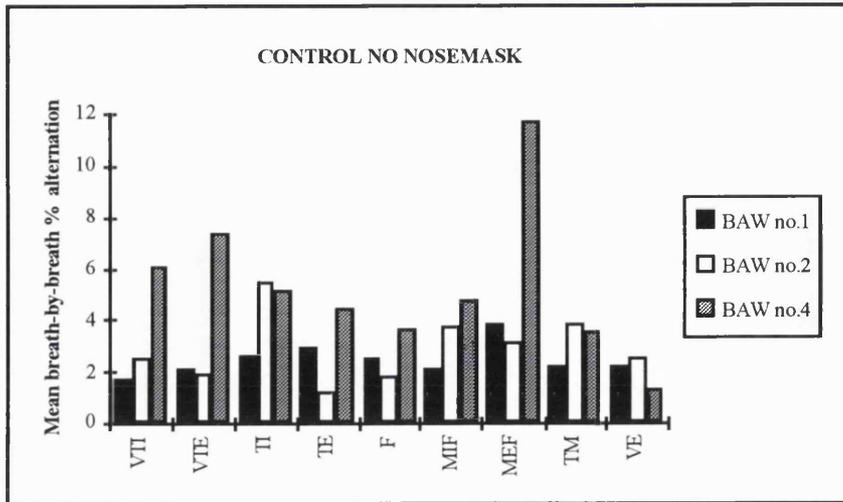


Figure 5.3.4.i.a Subject BAW control no nose mask on three separate days.

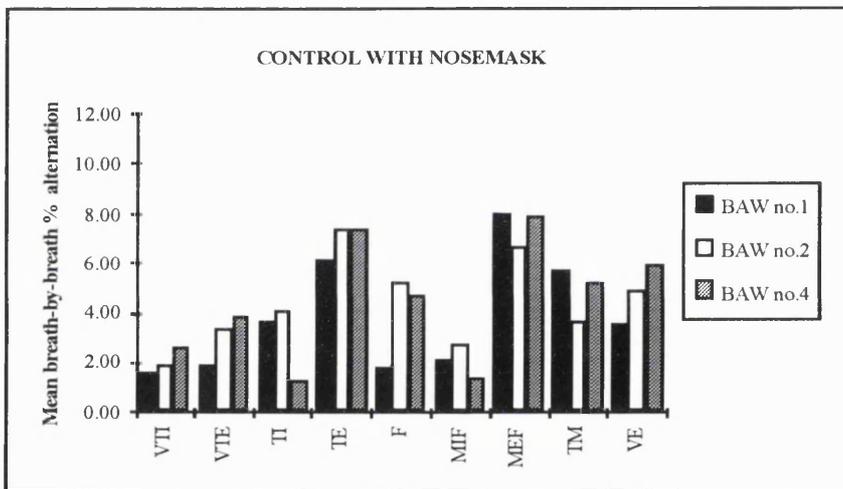


Figure 5.3.4.i.b Subject BAW control with nose mask on three separate days.

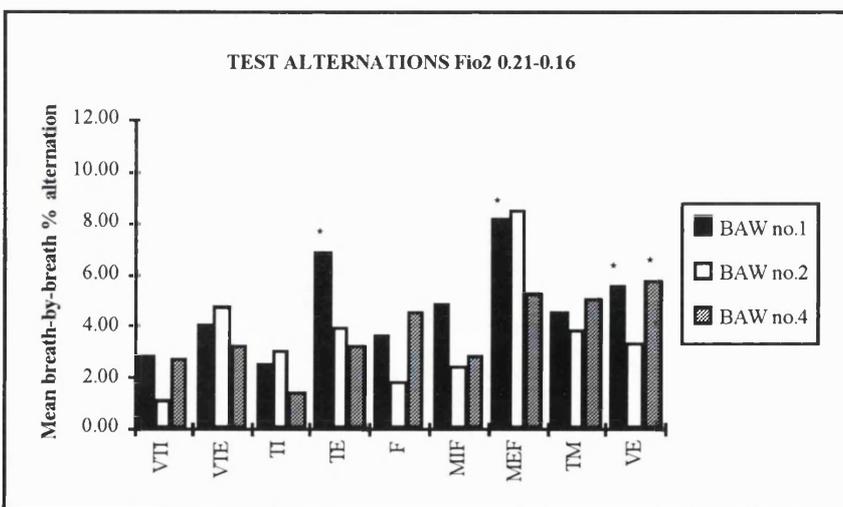


Figure 5.3.4.i.c Subject BAW test alternations Fio2 0.21-0.16 on three separate days. Asterisks denote variables that showed a significant chemoreflex response.

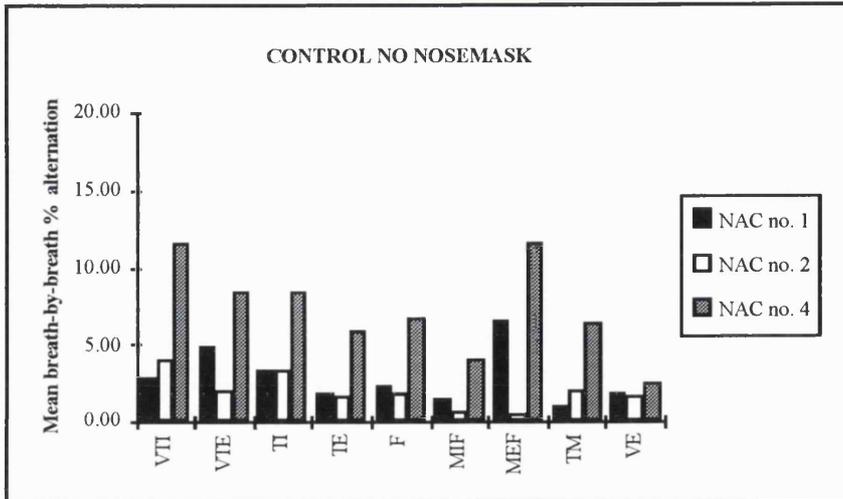


Figure 5.3.4.ii a Subject NAC control no nose mask on three separate days.

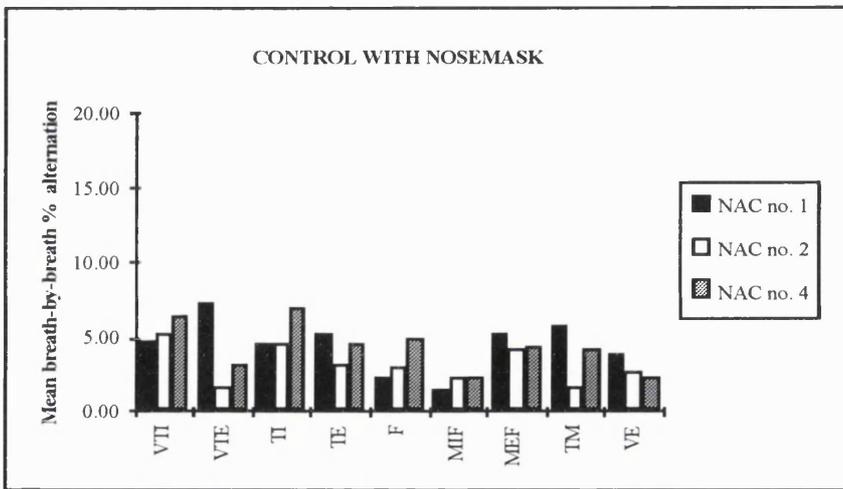


Figure 5.3.4.ii.b Subject NAC control with nose mask on three separate days.

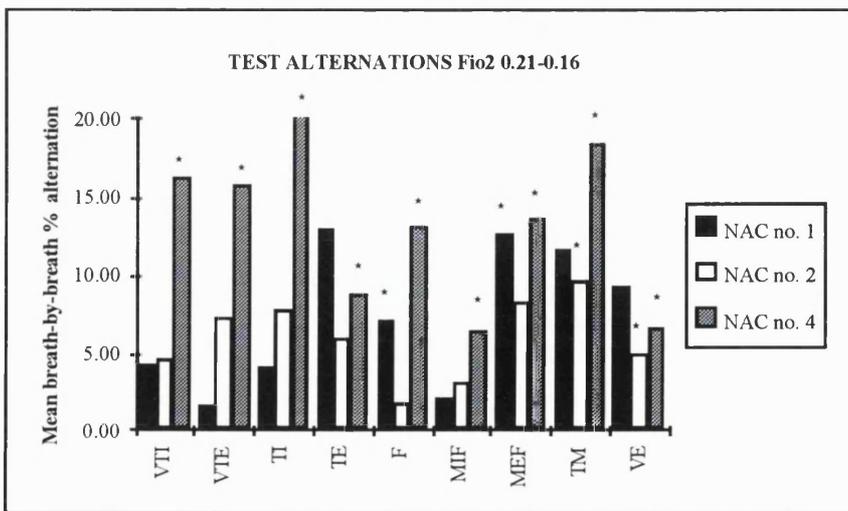


Figure 5.3.4.ii.c Subject NAC test alternations Fio<sub>2</sub> 0.21-0.16 on three separate days. Asterisks denote variables that showed a significant chemoreflex response.

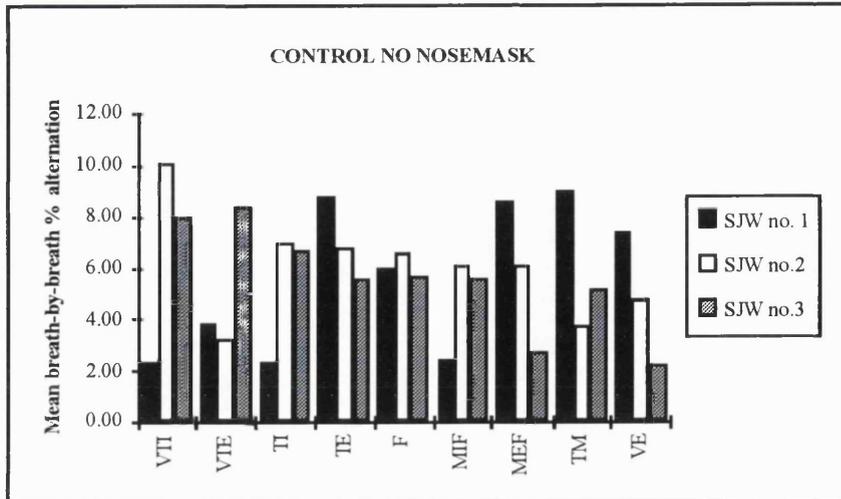


Figure 5.3.4.iii.a Subject SJW control no nose mask on three separate days.

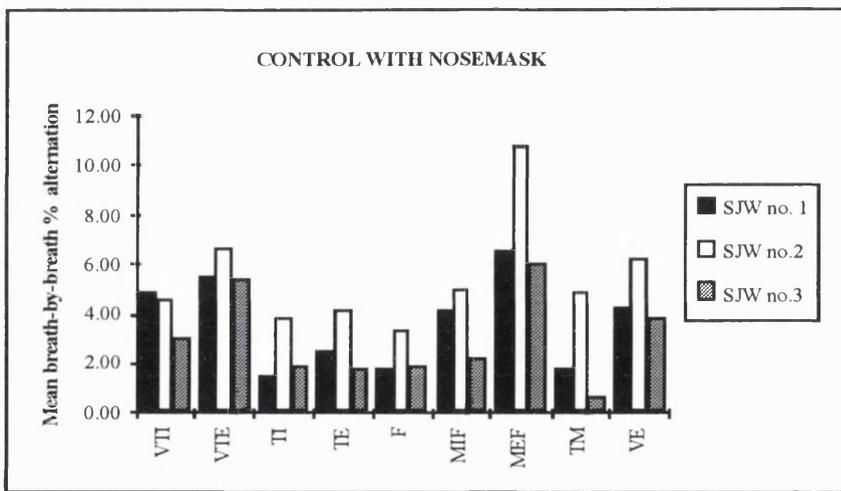


Figure 5.3.4.iii.b Subject SJW control with nose mask on three separate days.

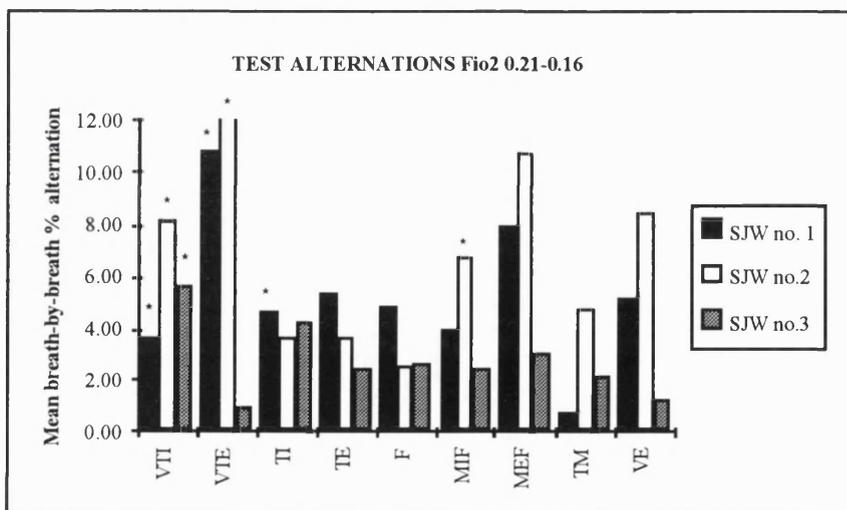


Figure 5.3.4.iii.c Subject SJW test alternations Fio<sub>2</sub> 0.21-0.16 on three separate days. Asterisks denote variables that showed a significant chemoreflex response.

### 5.3.5 Breath-by-breath alternations in etO<sub>2</sub> during test runs alternating between Fio<sub>2</sub> 0.21 and 0.16, and Fio<sub>2</sub> 0.26 and 0.16

The alternation in Fio<sub>2</sub> between 0.21 and 0.16 produced an alternation in etO<sub>2</sub> that was related to the stimulus. The change in etO<sub>2</sub> from one breath to the next confirmed that the alternations in Fio<sub>2</sub> were transmitted to the blood and indicated the strength of the stimulus. The breath-by-breath change in etO<sub>2</sub> was averaged over the duration of the test run and used to compare responses between individuals. Figure 5.3.5.i shows the chemoreflex respiratory response to the alternations in Fio<sub>2</sub> with the accompanying breath-by-breath changes in etO<sub>2</sub> for a subject classified as a responder, and in contrast figure 5.3.5.ii shows a subject who did not respond to the alternations in Fio<sub>2</sub>.

The responder shown in figure 5.3.5.i shows a regular alternation in etO<sub>2</sub>, and in contrast to the non-responder shown in figure 5.3.5.ii, a much larger breath-by-breath alternation in etO<sub>2</sub>. To observe in all subjects if responders showed a larger breath-by-breath alternation in etO<sub>2</sub> during test runs compared to non-responders, the mean percentage breath-by-breath alternation in etO<sub>2</sub> was plotted against test responses for respiratory variables V<sub>ti</sub>, t<sub>I</sub>, t<sub>E</sub> and V<sub>E</sub> in figure 5.3.5.iii-5.3.5.vi (mean % breath-by-breath alternation). Each test run for each subject is shown (i.e. each subject is represented by more than one point). Responders showed a significantly greater breath-by-breath change in end-tidal O<sub>2</sub> ( $0.53 \pm 0.04\%$ ; mean  $\pm$  S.E.M.) compared to non-responders ( $0.35 \pm 0.02\%$ ; mean  $\pm$  S.E.M.) in response to alternations between Fio<sub>2</sub> 0.21 and 0.16 (Mann-Whitney U test  $p < 0.01$ ).

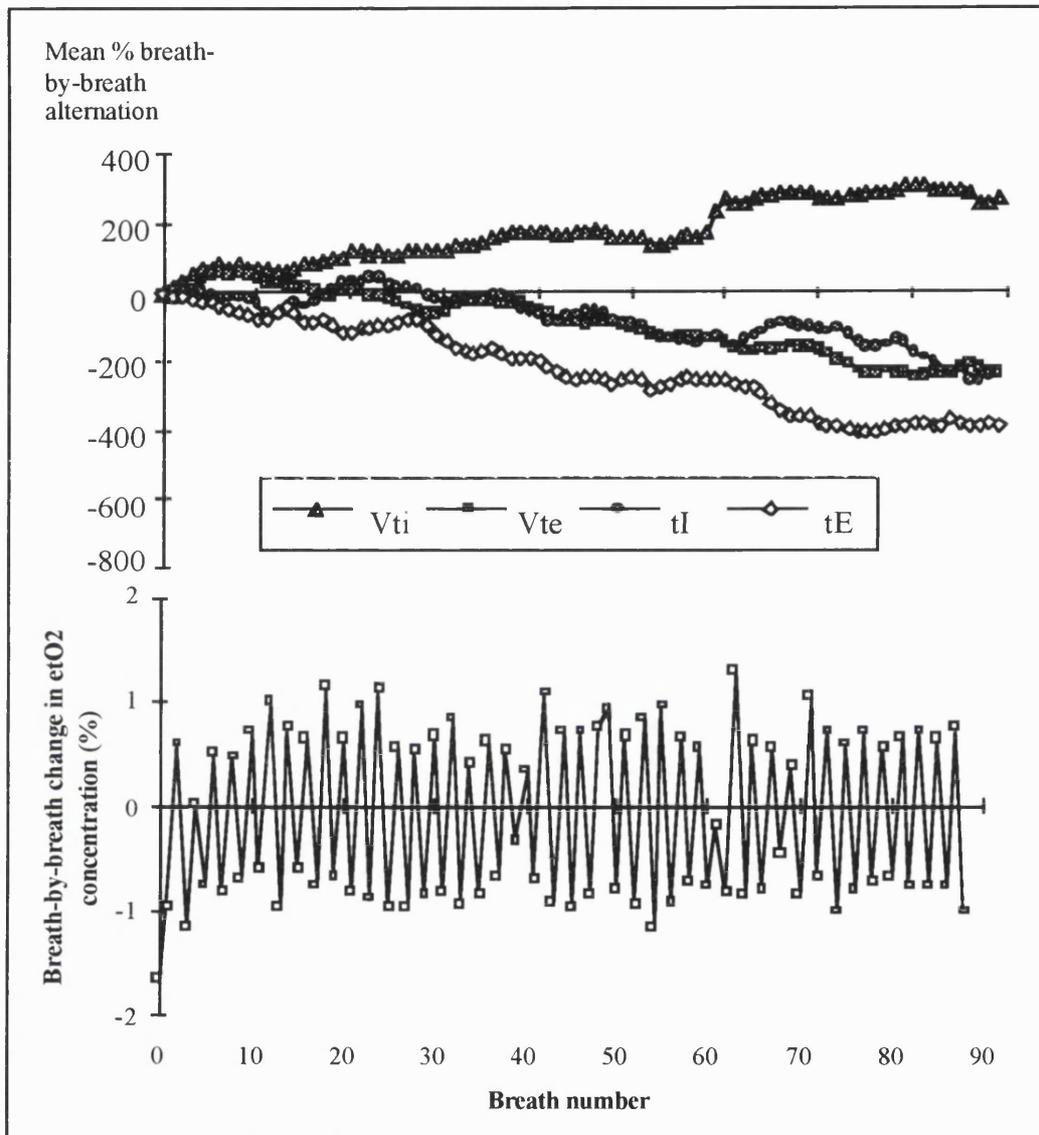


Figure 5.3.5.i Chemoreflex respiratory response and accompanying breath-by-breath changes in etO<sub>2</sub> for a responder.

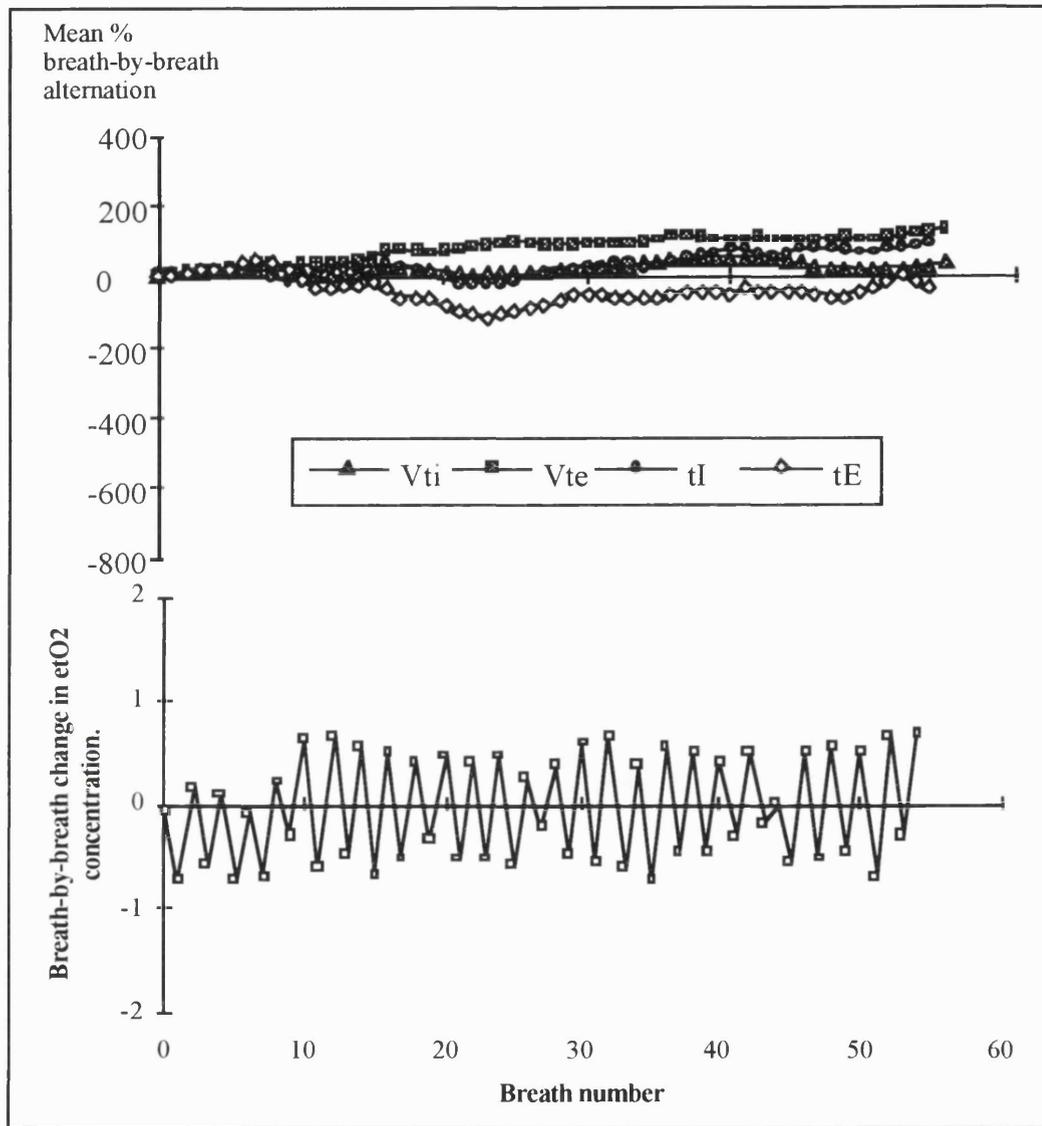


Figure 5.3.5.ii Chemoreflex respiratory response and accompanying breath-by-breath changes in etO<sub>2</sub> for a non-responder.

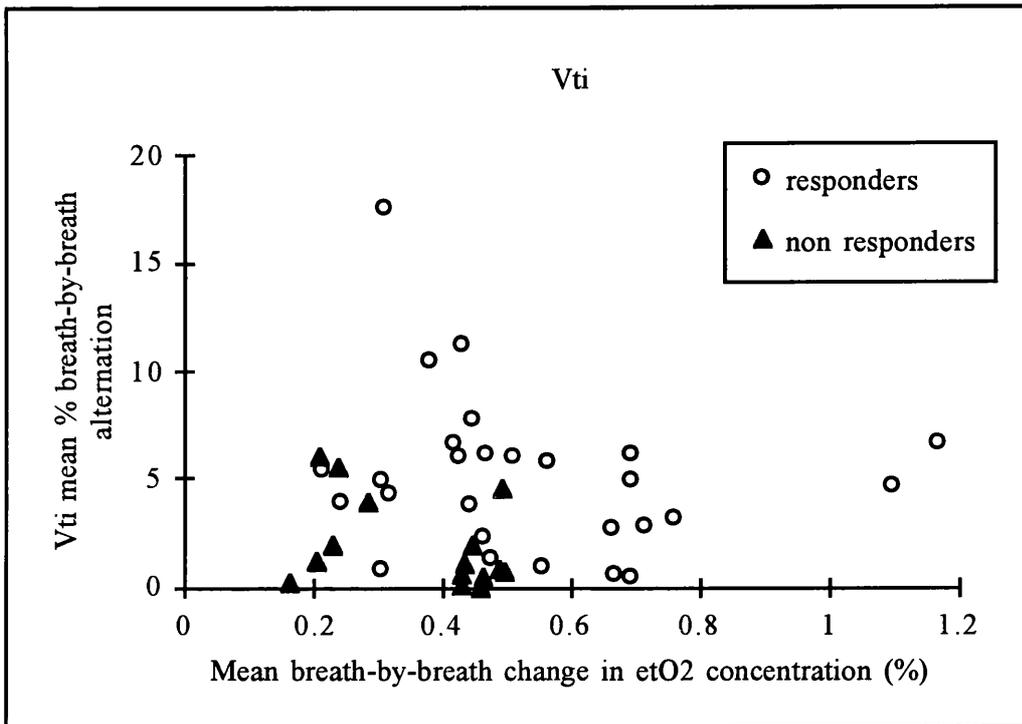


Figure 5.3.5.iii  $V_{ti}$  mean percentage breath-by-breath alternation plotted against the mean breath-by-breath change in  $etO_2$  during test runs alternating between  $F_{iO_2}$  0.21 and 0.16 for responders and non-responders.

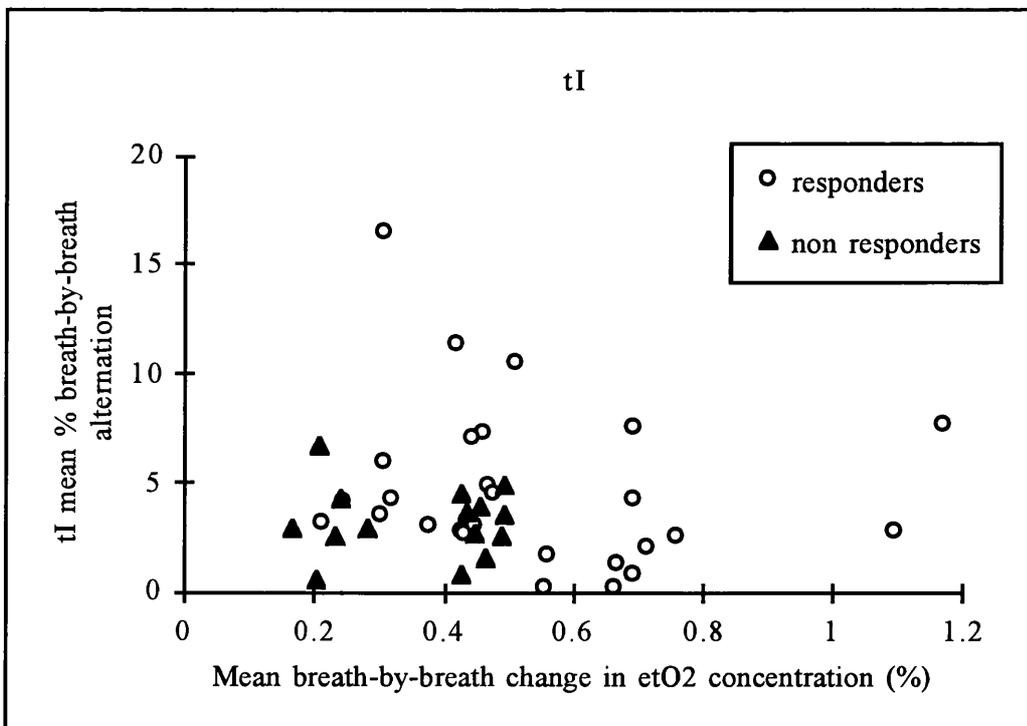


Figure 5.3.5.iv  $t_I$  mean percentage breath-by-breath alternation plotted against the mean breath-by-breath change in  $etO_2$  during test runs alternating between  $F_{iO_2}$  0.21 and 0.16 for responders and non-responders.

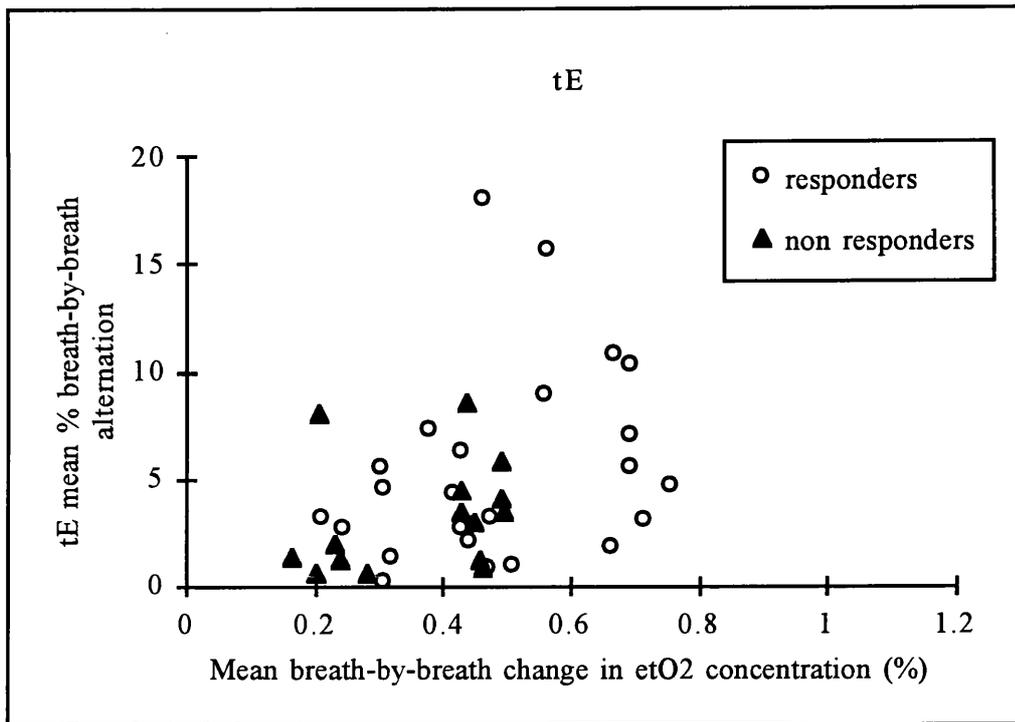


Figure 5.3.5.v t<sub>E</sub> mean percentage breath-by-breath alternation plotted against the mean breath-by-breath change in etO<sub>2</sub> during test runs alternating between Fio<sub>2</sub> 0.21 and 0.16 for responders and non-responders.

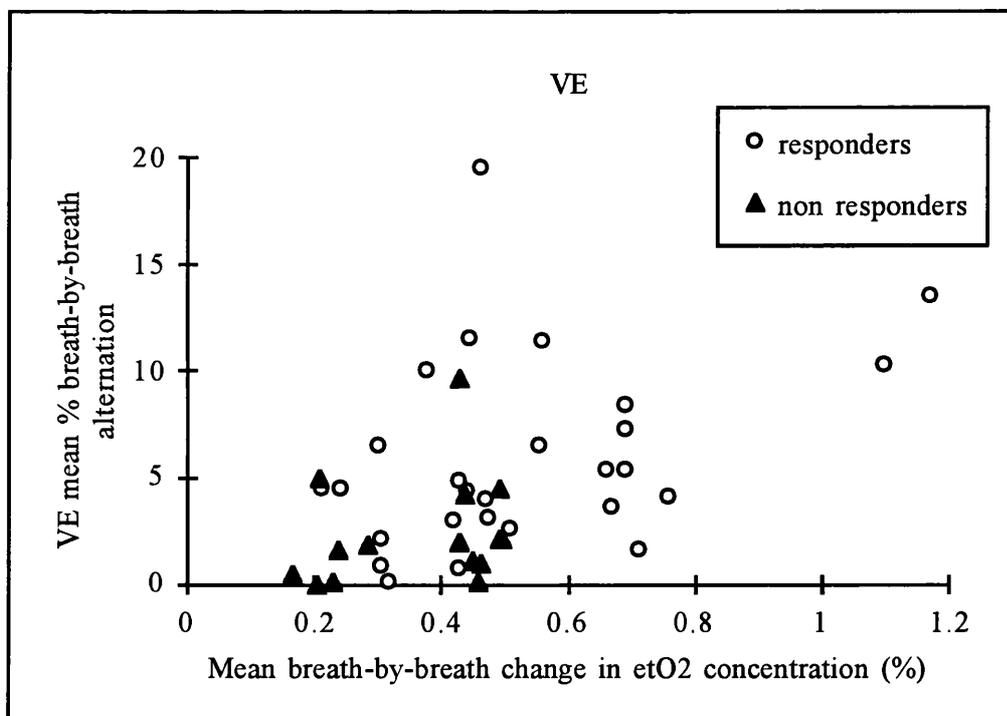


Figure 5.3.5.vi V<sub>E</sub> mean percentage breath-by-breath alternation plotted against the mean breath-by-breath change in etO<sub>2</sub> during test runs alternating between Fio<sub>2</sub> 0.21 and 0.16 for responders and non-responders.

I calculated the mean breath-by-breath change in  $etO_2$  for 12 subjects who were challenged by alternations between  $F_{iO_2}$  0.21-0.16, and also by alternations between  $F_{iO_2}$  0.26-0.16, and compared them. For alternations between  $F_{iO_2}$  0.26-0.16 the mean breath-by-breath change in  $etO_2$  was  $0.70 \pm 0.04\%$  (mean  $\pm$  S.E.M.), and for alternations between  $F_{iO_2}$  0.21-0.16 the mean breath-by-breath change in  $etO_2$  was  $0.45 \pm 0.04\%$  (mean  $\pm$  S.E.M.). This was significantly different by Wilcoxon matched pairs ( $p < 0.01$ ). The mean breath-by-breath change in  $etO_2$  for both test runs is shown in figure 5.3.5.vii. Only one subject did not show a greater breath-by-breath change in  $etO_2$  for alternations between  $F_{iO_2}$  0.26-0.16 compared to 0.21-0.16.

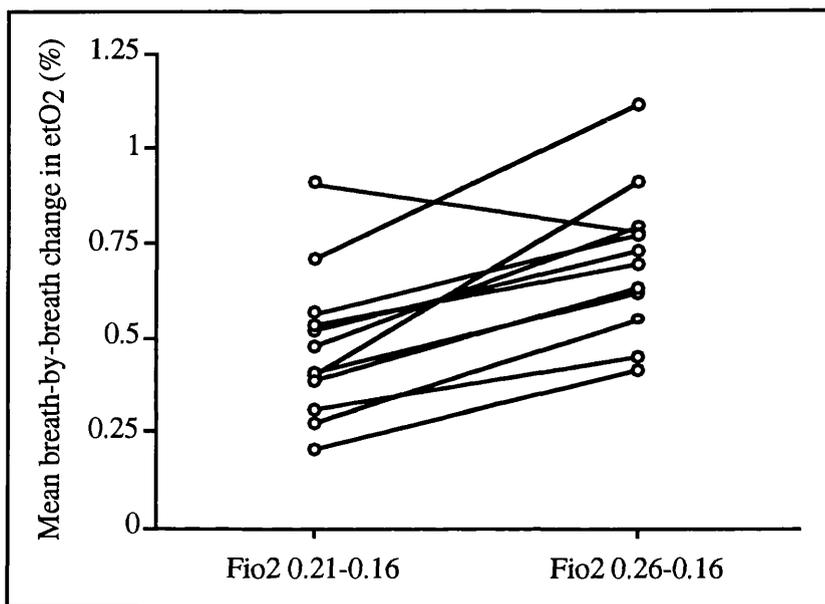


Figure 5.3.5.vii Mean breath-by-breath change in  $etO_2$  for test runs  $F_{iO_2}$  0.21 and 0.16 compared to  $F_{iO_2}$  0.26 and 0.16 in all subjects ( $n=12$ )

Likewise for alternations between  $F_{iO_2}$  0.26-0.16, I plotted the mean percentage breath-by-breath alteration in  $etO_2$  against test responses for respiratory variables  $V_{ti}$ ,  $t_I$ ,  $t_E$  and  $V_E$  in figure 5.3.5.viii-5.3.5.xi (mean % breath-by-breath alteration). However, in contrast to figures 5.3.5.ii-vi, responders to the  $F_{iO_2}$  0.26-0.16 alternations did not show a greater mean breath-by-breath change in  $etO_2$  ( $0.69 \pm 0.04\%$ ; mean  $\pm$  S.E.M.) compared to non-responders ( $0.71 \pm 0.05\%$ ; mean  $\pm$  S.E.M.). Therefore the mean breath-by-breath change in  $etO_2$  did not appear to account for the difference between responders and non-responders to the  $F_{iO_2}$  0.26-0.16 test. For *responders*, the mean  $\pm$  S.E.M. breath-by-breath oscillation in  $etO_2$  was not significantly different between the two types of tests;  $0.53 \pm 0.04\%$  for alternations between  $F_{iO_2}$  0.21-0.16, and  $0.69 \pm 0.04\%$  for alternations between  $F_{iO_2}$  0.26-0.16

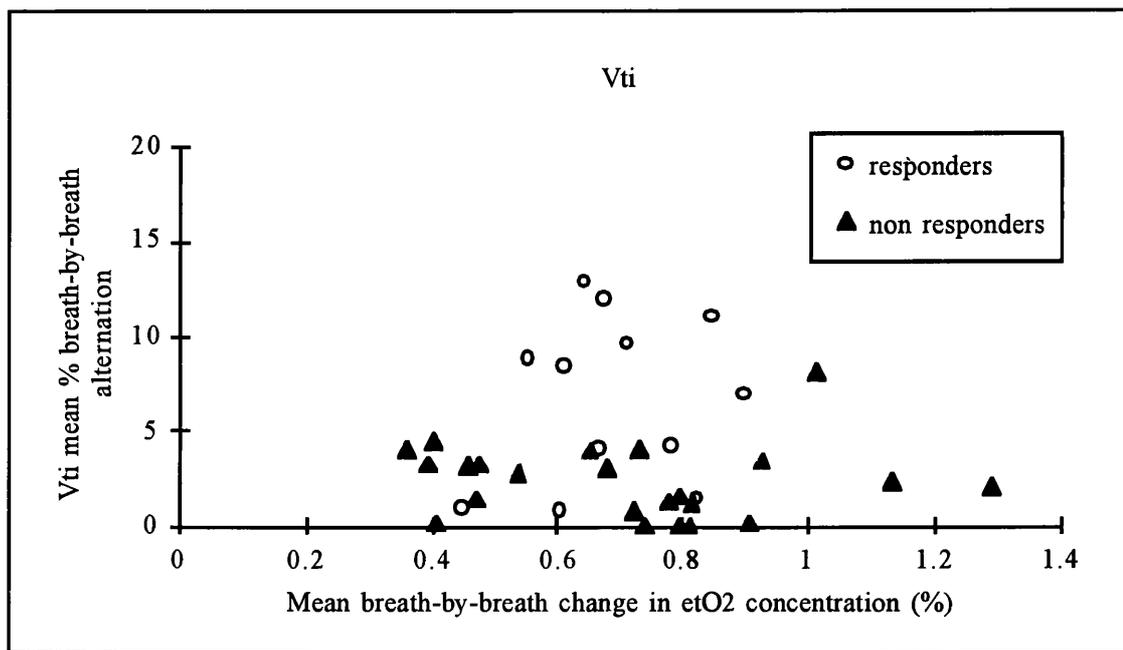


Figure 5.3.5.viii  $V_{ti}$  mean percentage breath-by-breath alternation plotted against the mean breath-by-breath change in  $etO_2$  during test runs alternating between  $F_{iO_2}$  0.26 and 0.16 for responders and non-responders.

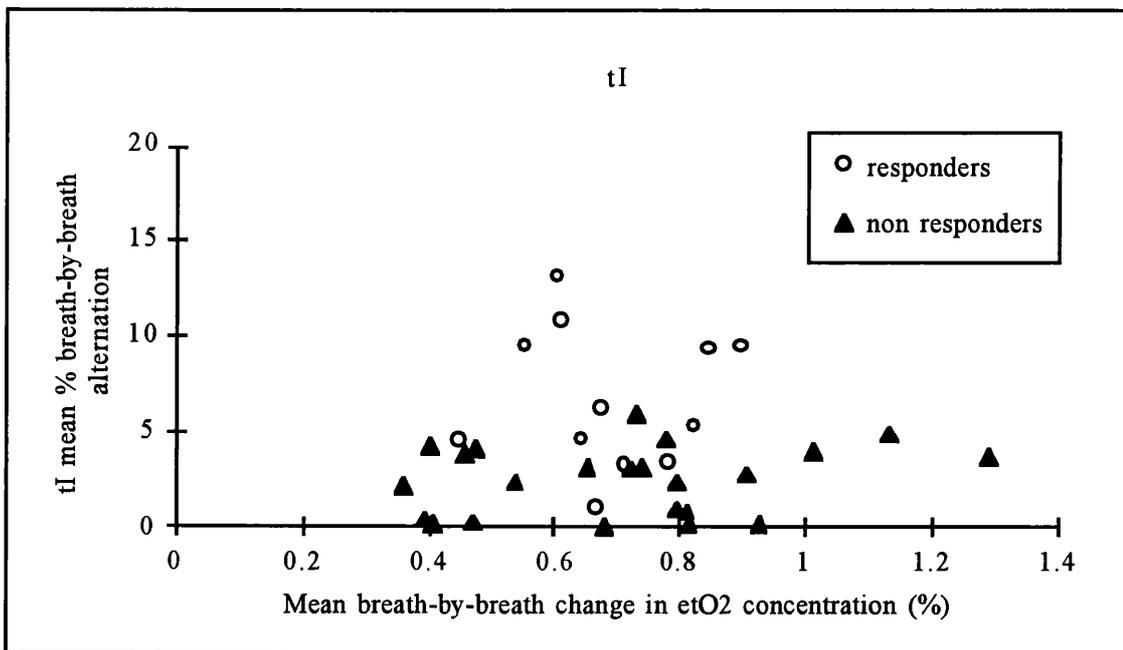


Figure 5.3.5.ix  $t_I$  mean percentage breath-by-breath alternation plotted against the mean breath-by-breath change in  $etO_2$  during test runs alternating between  $F_{iO_2}$  0.26 and 0.16 for responders and non-responders.

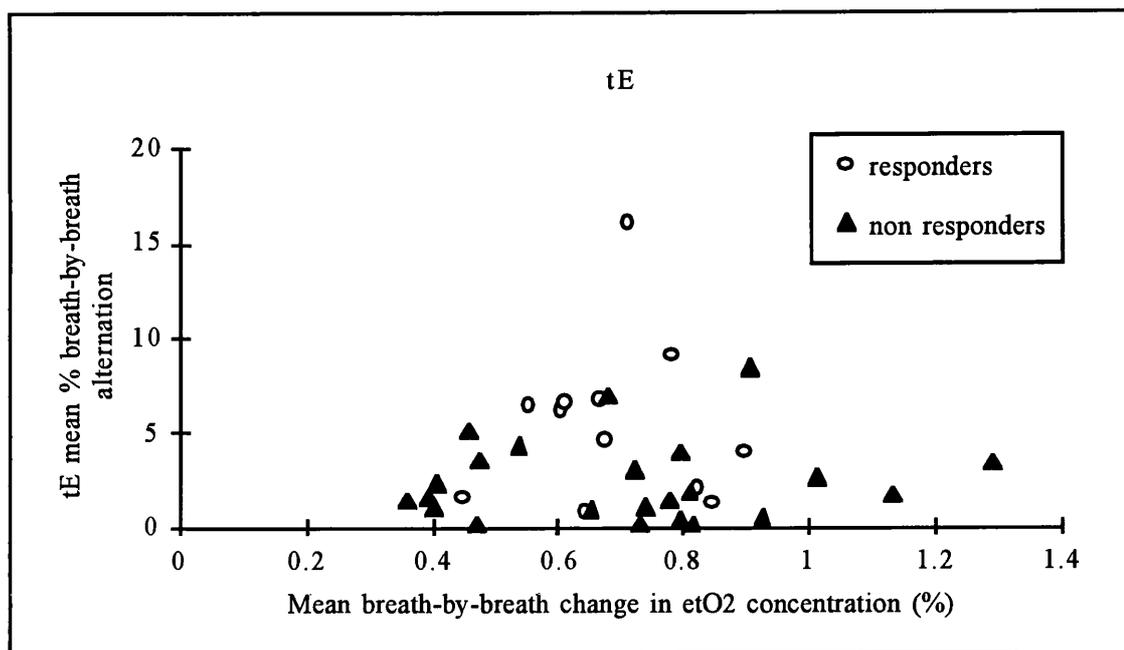


Figure 5.3.5.x tE mean percentage breath-by-breath alternation plotted against the mean breath-by-breath change in etO<sub>2</sub> during test runs alternating between Fio<sub>2</sub> 0.26 and 0.16 for responders and non-responders.

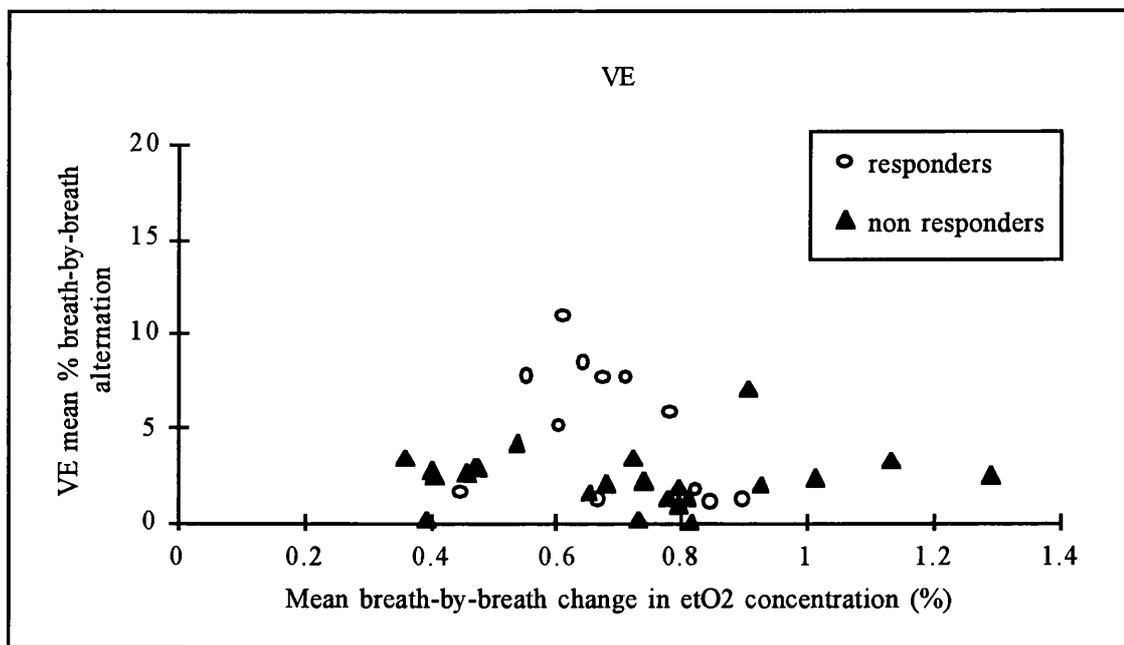


Figure 5.3.5.xi VE mean percentage breath-by-breath alternation plotted against the mean breath-by-breath change in etO<sub>2</sub> during test runs alternating between Fio<sub>2</sub> 0.26 and 0.16 for responders and non-responders.

### 5.3.6 Mean etO<sub>2</sub> concentration declines over the duration of test runs.

In the previous section I showed that the mean breath-to-breath change in etO<sub>2</sub> was greater during alternations to Fio<sub>2</sub> 0.26-0.16 compared to 0.21-0.16. Furthermore, whilst responders to the Fio<sub>2</sub> 0.21-0.16 alternation showed a greater change in etO<sub>2</sub> compared to non-responders, this was not the case for Fio<sub>2</sub> 0.26-0.16 alternations. I reasoned that the mean etO<sub>2</sub> about which the breaths oscillated may be important, so I compared mean levels between different runs. The mean etO<sub>2</sub> concentration was measured over the first and last 10 breaths for controls and both types of test runs, and this data is shown in table 5.3.6.i .

		Control runs Fio <sub>2</sub> 0.21 -0.21	Test runs Fio <sub>2</sub> 0.26 -0.16	Test runs Fio <sub>2</sub> 0.21 -0.16	p value
Mean etO <sub>2</sub> (%) first 10 breaths	mean ± S.E.M.	14.6 ± 0.2	16.3 ± 0.2*	13.4 ± 0.2†	* 0.02 † 0.02
Mean etO <sub>2</sub> (%) last 10 breaths	mean ± S.E.M.	14.6 ± 0.2	14.3 ± 0.2	12.4 ± 0.2†	NS † 0.02
	p value	0.64	0.02	0.02	

Table 5.3.6.i Mean ± S.E.M. etO<sub>2</sub> concentration over the first and last 10 breaths during control and test runs alternating between Fio<sub>2</sub> 0.26-0.16 and 0.21-0.16. Symbols refer to p values for comparisons between tests and controls. For further description see text.

There was a significant fall in the mean etO<sub>2</sub> concentration over the duration of both types of test runs. Test runs alternating between Fio<sub>2</sub> 0.26-0.16 showed a significantly greater mean etO<sub>2</sub> concentration over the first 10 and last breaths compared to test runs alternating between Fio<sub>2</sub> 0.21-0.16. When the mean etO<sub>2</sub> concentration over the *first 10 breaths* for both test runs was compared to control, the mean etO<sub>2</sub> for alternations between Fio<sub>2</sub> 0.26-0.16 was significantly greater, and that for alternations between Fio<sub>2</sub> 0.21-0.16 was significantly less, than control. In contrast, when the mean etO<sub>2</sub> concentration over the *last 10 breaths* for each type of test run was compared to control, the mean etO<sub>2</sub> for alternations between Fio<sub>2</sub> 0.21-0.16 was significantly less than control, and that for alternations between Fio<sub>2</sub> 0.26-0.16 was not significantly different from control. So over the duration of a test run, alternations between Fio<sub>2</sub> 0.26-0.16 oscillated about a mean etO<sub>2</sub> similar to that of control and alternations between Fio<sub>2</sub> 0.21-0.16 oscillated at a lower mean etO<sub>2</sub> level.

### 5.3.7 End-tidal CO<sub>2</sub> concentration measured during test runs

End-tidal CO<sub>2</sub> concentration was measured over the first and last 10 breaths of test runs to compare between responders and non-responders. There was no significant fall in the mean etCO<sub>2</sub> measured over the first 10 breaths compared to the last 10 breaths in non-responders, or in responders, or indeed for the whole group for test runs alternating between Fio<sub>2</sub> 0.21 and 0.16 (table 5.3.7.i) or for test runs alternating between Fio<sub>2</sub> 0.26 and 0.16 (table 5.3.7.ii).

Fio <sub>2</sub> 0.21 and 0.16		Group n=14	Non-responders n=5	Responders n=9
Mean etCO <sub>2</sub> (%) first 10 breaths	mean ± S.E.M.	5.4 ± 0.1	5.4 ± 0.1	5.5 ± 0.1
Mean etCO <sub>2</sub> (%) last 10 breaths	mean ± S.E.M.	5.4 ± 0.1	5.3 ± 0.1	5.5 ± 0.1

Table 5.3.7.i Mean ± S.E.M. etCO<sub>2</sub> concentration (%) over the first and last 10 breaths of test runs alternating between Fio<sub>2</sub> 0.21 and 0.16.

Fio <sub>2</sub> 0.26 and 0.16		Group n=12	Non-responders n=8	Responders n=4
Mean etCO <sub>2</sub> (%) first 10 breaths	mean ± S.E.M.	5.4 ± 0.1	5.4 ± 0.1	5.3 ± 0.1
Mean etCO <sub>2</sub> (%) last 10 breaths	mean ± S.E.M.	5.4 ± 0.1	5.4 ± 0.1	5.2 ± 0.1

Table 5.3.7.ii Mean ± S.E.M. etCO<sub>2</sub> concentration (%) over the first and last 10 breaths of test runs alternating between Fio<sub>2</sub> 0.26 and 0.16.

### 5.3.8 Chemoreflex respiratory response to 2 breath alternations between Fio<sub>2</sub> 0.00 and 0.40

The chemoreflex respiratory response to 2 breath alternations between Fio<sub>2</sub> 0.00 and 0.40 was measured in one subject. Figure 5.3.8.i shows an example of the raw data. The mean breath-by-breath % alternation for respiratory variables during control, test runs Fio<sub>2</sub> 0.21-0.16 and Fio<sub>2</sub> 0.40-0.00 measured on the same day are shown in figure 5.3.8.ii. The response to 2 breath alternations between Fio<sub>2</sub> 0.40 and 0.00 is much larger than the response to 1 breath alternations between Fio<sub>2</sub> 0.21 and 0.16 when

compared to runs measured on the same day, and all other test responses measured for this subject (see figure 5.3.4.i.c). Alternations in respiratory variables for the  $F_{iO_2}$  0.21 and 0.16 test were not significant (compared to control) on this occasion.  $SaO_2$  was 97%, however the subject complained of dizziness and disorientation. It must be noted that although the response to the  $F_{iO_2}$  0.00- 0.40 stimulus is increased compared to the  $F_{iO_2}$  0.21- 0.16 stimulus, the discomfort experienced by the subject may also have an effect on the response.

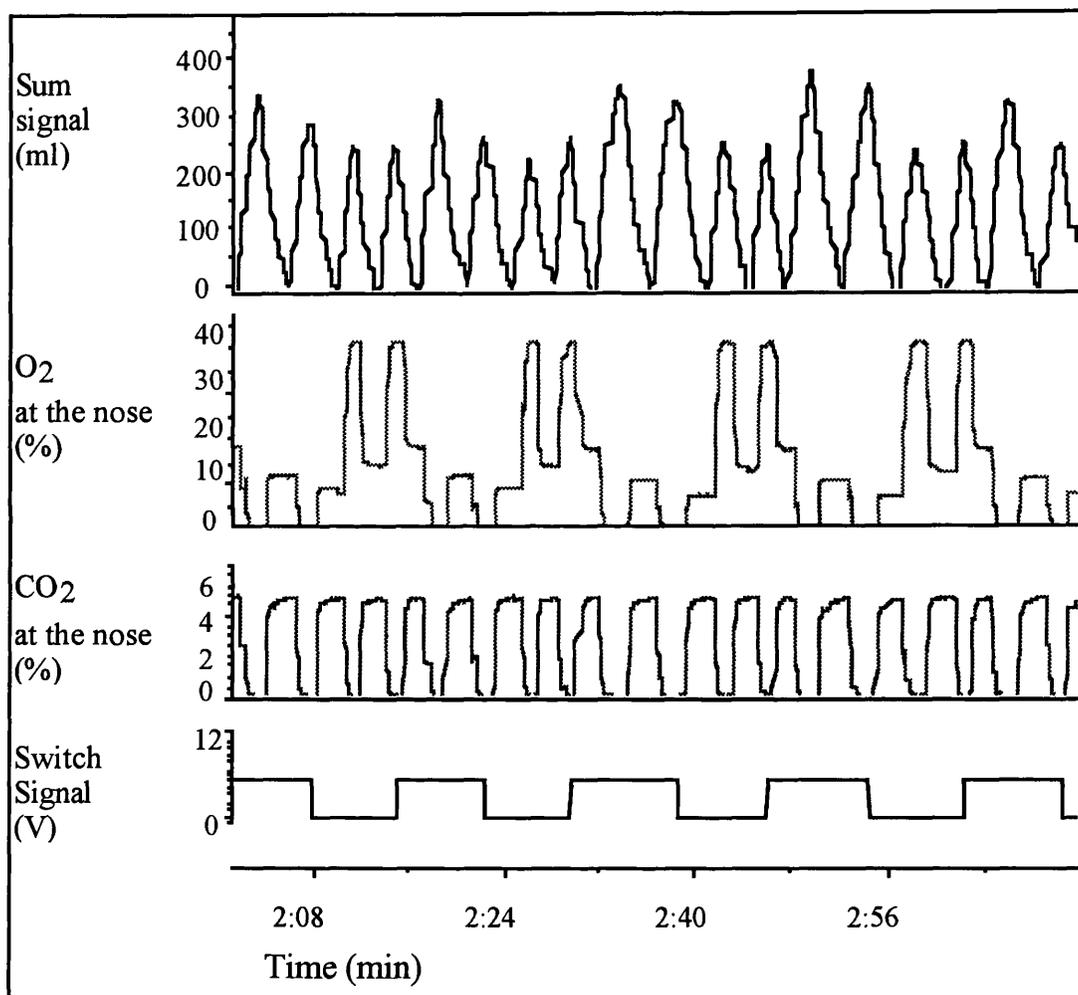


Figure 5.3.8.i The chemoreflex respiratory response to 2 breath alternations in  $F_{iO_2}$  between 0.40 and 0.00. Sum Respirance signal (ml),  $O_2$  at the nose (%),  $CO_2$  at the nose (%) and the switch signal (V).

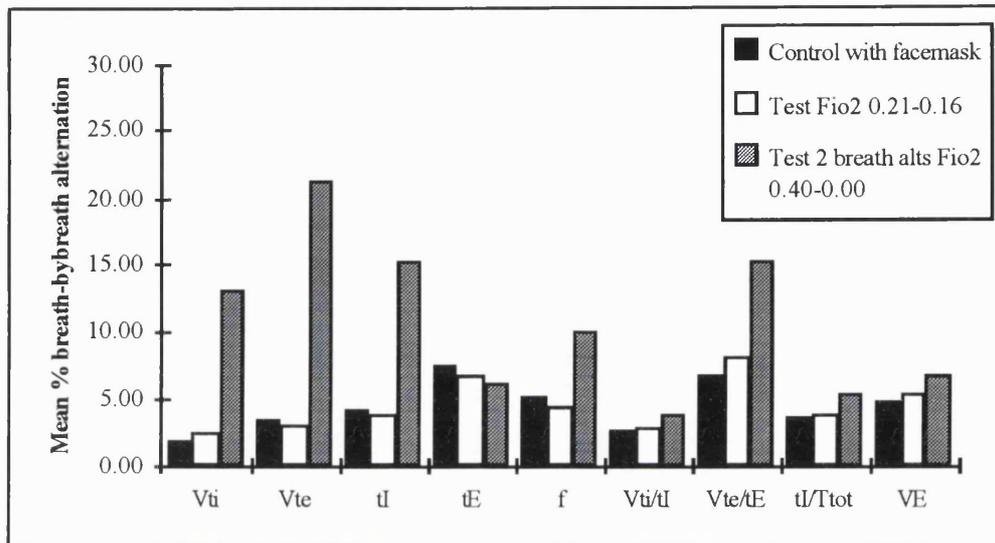


Figure 5.3.8.ii Summary of chemoreflex respiratory responses to single breath alternations between Fio<sub>2</sub> 0.21-0.21 (control) and 0.21-0.16 (test), and 2 breath alternations Fio<sub>2</sub> 0.40-0.00 (test) for one subject.

### 5.3.9 Comparison of chemoreflex respiratory responses between adults and infants at 2 months of age

Adults who showed a significant chemoreflex respiratory response and were classified as responders (9 of 14 subjects) were used to compare chemoreflex responses to those of infants aged 2 months of age ( $n=13$ ). The results of infant chemoreflex responses are presented in Chapter 3.

Tests were compared to control for statistical significance as outlined in section 2.3.4. All 13 infants showed a significant chemoreflex response at 2 months of age during quiet sleep, whilst 9 of 14 adults showed a significant chemoreflex response during wakefulness. The number of infants who showed a significant chemoreflex response is compared to the number of adults who showed a significant chemoreflex response in table 5.3.9.i. For each respiratory variable, a greater proportion of infants showed significant alternations than adults.

Respiratory variable	$V_{ti}$	$V_{te}$	$t_I$	$t_E$	$f$	$V_{ti}/t_I$	$V_{te}/t_E$	$t_I/T_{Tot}$	$V_E$
Number of infants (n=13) showing significant responses	8 62%	8 62%	7 54%	6 46%	8 62%	4 31%	7 54%	4 31%	6 46%
Number of adults (n=9) showing significant responses	3 33%	4 44%	3 33%	4 44%	3 33%	4 44%	4 44%	3 33%	3 33%

Table 5.3.9.i Number of infants and adults (n and percentage) showing significant chemoreflex respiratory responses to breath-by-breath alternations in  $F_{iO_2}$  between 0.21 and 0.16 for each respiratory variable.

		$V_{ti}$	$V_{te}$	$t_I$	$t_E$
INFANTS (n=13)	median	2.54	1.66	0.81	2.99
	25th-75th percentile	0.06-4.61	0.28-7.31	-0.98-2.27	0.64-3.71
ADULTS (n=9)	median	1.09	2.69	0.27	0.85
	25th-75th percentile	-0.56-3.57	2.10-5.44	-0.31-1.80	0.74-3.37
	p value	0.82	0.49	0.92	0.57

	$f$	$V_{ti}/t_I$	$V_{te}/t_E$	$t_I/T_{tot}$	$V_E$
median	1.59	1.64	2.12	0.71	0.78
25th-75th percentile	0.25-2.85	1.20-2.60	-0.03-3.66	0.30-2.80	0.43-4.83
median	1.71	1.62	2.14	1.12	1.96
25th-75th percentile	0.52-2.96	-0.26-2.68	-0.07-5.77	-1.19-2.40	1.65-3.96
p value	0.76	0.66	0.76	0.71	0.92

Table 5.3.9.ii Chemoreflex respiratory responses (average test % breath-by-breath alternation - average control % breath-by-breath alternation) for infants and adults.

Average control responses were subtracted from average test responses for each subject, and the alternation in respiratory variables compared between the two ages. Only adults that responded to the test (showed a significant chemoreflex response in at least one respiratory variable) were used to compared to infants. Chemoreflex respiratory responses are summarised in table 5.3.9.ii for both adults and infants. There was no significant difference between infants and adults in the magnitude of the chemoreflex response (average test - average control response) for any of the respiratory variables (Mann-Whitney U test,  $p > 0.05$ ). The magnitude of the chemoreflex response (mean

test % breath-by-breath alternation - mean control % breath-by-breath alternation) for each subject is shown in figure 5.3.9.i-iii. The range of responses is similar between infants and adults for all respiratory variables.

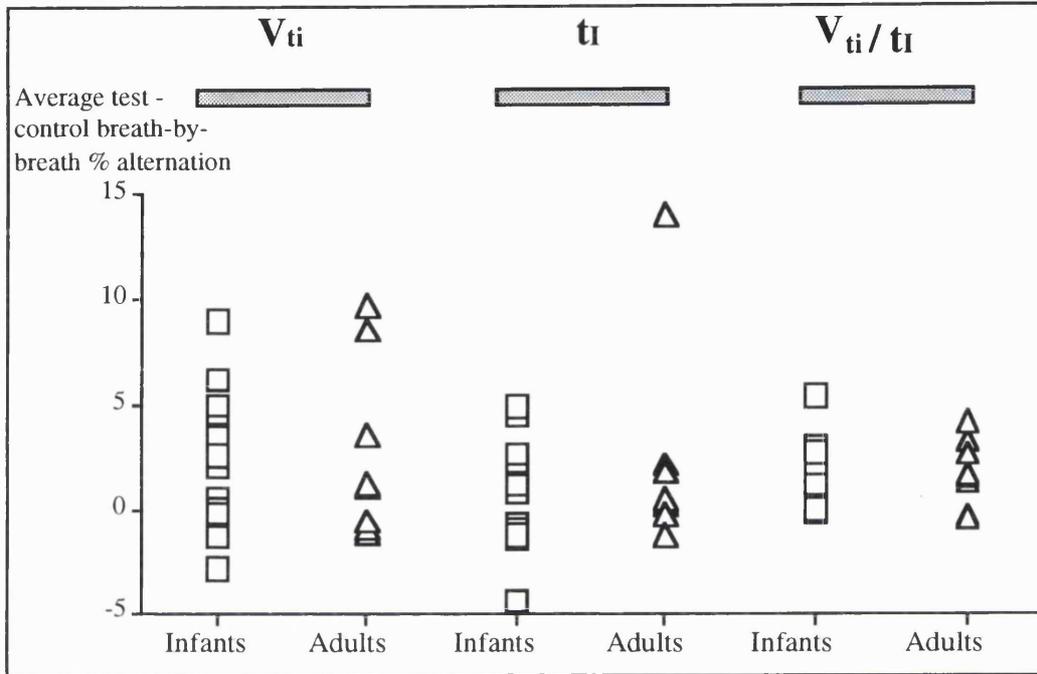


Figure 5.3.9.i  $V_{ti}$ ,  $t_I$ , and  $V_{ti}/t_I$  chemoreflex respiratory responses for infants and adults. Average test breath-by-breath % alternation - average control breath-by-breath % alternation.

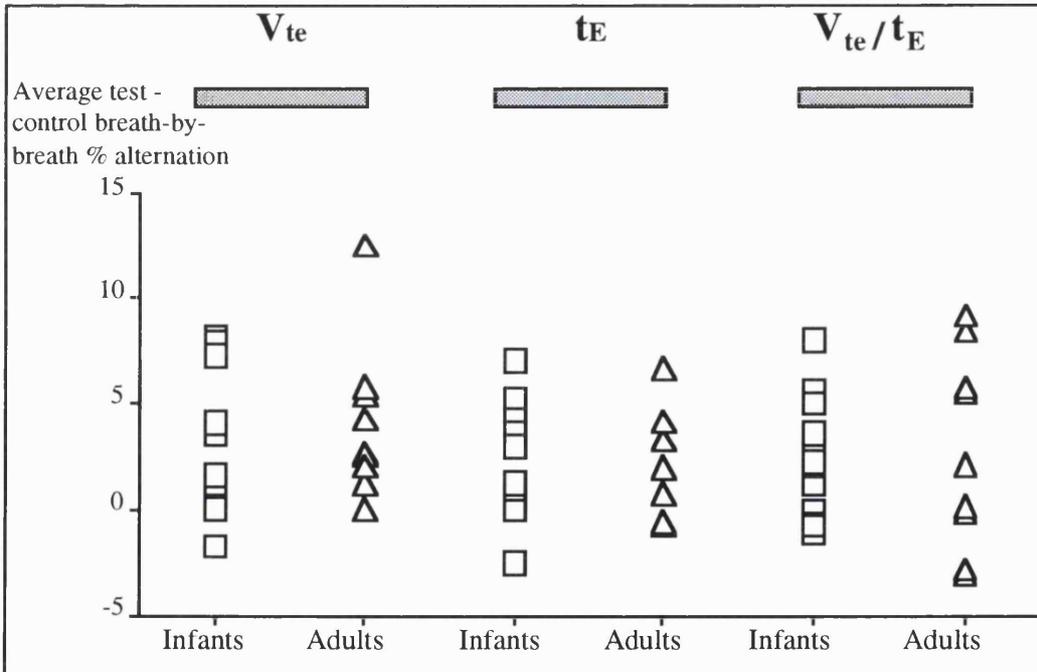


Figure 5.3.9.ii  $V_{te}$ ,  $t_E$ , and  $V_{te}/t_E$  chemoreflex respiratory responses for infants and adults. Average test breath-by-breath % alternation - average control breath-by-breath % alternation.

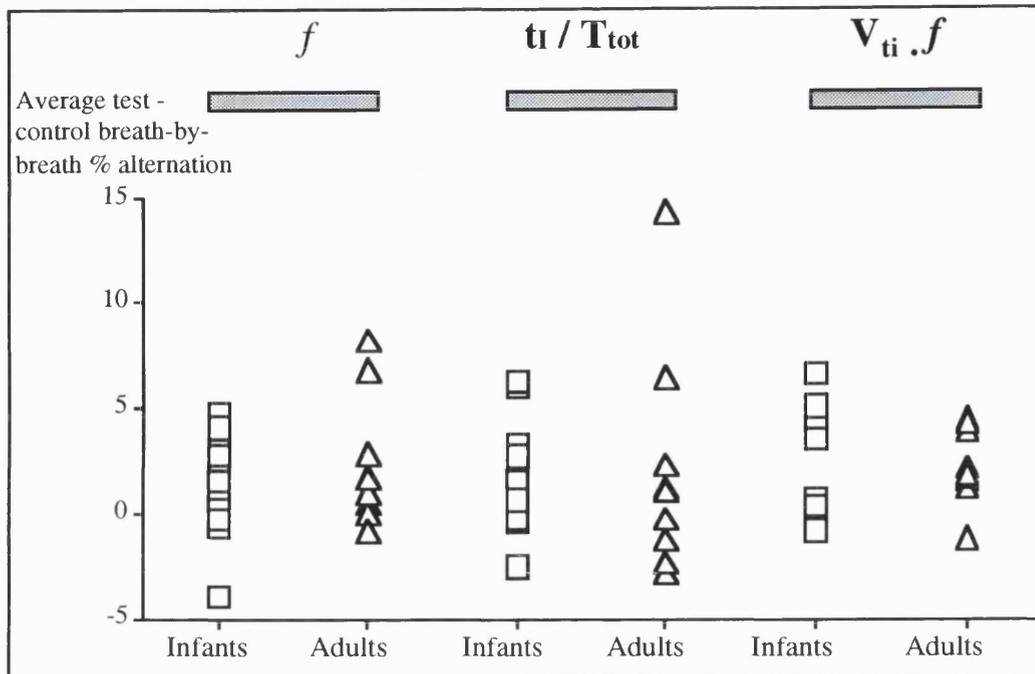


Figure 5.3.9.iii  $f$ ,  $t_i/T_{tot}$ , and  $V_E$  chemoreflex respiratory responses for infants and adults. Average test breath-by-breath % alternation - average control breath-by-breath % alternation.

## 5.4 Discussion

### 5.4.1 Overview

I have measured the chemoreflex respiratory response to single breath alternations between  $F_{iO_2}$  0.21-0.16 in 14 healthy adults during wakefulness. I have confirmed that these alternations produced a breath-by-breath oscillation in end-tidal  $O_2$ . Significant chemoreflex respiratory responses were present in 9 subjects (64%). Responders showed a significantly greater mean breath-to-breath change in  $etO_2$  than non-responders. There was no significant fall in  $etCO_2$  during  $F_{iO_2}$  alternations, and non-responders were not accounted for on the basis of a fall in  $etCO_2$  during test runs.

In 12 of the 14 subjects I measured the chemoreflex respiratory response to single breath alternations in  $F_{iO_2}$  between 0.26 and 0.16. Significant responses were present in 4 subjects (33%). The mean breath-to-breath change in  $etO_2$  during alternations between  $F_{iO_2}$  0.26-0.16 was greater than alternations between  $F_{iO_2}$  0.21-0.16. However, responders to the  $F_{iO_2}$  0.26-0.16 test did not show a greater breath-to-breath

change in  $\text{etO}_2$  compared to non-responders. Neither was there a fall in  $\text{etCO}_2$  during test runs for non-responders.

Adult chemoreflex responses (to  $\text{Fio}_2$  0.21-0.16 alternations) were compared to infant chemoreflex responses by subtracting the average control from the average test response for an individual. There was a similar range of chemoreflex responses for adults and infants. Furthermore, there was no significant difference in the degree of alternation between the two groups for any respiratory variable. This is further evidence that the chemoreflex response of infants at *ca.* 6wks to hypoxia is mature (see Chapter 3).

#### **5.4.2 Effect of wearing a nose mask on breathing**

I compared the effect of wearing a nose mask on the baseline respiratory pattern and on the degree of alternation when the mean breath-by-breath percentage alternation was calculated. I found that there was a significant increase in  $V_{\text{ti}}$  and  $t_{\text{I}}$ , and a significant decrease in  $t_{\text{E}}$  when subjects breathed via a nose mask. This is not a new finding, and could be explicable in terms of an increased dead space, mechanical stimulation of the face, nose or mouth, or an increased awareness of breathing. Askanazi, Silverberg, Foster, Hyman, Milic-Emili & Kinney (1980) found that there was a 32% and 15% increase in  $V_{\text{t}}$  from breathing through a mask and mouthpiece plus noseclip respectively, but that  $f$ ,  $t_{\text{I}}$  and  $t_{\text{E}}$  were unchanged in adult man. A subsequent study (Weissman, Askanazi, Milic-Emili & Kinney, 1984) observed that in addition to increases in  $V_{\text{t}}$ ,  $V_{\text{E}}$  and  $V_{\text{ti}}/t_{\text{I}}$  caused by breathing through a facemask, that inhalation of 4%  $\text{CO}_2$  attenuated the effect on respiratory pattern. Fleming et al. (1982) and Dolfin et al. (1983) suggested that this effect on breathing was mediated via trigeminal stimulation to the face in sleeping newborn infants. They compared the effect on respiration of breathing through a facemask attached to a pneumotachometer to breathing through a facemask alone. Tidal volume was increased, and respiratory frequency decreased with both methods, so it was concluded that the increased dead space of the pneumotachometer was not the main factor in alteration of the breathing pattern. Maxwell, Cover & Hughes (1985) found in adult man that  $V_{\text{t}}$  was increased, and  $f$  reduced, during tube breathing and whilst wearing a nose clip. Furthermore, the effect on respiratory pattern could be removed when breathing through a facemask at very high flows which reduced the dead space to *ca.* zero. They concluded that the application of the noseclip alone could stimulate trigeminal receptors in the nasal area, and that an increased deadspace also contributed to the greater  $V_{\text{t}}$  seen during facemask application. Western & Patrick (1988) investigated the effect of an increased awareness of breathing on respiratory pattern in man. Focusing attention on breathing for 5min

prolonged  $t_I$  whilst  $V_{tI}/t_I$  was constant and  $t_E$  lengthened. Tidal volume was also increased, but not  $V_E$ . They found no effect on breathing of the facemask rim on the face, and concluded that a large component of the effect of respiratory apparatus on breathing was due to increased awareness of the subject.

I also found that there was a significant decrease in the breath-by-breath % alternation for respiratory variables for  $V_{tI}$ ,  $V_{te}$ ,  $f$ ,  $V_{tI}/t_I$  and  $V_E$ . Thus it appeared that wearing a nose mask had a regularizing effect on the respiratory pattern which could be explained by a conscious awareness of wearing a nose mask.

That there is an alteration in breathing pattern caused by the wearing of instrumentation is not questionable. This effect on breathing of respiratory apparatus reiterates the need to compare test responses to controls which use the same method. I have employed this approach for the controls used in this study, so that the only difference between controls and test is the concentration of the inspired gases.

### 5.4.3 Chemoreflex responses to alternations between $F_{iO_2}$ 0.21 and 0.16, and $F_{iO_2}$ 0.26 and 0.16

I measured the chemoreflex respiratory response to alternations between  $F_{iO_2}$  0.21 and 0.16 in 14 subjects, and to alternations between  $F_{iO_2}$  0.26 and 0.16 in 12 of the 14 subjects. I found that 9 (64%) subjects showed a significant chemoreflex respiratory response for alternations between  $F_{iO_2}$  0.21 and 0.16, and 4 subjects (33%) for alternations between  $F_{iO_2}$  0.26 and 0.16. The largest mean alternations to  $F_{iO_2}$  alternations between 0.21-0.16 were  $6.76 \pm 1.11\%$  for  $V_{te}/t_E$ ,  $5.21 \pm 0.97\%$  for  $t_I$ ,  $5.15 \pm 0.71\%$  for  $V_{te}$  and  $5.06 \pm 0.77\%$  for  $t_I/T_{tot}$ . The largest mean alternations to  $F_{iO_2}$  alternations between 0.26-0.16 were  $4.00 \pm 0.69\%$  for  $V_{te}$  and  $5.37 \pm 0.9\%$  for  $V_{te}/t_E$ . Thus both the degree of reflex alternation and the incidence of significant responses was less for the stimulus alternating between  $F_{iO_2}$  0.26-0.16. Possible explanations of this finding are discussed in detail in section 5.4.5.

#### 5.4.3.a Comparison with previous studies

Ward et al. (1979) measured the chemoreflex response to single breath alternations in  $PET_{O_2}$  between 80 and 45 torr with  $PET_{CO_2}$  held 10 torr above the eupnoeic level. They observed an alternation in respiratory variables of some kind (details not given) in half to three quarters of the runs. No information is given on the degree of reflex alternation, i.e. the breath-by-breath percentage alternation. What is interesting in that study is that

the stimulus was very large and the mean  $PET_{O_2}$  hypoxic (*ca.* 60 torr), and was delivered against an elevated  $PET_{CO_2}$ . I observed significant chemoreflex responses in a similar proportion of subjects (64%), yet the stimulus I used was smaller. The alternation in my study alternated around a mean  $etO_2$  of  $12.4 \pm 0.2\%$  (*ca.* 88 torr) with a mean breath to breath oscillation in  $etO_2$  of  $0.47 \pm 0.06\%$  (*ca.* 3 torr). This suggests that the method used for detecting chemoreflex responses was more sensitive. However, it is not possible to compare the degree of reflex alternation, and hence the effect of the stimulus used, between the two studies as Ward et al. (1979) did not report this.

Metias et al. (1981) alternated  $etCO_2$  between eucapnia and values which were 8-9torr higher in hypoxia ( $etO_2$  50-60torr). When switching occurred during inspiration, the alternations produced were 6.4% for  $V_t$ , 4.7% for  $t_I$  and 4.7% for  $t_E$ . Alternations were 25% greater when switching of the gases occurred between breaths, i.e. at the start of expiration (analogous to the method I have used). Significant alternations (compared to zero by a *t*-test) were present in at least one variable for all subjects ( $n=4$ ). It is not surprising that so many alternations were significant when compared to zero, as this does not take into account the degree of alternation in control periods. Similarly, if I compared test alternation responses to zero, I would have found many more 'significant responses'. I chose not to compare responses in this way, and adopted a more rigorous approach. The degree of reflex alternation observed by Metias et al. (1981) in respiratory variables is comparable to the values I have found in adults, although the stimuli are different.

Cunningham et al. (1986) alternated  $etCO_2$  between eucapnia and values which were 7-10 torr higher in hypoxia ( $etO_2$  50-70torr) and report mean alternations of about 5% for  $V_{ti, f}$  and  $V_{ti}/t_I$ . They also illustrate the chemoreflex response for one subject that shows *ca.* 10% breath-by-breath alternation in  $V_{ti}$ . Due to the lack of similarity between my experiments and the observations from other workers, either in the method of analysis or the stimuli used, it is not possible to make any further comparisons.

#### **5.4.4 Possible reasons for non-responders not showing significant alternations.**

I found that only 9 of 14 subjects showed significant alternations to the  $F_{iO_2}$  0.21-0.16 alternating stimulus, and 4 of 12 subjects showed significant alternations to the  $F_{iO_2}$  0.26-0.16 alternating stimulus. This is not altogether surprising, as previous studies have selected subjects on the criteria that they exhibit a respiratory alternation (Ward &

Cunningham, 1977a, 1977b; Ward et al, 1979). I have investigated possibilities which may explain this finding.

#### **5.4.4.a Responders showed a greater breath-to-breath change in etO<sub>2</sub> than non-responders**

I found that responders to the alternating stimulus Fio<sub>2</sub> 0.21-0.16 showed a mean  $\pm$  S.E.M. breath-to-breath oscillation in etO<sub>2</sub> of  $0.53 \pm 0.04\%$  which was significantly greater than non-responders  $0.35 \pm 0.02\%$ . This was not the case for the alternating stimulus Fio<sub>2</sub> 0.26-0.16, when the mean  $\pm$  S.E.M. breath-to-breath oscillation in etO<sub>2</sub> was  $0.69 \pm 0.04\%$  for responders and  $0.71 \pm 0.05\%$  for non-responders. Thus it appears for Fio<sub>2</sub> alternations between 0.21 and 0.16 that a smaller stimulus was received by non-responders compared to responders, which could explain the non-significant chemoreflex responses. However for Fio<sub>2</sub> alternations between 0.26 and 0.16, another reason must exist to account for the greater proportion of non-responders.

I am confident that the smaller stimulus received by non-responders is not due to inadequacy of flow of inspired gas. All subjects received a flow of 25l/min which was in excess of peak inspiratory flow, and I was able to check their peak inspiratory flow values using Maclab software. It is possible that there may have been some stimulus dilution if the nose mask was poorly fitting, and hence room air may have been inspired. However, at such high flows it seems unlikely that air would be inspired from around the mask instead of the port where gas entered the mask. This high flow of gas would also clear the dead space of the gas delivery lines, the mask and pneumotachometer (total estimate *ca.* 150ml) in *ca.* 0.36sec. Nor is it likely that the subjects breathed via the mouth, as this would have been indicated by a perturbation of the etO<sub>2</sub> and etCO<sub>2</sub> signals. Another possibility is dilution of the stimulus in the lungs. I have no information on FRC in these subjects, so I am unable to speculate whether non-responders had stimulus dilution due to an increased FRC. These subjects had no history of obstructive respiratory disease so there was no reason to assume that any were breathing at an increased FRC.

There is no obvious reason to explain why some subjects received a smaller stimulus. Non-responders did show a regular alternation in etO<sub>2</sub>, albeit smaller than non-responders (see figures 5.3.5.i-ii). Without repeating the study in non-responders using a more severe level of hypoxia to achieve a greater oscillation in etO<sub>2</sub>, it is not possible to determine whether the reduction in stimulus size was the reason for the absence of any response.

#### **5.4.4.b Non-responders did not show a fall in etCO<sub>2</sub> during test runs**

In section 5.3.7 I measured the mean etCO<sub>2</sub> over the first and last 10 breaths for test runs alternating between Fio<sub>2</sub> 0.21-0.16 and 0.26-0.16. For the group as a whole, there was no decrease in etCO<sub>2</sub> during test runs for either the Fio<sub>2</sub> 0.21-0.16 or 0.26-0.16 alternating stimulus. Nor did responders or non-responders show a fall in etCO<sub>2</sub> during test runs when grouped separately. Therefore, the lack of chemoreflex response was not due to a fall in central respiratory drive caused by hyperventilation.

#### **5.4.4.c Effect of sleep state on chemoreflex response**

Chemoreflex studies were performed during wakefulness on adults, and during quiet sleep in newborn infants (Chapter 3 and 4). I did not have the facilities to study chemoreflex responses in a sleep laboratory for adults, so experiments were carried out with the subject seated during wakefulness. There is conflicting literature as to whether hypoxia chemosensitivity is enhanced, reduced or unchanged during NREM compared to wakefulness (see below).

Read & Kellogg (1977) measured the ventilatory response to 20mmHg steps of PAO<sub>2</sub> between 60-120 mmHg at sea level and at altitude. They assessed sleep state by behavioural observations and found that there was no difference in the response to hypoxia between wakefulness and NREM sleep. Douglas, White, Weil, Pickett, Martin, Hudgel & Zwillich (1982) measured the hypoxic ventilatory response during wakefulness and NREM sleep in adult man, sleep-staged by electroencephalogram recordings (EEG). The hypoxic ventilatory response to PETo<sub>2</sub> <50mmHg was reduced by 33% in NREM sleep compared to wakefulness. Attention was paid to PETco<sub>2</sub> during these experiments, and was maintained within 2mmHg of awake values during sleep. They criticised the earlier findings of Read & Kellogg (1977) who failed to observe a difference in the response to hypoxia between sleep states. Douglas et al. (1982) postulated that the level of hypoxia used was not severe enough and that no effort was made to maintain isocapnia.

In adult rats, the response to 10% hypoxia was greater in NREM sleep than during wakefulness, staged by EEG (Pappenheimer, 1977). Respiratory frequency increased 24% during wakefulness and 54% during NREM sleep, V<sub>E</sub> increased 47% during wakefulness and 74% during NREM sleep. In adult dogs, the response to progressive eucapnic hypoxia was the same during wakefulness and NREM sleep (Phillipson, Sullivan, Read, Murphy & Kozar, 1978). Sleep was staged by EEG in addition to behavioural observations.

In summary, it is difficult to speculate on the effect of wakefulness on the chemoreflex response for my experiments. The literature reports both no change in hypoxia sensitivity between wakefulness and NREM sleep, and either an increased or decreased sensitivity in NREM sleep. It appears that the maintenance of eucapnia is critical in determining a change in sensitivity between sleep states. I have no reason to suspect that any of my subjects were studied during NREM sleep, so it is unlikely that a difference in sleep state accounted for the difference between chemoreflex responses in responders and non-responders. In the future, it may be possible to perform these experiments during NREM sleep so that conditions between studies in the newborn baby are comparable to those in the adult.

#### **5.4.4.d Effect of smoking on chemoreflex response**

Five of 14 subjects did not show a significant chemoreflex response. Of these, 3 were non-smokers and 2 smoked less than 5 cigarettes per day. For the responders, 5 of the 9 subjects were smokers, 4 smoked less than 5 cigarettes per day and 1 smoked 11-15 cigarettes per day. There is some evidence that smoking enhances hypoxia chemosensitivity in man which may be important when considering chemoreflex responses (Yamamoto, Inaba, Nishiura, Kishi & Kawakami, 1985).

In adult men, Yamamoto et al. (1985) measured the ventilatory response to hypoxia whilst inhaling cigarette smoke containing nicotine and cigarette smoke containing minimum-level nicotine. The ventilatory response to hypoxia was increased by 19% during inhalation of the nicotine smoke, but was unaffected by minimum-level nicotine smoke. They presumed that nicotine acted on the peripheral chemoreceptors to augment hypoxic chemosensitivity. However, there was no evidence that smokers *per se* showed increased hypoxia chemosensitivity during periods when nicotine was not inhaled. It has been shown in adult cats that nicotine produces a hyperventilation by excitation of the carotid chemoreceptors (Zapata, Zuazo & Lladós, 1976). Mulligan & Lahiri (1987) have suggested that nicotine increased chemoreceptor discharge by stimulation of ganglionic-nicotinic receptors.

In newborn rats, nicotine exposure appears to reduce hypoxia chemosensitivity. Holgert, Hokfelt, Hertzberg & Lagercrantz (1995) measured the ventilatory response to 100% O<sub>2</sub> breathing in 3d old rat pups. Nicotine exposure caused a 70% reduction in the ventilatory response, and this effect was prevented by pharmacological blockade of peripheral dopamine type 2 receptors. Nicotine also reduced dopamine content of the carotid body and increased expression of tyrosine hydroxylase, which suggests an increased dopamine release to hypoxia and an up-regulation of dopamine synthesis

(refer also to section 1.3.4 for reference to tyrosine hydroxylase). The authors postulated that the prolonged release of dopamine, and the increase in tyrosine hydroxylase, could interfere with postnatal resetting of hypoxia sensitivity.

In newborn lambs, the effects of nicotine exposure on the ventilatory response to hypoxia and hyperoxia appears to be paradoxical (Milerad, Larsson, Lin & Sundell, 1995). Nicotine augmented the fall in  $V_E$  observed during 20sec of 100% O<sub>2</sub> at 7, 17 and 27d, but was only significant in the oldest age group. In contrast the early response to 10% O<sub>2</sub> was significantly reduced at 7 and 27d. These responses can be explained in terms of nicotine interfering with chemoreceptor responses at a time when resetting occurs. Milerad et al. (1995) also suggest a possible role for dopamine in mediating this response.

The inhibitory effect that nicotine has on hypoxia sensitivity in the neonate can be explained in terms of chemoreceptor resetting to hypoxia, as it is likely that nicotine interferes with resetting and reduces the reflex response to hypoxia in the newborn (Holgert et al, 1995). Dopamine is an inhibitory neuromodulator of carotid body function in the newborn, as in the adult (Bisgard, Forster, Klein, Manohar & Bullard, 1980; Maycock et al, 1983; Marchal et al, 1992b; refer also to section 1.5.3). At birth, there is a large increase in carotid body dopamine turnover, and the concentration of dopamine falls postnatally (Hertzberg et al, 1990; Hertzberg et al, 1992; Lagercrantz, Pequignot, Hertzberg, Holgert & Ringstedt, 1994). Cat carotid bodies studied *in vitro* show not only that dopamine was released in response to hypoxia and nicotine, but that nicotine increased the synthesis of dopamine (Dinger, Gonzalez, Yoshizaki & Fidone, 1985). It is likely that nicotine exposure in the newborn increases the dopamine content of carotid bodies, and prevents the normal decline observed postnatally, thus prolonging the inhibitory effect of dopamine on the chemoreflex. Nicotine exposure before, after or around the time of birth may irreversibly disrupt the process of chemoreceptor resetting.

For comparisons with my experiments it is most useful to use the observations of Yamamoto et al. (1985). This observation could be used to explain greater than expected chemoreflex responses in the case of nicotine exposure. It does not offer an explanation for the lack of response observed in non-responders, so in this context it seems probable that there was no influence of the smoking in 2 of the 5 non-responders.

#### 5.4.4.e Possibility of a type 2 error: accepting the null hypothesis when in fact a significant alternation exists.

In section 3.4.2 I discussed the method of analysis I have used for respiratory chemoreflex responses, and that the criteria I have used to detect significant alternations were in fact quite stringent. Similarly, it is possible that these criteria have in fact increased the likelihood of a type 2 error, i.e. that I have rejected a response as non-significant when it is fact significant. Due to the approach I have used in analysis, a type 2 error could occur if a subject showed a large alternation to a test run, but that the alternation shown during control runs was also large. Whilst a large alternation may have been greater than the critical value for that respiratory variable (see table 2.3.4.d.i), if the alternation during test runs was not twice as large as the greatest control alternation, the response was not deemed to be significant. Clearly, there may be occasions where control responses are not ideal because the subject exerted a greater behavioural control of breathing which increased perturbations in the respiratory pattern. So this may have been a factor determining the absence of significant responses, for example in subjects NGSC (in respiratory variables  $f$  and  $V_E$ ) and PDB (in  $V_{te}/t_E$ ; see table 5.3.2.ii). If I had used the methods of Metias et al. (1981) and Cunningham et al. (1986) who compared the value of the alternation to zero by  $t$ -test, then these subjects would have shown significant responses. However, I prefer to use the method of analysis described in this thesis, and it may be possible in the future to reduce the chance of a type 2 error by refining the criteria for significant responses.

#### 5.4.5 Which test is a more effective stimulus?

I found that 64% of subjects responded to alternations in  $F_{iO_2}$  between 0.21 and 0.16, and only 33% of subjects responded to alternations in  $F_{iO_2}$  between 0.26 and 0.16. For responders the mean  $\pm$  S.E.M. breath-by-breath oscillation in  $etO_2$  was not significantly different between the two types of tests;  $0.53 \pm 0.04\%$  for alternations between  $F_{iO_2}$  0.21-0.16, and  $0.69 \pm 0.04\%$  for alternations between  $F_{iO_2}$  0.26-0.16. In section 5.3.6 I measured the mean  $etO_2$  at the beginning and end of test runs. At the end of a test run, mean  $etO_2$  was significantly less than control values ( $14.6 \pm 0.2\%$ ) for the alternating stimulus  $F_{iO_2}$  0.21-0.16 ( $12.4 \pm 0.2\%$ ) but not for alternating stimulus  $F_{iO_2}$  0.26-0.16 ( $14.2 \pm 0.2\%$ ).

Due to the hyperbolic relationship between  $P_{O_2}$  and chemoreceptor discharge, a stimulus that lowers mean  $etO_2$  has important consequences for the respiratory response. At a higher  $etO_2$  and hence  $PaO_2$ , the chemoreceptor response curve is

flatter compared to at a lower PaO<sub>2</sub>. At a mean etO<sub>2</sub> of 12.4%, PaO<sub>2</sub> would be *ca.* 90mmHg with an oscillation of  $\pm 5$ mmHg, and for a mean etO<sub>2</sub> of 14.6%, PaO<sub>2</sub> would be *ca.* 100mmHg with an oscillation of  $\pm 5$ mmHg. For an equivalent breath-by-breath oscillation in etO<sub>2</sub>, a greater oscillation in discharge will be produced at the lower mean etO<sub>2</sub>. So I would expect that the test response alternating between Fio<sub>2</sub> 0.21-0.16 produced a greater oscillation in discharge. I am not able to confirm this from my observations, and nerve recordings of chemoreceptor activity would be required to substantiate this.

Ideally, it would be advantageous to perform an additional set of experiments whereby the mean etO<sub>2</sub> was held at the same level as for alternations between Fio<sub>2</sub> 0.21-0.16, but the amplitude of the oscillation was increased. Unfortunately I was unable to do this. Clearly, in these experiments alternations between Fio<sub>2</sub> 0.21-0.16 were more effective in eliciting a chemoreflex response than alternations between Fio<sub>2</sub> 0.26-0.16. I speculate that this was due to a lower mean etO<sub>2</sub> level in the first instance which augmented the oscillation in chemoreceptor discharge for the same amplitude of oscillation in etO<sub>2</sub>.

#### **5.4.6 Variability of the chemoreflex response**

In section 5.3.4 I compared the chemoreflex responses of 3 subjects measured on 3 different days. I found that both the magnitude of the response, and the number of respiratory variables that showed a significant alternation was different between different studies in the same individual. However, it was possible to find a significant alternation in at least one respiratory variable on each occasion.

This is not the first occasion that variability of the respiratory chemoreflex response has been reported in adult man. It is important to mention the studies of Ward & Cunningham (1977a, 1977b) and Ward et al. (1979) who selected their subjects on the basis that they showed a chemoreflex response. Under these conditions variability of the chemoreflex response may be reduced compared to an un-selected sample. These workers report, as does Metias et al. (1981), that an alternation of some sort was present in at least one respiratory variable. The chemoreflex response was not necessarily manifested in the same variable on repeated occasions or between different individuals.

There have been a number of studies that have demonstrated the relationship between the phase of the respiratory cycle in which a stimulus is delivered to the carotid body

and the effect exerted on respiratory pattern (Band, Cameron & Semple, 1970; Black & Torrance, 1971; Eldridge, 1972; Nye, Hanson & Torrance, 1981). Band et al. (1970) made injections of 100% CO<sub>2</sub> equilibrated saline into the carotid of adult cats, and observed that the effect on respiration was determined by the phase in the respiratory cycle in which the stimulus was delivered. During expiration the effects were variable and occasionally t<sub>E</sub> was prolonged, but expiratory volume or the next inspiration was unaffected. Injections made early in inspiration produced an increase in tidal volume. Black & Torrance (1971) observed similar effects to Band et al. (1970). Injections of 100% CO<sub>2</sub> equilibrated saline made early in inspiration increased the depth of the inspiration, but injections late in inspiration may be without effect. Injections during expiration were less effective; during early expiration t<sub>E</sub> was prolonged and during late expiration injections might evoke the next inspiration prematurely. Black & Torrance (1971) also produced these observations by electrically stimulating the carotid sinus nerve. Eldridge (1972) investigated the effect of several agents on respiratory pattern: venous blood, 100% CO<sub>2</sub> equilibrated saline, 100% CO<sub>2</sub> equilibrated NaHCO<sub>3</sub>, saline equilibrated with N<sub>2</sub>, NaCN and NH<sub>4</sub>OH. The most repeatable effect on respiration of carotid body responses was with NaHCO<sub>3</sub>. Early in inspiration stimulation of the carotid chemoreceptors increased mean inspiratory flow and shortened t<sub>I</sub>, however tidal volume was only increased by stimulation later in inspiration when t<sub>I</sub> increased. During expiration, t<sub>E</sub> was increased by stimulation and the effect was greater later in expiration. Bilateral carotid body denervation abolished these responses. Eldridge (1972) concluded that these effects on respiration of changing the phase of carotid chemoreceptor stimulation were mediated by central respiratory neurones because the increase in CSN discharge was equivalent at the different times of stimulation.

Nye et al. (1981) produced similar effects on respiration by making injections of 100% O<sub>2</sub> equilibrated Ringer's solution into the carotid artery. This method of carotid chemoreceptor stimulation, or perhaps more appropriately quiescence, was adopted so as to produce more of a step change at the carotid body by abruptly stopping chemoreceptor discharge. Injections made during inspiration reduced its volume and prolonged the immediately following expiration. During expiration, injections in early expiration prolonged t<sub>E</sub> and during late expiration they shortened it.

Small changes in the respiratory frequency will alter the relationship between the phase of respiration in which the carotid body is stimulated and the effect produced in respiration. The respiratory phase at which an oscillation in chemoreceptor discharge arrives at the CNS will influence the chemoreflex response. Thus, it is not surprising that alternations in different respiratory variables were seen between successive periods of alternate breath stimulation. Black & Torrance (1971) pointed out the particular

significance of this effect during exercise, whereby a change in respiratory frequency will affect the relationship between the oscillations in chemoreceptor discharge and respiratory pattern. It does in part explain the variation in response within individuals. It is not possible to standardize the arrival of the alternation in PaO<sub>2</sub> at the carotid body, so variability in the pattern of the chemoreflex response is unavoidable.

It is also important to consider the sensitivity of the carotid body to hypoxia to explain the variation in response between subjects. From figures 5.3.4.i-iii, it can be seen that larger reflex alternations were found for NAC compared to SJW and BAW, and for SJW compared to BAW. Vizek, Pickett & Weil (1987) postulated that variability in the hypoxic ventilatory response between adult cats was due to differences in peripheral chemoreceptor sensitivity. Chemoreceptor responses to hypoxia were positively correlated with ventilatory responses, however there was no relationship between the increment in V<sub>E</sub> with the increment in CSN discharge for the same reduction in PETO<sub>2</sub> (150 to 40 torr). Studies in adult humans have recognised genetic factors as a major determinant in inter-individual differences in hypoxia chemosensitivity (Collins, Scoggin, Zwillich & Weil, 1978; Kawakami, Yoshikawa, Shida, Asanuma & Murao, 1982; Kawakami, Yamamoto, Yoshikawa & Shida, 1984).

#### **5.4.7 Comparison of infant and adult chemoreflex responses**

The purpose of these experiments was to establish a range of responses in the adult to alternate breaths of Fio<sub>2</sub> 0.21 and 0.16. I was interested in comparing the degree of reflex alternation in respiratory variables for adults to responses previously measured in infants. In Chapter 3 I speculated that the human infant at *ca.* 6wks of age showed a mature respiratory chemoreflex to hypoxia. In section 5.3.9 I compared adult and infant chemoreflex responses, and found that they occurred over a similar range. Furthermore, there was no significant difference between the magnitude of the chemoreflex response for any of the respiratory variables in infants and adults.

To facilitate comparison of chemoreflex responses between adults and infants, I averaged responses for each respiratory variable and subtracted the degree of alternation during control runs from test runs. I found that this was necessary because adults showed greater alternation during control runs than infants, which may be accounted for on the basis of a difference in sleep states (as mentioned in section 5.4.4.c) and a greater behavioural control of breathing in adults. It could be argued that test responses should be compared without this modification, however then the information derived from performing control experiments is essentially neglected. I felt that I needed to take into

account the variation in respiratory variables during control to be able to compare between the ages. If I had the facilities to perform sleep studies in adults, then it may have been appropriate to compare between test alternations directly if it could be shown that a similar degree of alternation occurred during control runs in both adults and infants in quiet sleep.

There have been some reports in adult humans of a decrease in hypoxia chemosensitivity with age. Marcus, Glomb, Basinki, Davidson Ward & Keens (1994) measured the hypoxic ventilatory response to isocapnic hypoxic rebreathing in children ( $10 \pm 3$  yr;  $n=35$ ) and in adults ( $36 \pm 8$  yr;  $n=24$ ). When ventilatory responses were corrected for body weight, there was a significant reduction with increasing age. Furthermore in children the hypoxic ventilatory response was 46% greater than in adults. Children also showed significantly greater baseline values for  $V_E, f$  and  $V_t/t_I$  compared to adults. The authors speculated that the difference in response between children and adults was due to an elevated metabolic rate in children. Kawakamai, Yamamoto, Yoshikawa & Shida (1985) observed greater ventilatory responses to hypoxia in children aged  $16.3 \pm 0.9$  yr (mean  $\pm$  S.E.M.) compared to adults aged  $29.8 \pm 6.0$  yr and  $46.0 \pm 7.2$  yr.

Other studies report a decrease in hypoxia chemosensitivity throughout adulthood. Kronenberg & Drage (1973) found that the hypoxic ventilatory response to hypoxia was greater in young adults (22-30yr) compared to older adults (64-73yr). In a longitudinal study, Nishimura, Yamamoto, Yoshioka, Akiyama, Kishi & Kawakami (1991) measured the hypoxic ventilatory response to progressive lowering of  $PET_{O_2}$  from 120 to 40mmHg whilst maintaining isocapnia in men ( $n=32$ ) aged  $32 \pm 1.4$  yr and again at  $42.2 \pm 1.4$  yr. Ventilatory responses were correlated between the two studies, and there was a significant reduction in hypoxia sensitivity (27%) with age. There were also small, but significant decreases in vital capacity,  $FEV_1/FVC$  and maximal voluntary ventilation. However the authors suggest that the reduction in the hypoxic ventilatory response was due to a reduction in hypoxia chemosensitivity because the fall in the HVR was greater than could be explained by the fall in vital capacity,  $FEV_1/FVC$  and maximal voluntary ventilation.

These studies provide evidence for a reduction in the hypoxic ventilatory response between childhood and adulthood, perhaps in part mediated by a fall in hypoxia chemosensitivity. It could be argued from my results that there are also changes in chemosensitivity with age between infancy and adulthood. Two possibilities exist, first that hypoxia chemosensitivity increases between infancy and childhood, or secondly that if respiratory chemoreflexes to hypoxia show no change between infancy and

childhood, a decrease in hypoxia chemosensitivity may be observed in adulthood or later life. The age range in my study is too large to speculate on the likelihood of these possibilities, although it would be interesting to assess the respiratory chemoreflex to the alternate breath test in childhood to document changes after infancy.

#### **5.4.8 Summary**

The purpose of these experiments was to acquire a range of responses to the alternate breath test in the adult, which would provide a useful comparison when assessing chemoreflex hypoxia sensitivity in the newborn. I have shown that adults exhibit a respiratory chemoreflex to single breath alternations in  $F_{iO_2}$ , and that the response was greater for alternations between  $F_{iO_2}$  0.21-0.16 compared to  $F_{iO_2}$  0.26-0.16. My method of analysis has shown that the degree of reflex alternation to the hypoxic stimulus is no greater in adults compared to the infants reported in Chapter 3. This provides evidence for infants showing a mature chemoreflex response to hypoxia at 6wks of age, however further studies during childhood are necessary to rule out the possibility that hypoxia chemosensitivity increases between infancy and childhood, and then declines between childhood and adulthood.

## **6.0 MATURATION OF CAROTID CHEMORECEPTOR STEADY STATE CO<sub>2</sub> RESPONSES**

### **6.1 Introduction**

During fetal life arterial blood gas content is determined by the oxygenation of maternal arterial blood, blood supply to and gaseous exchange by the placenta. Fetal PaCO<sub>2</sub> is a few mm Hg higher than maternal PaCO<sub>2</sub>, however the difference between fetal and maternal PaCO<sub>2</sub> is not nearly as great as that for PaO<sub>2</sub>. The transition between fetal and neonatal hypoxia sensitivity of the carotid body has been well documented (see Chapter 3), and the time course for postnatal resetting of hypoxia chemosensitivity measured in several species. In contrast, sensitivity of the carotid body to CO<sub>2</sub> in the neonatal period has been much less investigated and there is little information available on the postnatal changes in CO<sub>2</sub> sensitivity. The majority of studies have focused on the ventilatory response to inspired CO<sub>2</sub> and measured the postnatal maturation of the respiratory chemoreflex. Thus there was a need to define steady state chemoreceptor responses to CO<sub>2</sub> in the newborn period and measure CO<sub>2</sub> sensitivity at different ages and at a range of PaO<sub>2</sub>s.

A few studies have measured steady state chemoreceptor responses to CO<sub>2</sub> in the newborn period. Marchal et al. (1992a) recorded from single chemoreceptor fibres in anaesthetised newborn kittens at two different ages and at three levels of inspired P<sub>CO2</sub> in oxygen. They found an increase in chemoreceptor discharge with an increase in inspired P<sub>CO2</sub>, and the response of kittens aged 10d or older was greater than the response for kittens under 10d of age. Arterial blood gas samples were taken in some of these kittens, and when chemoreceptor discharge was plotted as a function of PaCO<sub>2</sub>, the chemoreceptor response curve for older kittens was steeper than for younger kittens. The chemoreceptor response curve for kittens less than 10d of age was also displaced to the right compared to older kittens. Although Marchal et al. (1992a) found a postnatal increase in the chemoreceptor response to CO<sub>2</sub> when measured during oxygen, there was no attempt made to measure chemoreceptor responses to CO<sub>2</sub> at any other P<sub>O2</sub>s.

Carroll et al. (1993) measured chemoreceptor responses to CO<sub>2</sub> in anaesthetised kittens aged 1, 4 and 8wk compared these responses to those in anaesthetised adult cats. Their preparation involved recording from the whole CSN at three levels of PaO<sub>2</sub>(40-50, 90-100 and >300 torr), normalized to a percentage of discharge in normocapnic normoxia. They claimed to remove baroreceptor activity by 'mechanical and thermal modification', which seems likely to risk causing damage to chemoreceptor fibres. They found that

chemoreceptor responses to CO<sub>2</sub> were smallest in the youngest kittens compared to older kittens and adult cats at any PaO<sub>2</sub>. Chemoreceptor responses to CO<sub>2</sub> were greater during hypoxia than in hyperoxia or normoxia, but this interaction between CO<sub>2</sub> and O<sub>2</sub> was only significant in 8wk old kittens and adult cats. They found that the adult CO<sub>2</sub> chemoreceptor responses were steeper at all PaO<sub>2</sub>s and in addition showed a plateauing of discharge at Pco<sub>2</sub>s greater than 60 torr. These observations are in agreement with those of Marchal et al. (1992a), in that there is a postnatal maturation of the steady state chemoreceptor response to CO<sub>2</sub>. However, chemoreceptor recordings made from whole CSN are difficult to interpret and there is always the possibility of some residual baroreceptor activity.

Another approach taken by Pepper et al. (1995) was to measure the single fibre chemoreceptor responses to CO<sub>2</sub> in the rat carotid body *in vitro*. CO<sub>2</sub>-O<sub>2</sub> interaction in adult rat carotid bodies was compared to rat pups aged 5-7d. Rat pups showed an increase in discharge for increases in Pco<sub>2</sub> as for the adult rats, however there was no significant increase in CO<sub>2</sub> sensitivity at lower Po<sub>2</sub>s as demonstrated in the adult rat. They suggested that the postnatal maturation of carotid body hypoxia sensitivity could be due to the development of interaction between CO<sub>2</sub> and O<sub>2</sub>. One problem with this preparation is the lack of information available on tissue Po<sub>2</sub> and Pco<sub>2</sub>, and as it is not possible to take blood gas samples, conclusions on chemoreceptor activity must be based on the Po<sub>2</sub> and Pco<sub>2</sub> of the perfusate.

Ventilatory studies that have measured steady state CO<sub>2</sub> sensitivity of the carotid body are frequently complicated by the fact that the respiratory response may reflect, to a certain extent, stimulation of the central medullary chemoreceptors. There are however a few studies that have aimed to dissociate the peripheral and steady state components of the respiratory chemoreflex.

Wolsink et al. (1993) used the dynamic end-tidal forcing technique to assess the relative contribution the peripheral and central chemoreceptors made to the ventilatory response to CO<sub>2</sub> in anaesthetised newborn piglets. They reported a postnatal increase in CO<sub>2</sub> sensitivity, when the peripheral chemoreceptor component was expressed as a proportion of total CO<sub>2</sub> sensitivity. Neither the peripheral chemoreceptor component *per se*, nor the total ventilatory response showed a significant increase with age, and responses varied greatly at any age. This implies that the central component of the total respiratory response decreased with age if the peripheral chemoreceptor response increased with age. This seems unlikely as no such reports have been made elsewhere.

There have also been several reports that CO<sub>2</sub> sensitivity does not change postnatally. Elnazir & Kumar (1993) measured the ventilatory response of conscious newborn rats to 0.03 and 0.06 Fico<sub>2</sub> over the first minute of exposure. In this way they made a comparison to the biphasic ventilatory response of the neonate to hypoxia. They observed no increase in CO<sub>2</sub> sensitivity between 1-2 days and 8-10 days, however found a maturation of hypoxia sensitivity over the same period.

Jansen, Ioffe & Chernick (1992) measured phrenic activity to inspired CO<sub>2</sub> in anaesthetised, vagotomised and artificially ventilated lambs at three ages. To determine the apnoeic threshold, lambs were hyperventilated in oxygen until phrenic nerve activity ceased, and then etCO<sub>2</sub> was raised in 0.5% steps until respiratory activity was re-established. CO<sub>2</sub> sensitivity was compared before and after carotid sinus nerve denervation. In intact lambs, there was no difference in the mean apnoeic threshold with age. CSN denervation increased the apnoeic threshold, although there was still no difference between lambs of different ages. They concluded that there is no postnatal increase in carotid body steady state CO<sub>2</sub> sensitivity, and that central chemoreceptors are functionally mature shortly after birth.

Moss, Jakubowska, McCrabb, Billings & Harding (1995) measured ventilatory responses to progressive hypercapnia using a rebreathing technique in newborn lambs from birth to six weeks and also in ewes. Lambs rebreathed CO<sub>2</sub> via a rubber bag for 2-3 min, and O<sub>2</sub> was added to the system to maintain an Fio<sub>2</sub> of approximately 0.21. CO<sub>2</sub> sensitivity was measured as the slope of the ventilatory response, and there was no change in CO<sub>2</sub> sensitivity with postnatal age. However, there was an increase in hypoxia sensitivity over this period.

Thus, to make any firm conclusions on the maturation of carotid body steady state CO<sub>2</sub> sensitivity it was necessary to record directly from the carotid sinus nerve. There was a lack of information available on CO<sub>2</sub> sensitivity at a range of PaO<sub>2</sub>s, and interaction between CO<sub>2</sub> and O<sub>2</sub> had been poorly investigated in the neonate. Therefore, the purpose of these experiments was to measure steady state CO<sub>2</sub> sensitivity at several different PaO<sub>2</sub>s over a range of ages in the lamb. Some of these observations have been published in brief (Calder, Kumar & Hanson, 1995a).

## 6.2 Methods

### 6.2.1 Comparison of PaCO<sub>2</sub> and etCO<sub>2</sub> for increases in steady state CO<sub>2</sub>

Steady state CO<sub>2</sub> levels were quantified both by blood gas analysis and by mass spectrometry. In Chapter 7, it was only possible to measure CO<sub>2</sub> during alternations in inspired CO<sub>2</sub> by mass spectrometry. I compared PaCO<sub>2</sub> measured from blood gas analysis to etCO<sub>2</sub> measured from mass spectrometry as an estimate of PaCO<sub>2</sub> in figure 6.2.1.i. Data points for all steady state CO<sub>2</sub> levels at all ages have been included. Linear regression was used to describe the relationship between PaCO<sub>2</sub> and etCO<sub>2</sub> and the correlation coefficient was 0.883. The 2 variables were well correlated between PaCO<sub>2</sub> 30-75 mmHg, and outside of this range there were outliers that were not well described by the regression line.

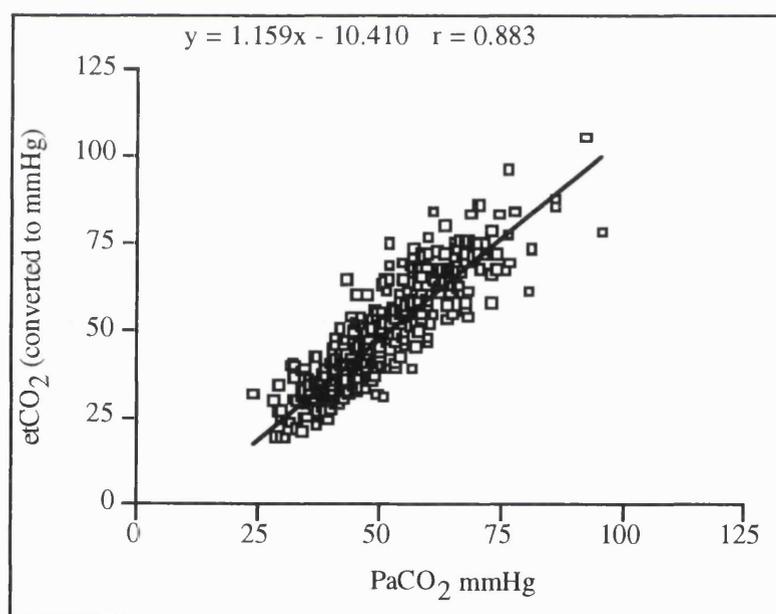


Figure 6.2.1.i Comparison of PaCO<sub>2</sub> (mmHg) measured by blood gas analyser and etCO<sub>2</sub> (measured by mass spectrometer as a percentage and then converted to mmHg) for steady state CO<sub>2</sub>. Curve of best fit and correlation coefficient by linear regression.

## 6.3 Results

### 6.3.1 Steady state chemoreceptor responses to CO<sub>2</sub>

Steady state chemoreceptor responses in few or multi-fibre chemoreceptor preparations are summarised in table 6.3.1.i. There was a total of 56 fibres (43 lambs); 16 fibres at 3-4d, 19 fibres at 5-9d and 21 fibres at 10-24d. It was not possible to make recordings for all fibres at all PaO<sub>2</sub>s.

	Total n	HYP	NX	MOD HX	SVHX
3-4d fibres	16	2	9	10	5
lambs	13	2	8	8	5
5-9d fibres	19	5	14	9	5
lambs	13	5	10	7	4
10-24d fibres	21	5	13	11	4
lambs	17	5	12	10	4

Table 6.3.1.i Summary of protocols for all fibre preparations.

A typical response of a chemoreceptor fibre to increasing PaCO<sub>2</sub> is shown as an example in figure 6.3.1.i. This lamb aged 8d showed an increase in both raw and integrated CSN discharge with PaCO<sub>2</sub> during normoxia. There was no change in blood pressure for an increase in PaCO<sub>2</sub>.

Steady state chemoreceptor responses to CO<sub>2</sub> were measured at several etCO<sub>2</sub> levels in hyperoxia (HYP), normoxia (NX), moderate hypoxia (MOD HX) and severe hypoxia (SVHX). Four examples of chemoreceptor responses to steady state CO<sub>2</sub> at four different ages are shown in figures 6.3.1.ii-iii. In figure 6.3.1.ii the chemoreceptor response to CO<sub>2</sub> is shown for one lamb at 3d during HYP, NX, MOD HX and SVHX, and another lamb aged 4d during NX, MOD HX and SVHX. In both lambs CSN discharge increased when PaCO<sub>2</sub> increased, however the 3d old lamb was unable to sustain an increase in discharge during SVHX. The 4d lamb showed an increase in discharge with PaCO<sub>2</sub> during SVHX, and the slope of this chemoreceptor response curve was greater than NX or MOD HX.

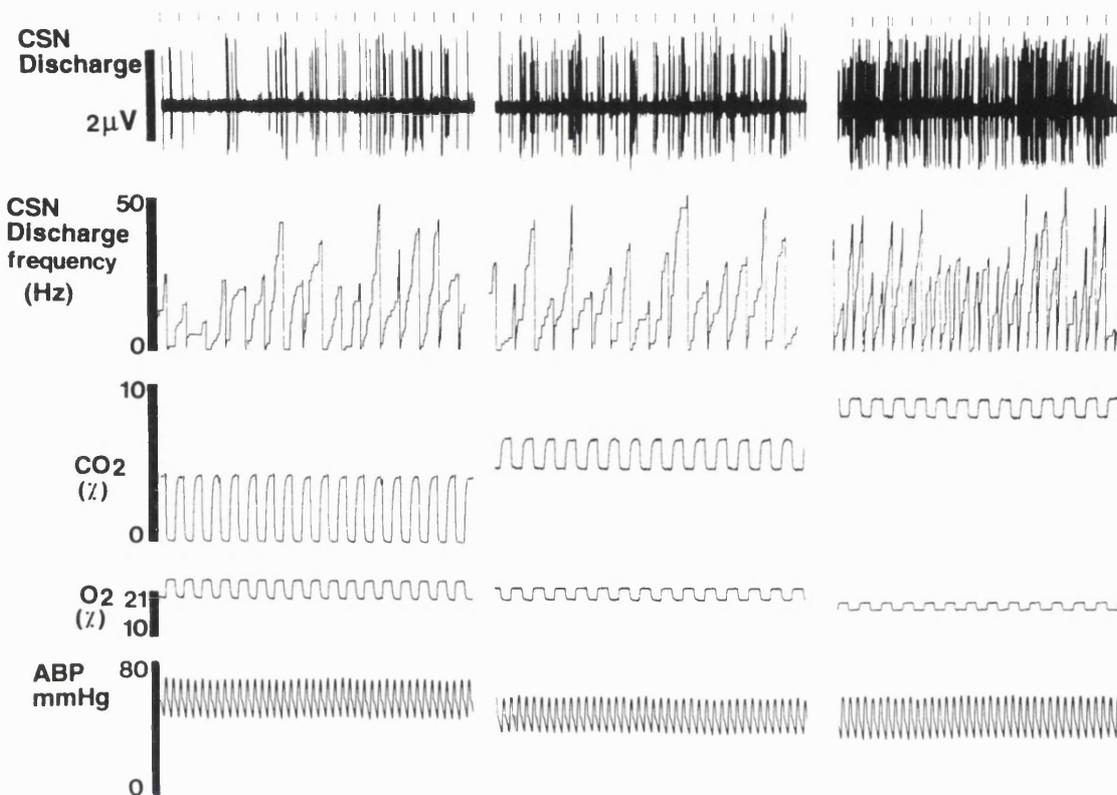


Figure 6.3.1.i. Steady state chemoreceptor responses for a lamb aged 8d during NX. Raw ( $\mu\text{V}$ ) and integrated CSN discharge (Hz), CO<sub>2</sub> (%), O<sub>2</sub> (%) and arterial blood pressure (ABP mmHg).

Blood gas analysis:      Left: pH- 7.464; PaCO<sub>2</sub>- 35.2 mmHg; PaO<sub>2</sub>- 95 mm Hg  
    Centre: pH- 7.321; PaCO<sub>2</sub>- 50.0 mmHg; PaO<sub>2</sub>- 105 mm Hg  
    Right: pH- 7.226; PaCO<sub>2</sub>- 62.9 mmHg; PaO<sub>2</sub>- 105 mm Hg

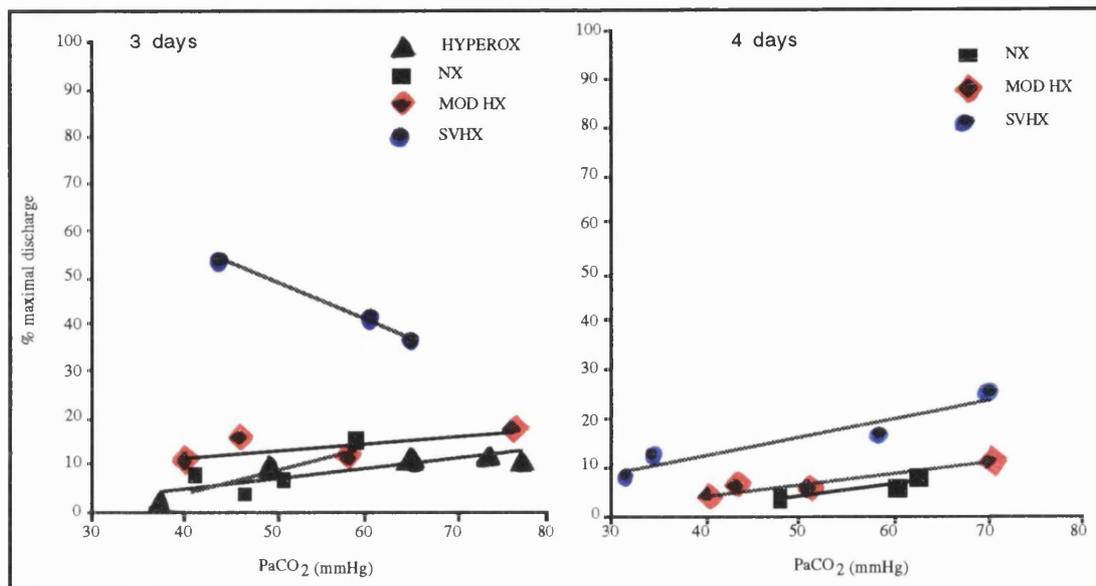


Figure 6.3.1.ii An example in one lamb aged 3d (left) and another lamb aged 4d (right) of CO<sub>2</sub> chemoreceptor response curves (% maximal discharge) as a function of PaCO<sub>2</sub> (mmHg) during HYP, NX, MOD HX and SVHX. The lamb aged 3d shows does not sustain discharge in SVHX, whereas the lamb at 4d does show a sustained increase in discharge in SVHX with increasing CO<sub>2</sub>.

In figure 6.3.1.iii the chemoreceptor response to CO<sub>2</sub> is shown for one lamb at 11d during NX, MOD HX and SVHX, and another lamb aged 8d during HYP, NX, MOD HX and SVHX. The 11d old lamb showed an increase in CSN discharge with PaCO<sub>2</sub> which was sustained during SVHX. The 8d old lamb showed an increase in discharge during HYP, NX and MOD HX with PaCO<sub>2</sub> but this was not sustained during SVHX. The slope of the chemoreceptor response curve was similar during HYP and NX, but was increased in MOD HX. I frequently found during SVHX that chemoreceptor fibres were unable to sustain an increase in discharge for an increase in PaCO<sub>2</sub>. This was more common in older lambs than younger lambs and is discussed further in section 6.3.5.

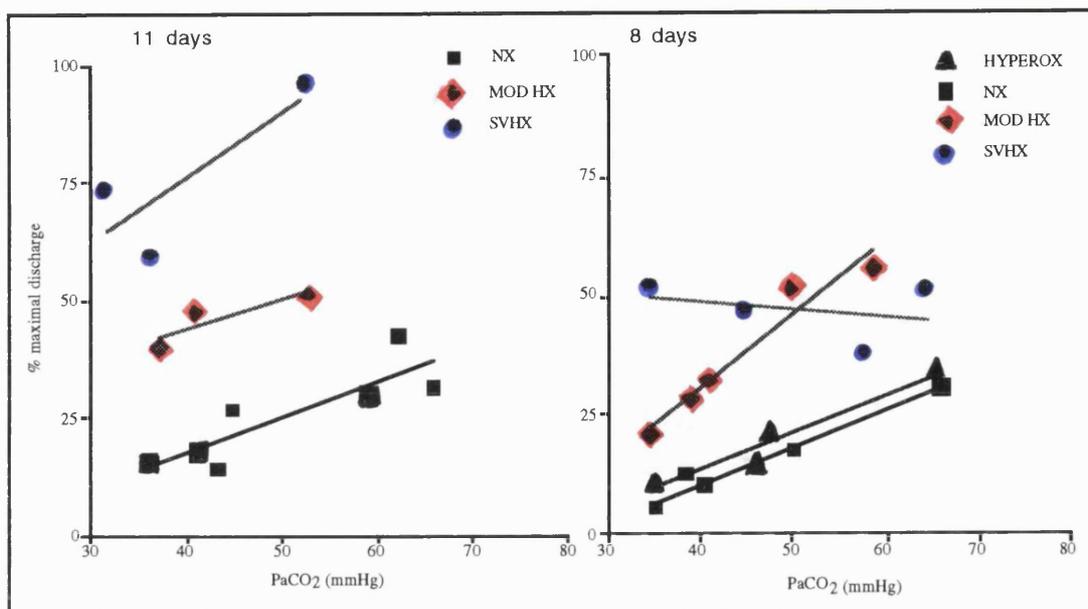


Figure 6.3.1.iii An example in one lamb aged 11d (left) and another lamb aged 8d (right) of CO<sub>2</sub> chemoreceptor response curves (% maximal discharge) as a function of PaCO<sub>2</sub> (mmHg) during HYP, NX, MOD HX and SVHX. The lamb aged 8d shows does not sustain discharge in SVHX, whereas the lamb at 11d does show a sustained increase in discharge in SVHX with increasing CO<sub>2</sub>.

### 6.3.2 Chemoreceptor CO<sub>2</sub> responses in Hyperoxia

Steady state chemoreceptor responses to CO<sub>2</sub> during hyperoxia are plotted for all fibres as a function of PaCO<sub>2</sub> (figure 6.3.2.i) and etCO<sub>2</sub> (figure 6.3.2.ii). Linear regression lines have been fitted for each distribution of points. For lambs aged 3-4d and in older lambs, there was increase in CSN discharge when PaCO<sub>2</sub> increased. The slope of the chemoreceptor CO<sub>2</sub> response curve increased in both older age groups. This suggests an increased CO<sub>2</sub> chemosensitivity in older lambs.

For each fibre linear regression was used to fit the chemoreceptor response and they were then grouped according to age. This corrected for each fibre being represented by more than one point as in figure 6.3.2.i. The data is given in table 6.3.2.i. There was an effect of age on chemoreceptor CO<sub>2</sub> responses between some ages. The chemoreceptor response curve for lambs at 10-24d was significantly steeper compared to 5-9d lambs (Mann-Whitney U test,  $p < 0.04$ ) but not to lambs aged 3-4d.

	slope	y intercept	r		slope	y intercept	r	
3-4days n=2	0.31	-8.38	0.83	10-24 days n=6	1.09	-26.40	0.93	
	0.55	-25.06	1.00			0.99	-21.50	1.00
mean	<b>0.43</b>	<b>-16.72</b>	<b>0.91</b>		0.65	-20.60	1.00	
5-9days n=5	0.49	-1.37	1.00	mean	0.69	-24.54	0.89	
	0.40	-8.44	0.41			0.41	-12.13	1.00
	0.16	-1.79	0.91		S.E.M	<b>0.77</b>	<b>-21.04</b>	<b>0.97</b>
	0.77	-16.87	0.98			<b>0.12</b>	<b>2.46</b>	<b>0.02</b>
	0.24	-1.85	0.85					
mean	<b>0.41</b>	<b>-6.06</b>	<b>0.83</b>					
S.E.M	<b>0.11</b>	<b>3.00</b>	<b>0.11</b>					

Table 6.3.2.i Summary of linear regression analysis for chemoreceptor responses to CO<sub>2</sub> in hyperoxia.

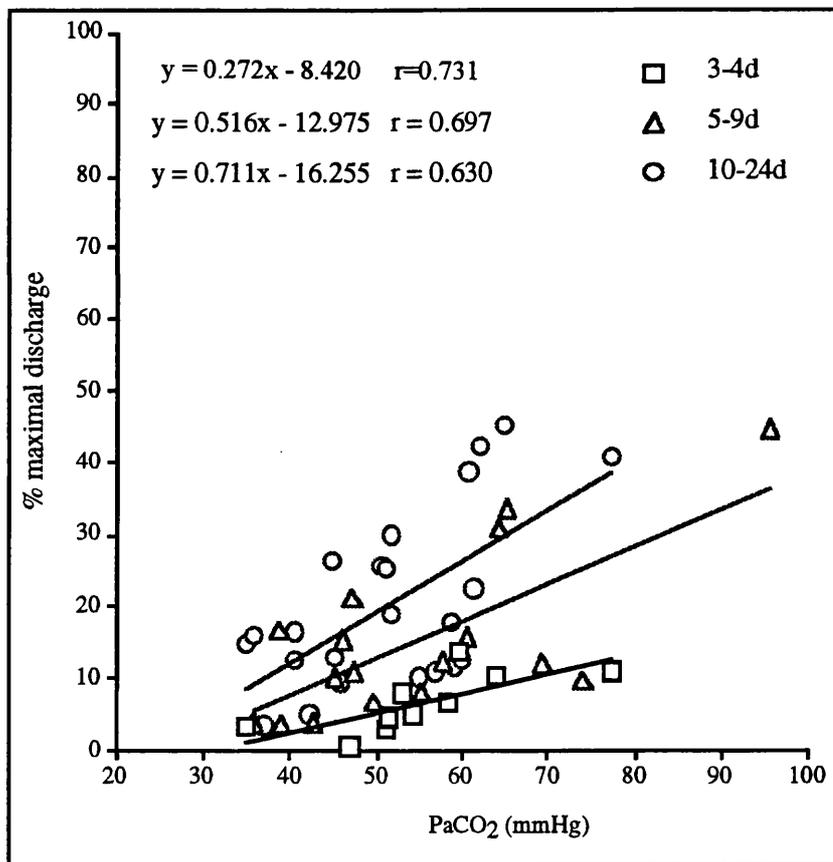


Figure 6.3.2.i Steady state chemoreceptor responses (% maximal discharge) plotted against PaCO<sub>2</sub> (mmHg) during hyperoxia for lambs aged 3-4d, 5-9d and 10-24d.

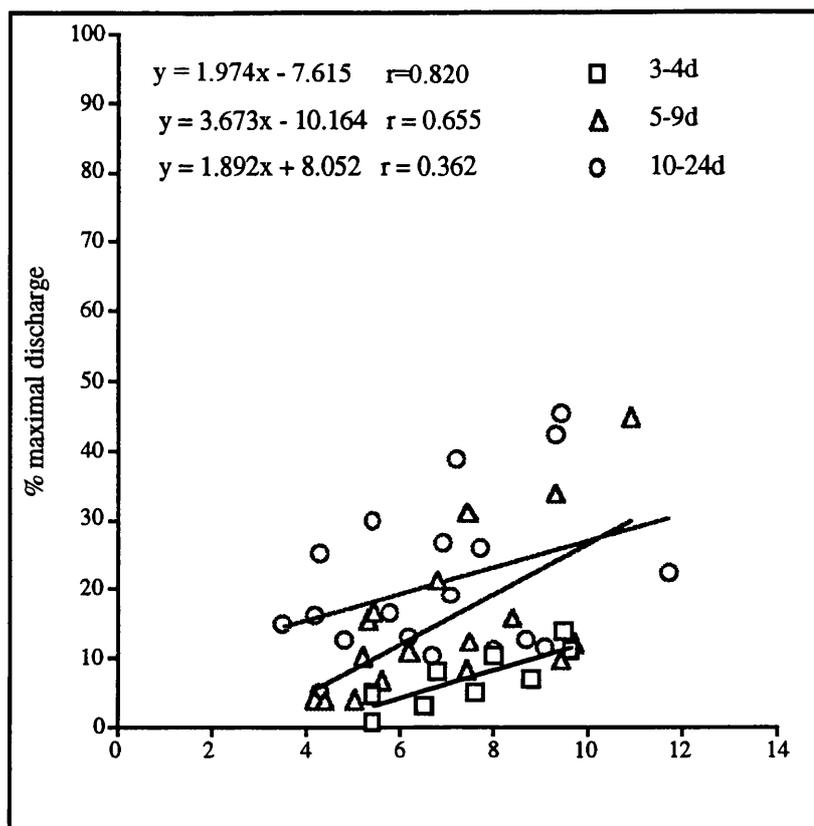


Figure 6.3.2.ii Steady state chemoreceptor responses (% maximal discharge) plotted against etCO<sub>2</sub> (%) during hyperoxia for lambs aged 3-4d, 5-9d and 10-24d.

### 6.3.3 Chemoreceptor CO<sub>2</sub> responses in Normoxia

Steady state chemoreceptor responses to CO<sub>2</sub> during normoxia are plotted for all fibres as a function of PaCO<sub>2</sub> (figure 6.3.3.i) and etCO<sub>2</sub> (figure 6.3.3.ii) and described by linear regression. There was an increase in CSN discharge for an increase in PaCO<sub>2</sub> in all age groups. There was also a tendency for chemoreceptor responses to be greater in older lambs, and this was most marked between ages 3-4d and 10-24d. Individual fibres were fitted for linear regression and this data is shown in table 6.3.3.i. Lambs 10-24d and 5-9d showed significantly greater chemoreceptor responses than at 3-4d ( $P < 0.001$  in each case by Mann-Whitney U test), but was not significant between 10-24d and 5-9d.

	slope	y intercept	r		slope	y intercept	r
3-4days n=9	0.21	-3.10	0.87	10-24 days n=13	0.97	8.94	0.90
	0.26	-2.59	1.00		0.89	-19.34	1.00
	0.16	-4.17	1.00		1.27	-30.14	0.99
	0.19	0.59	0.92		0.89	4.12	0.80
	0.15	9.67	1.00		0.79	-19.58	0.99
	0.35	-0.55	0.91		2.26	-79.62	1.00
	0.42	-13.10	0.88		0.50	-1.14	1.00
	0.74	-22.17	0.99		1.95	-79.60	0.99
	0.25	-8.66	0.60		1.45	-29.59	1.00
mean	<b>0.30</b>	<b>-4.90</b>	<b>0.91</b>	1.09	-24.24	0.69	
S.E.M	<b>0.06</b>	<b>3.01</b>	<b>0.04</b>	1.35	-42.72	1.00	
5-9days n=14	0.47	-10.39	1.00	mean S.E.M	0.90	-20.42	1.00
	0.67	-15.77	1.00		0.62	-23.38	1.00
	1.57	-48.10	1.00		<b>1.15</b>	<b>-27.44</b>	<b>.95</b>
	0.31	-11.12	0.95		<b>0.14</b>	<b>7.56</b>	<b>0.03</b>
	0.76	-19.26	0.99				
	0.59	14.74	1.00				
	0.80	-7.41	0.96				
	0.97	-12.39	0.74				
	0.33	-12.74	1.00				
	1.66	-53.15	0.99				
	2.59	-109.80	0.94				
	1.29	-46.38	1.00				
	1.11	-33.59	0.96				
0.33	-1.64	0.98					
mean	<b>0.96</b>	<b>-26.21</b>	<b>0.96</b>				
S.E.M	<b>0.17</b>	<b>8.22</b>	<b>0.02</b>				

Table 6.3.3.i Summary of linear regression analysis for chemoreceptor responses to CO<sub>2</sub> in normoxia.

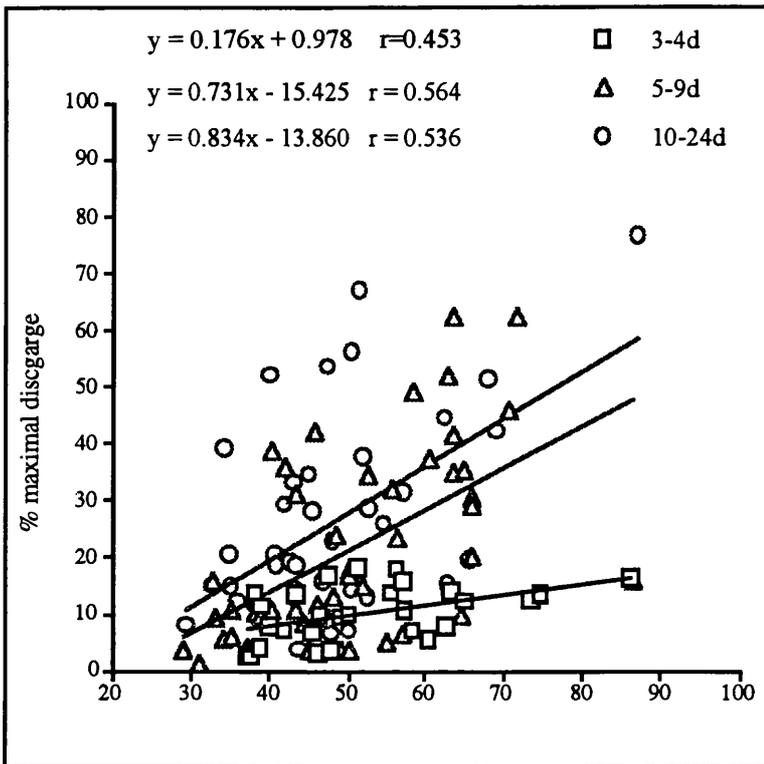


Figure 6.3.3.i Steady state chemoreceptor responses (% maximal discharge) plotted against PaCO<sub>2</sub> (mmHg) during normoxia for lambs aged 3-4d, 5-9d and 10-24d.

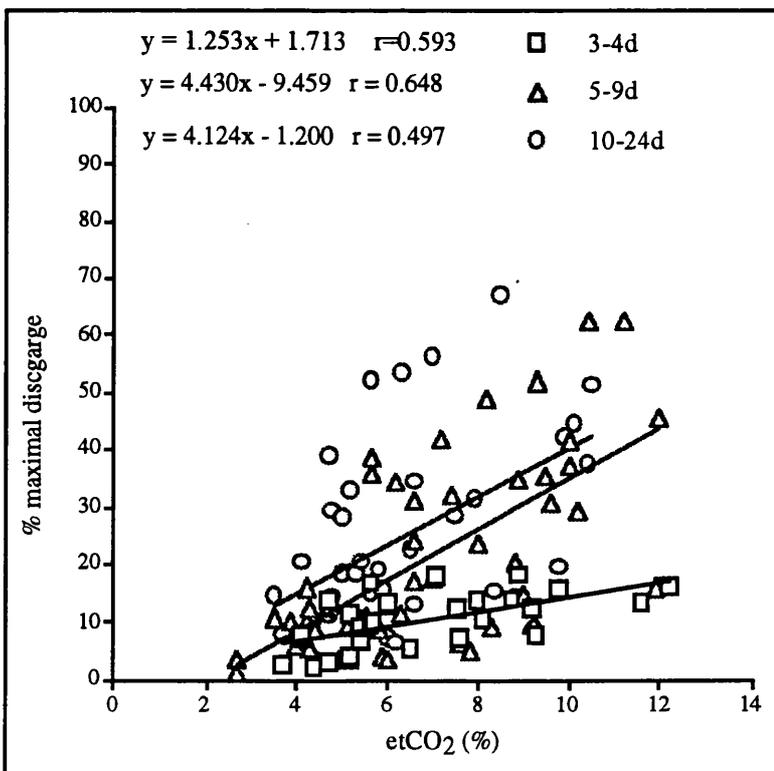


Figure 6.3.3.ii Steady state chemoreceptor responses (% maximal discharge) plotted against etCO<sub>2</sub> (%) during normoxia for lambs aged 3-4d, 5-9d and 10-24d.

### 6.3.4 Chemoreceptor CO<sub>2</sub> responses in Moderate hypoxia

Steady state chemoreceptor responses to CO<sub>2</sub> during moderate hypoxia were plotted as a function of PaCO<sub>2</sub> (figure 6.3.4.i) and etCO<sub>2</sub> (figure 6.3.4.ii) for all fibres. Once again, there was an increase in CSN discharge for an increase in PaCO<sub>2</sub>. Although there was a large scatter within age groups for chemoreceptor responses, a general tendency for an increase in CSN discharge for increases in CO<sub>2</sub> was observed. Linear regression lines fitted to chemoreceptor responses are shown in table 6.3.4.i. Lambs aged 10-24d showed significantly greater chemoreceptor responses to CO<sub>2</sub> than lambs aged 3-4d ( $p < 0.03$ , Mann-Whitney U test), but not for lambs 5-9d compared to 3-4d or 10-24d.

	slope	y intercept	r		slope	y intercept	r
3-4days n=10	1.50	-32.04	0.92	10-24 days n=10	1.44	9.44	1.00
	0.14	6.50	0.65		1.19	-30.43	0.99
	0.75	-16.99	0.99		3.27	-82.98	0.80
	0.50	-7.71	0.81		0.48	6.83	0.91
	0.99	-28.56	0.86		2.08	-39.87	0.91
	0.71	-2.56	0.82		0.71	-0.95	0.96
	0.21	-3.69	0.95		1.00	-16.43	0.98
	1.46	-35.16	0.99		1.67	-37.00	0.70
	0.77	-7.88	0.92		0.85	-16.49	1.00
	0.46	-8.70	0.89	2.97	-83.33	1.00	
mean	<b>0.75</b>	<b>-13.68</b>	<b>0.88</b>	mean	<b>1.57</b>	<b>-29.12</b>	<b>0.92</b>
S.E.M	<b>0.15</b>	<b>4.42</b>	<b>0.03</b>	S.E.M	<b>0.30</b>	<b>10.50</b>	<b>0.03</b>
5-9days n=9	1.26	-35.41	0.97				
	0.41	9.92	0.90				
	1.19	-5.60	1.00				
	0.81	-22.74	0.45				
	1.53	-30.35	0.97				
	0.62	-17.98	0.79				
	0.67	-14.72	0.74				
	3.80	-141.71	1.00				
	0.41	-8.54	0.97				
mean	<b>1.19</b>	<b>-29.68</b>	<b>0.86</b>				
S.E.M	<b>0.35</b>	<b>14.71</b>	<b>0.06</b>				

Table 6.3.4.i Summary of linear regression analysis for chemoreceptor responses to CO<sub>2</sub> in moderate hypoxia.

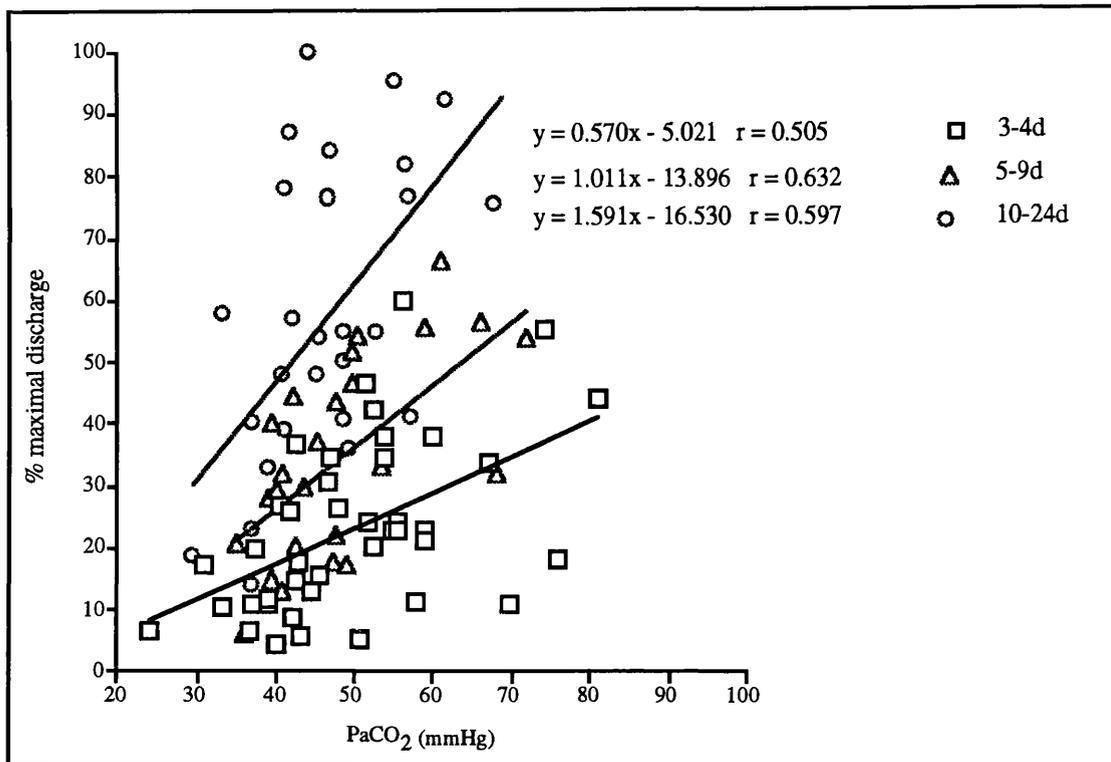


Figure 6.3.4.i Steady state chemoreceptor responses (% maximal discharge) plotted against PaCO<sub>2</sub> (mmHg) during moderate hypoxia for lambs aged 3-4d, 5-9d and 10-24d.

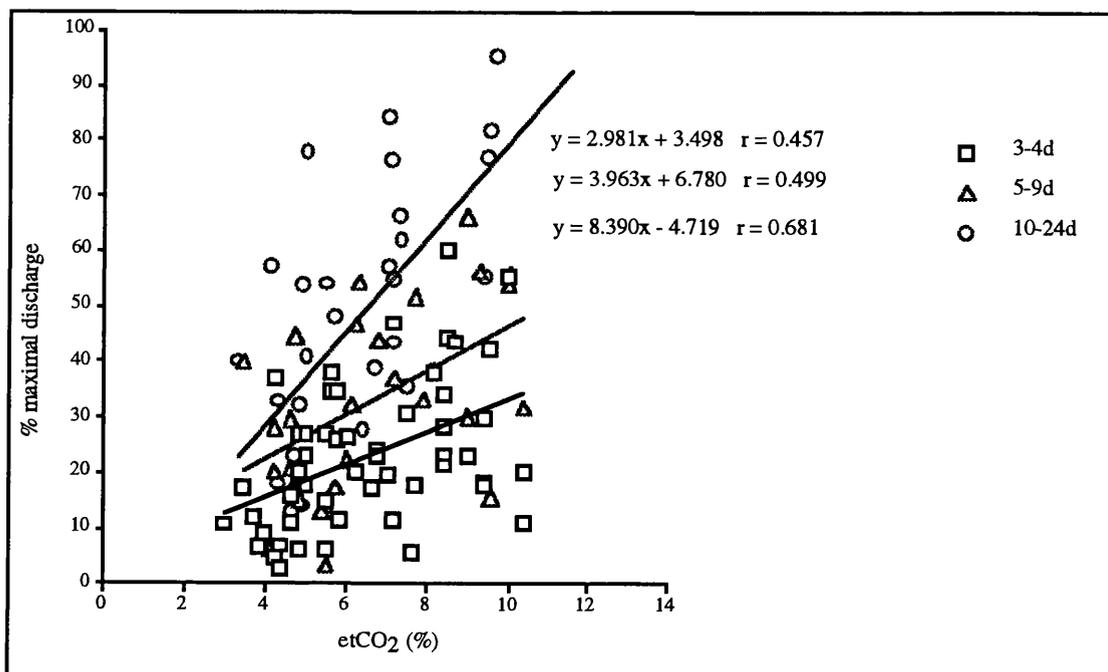


Figure 6.3.4.ii Steady state chemoreceptor responses (% maximal discharge) plotted against etCO<sub>2</sub> (%) during moderate hypoxia for lambs aged 3-4d, 5-9d and 10-24d.

### 6.3.5 Chemoreceptor CO<sub>2</sub> responses in Severe hypoxia

Steady state chemoreceptor responses to CO<sub>2</sub> during severe hypoxia were plotted as a function of PaCO<sub>2</sub> (figure 6.3.5.i) and etCO<sub>2</sub> (figure 6.3.5.ii) for all fibres. Chemoreceptor responses during SVHX were more variable than at any other PaO<sub>2</sub>. Linear regression fitted to these responses for the whole group show that whilst lambs at 3-4d increased CSN discharge when PaCO<sub>2</sub> increased, older lambs showed a fall in chemoreceptor discharge frequency as PaCO<sub>2</sub> increased. However, even in lambs at 3-4d the responses were widely scattered and chemoreceptor fibres would show a fall in discharge frequency if data points PaCO<sub>2</sub> < 40mmHg were excluded. Linear regression of the individual fibres showed that 3 of 5 fibres at 3-4d, 2 of 3 fibres at 5-9d and 3 of 4 fibres at 10-24d were unable to sustain an increase in discharge frequency as PaCO<sub>2</sub> increased. There were no significant differences between chemoreceptor responses with age.

	slope	y intercept	r		slope	y intercept	r
3-4days n=5	-0.82	89.91	1.00	10-24 days n=4	1.40	20.74	0.82
	-1.66	143.56	0.97		-3.89	114.99	0.80
	1.22	-23.37	0.98		-2.39	171.88	0.76
	-0.99	111.62	0.98		-0.07	29.87	0.22
	0.38	-2.97	0.95				
mean	<b>-0.37</b>	<b>63.75</b>	<b>0.98</b>	mean	<b>-1.23</b>	<b>84.37</b>	<b>0.65</b>
S.E.M	<b>0.52</b>	<b>32.70</b>	<b>0.01</b>	S.E.M	<b>1.18</b>	<b>36.07</b>	<b>0.14</b>
5-9days n=5	-0.29	85.05	0.76				
	-0.21	42.56	1.00				
	0.41	50.50	0.79				
	-0.17	55.81	0.35				
	1.15	53.70	1.00				
mean	<b>0.18</b>	<b>57.53</b>	<b>0.78</b>				
S.E.M	<b>0.27</b>	<b>7.24</b>	<b>0.12</b>				

Table 6.3.5.i Summary of linear regression analysis for chemoreceptor responses to CO<sub>2</sub> in severe hypoxia.

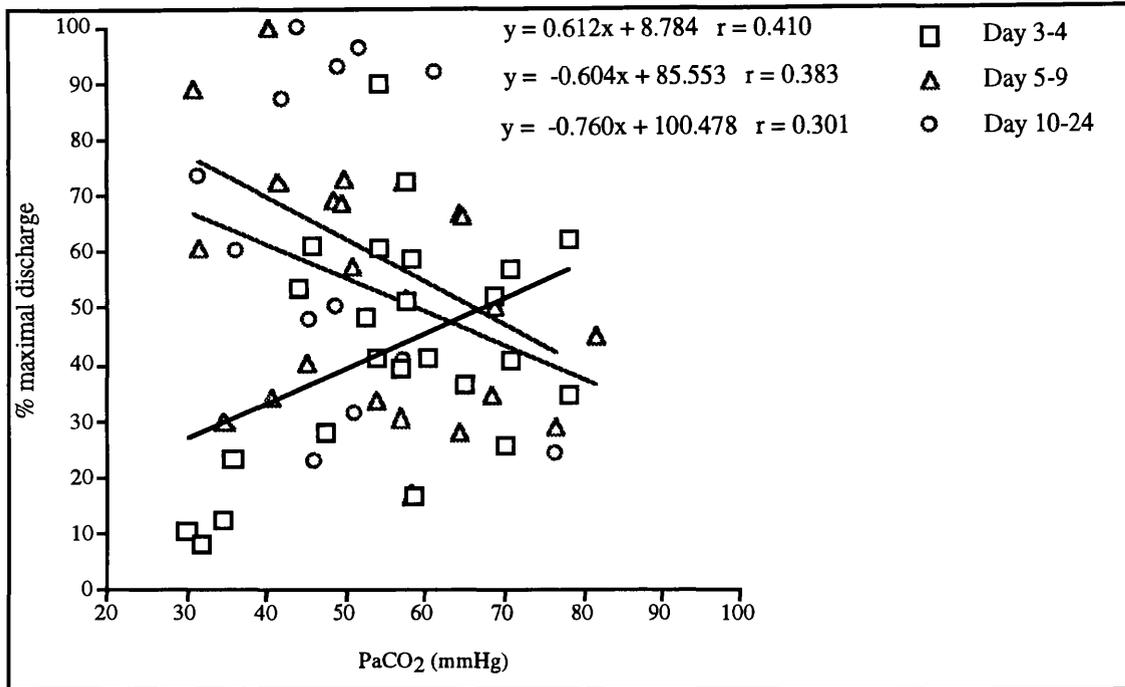


Figure 6.3.5.i Steady state chemoreceptor responses (% maximal discharge) plotted against PaCO<sub>2</sub> (mmHg) during severe hypoxia for lambs aged 3-4d, 5-9d and 10-24d.

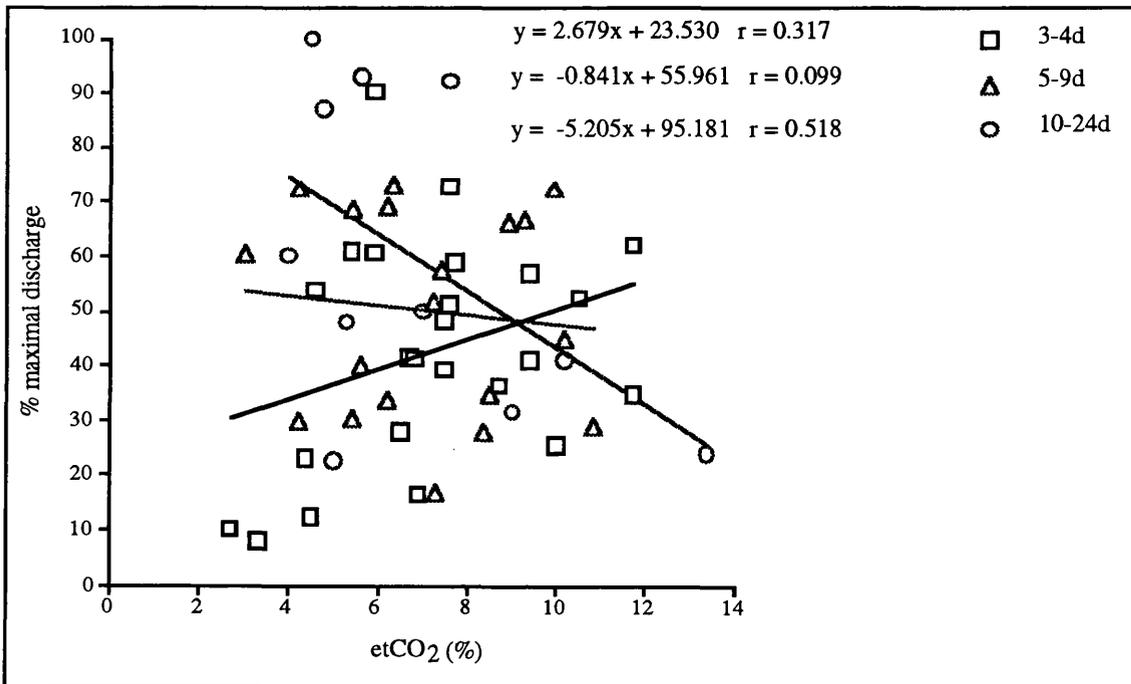


Figure 6.3.5.ii Steady state chemoreceptor responses (% maximal discharge) plotted against etCO<sub>2</sub> (%) during severe hypoxia for lambs aged 3-4d, 5-9d and 10-24d.

### **6.3.6 Maturation of chemoreceptor CO<sub>2</sub> responses**

Chemoreceptor responses to CO<sub>2</sub> in HYP, NX, MOD HX and SVHX have been shown for 3 different age groups in sections 6.3.2 - 5. These figures summarise CSN discharge for steady state increases in PaCO<sub>2</sub>. Chemoreceptor discharge frequency increased when PaCO<sub>2</sub> increased, and this effect was greater in older lambs compared to younger lambs. This was observed during HYP, NX and MOD HX however during SVHX there was often a fall in discharge frequency as PaCO<sub>2</sub> continued to increase.

In figure 6.3.6.i I have summarised chemoreceptor responses in another way. Lambs were grouped into three age groups and discharge frequency was plotted against grouped CO<sub>2</sub> at several PaO<sub>2</sub>s. Lambs aged 3-4d showed similar responses to CO<sub>2</sub> during HYP and NX. During MOD HX CO<sub>2</sub> sensitivity was increased compared to HYP and NX, and was greater still during the initial phase of SVHX at 3-4d. There was a plateau reached during SVHX in 3-4d lambs at 40-50 mmHg PaCO<sub>2</sub>. In lambs aged 5-9d CO<sub>2</sub> sensitivity increased from HYP to NX, and in MOD HX the slope of the chemoreceptor response curve was similar to NX. Lambs at 5-9d showed a fall in discharge frequency during SVHX and there was no plateau after an initial increase as observed at 3-4d. Lambs aged 10-24d showed similar responses to CO<sub>2</sub> during HYP and NX. During MOD HX CO<sub>2</sub> sensitivity was increased compared to HYP and NX. During SVHX discharge frequency fell as PaCO<sub>2</sub> increased however this was not as pronounced as at 5-9d, although the errors about the mean were much greater in the older lambs. At any given PaO<sub>2</sub> level (HYP, NX and MOD HX) there was an obvious increase in CO<sub>2</sub> sensitivity with age. This was not the case during SVHX as only lambs at 3-4d showed an increase in discharge frequency as CO<sub>2</sub> increased and this then plateaued. In the older lambs the discharge frequency during SVHX at low PaCO<sub>2</sub>s was very high. Chemoreceptor fibres did not sustain this level of discharge when PaCO<sub>2</sub> increased, and a fall in discharge frequency followed. This initial discharge frequency at low PaCO<sub>2</sub>s for lambs aged 5-9d and 10-24d (*ca.* 70% maximal discharge) was considerably higher than for lambs aged 3-4d (*ca.* 10% maximal discharge).

Linear regression was used to describe the chemoreceptor response for each fibre. The mean CO<sub>2</sub> sensitivity and y-intercept were used to reconstruct the grouped response for all aged fibres at each PaO<sub>2</sub>, and is shown in figure 6.3.6.ii. In HYP, NX and MOD HX CO<sub>2</sub> sensitivity increased with age, but in SVHX chemoreceptor responses to CO<sub>2</sub> fell when PaCO<sub>2</sub> increased. For all aged lambs CO<sub>2</sub> sensitivity increased with a fall in PaO<sub>2</sub> between HYP, NX and MOD HX but not in SVHX.

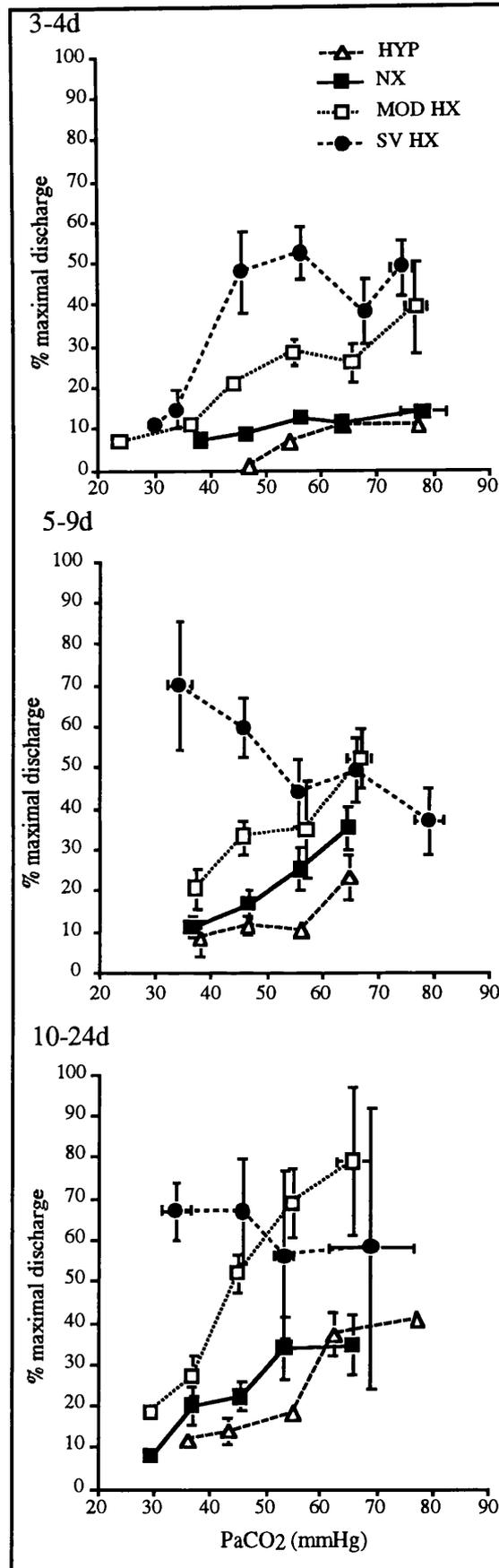


Figure 6.3.6.i Steady state chemoreceptor responses (% maximal discharge) to CO<sub>2</sub> (mmHg) plotted as PaCO<sub>2</sub> groups. Chemoreceptor responses shown during HYPEROX (open triangles), NX (closed squares), MOD HX (open squares) and SVHX (closed circles).

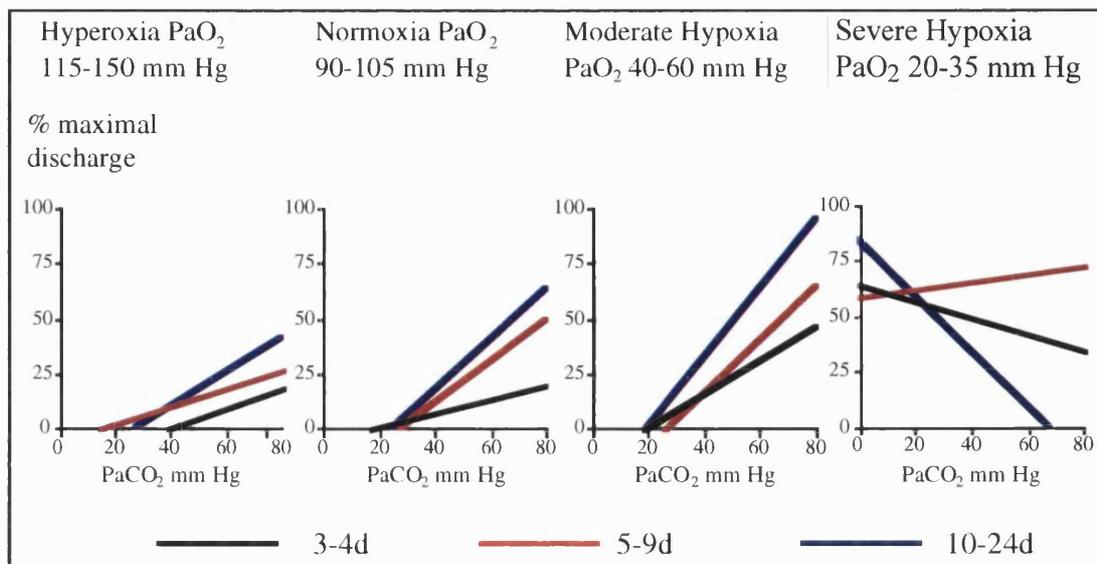


Figure 6.3.6.ii Mean chemoreceptor responses to increases in PaCO<sub>2</sub> for lambs 3-4d, 5-9d and 10-24d in HYP, NX, MOD HX and SVHX.

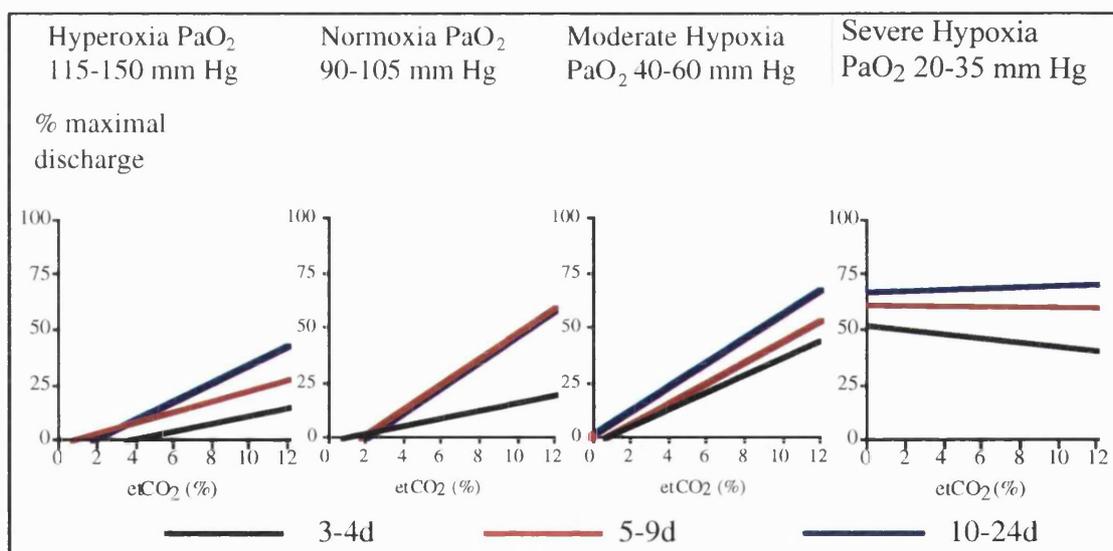


Figure 6.3.6.iii Mean chemoreceptor responses to increases in etCO<sub>2</sub> for lambs 3-4d, 5-9d and 10-24d in HYP, NX, MOD HX and SVHX.

Similarly, mean chemoreceptor responses for lambs grouped at three ages were plotted against etCO<sub>2</sub> in figure 6.3.6.iii. These were reconstructed in the same way as for figure 6.3.6.ii from the mean slopes and y-intercepts derived from linear regression. In HYP, NX and MOD HX chemoreceptor responses increased when etCO<sub>2</sub> increased, but fell in SVHX. CO<sub>2</sub> sensitivity was greater in older lambs than in younger lambs in HYP, NX and MOD HX. For any given age, CO<sub>2</sub> sensitivity increased between HYP and

MOD HX, but fell in SVHX. Chemoreceptor responses in figure 6.3.6.iii are similar to those in figure 6.3.6.ii, although in NX chemoreceptor responses between 5-9d and 10-24d lambs are almost superimposed when plotted against etCO<sub>2</sub> but not for PaCO<sub>2</sub>. In SVHX, the fall in discharge is most pronounced in figure 6.3.6.ii.

Multiple linear regression was used to describe the relationship between CO<sub>2</sub> sensitivity (% maximal discharge/mmHg PaCO<sub>2</sub>) with age and PaO<sub>2</sub>. Fibres that were unable to sustain an increase in discharge frequency during SVHX were excluded from multiple linear regression analysis at that PaO<sub>2</sub>, but not at all other PaO<sub>2</sub>s. CO<sub>2</sub> sensitivity was log transformed to fit a normal distribution (figure 6.3.6.iv). PaO<sub>2</sub> was also log transformed to linearize the relationship with CO<sub>2</sub> sensitivity. The correlation between each variable is given in table 6.3.6.i. Log-CO<sub>2</sub> sensitivity was strongly correlated with age (P<0.001), but not with log-PaO<sub>2</sub> (P<0.07). There was a significant correlation between log-PaO<sub>2</sub> and age (P<0.05).

Multiple linear regression showed that there was a significant effect of age (P<0.0001) and log-PaO<sub>2</sub> (P<0.025) on log-CO<sub>2</sub> sensitivity. The multiple linear regression equation is given by:

$$\text{Log-Steady state CO}_2 \text{ sensitivity} = (\{0.027 \pm 0.007\} \times \text{Age}) - (\{0.425 \pm 0.184\} \times \log\text{-PaO}_2) + 0.415 \pm 0.341.$$

Correlation coefficients	log-CO <sub>2</sub> sensitivity	Age	log-PaO <sub>2</sub>
log-CO <sub>2</sub> sensitivity	-	0.38, P<0.001	-0.16, P<0.07
Age	0.38, P<0.001	-	0.05, P<0.38
log-PaO <sub>2</sub>	-0.16, P<0.07	0.18, P<0.05	-

Table 6.3.6.i Correlation matrix (correlation coefficient, one-tailed significance) between age and log-PaO<sub>2</sub> and log-CO<sub>2</sub> sensitivity.

I also compared the effect of age and PaO<sub>2</sub> on CO<sub>2</sub> sensitivity by two-way ANOVA. This alternative method of statistical analysis similarly showed a significant effect of age (P<0.004) and PaO<sub>2</sub> (P<0.01) on CO<sub>2</sub> sensitivity. There was no significant interaction between age and PaO<sub>2</sub> (P>0.8).

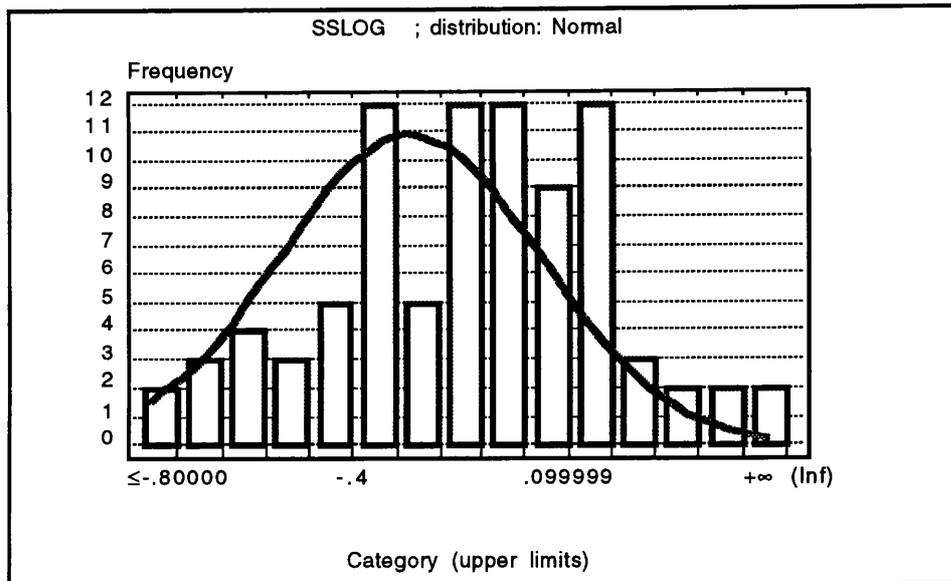


Figure 6.3.6.iv Frequency histogram for steady state log-CO<sub>2</sub> sensitivity (% maximal discharge / mmHg PaCO<sub>2</sub>). This data was normally distributed by X<sup>2</sup> test (P>0.35)

### 6.3.7. Interaction between CO<sub>2</sub> and O<sub>2</sub>

In figure 6.3.6.i chemoreceptor responses were plotted for lambs at three ages and several PaO<sub>2</sub>s. I found that, as PaO<sub>2</sub> fell from HYP to NX and then to MOD HX, the slope of the chemoreceptor response curve (CO<sub>2</sub> sensitivity) increased. To determine whether this interaction between CO<sub>2</sub> and O<sub>2</sub> was significant I used multiple linear regression to describe the relationship between discharge frequency, PaO<sub>2</sub>, PaCO<sub>2</sub>, PaO<sub>2</sub>-PaCO<sub>2</sub> interaction and age. It was necessary to take the square root of discharge frequency to normalize the data as shown in figure 6.3.7.i. Chemoreceptor responses to CO<sub>2</sub> measured during SVHX were excluded from analysis because I have shown that many fibres were unable to sustain an increase in discharge frequency during SVHX. The correlation matrix for the relationship between each of the variables is shown in table 6.3.7.i. (Square root)-discharge frequency was strongly correlated with age (P<0.001), PaO<sub>2</sub> (P<0.001) and PaCO<sub>2</sub> (P<0.001), but not with PaO<sub>2</sub>xPaCO<sub>2</sub> (P>0.4).

Multiple linear regression analysis showed that there was a significant effect of age (P<0.001), PaO<sub>2</sub> (P<0.03) and PaCO<sub>2</sub> (P<0.05) on (square root)-discharge frequency, but there was no significant interaction between PaO<sub>2</sub> and PaCO<sub>2</sub> (P>0.5). The multiple linear regression equation is given by:

$$\begin{aligned} \text{(Square root)-discharge frequency} = & \{0.121 \pm 0.017\} \times \text{Age} + \{0.047 \pm 0.023\} \times \\ & \text{PaCO}_2 - \{0.031 \pm 0.014\} \times \text{PaO}_2 + \{1.483 \times 10^{-4} \pm 2.656 \times 10^{-4}\} \times \text{PaCO}_2 \times \text{PaO}_2 \\ & + 3.35 \pm 1.20. \end{aligned}$$

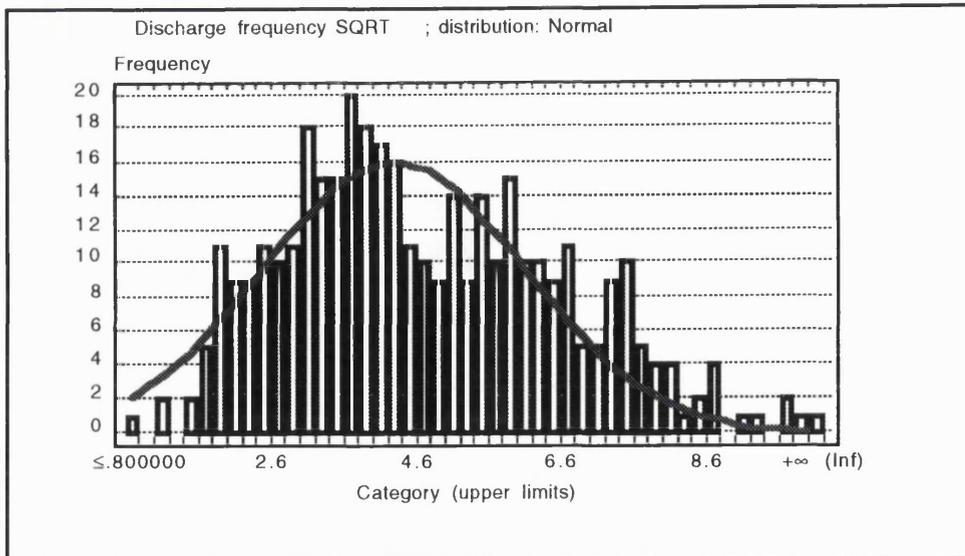


Figure 6.3.7.i Frequency histogram for steady state (square root)-chemoreceptor responses to CO<sub>2</sub> (% maximal discharge). This data was normally distributed by X<sup>2</sup> test (P>0.06)

Correlation coefficients	(square root)-discharge freq.	Age	PaCO <sub>2</sub>	PaO <sub>2</sub>	PaO <sub>2</sub> x PaCO <sub>2</sub>
(square root)-discharge freq.	-	0.248 P<0.001	0.341 P<0.001	-0.254 P<0.001	0.003 P>0.4
Age	0.248 P<0.001	-	-.001 P>0.4	0.187 P<0.001	0.144 P<0.005
PaCO <sub>2</sub>	0.341 P<0.001	-.001 P>0.4	-	0.094 P<0.040	0.621 P<0.001
PaO <sub>2</sub>	-0.254 P<0.001	0.187 P<0.001	0.094 P<0.040	-	0.819 P<0.001
PaO <sub>2</sub> x PaCO <sub>2</sub>	-0.003 P>0.4	0.144 P<0.005	0.621 P<0.001	0.819 P<0.001	-

Table 6.3.7.i Correlation matrix (correlation coefficient, one-tailed significance) between age, PaO<sub>2</sub>, PaCO<sub>2</sub>, PaO<sub>2</sub> x PaCO<sub>2</sub> and (square root)-discharge frequency.

When I analysed all chemoreceptor responses together in this manner, I could not find a significant effect of PaO<sub>2</sub>-PaCO<sub>2</sub> interaction on discharge frequency. Therefore using multiple linear regression, I compared PaO<sub>2</sub>, PaCO<sub>2</sub> and PaO<sub>2</sub>-PaCO<sub>2</sub> interaction to discharge frequency for the three age groups individually. I felt that this was warranted because the data presented in figure 6.3.6.i suggested the presence of O<sub>2</sub>-CO<sub>2</sub> interaction at 10-24d. Chemoreceptor discharge in figure 6.3.6.i is plotted as the mean

response for a PaCO<sub>2</sub> range. I have used these values to assess O<sub>2</sub>-CO<sub>2</sub> interaction at three ages.

On this occasion I was able to demonstrate a significant effect of CO<sub>2</sub>-O<sub>2</sub> interaction in older lambs. Chemoreceptor responses to CO<sub>2</sub> measured during SVHX were excluded from analysis as previously mentioned (i.e. fibres at 10-24d were unable to sustain an increase in discharge frequency during SVHX). In figure 6.3.7.ii I have shown that chemoreceptor discharge (% maximum) was normally distributed for lambs aged 10-24d (X<sup>2</sup> test; P>0.18). The correlation matrix for the relationship between each of the variables is shown in table 6.3.7.ii. Discharge frequency was correlated with PaO<sub>2</sub> (P<0.017) and PaCO<sub>2</sub> (P<0.010), but not with PaO<sub>2</sub> x PaCO<sub>2</sub> (P>0.38).

Multiple linear regression showed that there was a significant effect of PaO<sub>2</sub> (P<0.03), PaCO<sub>2</sub> (P<0.001) and PaO<sub>2</sub> x PaCO<sub>2</sub> (P<0.001) on discharge frequency for lambs aged 10-24d. The multiple linear regression equation is given by:

$$\text{Discharge frequency} = (\{2.80 \pm 0.33\} \times \text{PaCO}_2) + (\{0.437 \pm 0.173\} \times \text{PaO}_2) - (\{0.019 \pm 0.003\} \times \text{PaCO}_2 \times \text{PaO}_2) - (59.47 \pm 15.97).$$

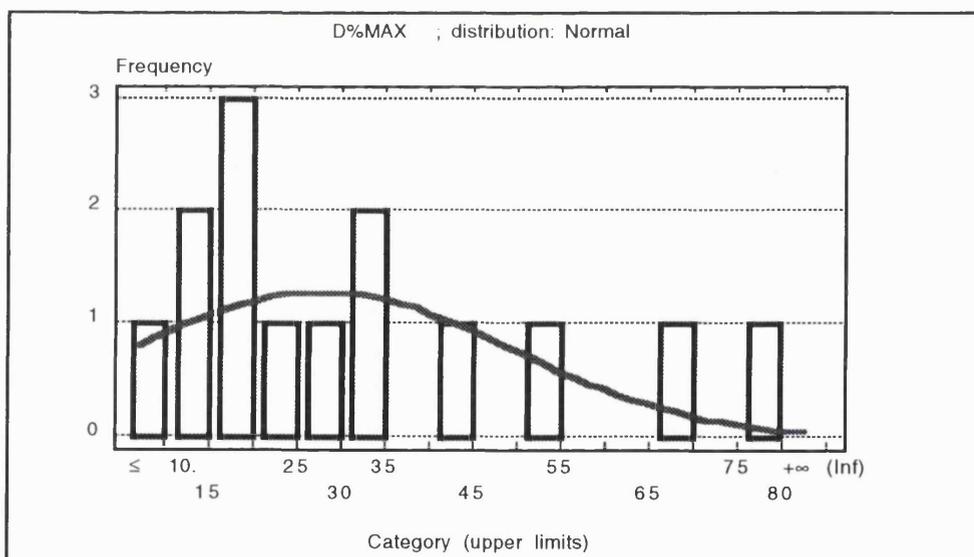


Figure 6.3.7.ii Frequency histogram for steady state chemoreceptor responses to CO<sub>2</sub> (% maximal discharge) in lambs aged 10-24d. This data was normally distributed by X<sup>2</sup> test (P>0.18).

Correlation coefficients	Discharge freq.	PaCO <sub>2</sub>	PaO <sub>2</sub>	PaO <sub>2</sub> x PaCO <sub>2</sub>
Discharge freq.	-	0.615 P<0.010	-0.570 P<0.017	-0.088 P>0.382
PaCO <sub>2</sub>	0.615 P<0.010	-	0.171 P<0.280	0.711 P<0.002
PaO <sub>2</sub>	-0.570 P<0.017	0.171 P<0.280	-	0.792 P<0.001
PaO <sub>2</sub> x PaCO <sub>2</sub>	-0.088 P>0.382	0.711 P<0.002	0.792 P<0.001	-

Table 6.3.7.ii Correlation matrix (correlation coefficient, one-tailed significance) between PaO<sub>2</sub>, PaCO<sub>2</sub>, PaO<sub>2</sub> x PaCO<sub>2</sub> and discharge frequency for lambs aged 10-24d.

In contrast, when I analysed chemoreceptor responses in the same manner for lambs aged 3-4d and 5-9d, I was unable to find a significant effect of CO<sub>2</sub>-O<sub>2</sub> interaction on chemoreceptor discharge. Thus it appeared that CO<sub>2</sub>-O<sub>2</sub> interaction was only significant in older lambs, but not in younger lambs. This explains why CO<sub>2</sub>-O<sub>2</sub> interaction was not significant when chemoreceptor responses at all ages were analysed (see above).

CO<sub>2</sub> sensitivity was also compared within each of the three age groups at different levels of PaO<sub>2</sub> for any evidence of interaction. CO<sub>2</sub> sensitivity was compared in this way by linear regression describing individual chemoreceptor responses. The slope for each fibre was used as an index of CO<sub>2</sub> sensitivity (see tables 6.3.2.i - 5.i). They were compared between oxygen levels at three ages using Mann-Whitney U test. As more than half of chemoreceptor fibres at any age showed a fall in discharge during SVHX, comparisons were only made between HYP, NX and MOD HX to detect CO<sub>2</sub>-O<sub>2</sub> interaction.

In lambs aged 3-4d CO<sub>2</sub> sensitivity during MOD HX was significantly greater than that in NX ( $p<0.03$ , Mann-Whitney U test), but not in HYP. In lambs aged 5-9d CO<sub>2</sub> sensitivity during NX ( $p<0.05$ , Mann-Whitney U test) and MOD HX ( $p<0.03$ , Mann-Whitney U test) was significantly greater than that during HYP. CO<sub>2</sub> sensitivity during MOD HX at 5-9d was not significantly greater than in NX. In lambs aged 10-24d CO<sub>2</sub> sensitivity during MOD HX was significantly greater than that in HYP ( $p<0.03$ , Mann-Whitney U test), but not to NX. There is some evidence of interaction at each age group of lambs between different PaO<sub>2</sub> levels, but it is not uniform.

Another method used to investigate the presence of O<sub>2</sub>-CO<sub>2</sub> interaction at different ages was to plot CO<sub>2</sub> sensitivity against PaO<sub>2</sub>. The slopes derived from linear regression analysis for the individual fibres were used as an index of CO<sub>2</sub> sensitivity (mean  $\pm$  S.E.M) and plotted against PaO<sub>2</sub> (mean  $\pm$  S.E.M) for different age groups (figure 6.3.7.iii). Interaction between O<sub>2</sub> and CO<sub>2</sub> is evident from an increase in CO<sub>2</sub> sensitivity as PaO<sub>2</sub> falls. O<sub>2</sub>-CO<sub>2</sub> interaction can be observed in 10-24d lambs in MOD HX compared to NX and HYP. Interaction between the two stimuli can also be seen in 3-4d lambs in NX compared to MOD HX and in 5-9d lambs in HYP compared to NX. It is difficult to comment on the effect of age on O<sub>2</sub>-CO<sub>2</sub> interaction with age from figure 6.3.7.iii. There does not appear to be any clear relationship between age and O<sub>2</sub>-CO<sub>2</sub> interaction when the data is presented in this way.

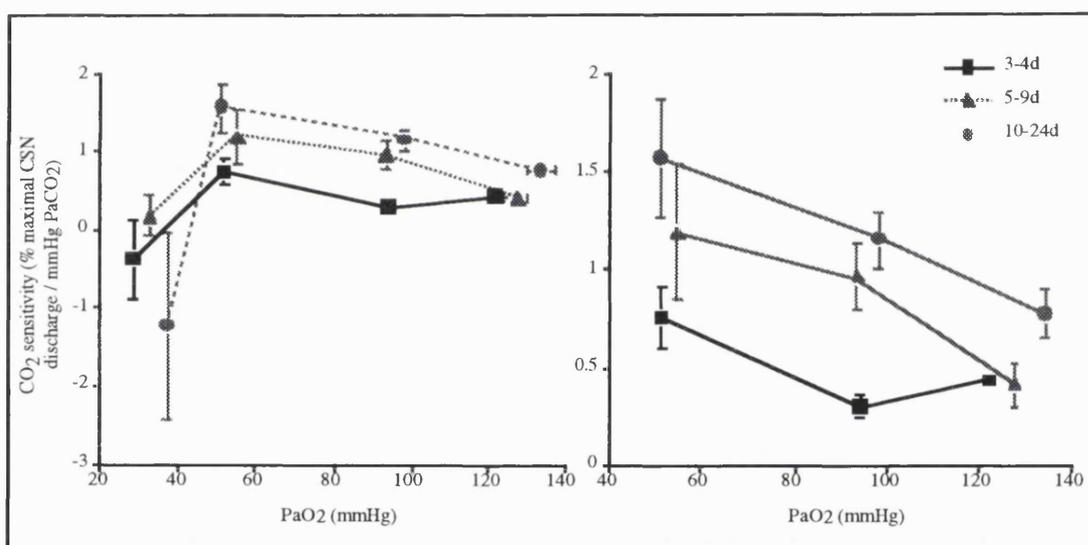


Figure 6.3.7.iii Summary of chemoreceptor CO<sub>2</sub> sensitivity (mean  $\pm$  S.E.M) against PaO<sub>2</sub> (mean  $\pm$  S.E.M) for lambs at three ages. Left: CO<sub>2</sub> sensitivity during SVHX is included; it is excluded on the right.

In summary, there is strong evidence to show CO<sub>2</sub>-O<sub>2</sub> interaction in the older lambs from multiple linear regression and from the comparison of CO<sub>2</sub> sensitivities with non-parametric methods (Mann-Whitney U test). However, the evidence for CO<sub>2</sub>-O<sub>2</sub> interaction in lambs aged 3-4d and 5-9d was less convincing. Although there is the suggestion of some interaction from the Mann-Whitney U test analysis, this is not supported by multiple linear regression, hence it is not significant.

## **6.4 Discussion**

### **6.4.1 Overview**

I have measured carotid body steady state CO<sub>2</sub> sensitivity in newborn lambs. I found that chemoreceptor fibres increased discharge frequency when CO<sub>2</sub> increased. There was a significant effect of PaCO<sub>2</sub> ( $P < 0.05$ ), age ( $P < 0.001$ ) and PaO<sub>2</sub> ( $P < 0.03$ ) on chemoreceptor discharge. Chemoreceptor responses were analysed in terms of CO<sub>2</sub> sensitivity and this was significantly increased at lower PaO<sub>2</sub>s ( $P < 0.025$ ) and in older lambs ( $P < 0.0001$ ). The effect of PaO<sub>2</sub> on CO<sub>2</sub> sensitivity was observed between hyperoxia, normoxia and moderate hypoxia, but during severe hypoxia chemoreceptor fibres were unable to increase discharge frequency as PaCO<sub>2</sub> continued to increase.

I found in some chemoreceptor fibres the suggestion of CO<sub>2</sub> and O<sub>2</sub> interaction (i.e. CO<sub>2</sub> sensitivity increased at lower PaO<sub>2</sub>s) using Mann Whitney U test to make comparisons between CO<sub>2</sub> sensitivities. At 3-4d CO<sub>2</sub> sensitivity during MOD HX was significantly greater compared to NX; at 5-9d CO<sub>2</sub> sensitivity during NX and MOD HX were significantly greater than during HYP; and at 10-24d CO<sub>2</sub> sensitivity during MOD HX was significantly greater compared to HYP. However, this did not provide firm evidence for CO<sub>2</sub>-O<sub>2</sub> interaction. I also compared chemoreceptor responses using multiple linear regression. When all chemoreceptor responses were pooled there was no evidence of interaction. When I analysed chemoreceptor responses at three ages, older lambs (10-24d) did show evidence for CO<sub>2</sub>-O<sub>2</sub> interaction. In younger lambs there was no significant effect of interaction on chemoreceptor response. Thus for lambs aged 10-24d, there was a significant effect of PaO<sub>2</sub> ( $P < 0.03$ ), PaCO<sub>2</sub> ( $P < 0.001$ ) and PaO<sub>2</sub> x PaCO<sub>2</sub> ( $P < 0.001$ ) on chemoreceptor discharge. This is the first evidence for an interaction between the two stimuli at this age.

### **6.4.2 Recording from chemoreceptor fibres as a technique to assess CO<sub>2</sub> sensitivity**

#### **6.4.2.a Standardizing and transforming chemoreceptor responses**

To determine carotid body steady state CO<sub>2</sub> sensitivity, it is necessary to record from single chemoreceptor fibres. I found that I was only able to dissect to the level of few or multi-chemoreceptor fibres which necessitated normalizing my data to some standard level. I chose to normalize chemoreceptor discharge to maximal discharge, in preference to a normocapnic normoxic level, because it was difficult to set the criteria for the latter.

If a normocapnic normoxic level was chosen on the basis of blood gas analysis, then a PaCO<sub>2</sub> of 40 mmHg and PaO<sub>2</sub> of 100 mmHg may be normocapnic and normoxic for a lamb aged 12d, but not for one aged 3d. Thus to set a level of chemoreceptor discharge for standardization which was independent of the postnatal increase in hypoxia sensitivity, I decided that it was more accurate to normalize my results to maximal discharge. In some preliminary experiments I tried to record the chemoreceptor response to 100% CO<sub>2</sub>-equilibrated saline injected via the lingual artery. However I found that the chemoreceptor recording became contaminated because the pressure pulse could evoke discharge from quiescent baroreceptors. Thus, I preferred to standardize my results to the maximal chemoreceptor response to an inspired hypoxic stimulus.

I have used multiple linear regression to assess the effect of age and PaO<sub>2</sub> on CO<sub>2</sub> sensitivity, hence the assumption is made that the data are normally distributed. For the purpose of statistical analysis it was necessary to transform the data to fit a normal distribution. CO<sub>2</sub> sensitivity was log-transformed to fit a normal distribution (see figure 6.3.6.iv). To assess CO<sub>2</sub>-O<sub>2</sub> interaction it was also necessary to transform chemoreceptor discharge (see section 6.3.7). Log-transformation did not normalize the data, so it was necessary to try alternative transformation. I found that the only possibility to normalize the data was to take the square root of discharge (figure 6.3.7.i). Whilst this approach is slightly less conventional than log-transformation, I had already shown that PaO<sub>2</sub> and age had an effect on CO<sub>2</sub> sensitivity, and the purpose of this analysis was to address CO<sub>2</sub>-O<sub>2</sub> interaction. Once again I confirmed that PaCO<sub>2</sub>, PaO<sub>2</sub> and age all had an effect on (Square root)-discharge, but there was no effect of CO<sub>2</sub>-O<sub>2</sub> interaction for the groups taken together. So the transformed data all show the same effect.

#### **6.4.2.b Few-fibre vs. multi-fibre chemoreceptor preparations: Are they a representative sample?**

In analysing the recordings from few or multi- chemoreceptor fibres, I have made the assumption that my results are representative of the entire population of chemoreceptor fibres. This is not necessarily valid as, for example, I had no means of determining if the fibres I recorded from were myelinated or unmyelinated. It is likely that recordings were biased in the favour of myelinated fibres as these would show the largest action potentials. In the interest of a good signal to noise ratio, my recordings favoured the larger action potentials so that I need not consider if smaller spikes were in fact chemoreceptor activity or noise. Neither do I know if the sensitivity to CO<sub>2</sub> and O<sub>2</sub> varied between different fibres of the same preparation, or between preparations, or if

the threshold for action potential discharge was different. Particularly with the multi-fibre chemoreceptor recordings, I am unable to comment on the level of recruitment that occurred at lower PaO<sub>2</sub>s. I would expect that recruitment of chemoreceptor fibres at low PaO<sub>2</sub>s occurred to a greater degree in multi- compared to few chemoreceptor fibres. Assessing the degree of recruitment that occurs at lower PaO<sub>2</sub>s is more important when the purpose of the experiment is to understand carotid body chemo-sensing mechanisms. However, my experiments have emanated from the respiratory side of the chemoreflex, in that I have used a method to deliver the stimulus similar to that used in human infants. As such, the afferent input to the CNS is relevant in terms of the respiratory response, and multi-fibre preparations will give a better indication of this afferent discharge. Thus, multi-fibre preparations are valid in the context of understanding reflexes in the whole animal, and facilitate extrapolation to my observations of the respiratory response in babies.

#### **6.4.2.c Elimination of baroreceptor discharge**

Although it is impossible to rule out the effect of baroreceptor contamination, I am confident that the effect of baroreceptor discharge on these chemoreceptor recordings was kept to a minimum. Chemoreceptor recordings were always made when there was an intra-arterial catheter, and so it was immediately obvious from the chart record (at fast speed) and from the raw discharge audible via an amplifier and loud speaker, if baroreceptor contamination was present. In the instance of baroreceptor contamination the chemoreceptor recording was rejected, or recounted so that baroreceptor activity was removed. On some occasions I recounted chemoreceptor activity after the experiment if there was question of baroreceptor contamination. I observed in a few experiments that very small baroreceptor action potentials were audible close to the level of noise, whilst the chemoreceptor action potentials were of a much greater amplitude. The baroreceptor discharge could be eliminated by raising the window height of the spike discriminator when I recounted discharge so that only chemoreceptor activity was recorded.

#### **6.4.2.d Sympathetic innervation of the carotid body**

I chose not to section the efferent sympathetic innervation of the carotid body from the superior cervical ganglion, namely the ganglio-glomerular nerves. In the context of respiratory chemoreflexes, of which much of this thesis is concerned, it made little sense to remove sympathetic innervation of the carotid body. Once again, this is the situation in the whole animal, and *in vitro* experiments provide a better method to study the

carotid body in isolation. Furthermore, section of the ganglio-glomerular nerves could increase the likelihood of damage to the carotid body.

Electrical stimulation of the sympathetic fibres innervating the carotid body causes a decrease in carotid body blood flow (de Burgh Daly et al, 1954; Purves, 1970) and an increase in chemoreceptor discharge (Floyd & Neil, 1952; Eyzaguirre & Lewin, 1961; Jansen, Purves & Tan, 1980; O'Regan, 1981). Moreover, there is evidence that sympathetic innervation of the carotid body has a negligible role in determining steady state sensitivity to hypoxia and CO<sub>2</sub>. Davies, Nishino & Lahiri (1981) measured the response of single chemoreceptor fibres to four steady state levels of oxygen in the adult cat. They found that steady state chemoreceptor responses were unchanged after section of the carotid body sympathetic nerves, and concluded that the effect of carotid body sympathetic innervation was small in determining steady state chemoreceptor responses to O<sub>2</sub>. McQueen, Evrard, Gordon & Campbell (1989) recorded chemoreceptor fibres in anaesthetised cats, and showed that the response to hypoxia and hypercapnia was unchanged after section of the ganglio-glomerular nerves. There was a slight reduction in the chemoreceptor response to CO<sub>2</sub> after section, however this was not significant. Prabhakar & Kou (1994) measured the chemoreceptor response to sustained hypoxia in anaesthetised cats before and after section of sympathetic innervation of the carotid body. They found that the chemoreceptor response during the first 10min of isocapnic hypoxia was the same before and after section. It was only over the following 20min (i.e. total duration 30min) that the chemoreceptor response to sustained hypoxia was greater in the denervated carotid bodies. They concluded that sympathetic innervation of the carotid body exerts an inhibitory influence on the chemoreceptor response to sustained hypoxia but that this effect was not evident in the first 10min.

In summary, there is evidence in support of sympathetic innervation of the carotid body modifying chemoreceptor responses. However, this inhibitory effect on carotid body discharge is likely to be small, and should not be a major determinant of the results of my experiments. In addition, my results are not qualitatively different from those of Carroll et al. (1993) who sectioned the ganglio-glomerular nerves in newborn kittens.

### **6.4.3 Effect of age on steady state CO<sub>2</sub> sensitivity**

Lambs aged 3-24d were studied to measure carotid body steady state CO<sub>2</sub> sensitivity. I found a significant effect of age ( $P < 0.0001$ ) on CO<sub>2</sub> sensitivity so that older lambs showed a significantly greater CO<sub>2</sub> sensitivity compared to younger lambs. This is in

agreement with the observations of Marchal et al. (1992a) and Carroll et al. (1993) in the kitten *in vivo*, and with the observations of Pepper et al. (1995) in the rat *in vitro*. These workers report a greater CO<sub>2</sub> sensitivity in older compared to younger animals. Marchal et al. (1992a) showed a steeper CO<sub>2</sub> response curve that was displaced to the left in older kittens compared to younger kittens. I similarly found that the chemoreceptor CO<sub>2</sub> response curve was steeper in older lambs.

The greatest increase in CO<sub>2</sub> sensitivity observed by Carroll et al. (1993) occurred between kittens studied at 1wk and 4wks of age. Kittens aged 8wk showed chemoreceptor responses to CO<sub>2</sub> similar to kittens aged 4wks, but they were less than responses in adult cats. I analysed chemoreceptor responses in newborn lambs at three age groups, all younger than 4wks of age. Looking qualitatively at the data in figure 6.3.6.i, my results show that the greatest increase in CO<sub>2</sub> sensitivity occurred between 5-9d and 10-24d. Whilst the age groups I have chosen are different from those in the study of Carroll et al. (1993), they show the same trend of an effect of age on CO<sub>2</sub> sensitivity.

Pepper et al. (1995) measured chemoreceptor responses in neonatal rats at only one age, 5-7d, and compared them to adult rats. They observed in adult rats that an increase in P<sub>CO<sub>2</sub></sub> shifted the hypoxia chemoreceptor response curve to the right, but that this effect was not present in the neonate. This reflects an increase in hypoxia or CO<sub>2</sub> sensitivity, or both, between rat pups aged 5-7d and adulthood, but does not provide any additional information on the time course of postnatal changes in CO<sub>2</sub> sensitivity.

Two ventilatory studies have reported no change in carotid body steady state CO<sub>2</sub> sensitivity postnatally (Jansen et al, 1992; Moss et al, 1995). Ventilation (Moss et al, 1995) or phrenic nerve activity (Jansen et al, 1992) was plotted as a function of PaCO<sub>2</sub> during CO<sub>2</sub> breathing. CO<sub>2</sub> sensitivity was measured as the slope of this response, and there was no increase with age. That there was no increase in CO<sub>2</sub> sensitivity postnatally is surprising, when such a clear effect has been demonstrated from my experiments. Wolsink et al. (1993) observed a maturation in CO<sub>2</sub> sensitivity in anaesthetised piglets aged 0.5-11d, but did not find a postnatal increase in their previous study when only piglets aged 2-11d were studied (Wolsink et al, 1991). It is difficult to interpret from the two studies of Wolsink et al. (1991; 1993) if the ventilatory response measured reflects steady state or dynamic CO<sub>2</sub> sensitivity, or both. It is also surprising that it was necessary to study piglets from 0.5d, and that no increase in CO<sub>2</sub> sensitivity was observed between 2 and 11d. In view of the evidence that supports a postnatal increase in CO<sub>2</sub> sensitivity, the method used by Wolsink et al. (1993) may not be sufficiently sensitive to detect changes in CO<sub>2</sub> sensitivity after the first postnatal day.

The most direct method to measure carotid body CO<sub>2</sub> sensitivity is to record from the carotid sinus nerve. It is difficult to rule out a central component in the ventilatory response to CO<sub>2</sub>, and postnatal changes in respiratory mechanics will also influence the ventilatory response to CO<sub>2</sub>. Therefore to comment on the effect of age on chemoreceptor responses to CO<sub>2</sub>, I prefer to compare my results with the studies of Marchal et al. (1992a) and Carroll et al. (1993).

#### **6.4.4 Effect of PaO<sub>2</sub> on steady state CO<sub>2</sub> sensitivity**

I found that there was a significant effect of PaO<sub>2</sub> on chemoreceptor discharge ( $P < 0.03$ ), and in addition a significant effect of PaO<sub>2</sub> on carotid body steady state CO<sub>2</sub> sensitivity ( $P < 0.025$ ), so that at lower oxygen levels CO<sub>2</sub> sensitivity was increased. The implications of these observations are discussed in relation to CO<sub>2</sub>-O<sub>2</sub> interaction in section 6.4.6.

The effect of PaO<sub>2</sub> on chemoreceptor responses was observed during HYP, NX and MOD HX. I found it was necessary to exclude chemoreceptor responses obtained during SVHX from statistical analysis as many fibres were unable to sustain chemoreceptor discharge at a certain frequency, and showed a fall in discharge as PaCO<sub>2</sub> increased. This is discussed further in section 6.4.5.

#### **6.4.5 Chemoreceptor responses to CO<sub>2</sub> during severe hypoxia**

Chemoreceptor responses to CO<sub>2</sub> during SVHX (PaO<sub>2</sub> 20-35 mmHg) differed to responses obtained during HYP, NX and MOD HX in that 3 of 5 fibres at 3-4d, 3 of 5 fibres at 5-9d and 3 of 4 fibres at 10-24d did not show an increase in chemoreceptor discharge when PaCO<sub>2</sub> increased. These fibres were unable to sustain the initial discharge frequency reached and showed a subsequent fall as PaCO<sub>2</sub> increased further. When all chemoreceptor responses were grouped (see figure 6.3.6.i), lambs aged 3-4d showed an initial increase in discharge during SVHX, which then plateaued. Lambs aged 5-9d and 10-24d showed a fall in discharge as PaCO<sub>2</sub> increased. Hornbein et al. (1961) had observed in adult cats at PaO<sub>2</sub> < 35 mmHg during hypocapnia and PaO<sub>2</sub> < 25 mmHg during normocapnia that chemoreceptor discharge was less compared to higher PaO<sub>2</sub>s. This bears some resemblance to my observations, because as the PaO<sub>2</sub> is reduced and PaCO<sub>2</sub> increased, chemoreceptor discharge is less. To my knowledge, this effect of CO<sub>2</sub> at low O<sub>2</sub> has not been reported in the neonate, however during sustained isocapnic hypoxia some workers report that chemoreceptor discharge is not maintained

(Kumar & Hanson, 1989; Mulligan & Bhide, 1989; Marchal et al, 1992a; Carroll et al, 1993).

The biphasic nature of the ventilatory response to hypoxia in the neonate has driven experiments designed to determine if a fall in chemoreceptor discharge is the cause for the fall in ventilation. There is evidence both for and against this argument. Schweiler (1968) recorded single fibre chemoreceptor activity in anaesthetised newborn kittens whilst breathing 10% oxygen in nitrogen. In six single chemoreceptor units from four kittens aged 1-2d, chemoreceptor discharge increased in hypoxia and was sustained for up to 5min.

Blanco et al. (1984b) simultaneously recorded ventilation and carotid chemoreceptor activity in kittens aged 5-34d during acute hypoxia episodes ( $F_{iO_2}$  0.06-0.12;  $PaO_2$   $34 \pm 6$  mmHg, mean  $\pm$  S.E.M). Both hypocapnic (when no attempt was made to control peak expired  $CO_2$ ) and isocapnic hypoxia produced a biphasic ventilatory response, however this was not accompanied by a fall in chemoreceptor discharge. Kittens showed sustained chemoreceptor discharge during the hypoxic episode.

In contrast to these studies, other workers have not found a sustained chemoreceptor response to acute isocapnic hypoxia ( $PaO_2$  40-45mmHg). Carroll et al. (1993) reported that in four of six kittens aged 1wk, and one of six kittens aged 4wks, the chemoreceptor response to hypoxia was biphasic. In an additional two kittens aged 1wk, the chemoreceptor response to hypoxia was not sustained and the protocol was not completed. The time course for the biphasic pattern of the chemoreceptor recording does not fit with that for the ventilatory response to hypoxia. Carroll et al. (1993) observed that discharge reached a peak within the first 30sec, and then adapted to a lower level by 2min. This is probably too fast to account for the biphasic ventilatory response which peaks at *ca.* 2min. Of particular consideration are the whole nerve recordings made in these experiments, and it is not possible to elucidate what the individual chemoreceptors fibres showed.

Marchal et al. (1992a) also report a biphasic chemoreceptor response to hypoxia ( $PaO_2$  55mmHg) in 8 of 15 kittens aged <10d, and 3 of 9 kittens aged >10d. They report peak chemosensory discharge was reached after 20sec, was maintained for *ca.* 15sec, and then gradually declined to a lower steady state level after another 15sec. They suggest that a fast excitatory, and a slow inhibitory component of  $O_2$  chemoreception may explain the biphasic nature of their recordings.

Mulligan & Bhide (1989) recorded single fibre chemoreceptor activity extracellularly from the petrosal ganglion in anaesthetised newborn piglets aged 1-21d. They report that in some piglets chemoreceptor activity was not sustained during sustained hypoxia, and after an initial increase it fell to a low level. No information is given on the number of piglets in which this occurred, the time course of these observations or on blood gas analysis. Furthermore, they were unable to stimulate chemoreceptor activity further by stimulation with CO<sub>2</sub>, cyanide or a further decrease in PaO<sub>2</sub>. Cyanide stimulates the chemoreceptors by blocking cytochrome c oxidase and reducing O<sub>2</sub> availability (Jones et al, 1984), which suggests that if cyanide was unable to increase discharge, that PaO<sub>2</sub> had already fallen to a very low level. Following recovery, chemoreceptors were again responsive to the stimuli, which implies that PaO<sub>2</sub> had been reduced to a level which caused chemoreceptor failure.

My observations are similar in nature to those of Kumar & Hanson (1989) who recorded aortic chemoreceptor fibres in anaesthetised lambs at each of two ages, 1-4d (n=15) and 10-19d (n=15). Chemoreceptor activity was recorded at several inspired P<sub>O<sub>2</sub></sub>s and a hyperbolic hypoxic response curve fitted to the data. Lambs in both age groups showed an increase in discharge frequency for a fall in PaO<sub>2</sub>, however older lambs were unable to sustain discharge below 30mmHg. In contrast, younger lambs were able to show an increase in chemoreceptor discharge for reductions in PaO<sub>2</sub> to ca. 25 mmHg. Hypoxia was isocapnic for all these recordings.

My observations are also supported by Kholwadwala & Donnelly (1992). They found evidence for a fall in chemoreceptor discharge during prolonged periods of anoxia and severe hypoxia recorded from the rat carotid body *in vitro*. Carotid bodies from rat pups aged 1-2d, 4-7d, 10-15d and adult rats were exposed to anoxia and the reduction from peak discharge measured 2min into the anoxic period. Adult rats showed a greater fall from peak discharge (70-90% reduction) than rats pups aged 1-2d (47% reduction).

Whilst the evidence is conflicting, there are two fundamental differences between my results which show a fall in chemoreceptor discharge and those of other workers who report a similar finding at mild or moderate levels of hypoxia in the neonate. First, the hypoxia level that I allocated as severe was PaO<sub>2</sub> 20-35 mmHg, and so was lower than the studies of Marchal et al. (1992a) and Carroll et al. (1993). I then superimposed asphyxia on this low oxygen level; Marchal et al. (1992a) and Carroll et al. (1993) do not report that the observed decrease in chemoreceptor discharge occurred when CO<sub>2</sub> was increased. Secondly, I averaged chemoreceptor discharge 3min after a change in F<sub>IO<sub>2</sub></sub> and/or F<sub>ICO<sub>2</sub></sub>, so the time course I observed for the fall in chemoreceptor discharge is considerably slower than the 20-30sec reported in the kitten (Marchal et al, 1992a).

My observations share a similarity with the observations of Kumar & Hanson (1989) and Kholwadwala & Donnelly (1992), and are most likely to be due to hypoxia resetting of the carotid body from the fetal range for the youngest age group to the adult range in older lambs. The neonatal lamb is unable to sustain a given level of chemoreceptor discharge at a PaO<sub>2</sub> that is within the fetal range. This contrasts to the environment in the fetus when PaO<sub>2</sub> must be reduced below 20 mmHg to produce an increase in chemoreceptor discharge (Blanco et al, 1984a). This supports the idea that chemoreceptor discharge 'fails' in older lambs, and that the observed fall in chemoreceptor discharge it is not due to adaptation. Chemoreceptor adaptation implies that a maximal response is reached after which time discharge falls to a lower level. Chemoreceptors adapt to a dynamic stimulus of CO<sub>2</sub> with a half time of 5-10sec (Black et al, 1971), but my observations were made 3min after a change in PaCO<sub>2</sub> and PaO<sub>2</sub> and so are not a dynamic stimulus. Furthermore, if chemoreceptors adapted to CO<sub>2</sub> during severe hypoxia, then it might be expected for discharge to plateau but not to fall. The response to CO<sub>2</sub> should elicit a rapid increase in discharge which then adapts to a steady state level determined by the PaO<sub>2</sub>. If the chemoreceptor fibre is unable to increase activity to the CO<sub>2</sub> stimulus, then discharge should remain constant as determined by the level of oxygen. The chemoreceptor response for lambs aged 3-4d resembles such a phenomenon, but I did not observe this in lambs aged 5-9d and 10-24d (see figure 6.3.6.i). The fall in discharge was not related to a rise in PaO<sub>2</sub> as PaCO<sub>2</sub> increased. Moreover, the fact that this was observed primarily in older lambs and not in younger ones, supports the idea that it is linked to the postnatal resetting of hypoxia sensitivity. Thus, it seems sensible to describe this fall in chemoreceptor activity observed in older lambs as chemoreceptor failure.

There are several possibilities to consider if the assumption is made that my experiments show chemoreceptor failure during severe hypoxia in older lambs. The first possibility is that the chemoreceptor fibre degenerated throughout the protocol, and acidification of the blood and extracellular fluid may have enhanced this process. I was very cautious of retaining chemoreceptor fibre recordings that demonstrated a decline in the signal to noise ratio over the duration of the experiment. If I had reason to suspect that the fall in chemoreceptor discharge was not 'failure' but degeneration of the fibre (for instance if the fibre did not recover after 15-20min in normoxia/hyperoxia), then I did not include the data in the analysis. I do not believe that this was the explanation for my observations, and it does not explain why this fall in chemoreceptor was greater in the older lambs.

The bicarbonate hypothesis (see section 1.4.3) suggests that CO<sub>2</sub> and O<sub>2</sub> interact by convergence at some common pH<sub>i</sub> in the type I cells. The degree of adaptation is

determined by the pumping of H<sup>+</sup> out of the cell to a level determined by the PaO<sub>2</sub>. If I were to explain my observations in terms of this theory, then it implies that the action of the pump is enhanced under severely asphyxic conditions. That is, discharge falls due to a fall in pH<sub>i</sub>. It could be argued that this is a protective mechanism for the cell to prevent further acidification when pH<sub>i</sub> is low. The Na<sup>+</sup>-H<sup>+</sup> ion exchange has been proposed as a mechanism for the type I cell to regulate pH<sub>i</sub>. It is feasible that under acid conditions the driving force for H<sup>+</sup> to leave the cell is increased, resulting in a rise in pH<sub>i</sub> and [Na<sup>+</sup>]<sub>i</sub>. The only problem with this theory is that the cell would become more depolarized, which would not explain a fall in discharge, hence it is difficult to reconcile a protective mechanism to prevent fatal acidification with the effect of membrane potential and neurotransmitter release.

There is some evidence in support of acidification of type I cells increasing the activity of the Na<sup>+</sup>-H<sup>+</sup> exchanger (for review see Buckler & Vaughan-Jones, 1994c). A reduction in extracellular pH can inhibit the activity of the Na<sup>+</sup>-H<sup>+</sup> exchanger, whilst a reduction in pH<sub>i</sub> can stimulate it. Under normal circumstances, this means that a reduction in extracellular pH will inhibit Na<sup>+</sup>-H<sup>+</sup> exchange and acidify the cell, which is important in terms of extracellular pH exerting an effect on intracellular pH. As pH<sub>i</sub> falls the Na<sup>+</sup>-H<sup>+</sup> exchanger is stimulated and pH<sub>i</sub> is stabilized, although it is lower. In theory, this mechanism should allow an equilibrium to be reached, but perhaps during severe hypoxia and asphyxia the Na<sup>+</sup>-H<sup>+</sup> exchanger is over stimulated. The result effect would be an increase in pH<sub>i</sub> and a fall in chemoreceptor discharge.

The effect on ATP production is important to consider. Under conditions of severe hypoxia ATP production may be substantially reduced, perhaps to levels that cause cellular dysfunction. As reviewed in section 1.4.2.d, Obeso et al. (1992) showed in adult rabbits that the hypoxia-induced release of dopamine was dihydropyridine sensitive. In contrast, they found that dopamine release was not dihydropyridine sensitive to high P<sub>CO2</sub>/low pH. They suggested that the presence of a Na<sup>+</sup>-Ca<sup>2+</sup> ion exchange was responsible for Ca<sup>2+</sup> influx during severe hypoxia and hypercapnia / low pH. So during moderate hypoxia, Ca<sup>2+</sup> influx occurred predominantly via the voltage-gated Ca<sup>2+</sup> channels, however in severe hypoxia Ca<sup>2+</sup> influx also occurred via the Na<sup>+</sup>-Ca<sup>2+</sup> ion exchange. It is possible that very low levels of oxygen reduce cellular ATP so that the voltage-gated Ca<sup>2+</sup> channels are almost inactivated, and Ca<sup>2+</sup> influx occurs only via the Na<sup>+</sup>-Ca<sup>2+</sup> ion exchange. During periods of prolonged anoxia, [Ca<sup>2+</sup>]<sub>i</sub> will fall as the Na<sup>+</sup>-Ca<sup>2+</sup> ion exchange is less efficient and dependent on the concentration gradient across the cell membrane. A fall in [Ca<sup>2+</sup>]<sub>i</sub> will reduce dopamine release and this would have an effect on chemoreceptor discharge. So, it is quite possible that a reduction in cellular ATP will reduce chemoreceptor discharge by inactivation of the voltage-gated

Ca<sup>2+</sup> channels. It is more difficult however to ascribe this theory to a differential effect between ages. It implies that the newborn is less affected by severely low oxygen levels reducing the ability to make ATP. The persistence of fetal haemoglobin in lambs at 3-4d would increase the oxygen carrying capacity of the blood, and could be one explanation for ATP production being less affected by very low oxygen levels in the first few days following birth.

A reduction in ATP is less likely to have an effect on K<sup>+</sup> channels, because although they have been shown to be inhibited by low O<sub>2</sub> (López-López et al, 1989; Ganfornina & López-Barneo, 1991, 1992) and acid stimuli (Peers, 1990a; Peers & Green, 1991), the effect is ATP independent (López-López et al, 1989; Ganfornina & López-Barneo, 1991, 1992).

It is also possible that prolonged periods of anoxia and asphyxia depleted dopamine stores in the type I cells, so that the effect at the nerve ending was less and chemoreceptor discharge was reduced. However, I was able to make further recordings of chemoreceptor activity after periods of recovery from severe hypoxia so this seems unlikely.

To answer the question of which mechanism underlies the 'failure' in chemoreceptor discharge observed in older lambs, it is necessary to study the carotid body in isolation *in vitro* at different ages. Ironically, it is not possible to measure tissue P<sub>O<sub>2</sub></sub> *in vitro*, and only estimates can be made from the P<sub>O<sub>2</sub></sub> of the perfusate. Consequently, it is only possible to speculate at present on the possible mechanisms involved in chemoreceptor failure.

#### 6.4.6 Interaction between CO<sub>2</sub> and O<sub>2</sub> in the neonate

Hornbein et al. (1961) first reported that there was a "*marked potentiation of chemoreceptor activity produced by combination of (H<sup>+</sup>)-P<sub>CO<sub>2</sub></sub> and hypoxia*". The hypoxic response curve was shifted upwards and was steeper at higher PaCO<sub>2</sub>s. Eyzaguirre & Lewin (1961) also found an interaction between CO<sub>2</sub> and O<sub>2</sub> in anaesthetised adult cats. The hyperbolic hypoxic response curve of the carotid chemoreceptors at PaCO<sub>2</sub>>30 mmHg was shifted upwards and was steeper as O<sub>2</sub> was reduced from 50 to 10% compared to when PaCO<sub>2</sub><30 mmHg. At high PaCO<sub>2</sub>s (>40 mmHg) the hypoxic response curve was initially quite steep, but as PaO<sub>2</sub> fell discharge reached a plateau and did not increase considerably at PaO<sub>2</sub>s below 30 mmHg.

Fitzgerald & Parks (1971) measured chemoreceptor response curves to steady state CO<sub>2</sub> in few or multi-carotid chemoreceptor fibres in five adult cats. Chemoreceptor activity was normalized to the maximal response to asphyxia. They observed at higher PaO<sub>2</sub>s that chemoreceptor activity to CO<sub>2</sub> was less than at lower PaO<sub>2</sub>s, but that chemoreceptor discharge continued to show an increase for increases in PaCO<sub>2</sub> even when PaO<sub>2</sub> was 450 mmHg. At low PaO<sub>2</sub>s, chemoreceptor discharge reached a peak at a lower PaCO<sub>2</sub> and then tended to plateau. These observations showed that carotid chemoreceptor responses to steady state CO<sub>2</sub> approximated to a straight line, and that they produced a fan of response curves as PaO<sub>2</sub> fell with a threshold for discharge at *ca.* PaCO<sub>2</sub> 15-20 mmHg. The CO<sub>2</sub> response curve was steepest, prior to reaching a plateau, for when PaO<sub>2</sub> was 33.5-55.5 mmHg.

Lahiri & Delaney (1975) recorded from single carotid chemoreceptor fibres and showed an interaction between CO<sub>2</sub> and O<sub>2</sub>. They demonstrated progressive increases in the slope of the CO<sub>2</sub> chemoreceptor response curve for a fall in PaO<sub>2</sub>. A total of 38 single afferent fibres were recorded from, and all except 3 showed a multiplicative interaction between CO<sub>2</sub> and O<sub>2</sub>.

A number of studies have now investigated stimulus interaction between CO<sub>2</sub> and O<sub>2</sub> in the neonate. In an early study, Mulligan (1989) recorded chemoreceptor activity extracellularly from the petrosal ganglion in anaesthetised piglets to investigate stimulus interaction between CO<sub>2</sub> and O<sub>2</sub>. Mulligan (1989) observed an increase in steady state chemoreceptor activity for a fall in PaO<sub>2</sub> or a rise in PaCO<sub>2</sub>. Responses to CO<sub>2</sub> during hypoxia in the newborn piglet were compared to those in the adult cat, and it was concluded that interaction between CO<sub>2</sub> and O<sub>2</sub> was minimal.

Carroll et al. (1993) observations agree with Mulligan (1989), that there is no interaction between CO<sub>2</sub> and O<sub>2</sub> in the neonate. They measured steady state CO<sub>2</sub> chemoreceptor responses at several PaO<sub>2</sub> levels in anaesthetised kittens and cats. Chemoreceptor responses to CO<sub>2</sub> were increased at lower oxygen levels. This was attributable to an upward shift of the chemoreceptor CO<sub>2</sub> response curve in kittens aged 1wk and 4wks. For kittens aged 8wk and in adult cats the slope of the chemoreceptor CO<sub>2</sub> response curve was also increased. They observed in kittens aged 8wk and in adults that discharge frequency reached a plateau during hypoxia (PaO<sub>2</sub> 40-45 mmHg) after which further increases in PaCO<sub>2</sub> produced little or no further increase in discharge frequency. I observed some evidence of plateauing at higher PaCO<sub>2</sub>s during MOD HX in lambs 10-24d, and during SVHX in lambs aged 3-4d (see figure 6.3.6.i). Chemoreceptor discharge was also plotted against PaCO<sub>2</sub> and PaO<sub>2</sub> as a three dimensional function, previously described by Eyzaguirre & Lewin (1961). In kittens aged 1wk there was minimal, or no

effect, of hypoxia on the chemoreceptor response to CO<sub>2</sub>. The response for adult cats was qualitatively similar to that shown by Eyzaguirre & Lewin (1961). They concluded that multiplicative CO<sub>2</sub>-O<sub>2</sub> interaction developed with age.

More recently, Pepper et al. (1995) have postulated that the postnatal development of carotid body hypoxia sensitivity is due to the emergence of significant interaction between O<sub>2</sub> and CO<sub>2</sub>. Superperfused rat carotid bodies were studied *in vitro*, and at 5-7d the chemoreceptor response to CO<sub>2</sub> was increased during hypoxia by an upward shift in chemoreceptor discharge. However, CO<sub>2</sub> sensitivity was unchanged in hypoxia. Similarly from the hypoxia response curve, an increase in P<sub>CO2</sub> caused an upward shift of the hyperbola and baseline discharge was increased, however the rate constants describing the hyperbola were unchanged during hypoxia. This is the first evidence *in vitro* of a postnatal increase in CO<sub>2</sub>-O<sub>2</sub> interaction.

My results showed some evidence of interaction between CO<sub>2</sub> and O<sub>2</sub> when I compared the slope of the chemoreceptor response curves at three different ages. At 3-4d CO<sub>2</sub> sensitivity during MOD HX was significantly greater compared to NX; at 5-9d CO<sub>2</sub> sensitivity during NX and MOD HX were significantly greater than during HYP; and at 10-24d CO<sub>2</sub> sensitivity during MOD HX was significantly greater compared to HYP. There was however no significant interaction when all chemoreceptor responses were pooled across the ages. However, this is not surprising considering there is evidence to suggest that interaction develops postnatally.

My results show that interaction between CO<sub>2</sub> and O<sub>2</sub> was significant in lambs aged 10-24d, but not in younger lambs. In section 6.3.7 I compared the chemoreceptor responses at three ages for the effect of O<sub>2</sub>, CO<sub>2</sub> and CO<sub>2</sub> x O<sub>2</sub> on chemoreceptor discharge. Chemoreceptor responses were measured at different PaO<sub>2</sub>s and PaCO<sub>2</sub>s. There was a clear effect of CO<sub>2</sub>-O<sub>2</sub> interaction in the oldest lambs. This is a new finding and has not been reported previously. My findings are strengthened by the fact that the age ranges were narrow and concentrated on the first few weeks after birth. The observations of Pepper et al. (1995) do not permit any speculation for the time course of the development of interaction, as only one age group of neonates is compared to the adult. My results suggest that stimulus interaction is present at an earlier age than previously suggested by Carroll et al. (1993). Thus, in younger lambs the increase in discharge to CO<sub>2</sub> observed during hypoxia is best explained by an upward shift of the chemoreceptor response curve without a significant increase in CO<sub>2</sub> sensitivity. In older lambs, the increase in discharge to CO<sub>2</sub> during hypoxia can be attributed to CO<sub>2</sub>-O<sub>2</sub> interaction. This is an important new finding for the postnatal development of CO<sub>2</sub>-O<sub>2</sub> interaction.

### 6.4.7 Possible mechanisms for a postnatal increase in steady state CO<sub>2</sub> sensitivity

There are two possibilities to explain a postnatal increase in steady state CO<sub>2</sub> sensitivity. The first is that the chemotransduction mechanisms responsible for the sensing of CO<sub>2</sub>/pH show a developmental increase, the second that the increase in CO<sub>2</sub> sensitivity is mediated by the postnatal increase in O<sub>2</sub> sensitivity. To investigate the first possibility, it is important to consider the evidence the peripheral chemoreceptor responding to CO<sub>2</sub> *in utero*.

Blanco et al. (1984a) showed in the fetal lamb *in utero* (90-143d gestation) that the carotid chemoreceptors were responsive to CO<sub>2</sub>. Discharge increased when CO<sub>2</sub>-equilibrated saline was injected retrogradely into the lingual artery, which confirmed the presence of carbonic anhydrase in the fetal carotid body. Marchal et al. (1992a) speculated that there may be an increase in the expression of this enzyme from the fetus to the neonate, however to date, no studies have investigated developmental changes in carbonic anhydrase expression in the carotid body. There is evidence to suggest in the newborn rat that CA activity at different sites in the brain shows a developmental increase (Odarjuk, Lun, Moller, Pohle, Meyer & Gross, 1986; Endröczi, Sasváry & Simon, 1994). Odarjuk et al. (1986) found an increase in CA activity in homogenates of the striatum, hippocampus and cerebral cortex in newborn rats over the first 2-3wks. Hypoxia also increased CA activity which they assumed to be a catecholamine-mediated response to activation of the glia. Endröczi et al. (1994) showed an increase in CA activity in the neocortex and hippocampus in rat pups between 3 and 21d. They also found that noradrenaline increased CA activity. The findings of Blanco et al. (1984a) confirm the presence of CA in the fetal carotid body, however it would be interesting to look at the development of CA expression in the neonatal carotid body in the first few days of life. As the developmental studies of CA activity in the brain suggest that hypoxia (Odarjuk et al, 1986) and noradrenaline (Endröczi et al, 1994) increase CA activity, the changes in neonatal carotid body dopamine and noradrenaline content could have an effect on CA activity (refer to section 1.5.3).

Dynamic CO<sub>2</sub> sensitivity of the carotid body is dependent on the presence of CA (Black et al, 1971). Therefore, assessment of dynamic CO<sub>2</sub> sensitivity in the neonate allows some indication of postnatal development of CA activity. Torrance's bicarbonate hypothesis predicts that dynamic CO<sub>2</sub> sensitivity is independent of the background level of oxygen (Torrance, 1976; Torrance et al, 1993). So in the newborn when hypoxia sensitivity is increasing postnatally, there should be a dynamic CO<sub>2</sub> sensitivity present that is independent of O<sub>2</sub>. This was the objective of experiments in

Chapter 7. I have measured the dynamic chemoreceptor response to CO<sub>2</sub> in newborn lambs to determine if the increase in steady state CO<sub>2</sub> sensitivity could be explicable in terms of an increase in the CO<sub>2</sub> chemo-sensing mechanism postnatally. Steady state CO<sub>2</sub> sensitivity is obviously influenced by O<sub>2</sub>, but the basic CA sensing mechanism is not dependent on O<sub>2</sub>. Therefore if it is possible to demonstrate that there is no change in dynamic CO<sub>2</sub> sensitivity postnatally, then it is unlikely that the postnatal increase in steady state CO<sub>2</sub> sensitivity I have observed in newborn lambs is due to a development of CO<sub>2</sub> chemo-sensing.

The mechanism of O<sub>2</sub> chemotransduction is not well understood, and so the exact process of postnatal hypoxia resetting is currently unknown. A review of the current literature and popular theories at present for O<sub>2</sub> chemotransduction and hypoxia resetting can be found in Chapter 1. From studies in adult cats, Fitzgerald & Parks (1971) have demonstrated interaction between O<sub>2</sub> and CO<sub>2</sub> for the carotid chemoreceptors. As O<sub>2</sub> falls, CO<sub>2</sub> sensitivity increases. In the neonate, hypoxia sensitivity increases with age. At 1-2d, hypoxia sensitivity is low and so a fall in PaO<sub>2</sub> has a relatively small effect on chemoreceptor discharge and there is no effect on CO<sub>2</sub> sensitivity at a lower PaO<sub>2</sub>. At *ca.* 2wks when hypoxia sensitivity has reset, a reduction in PaO<sub>2</sub> will have a much greater effect on chemoreceptor discharge and CO<sub>2</sub> sensitivity is also increased, as I have shown in lambs aged 10-24d. This strongly suggests that the postnatal increase in CO<sub>2</sub> sensitivity is dependent on the increase in O<sub>2</sub> sensitivity. However, because the exact mechanism for hypoxia resetting is unknown, it is not possible to prevent this and observe if the development of CO<sub>2</sub> sensitivity is also disrupted. In the future, *in vitro* carotid body preparations may uncover the mechanism behind hypoxia resetting and it will be possible to test that it also determines the increase in CO<sub>2</sub> sensitivity.

Pepper et al. (1995) have suggested that resetting of hypoxia chemosensitivity may result from the development of CO<sub>2</sub>-O<sub>2</sub> interaction. I have found, as have others, that CO<sub>2</sub>-O<sub>2</sub> interaction is not significant in the immediate neonatal period (Carroll et al, 1993; Pepper et al, 1995). During this time when interaction is suggested to be minimal or absent, the newborn does show an increase in CO<sub>2</sub> sensitivity with age. Thus it could be argued that the increase in CO<sub>2</sub> sensitivity is not mediated by its interaction with O<sub>2</sub>, and so is possibly independent of hypoxia sensitivity resetting. It seems unlikely that the development of CO<sub>2</sub>-O<sub>2</sub> interaction would account for the increase in CO<sub>2</sub> sensitivity, because chronic hypoxia does not abolish CO<sub>2</sub> sensitivity. Chronically hypoxic animals (Landauer et al, 1995) and newborn infants at high altitude (Lahiri, 1978) that show blunted hypoxia responses are capable of responding to CO<sub>2</sub>.

However there have been no systematic studies of the development of CO<sub>2</sub> sensitivity in chronically hypoxic animals, so this theory remains to be consolidated.

### **6.4.8 Summary**

I have shown from few and multi-fibre chemoreceptor recordings in anaesthetized newborn lambs, that there is a CO<sub>2</sub> sensitivity present at 3-4d, and that it increases with age. Older lambs were unable to sustain an increase in chemoreceptor discharge during severe hypoxia (PaO<sub>2</sub> 20-35 mmHg) when PaCO<sub>2</sub> was increased. Lambs at 3-4d showed an initial increase in chemoreceptor discharge during severe hypoxia (PaO<sub>2</sub> 20-35 mmHg), which plateaued when PaCO<sub>2</sub> was increased. I have found that CO<sub>2</sub>-O<sub>2</sub> stimulus interaction is present in lambs at 10-24d, but not at 5-9d and 3-4d. This has not been reported previously.

## **7.0 DYNAMIC CHEMORECEPTOR RESPONSES TO CO<sub>2</sub> IN THE NEWBORN LAMB**

### **7.1 Introduction**

It was first suggested by Hornbein et al. (1961) that carotid body chemoreceptor discharge oscillated about a mean at the frequency of respiration in adult cats. Similarly Leitner & Dejourns (1968) demonstrated in the cat, and Biscoe & Purves (1967) in the newborn lamb that respiratory related oscillations in chemoreceptor discharge occurred about a mean level of discharge. These oscillations in chemoreceptor discharge were related to oscillations in arterial blood gas, produced in turn by oscillations in alveolar gas composition (Haldane & Priestly, 1935; Chilton & Stacey, 1952; DuBois et al. 1952; Yokota & Kreuzer, 1973). Respiratory induced oscillations in PaO<sub>2</sub> produced oscillations in chemoreceptor discharge in the adult cat (Purves, 1964; Purves, 1966a; Purves, 1966f). PaCO<sub>2</sub>, and hence pH, of arterial blood also oscillated at the same frequency as respiration and showed respiratory related oscillations in chemoreceptor discharge in adult cat and man (Band & Semple, 1966; Band & Semple, 1967). The amplitude of the oscillations in arterial blood gases at respiratory frequency were for pH 0.01-0.02 units (Honda & Veda, 1961; Band, Cameron & Semple, 1969), for PaCO<sub>2</sub> 1-3 mmHg (Honda & Veda, 1961) and for PaO<sub>2</sub> 1-2 mmHg. Whilst the oscillations in PaCO<sub>2</sub> and PaO<sub>2</sub> both contribute to an oscillation in chemoreceptor discharge, the primary determinant is the oscillation in alveolar CO<sub>2</sub> (Band et al, 1971; Goodman, Nail & Torrance, 1974; Band, McClelland, Phillips, Saunders & Wolff, 1978).

Oscillations in chemoreceptor discharge have been investigated by the summation of discharge from single fibre units from the carotid sinus nerve (CSN) over several respiratory cycles (Hornbein et al, 1961; Biscoe & Purves, 1967; Leitner & Dejourns, 1968). Goodman et al. (1974) showed that the amplitude of chemoreceptor oscillation increased by decreasing respiratory frequency, or by poisoning with DNP, so as to increase metabolism. They found that during hypercapnia or hypoxia the absolute amplitude (from peak to trough) of the oscillation remained approximately constant. However the relative amplitude (absolute amplitude as a percentage of the mean level of discharge) always decreased due to a rise in the mean level of discharge.

Adaptation in the chemoreceptor response to CO<sub>2</sub> has been observed *in vivo* (Black et al, 1971; Leitner & Dejourns, 1968; Black, McCloskey, Torrance, 1966). Black et al. (1971) observed from intra-arterial injections of 100% CO<sub>2</sub> equilibrated saline close to the carotid body there was a brisk increase in discharge frequency followed by an

adaptation to a lower steady level which occurred more slowly. Discharge reached a maximum within the first second, and adapted with a half time of 5-10sec. Upon removal of the stimulus, discharge fell to a minimum and then adapted to a higher steady level. They also found that the adaptation to an increase in PaCO<sub>2</sub> occurred more rapidly than when the stimulus was removed. This adaptation in the chemoreceptor response to CO<sub>2</sub> is mediated by carbonic anhydrase (CA) and abolished by acetazolamide (Black et al, 1971). Chemoreceptors do show some adaptation in response to PaO<sub>2</sub>, however they respond more slowly to oscillations in PaO<sub>2</sub> compared to PaCO<sub>2</sub> (Fitzgerald, Leitner, Liaubet, 1969; Kumar, Nye, Torrance, 1988). The contribution that PaO<sub>2</sub> oscillations make to oscillations in chemoreceptor discharge is small, but is increased in hypoxia compared to normoxia (Kumar et al, 1988; Kumar & Nye, 1985).

A comparison between the shape of the oscillation in chemoreceptor discharge and that for PaCO<sub>2</sub> led to the theory that chemoreceptor discharge responded to the rate of change in pH and not the level of acidity (Band, Saunders & Wolff, 1971; Goodman et al, 1974; Cross, Leaver, Semple & Stidwell, 1986). The amplitude of the oscillation in discharge was found to be too large (relative to mean discharge frequency) to be explained solely on the basis of a proportional increase in discharge for an increase in PaCO<sub>2</sub> (Goodman et al, 1974; Band et al, 1971). Based on the steady state relationship between chemoreceptor discharge and mean PaCO<sub>2</sub> (Fitzgerald & Parks, 1971; Lahiri & Delaney, 1975) the amplitude of the oscillation in discharge exceeded that predicted by the steady state CO<sub>2</sub> response curves. Goodman et al. (1974) found at very rapid respiratory frequencies that the oscillation in discharge became damped or disappeared completely. Linton & Band (1988) found that the amplitude of the oscillation in pH was reduced at faster respiratory frequencies, and this may or may not reduce the amplitude of the oscillation in discharge. At fast respiratory frequencies the oscillation was a sine wave, but as respiratory frequency slowed the sine wave became skewed and then saw-toothed (Goodman et al, 1974). The saw-tooth shaped oscillation showed a much more rapid rate of fall than rate of rise in discharge frequency, and the slow rate of rise in discharge frequency often plateaued after reaching an initial peak. The concept that chemoreceptors responded to the rate of change of pH, and by inference in PaCO<sub>2</sub>, was argued by the fact that at slow respiratory frequencies the adaptation to CO<sub>2</sub> was rapid enough to determine in part the shape of the oscillation. Investigation of the relationship between the oscillation in arterial pH and chemoreceptor discharge showed that the carotid chemoreceptor did not respond as a proportional receptor to oscillations in PaCO<sub>2</sub> (Cross et al, 1986; Band et al, 1971). The point of minimum discharge frequency coincided with the maximum point of pH oscillation (Cross et al, 1986), however, the maximum discharge frequency reached during the oscillation did not occur

with the acid trough of the oscillation in arterial pH (Cross et al, 1986; Band et al, 1971). The peak in discharge frequency preceded the trough in pH, and it appeared to occur at a time when the fall in pH was steepest. Thus, the carotid chemoreceptor responded to the rate of change in pH and not the pH minimum reached during the oscillation.

If the carotid chemoreceptor simply responded to the rate of change in pH, i.e. a rate receptor, then the greatest discharge would be observed when the rate of pH change was greatest. Kumar, Nye & Torrance (1994) showed that this was not the case by producing CO<sub>2</sub> ramps and measuring chemoreceptor discharge. CO<sub>2</sub> ramps at rapid respiratory frequencies reduced the amplitude of the chemoreceptor oscillation, compared to slower respiratory frequencies when the amplitude of the CO<sub>2</sub> ramp was constant. Thus when the rate of pH change was greatest, chemoreceptor discharge was not, and so was not described by a 'rate' receptor. Rather discharge was proportional to the steady state increase in CO<sub>2</sub>. They propose that carotid chemoreceptor dynamic sensitivity has two components; first it responds proportionally to the rate of change in arterial pH, and secondly it adapts to a lower level determined by the P<sub>O<sub>2</sub></sub>. In a further series of experiments Torrance, Iturriaga & Zapata (1994) showed in anaesthetised cats when t<sub>E</sub> was prolonged, chemoreceptor discharge increased and reached a plateau. Discharge did not fall towards the end of t<sub>E</sub> when the rate of rise in CO<sub>2</sub> was presumably less than at the start of t<sub>E</sub>. Again it was argued from these observations that the carotid chemoreceptor response to CO<sub>2</sub> was not described by a rate receptor, but that of an adapting proportional receptor (Black et al, 1971; Kumar et al, 1994).

The observed adaptation in the chemoreceptor response to CO<sub>2</sub> led to the theory that there was both steady state and dynamic sensitivity of the carotid body to CO<sub>2</sub> (Torrance, 1976). Band & Wolff (1973, 1978) investigated the effect of hypoxia on the oscillations in chemoreceptor discharge. They found no consistent trend between the absolute amplitude of the oscillation in discharge and the level of hypoxia. The amplitude of the oscillation was also expressed in relation to the mean rate of discharge, and in this way there was a relation with PaO<sub>2</sub>. As PaO<sub>2</sub> fell the amplitude to mean ratio also fell, and this was due to a rise in the mean rate of discharge during hypoxia. This was in agreement with the earlier findings of Goodman et al. (1974) who also observed no change in the absolute amplitude of the oscillation during hypoxia. Kumar et al. (1988) found in adult cats that the amplitude and shape of the oscillation in chemoreceptor discharge was not significantly altered by a change in PaO<sub>2</sub>, although mean discharge increased in hypoxia as expected. They also confirmed the findings of Cross et al. (1986) who had reported that raising the mean PaCO<sub>2</sub> level similarly had no effect on the amplitude of the oscillation in discharge. Kumar et al. (1988) showed,

by plotting the maximum and minimum frequencies for the oscillation in discharge as a function of PaCO<sub>2</sub>, that the transient responses were steeper than steady state response curves, and that they were parallel at different mean PaCO<sub>2</sub>s. Thus dynamic CO<sub>2</sub> sensitivity of the carotid body appeared to be independent of the mean level of PaCO<sub>2</sub> and PaO<sub>2</sub>. This fitted with Torrance's bicarbonate theory (Torrance, 1976; Torrance et al, 1993) and Band & Wolff's previous observations (1978) that the dynamic response to CO<sub>2</sub> was described by a family of parallel transient response curves.

The neonatal response to hypoxia and the postnatal resetting of hypoxia chemosensitivity has been extensively examined (see Chapter 2). In contrast, the dynamic response of the carotid body to CO<sub>2</sub> has been poorly investigated in the neonate. Marchal et al. (1992a) investigated the dynamic response to CO<sub>2</sub> in anaesthetised new born kittens from single fibre chemoreceptor recordings. They measured the time taken to reach a steady state level of chemoreceptor discharge after the application of 5% or 10% CO<sub>2</sub> in oxygen. They report an average time of 40sec to reach a steady level of discharge, and comment that no brisk response followed by an adaptation (as observed by Black et al, 1971) was observed. They inferred from these observations that the newborn kitten did not possess a dynamic chemosensitivity to CO<sub>2</sub>, or at least it was greatly reduced compared to the adult cat. This is problematic for two reasons. Firstly, because the time taken to reach a steady state after application of a stimulus is not an indication of dynamic sensitivity, rather it measures the time course of the continued effects of a dynamic response and of adaptation. Secondly, Marchal et al. (1992a) argued that the "*absence of overshoot was not due to the speed of delivery of the CO<sub>2</sub> stimulus, since its time constant was 4 sec, i.e. shorter than the time course for adaptation to CO<sub>2</sub> stimulus in the adult cat*". However, this application of the stimulus is much slower than that used by Black et al. (1971), as they saw a maximal response within the first second and their time course for adaptation was 5-10sec. If the time course for the delivery of the stimulus used by Marchal et al. (1992a) was at best 4sec, then it is very unlikely that an overshoot would ever be observed. Thus, they for the most part based their definition of dynamic sensitivity on the appearance of an overshoot in the chemoreceptor response to CO<sub>2</sub>, however they have failed to deliver the stimulus rapidly enough to observe such an effect. Therefore, direct evidence for the presence of dynamic chemoreceptor sensitivity to CO<sub>2</sub> in the neonate from chemoreceptor recordings remained lacking.

Ventilatory studies in the neonate designed to measure the dynamic sensitivity to CO<sub>2</sub> are also controversial. Several studies report no change in the dynamic CO<sub>2</sub> sensitivity after birth. Canet, Carroll, Praud, Delacourt & Bureau (1990) found using a two breath transient CO<sub>2</sub> test that the ventilatory response did not increase significantly between

day 1 and 14 in awake newborn lambs. In an earlier study Carroll & Bureau (1987b) found that the ventilatory response to a CO<sub>2</sub> step challenge was not significantly greater in 10d compared to 2d old lambs. Watanabe, Kumar & Hanson (1993b) alternated inspired gas between air and 5% CO<sub>2</sub> in air, or between air and 16% oxygen on a single breath basis in neonatal kittens during quiet sleep. They found that the respiratory response to CO<sub>2</sub> was present when the hypoxic response was weak in kittens at 2-7d, and concluded that the carotid body possessed a dynamic sensitivity to CO<sub>2</sub> at a time when hypoxia sensitivity was resetting.

Other researchers have found a maturation of CO<sub>2</sub> sensitivity postnatally. Wolsink et al. (1993) used the dynamic end-tidal forcing technique to assess the relative contribution the peripheral and central chemoreceptors made to the ventilatory response to CO<sub>2</sub> in anaesthetised newborn piglets. They concluded that the relative contribution (to total CO<sub>2</sub> sensitivity) the peripheral chemoreceptors made to the ventilatory response increased postnatally, however there was no maturation in the peripheral chemoreceptor component *per se* (see also section 6.1). It is difficult to interpret whether this peripheral component is a measure of dynamic or steady state CO<sub>2</sub> sensitivity of the carotid body, or whether it is a measure of both. Upton, Milner, Stokes & Wilson (1990) measured the dynamic ventilatory response to tube breathing in term infants during quiet sleep. Infants breathed through tubes that were equal to two anatomical dead spaces. Instantaneous ventilation was measured and plotted over time, and the time taken to reach 63% of the steady state ventilatory response was used as an index of the dynamic response to CO<sub>2</sub>. They observed a reduction in the time taken to reach this 63% level over the first 10 days of life. They concluded that these observations reflected a maturation of dynamic CO<sub>2</sub> sensitivity. However, it must be noted that even in infants aged 4d the time taken to reach this 63% level was 30-60sec, which would undoubtedly be influenced by the central chemoreceptor response to CO<sub>2</sub>.

Thus, given the conflicting observations from ventilatory studies investigating postnatal maturation of the dynamic response to CO<sub>2</sub>, and the virtual absence of any information about dynamic CO<sub>2</sub> sensitivity at the level of the carotid body, I measured dynamic CO<sub>2</sub> sensitivity in anaesthetized newborn lambs by making direct recordings from the carotid sinus nerve (CSN). I did this by measuring the chemoreceptor response to alternations in etCO<sub>2</sub> which were produced by adding CO<sub>2</sub> to the inspired gas on an alternate breath basis. The alternations were produced in one of two ways: first, lambs were ventilated at *ca.* 20 breaths/min and alternations were produced on a single breath basis, and secondly lambs were ventilated more rapidly at *ca.* 120 breaths/min and alternations were produced on a 15 breath basis. Some of these observations have been published in brief (Calder, Kumar & Hanson, 1995a).

## 7.2 Methods

### 7.2.1 Sheep husbandry

As detailed in section 2.4.1.

### 7.2.2 Animal preparation

As detailed in section 2.4.2. Ventilation was controlled by a purpose built switching ventilator (Birmingham University). The ventilator controlled respiratory frequency and set the ratio for inspiratory time to expiratory time. It also controlled three solenoids, one ventilating solenoid and two (two-way) solenoids that allowed inspired gas to switch between one of two gas delivery lines. The switching of inspired gas occurred at the start of expiration on a cyclical basis. When the two-way solenoid was open, gas in that delivery line was inspired by the lamb, and when the solenoid was closed it was diverted to atmospheric air. It was possible to set the number of breaths per alternation cycle from 1 to 99 breaths per alternations (see figure 7.2.2.i).

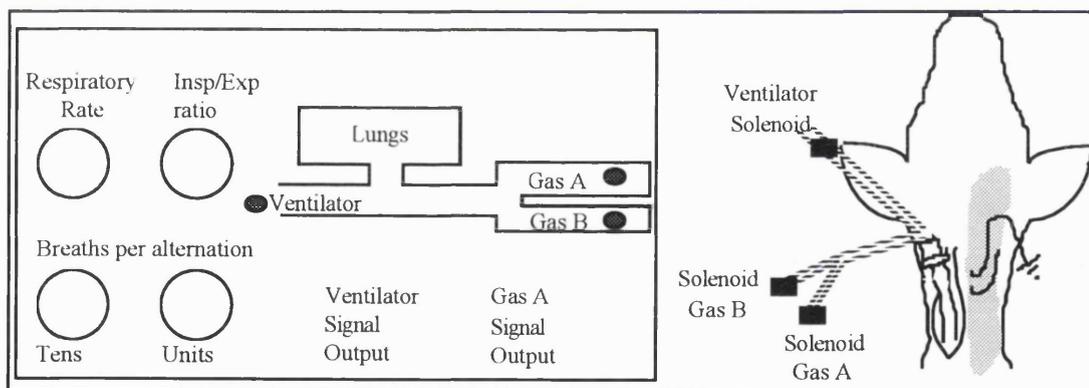


Figure 7.2.2.i Schematic diagram of alternating ventilator.

The alternating gas signal was used by the computer to detect the start of an alternation period. Summing of chemoreceptor discharge always started on inspiration. The number of breaths over which summation occurred was entered manually into the computer (i.e. for a 1 breath alternation discharge was summed over 2 breaths, for a 15 breath alternation discharge was summed over 30 breaths).

### **7.2.3 Protocol**

Carotid chemoreceptor dynamic responses to CO<sub>2</sub> were measured in 23 of the 43 lambs, and were also assessed for steady state chemoreceptor responses at the same PaO<sub>2</sub> after the alternation (as described in section 2.4.3). Two different approaches were used to measure dynamic chemoreceptor responses:

- (i) Lambs were ventilated at 0.3-0.5 Hz and inspired gas was switched at the start of expiration on a breath-by-breath basis between two gas lines (alternation period  $\approx$  6-8sec).
- (ii) Lambs were ventilated at *ca.* 2.0 Hz and inspired gas was switched at the start of expiration on a 15 breath cycle (alternation period  $\approx$  16 sec) between two gas lines.

One gas line contained no added CO<sub>2</sub>, and to the other 0.4-0.5l/min CO<sub>2</sub> was added (total flow 4.0-7.0l/min) in NX (PaO<sub>2</sub> 80-100 mmHg) and MOD HX (PaO<sub>2</sub> 40-60 mmHg). Arterial blood gas was sampled during the alternation period to determine PaO<sub>2</sub>. After the chemoreceptor response to 1 or 15 breath alternations in etCO<sub>2</sub> had been measured, the steady state chemoreceptor response was measured at the same PaO<sub>2</sub>.

### **7.2.4 Data Analysis**

#### **7.2.4.a Acquisition of CSN discharge**

Raw CSN discharge was converted to TTL pulse and captured digitally by a data acquisition system (NB-MIO-16 input/output board, National Instruments, TX, U.S.A.). Labview software (Apple Computer Inc., CA, U.S.A.) programmed in conjunction with Birmingham University was used to bin discharge in 200msec on a Macintosh Quadra 630 (Apple Computer Inc., CA, U.S.A.). Discharge was summed in 200msec bins over the period of the alternation cycle (i.e. over 2 breaths for a 1 breath alternation in etCO<sub>2</sub>, or over 30 breaths for a slower alternation). Summation of chemoreceptor discharge began approximately 3min after the alternation in etCO<sub>2</sub> was commenced. The alternating gas signal from the ventilator was used by the computer to recognise the start and end of the alternation period. The oscillation in CSN discharge was smoothed by moving average (3 point moving average for 1 breath alternations; 10 point moving average for 15 breath alternations), and the peak and trough of the oscillation was expressed as discharge frequency (Hz). The steepness of the slope formed between the peak and trough in CSN discharge was used as a measure of the dynamic response to CO<sub>2</sub>. Dynamic CO<sub>2</sub> sensitivity was expressed as a percentage of

maximal discharge (% maximal discharge / etCO<sub>2</sub> %). Maximal discharge was measured as described in section 2.4.2.

Following the alternations, steady state chemoreceptor responses to CO<sub>2</sub> were measured. Discharge was averaged over a 20sec period at least 3min after the change in etCO<sub>2</sub> from the chart record. Steady state chemoreceptor responses to CO<sub>2</sub> were also summed in 200msec as per the alternations.

#### 7.2.4.b Criteria for accepting oscillations in CSN discharge

It was necessary to set some criteria for what I considered to be an acceptable oscillation in CSN discharge. It was essential that there was a clearly defined peak and trough in the oscillation, and that the time between these two points in the oscillation approximated to half the inspired gas alternation period, that is the duration for which the animal received CO<sub>2</sub> in the inspire. Figure 7.2.4.b.i shows an example of an experiment that was not acceptable for inclusion in the analysis of oscillations in CSN discharge. Whilst there is variation in discharge frequency over the period of the alternation, it is not obvious that these occur in relation to changes in inspired CO<sub>2</sub>. For this alternation, gas supply would have switched at 0 and 8 sec, however there does not appear to be any relation with the timing of the alternation and the addition or removal of CO<sub>2</sub> from the inspire.

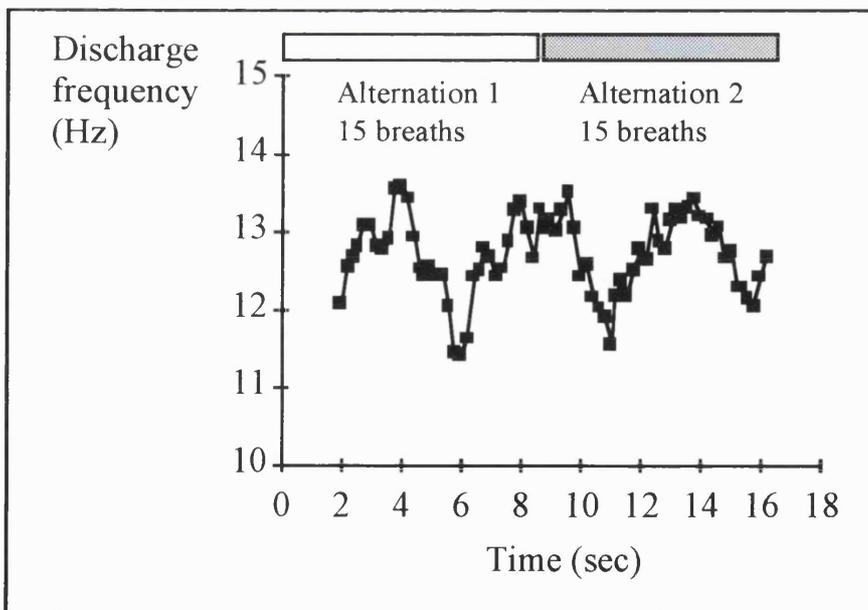


Figure 7.2.4.b.i CSN discharge in response to 15 breath alternation in etCO<sub>2</sub> during MOD HX. This response was not included in the analysis of oscillations in CSN discharge. Although a peak and trough could be detected from this response, it does not appear to correlate with alternation period.

**7.2.4.c Analysis of dynamic chemoreceptor responses and comparison to steady state**

Dynamic CO<sub>2</sub> responses were always compared to steady state CO<sub>2</sub> responses for the same fibre at the appropriate PaO<sub>2</sub>. To address the question of whether the chemoreceptor response to an alternation in etCO<sub>2</sub> was greater than the response to similar steady state level of CO<sub>2</sub> in the same fibre, the difference between steady state and dynamic responses were tested by a one sample *t*-test (Altman, 1991; pp 184-185). This approach was used upon recommendation from the Statistical Consultancy service in the department of Epidemiology and Public Health, University College London. Essentially, this tests the hypothesis that a sample from a population has a hypothesized mean. The value of *t* was calculated as:

$$t = \frac{\text{sample mean} - \text{hypothesized mean}}{\text{standard error of sample mean}}$$

In this instance the hypothesized mean is zero, in that is there is no difference between dynamic and steady state responses. The magnitude of *t* gives "*the average discrepancy of the sample values from the hypothetical mean, divided by the sample error of the sample mean*" (Altman, 1991; pp 184-185).

**7.2.4.d Effect of age and PaO<sub>2</sub> on dynamic chemoreceptor responses**

Two different approaches were used to determine the effect of age and PaO<sub>2</sub> on dynamic chemoreceptor responses. First, the slope of steady state CO<sub>2</sub> response curve (% maximal discharge / etCO<sub>2</sub> % concentration ) was compared to the slope of the dynamic CO<sub>2</sub> response ( peak - trough discharge as % maximal discharge / peak - trough etCO<sub>2</sub> ). Multiple linear regression was used to determine the effect of age and PaO<sub>2</sub> on chemoreceptor responses.

Secondly, the amplitude of the oscillation in discharge produced by the alternation in etCO<sub>2</sub> was compared to the amplitude of the naturally occurring oscillation in discharge at each of the steady state etCO<sub>2</sub> steps for the same PaO<sub>2</sub>.

## 7.3 Results

### 7.3.1. Possible sources of error when comparing dynamic to steady state chemoreceptor responses.

Linear regression was used to describe steady state chemoreceptor responses to CO<sub>2</sub> and allowed the comparison between steady state and dynamic chemoreceptor responses. Chemoreceptor curves will be poorly described by linear regression if there are only 2 steady state points on which to base that line, or if the correlation coefficient of the regression line is low. To investigate if steady state chemoreceptor responses were in fact poorly described by linear regression, they were scored according to their characteristics in table 7.3.1.i. There are no clear differences between the three age group, and fibres at all ages are described by steady state response curves that have correlation coefficients <0.90, or have only two points.

		Only 2 steady state points to describe curve No. fibres	Correlation coefficient > 0.90 No. fibres	Correlation coefficient > 0.80 No. fibres
3-4d	NX	2 of 6	0 of 6	0 of 6
	MOD HX	0 of 7	6 of 7	1 of 7
5-9d	NX	2 of 10	5 of 10	3 of 10
	MOD HX	3 of 5	0 of 5	0 of 5
10-17d	NX	3 of 8	0 of 8	0 of 8
	MOD HX	2 of 9	1 of 9	1 of 9

Table 7.3.1.i Summary of characteristics of steady state response curves as described by linear regression.

### 7.3.2 Oscillations in CSN discharge to alternations in etCO<sub>2</sub>

Alternations in etCO<sub>2</sub> were produced by one of two methods (see section 7.2.3) in either NX or MOD HX or both. A total of 8 fibres at 3-4d, 11 fibres at 5-9d and 12 fibres at 10-17d were tested for both dynamic and steady state chemoreceptor responses to CO<sub>2</sub> (table 7.3.2.i). The number of fibres in which 15 breath or 1 breath CO<sub>2</sub> alternations were measured is shown in table 7.3.2.ii.

Age	n	Total	NX	MOD HX	Both NX and MOD HX
3-4d	fibres	8	6	7	4
	lambs	7	5	6	4
5-9d	fibres	11	10	5	4
	lambs	7	6	4	3
10-17d	fibres	12	8	9	5
	lambs	9	7	8	5

Table 7.3.2.i Summary of chemoreceptor fibres measured for both dynamic and steady state chemoreceptor responses to CO<sub>2</sub>.

Age	Total fibres	15 breath alternations	1 breath alternations	Both 15 and 1 breath alternations	
3-4d	NX	6	5	2	1
	MOD HX	7	7	5	4
5-9d	NX	10	7	5	2
	MOD HX	5	5	5	4
10-17d	NX	8	7	7	6
	MOD HX	9	7	5	3

Table 7.3.2.ii Summary of CO<sub>2</sub> alternations performed in chemoreceptor fibres.

An example of the two types of oscillation produced are shown in figures 7.3.2.i.a-ii.a. Figure 7.3.2.i.a shows a 1 breath alternation in etCO<sub>2</sub>, and figure 7.3.2.ii.a shows a 15 breath alternation for the same lamb, both during NX at 14d. As detailed in section 7.2.4, CSN discharge was counted in 200msec bins, summed over the period of the alternation and smoothed by moving average. The accompanying oscillations in CSN discharge are shown in figures 7.3.2.i.b-ii.b and plotted over two alternation periods.

For 1 breath alternations, etCO<sub>2</sub> alternated around a mean  $\pm$  S.E.M level of  $7.56 \pm 0.15\%$ , with a mean  $\pm$  S.E.M amplitude of  $1.23 \pm 0.07\%$ . For 15 breath alternations, etCO<sub>2</sub> alternated around a mean  $\pm$  S.E.M level of  $7.20 \pm 0.23\%$ , with a mean  $\pm$  S.E.M amplitude of  $2.44 \pm 0.12\%$ . Hence, both protocols alternated about a similar mean etCO<sub>2</sub> of *ca.* 7%, however the magnitude of the alternation was twice as great for the 15 breath alternations compared to the single breath alternations.

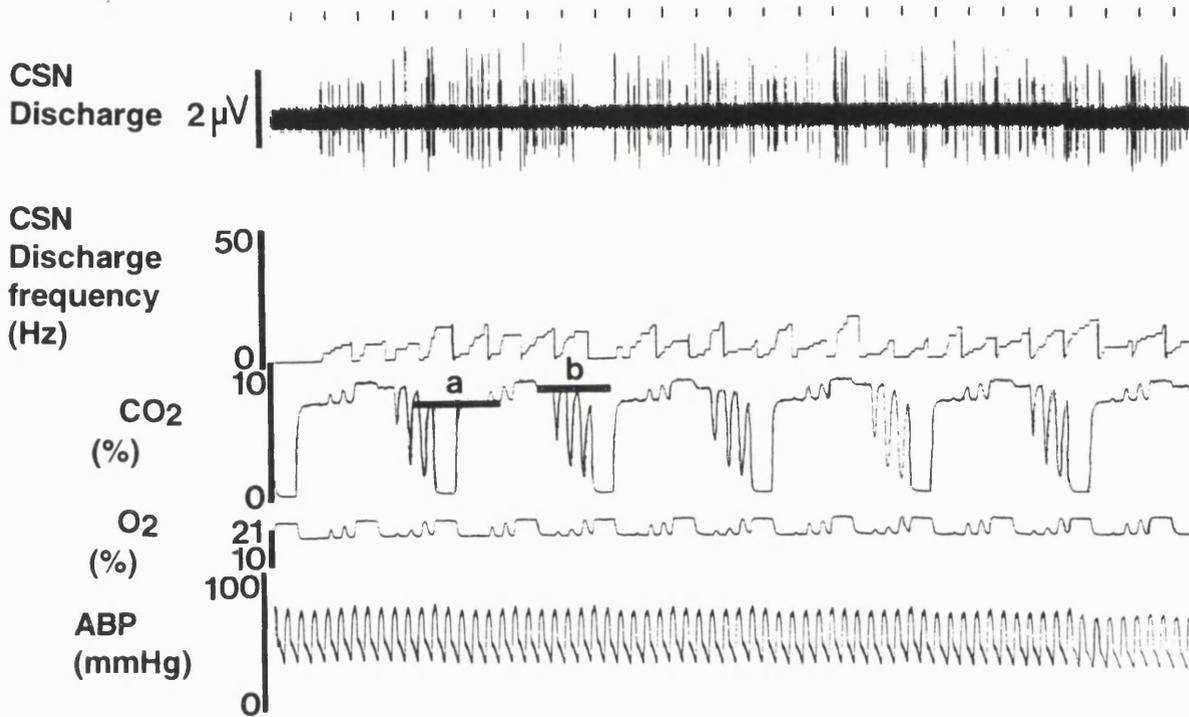


Figure 7.3.2.i.a Single breath alternation in etCO<sub>2</sub> during NX in a lamb aged 14d. A. and B. mark the peak and trough etCO<sub>2</sub> values to measure CO<sub>2</sub> sensitivity. Raw ( $\mu$ V) and integrated CSN discharge (Hz), CO<sub>2</sub> (%), O<sub>2</sub> (%) and arterial blood pressure (ABP mmHg).

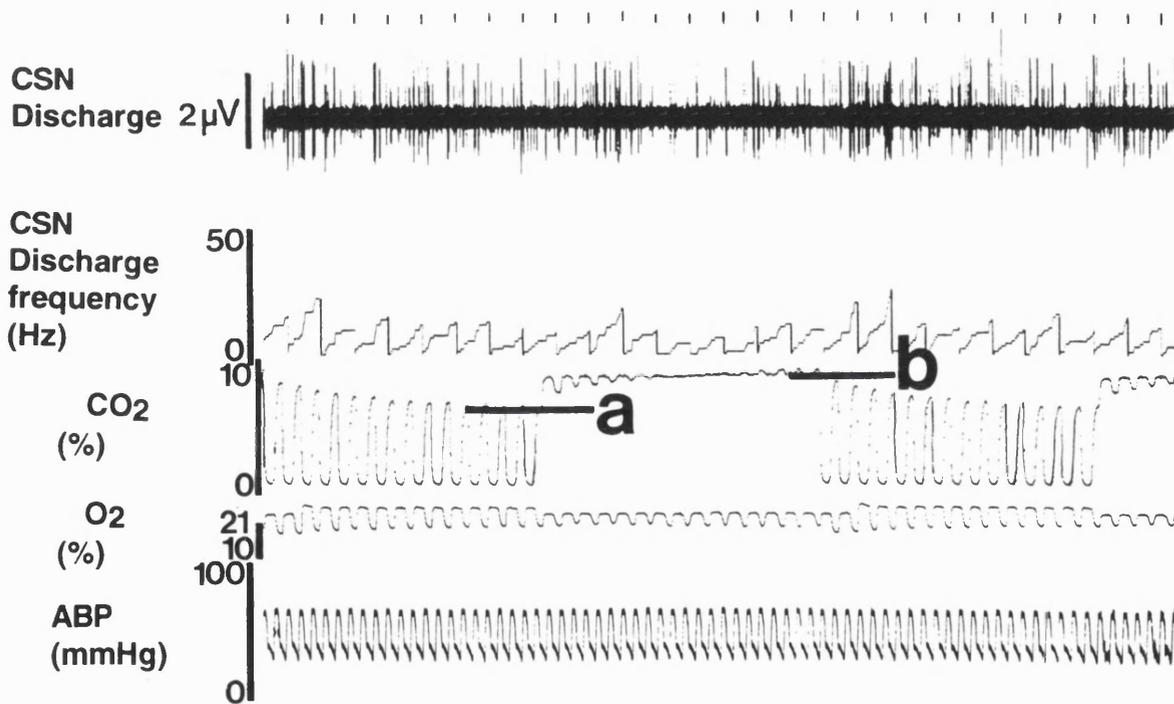


Figure 7.3.2.ii.a 15 breath alternation in etCO<sub>2</sub> during NX in a lamb aged 14d. A. and B. mark the peak and trough etCO<sub>2</sub> values to measure CO<sub>2</sub> sensitivity. Raw ( $\mu$ V) and integrated CSN discharge (Hz), CO<sub>2</sub> (%), O<sub>2</sub> (%) and arterial blood pressure (ABP mmHg)

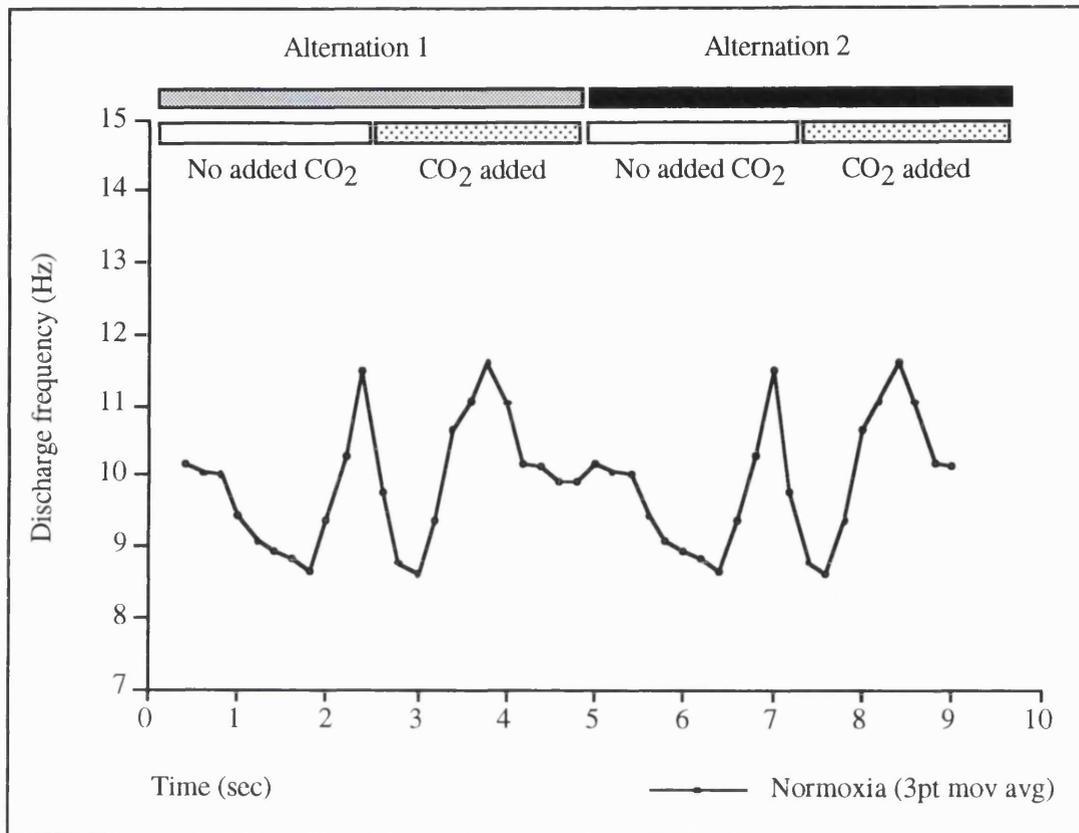


Figure 7.3.2.i.b. CSN discharge summed over 2 alternation periods for 1 breath alternation in ctCO<sub>2</sub> during NX at 14d.

The oscillation in CSN discharge in figure 7.3.2.i.b shows an increase in discharge frequency *ca.* 3-4 sec after the addition of CO<sub>2</sub> to the inspire. This delay in response time is only slightly longer than would have been expected. This alternation has been smoothed by a 3 point moving average, so as not to lose the initial increase in discharge frequency (*ca.* 2.0 and 6.5 sec), followed by a fall, which were less evident when smoothed by 5 point moving average. In this particular oscillation, the sharp rise in discharge with an appropriate delay in response time, probably reflects the dynamic response to an increase in CO<sub>2</sub>. Similarly the fall in discharge at *ca.* 4.0 sec to a relative plateau, probably reflects the dynamic response to a decrease in CO<sub>2</sub>.

The oscillation in CSN discharge in figure 7.3.2.ii.b shows an increase in discharge frequency *ca.* 6 sec after the addition of CO<sub>2</sub> to the inspire, which is longer than in figure 7.3.2.i.b. The increase in discharge in response to the increase in CO<sub>2</sub> at *ca.* 14 sec was very brisk over 4 sec, but did not adapt after the initial increase. Instead, discharge continued to rise more slowly over the following 4 sec. The fall in discharge frequency (*ca.* 6.0 and 22.0 sec) in response to the removal of CO<sub>2</sub> from the inspire is

initially quite steep, and then appears to reach a plateau for approximately 4.0 sec before falling again.

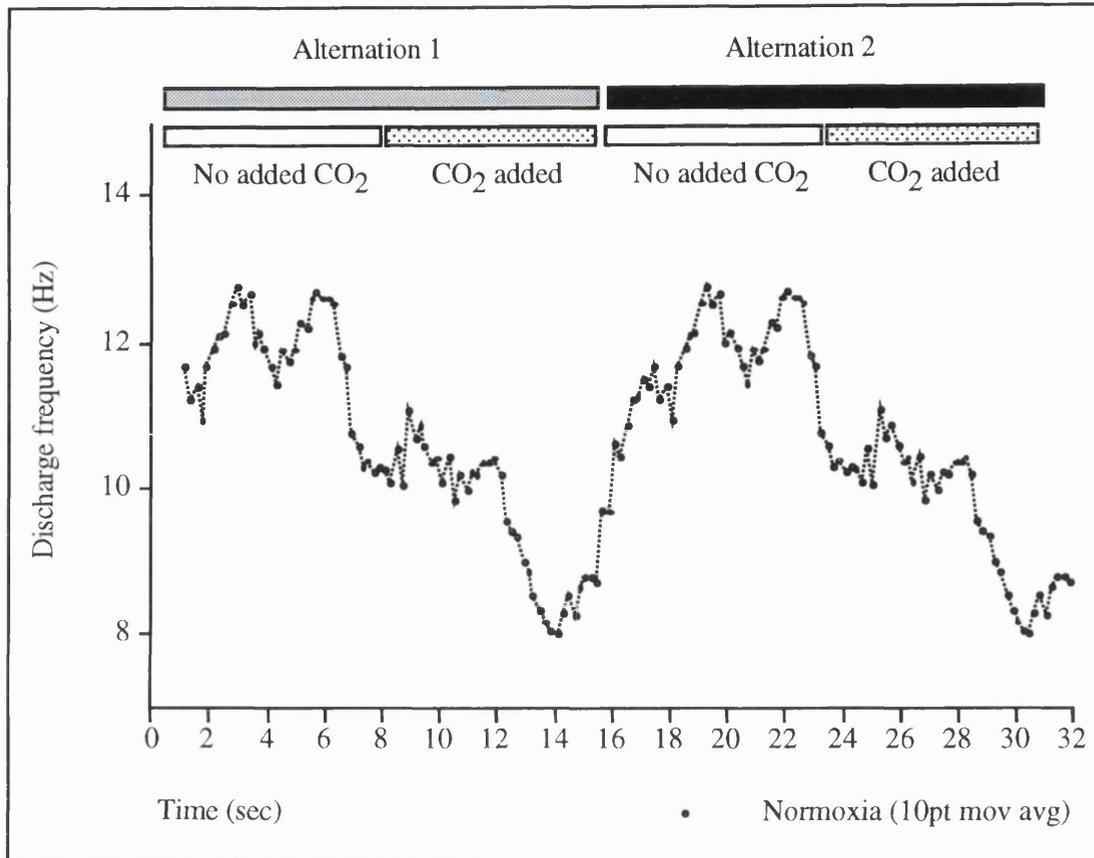


Figure 7.3.2.ii.b. CSN discharge summed over 2 alternation periods for 15 breath alternations in etCO<sub>2</sub> during NX at 14d.

Oscillations in CSN discharge produced by 1 breath (figure 7.3.2.iii) and 15 breath (figures 7.3.2.iv) alternations in CO<sub>2</sub> during NX and MOD HX are shown for a different fibre at 14 days. Mean and amplitude CSN discharge were greater in MOD HX than in NX for both types of alternations, as expected. For this particular fibre, the oscillation amplitude for a 1 breath alternation was similar to the oscillation amplitude for a 15 breath alternation in NX and MOD HX. Comparison of 1 and 15 breath alternations can be found in section 7.3.3.

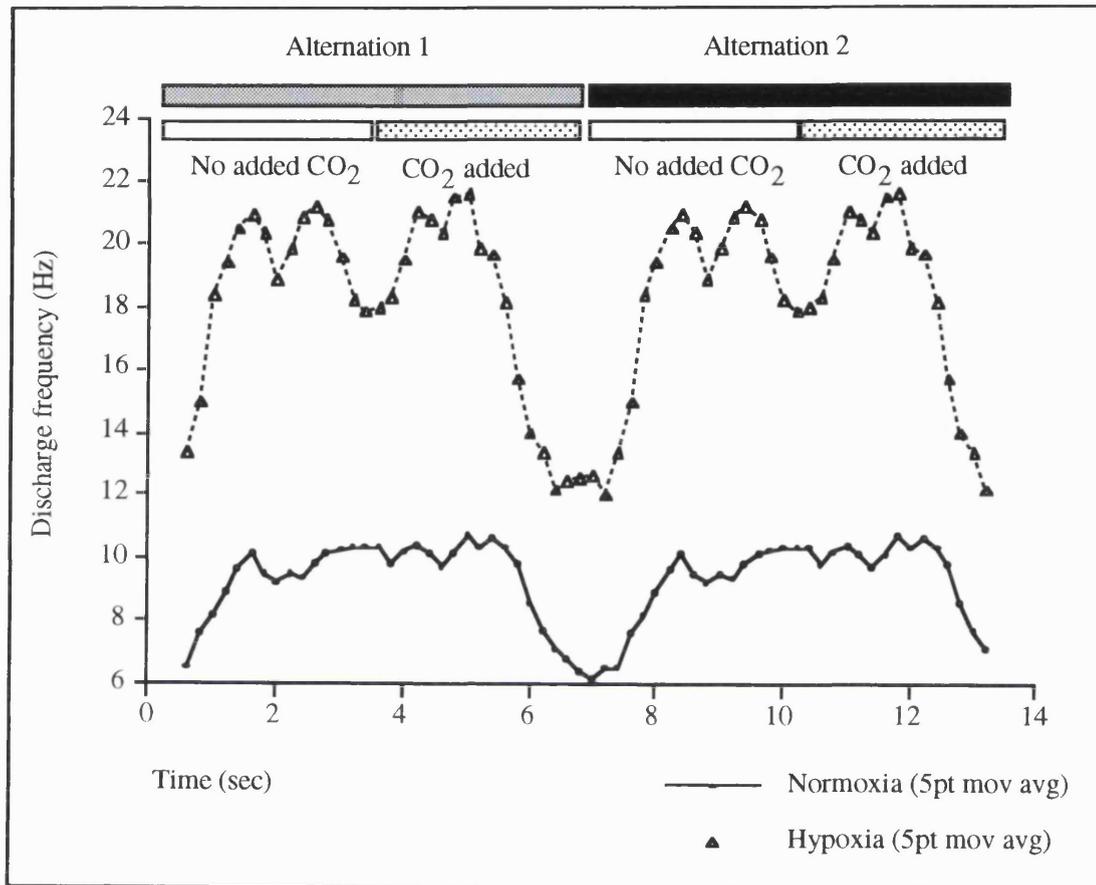


Figure 7.3.2.iii CSN discharge summed over 2 alternation periods for 1 breath alternations in etCO<sub>2</sub> during NX (squares) and MOD HX (triangles).

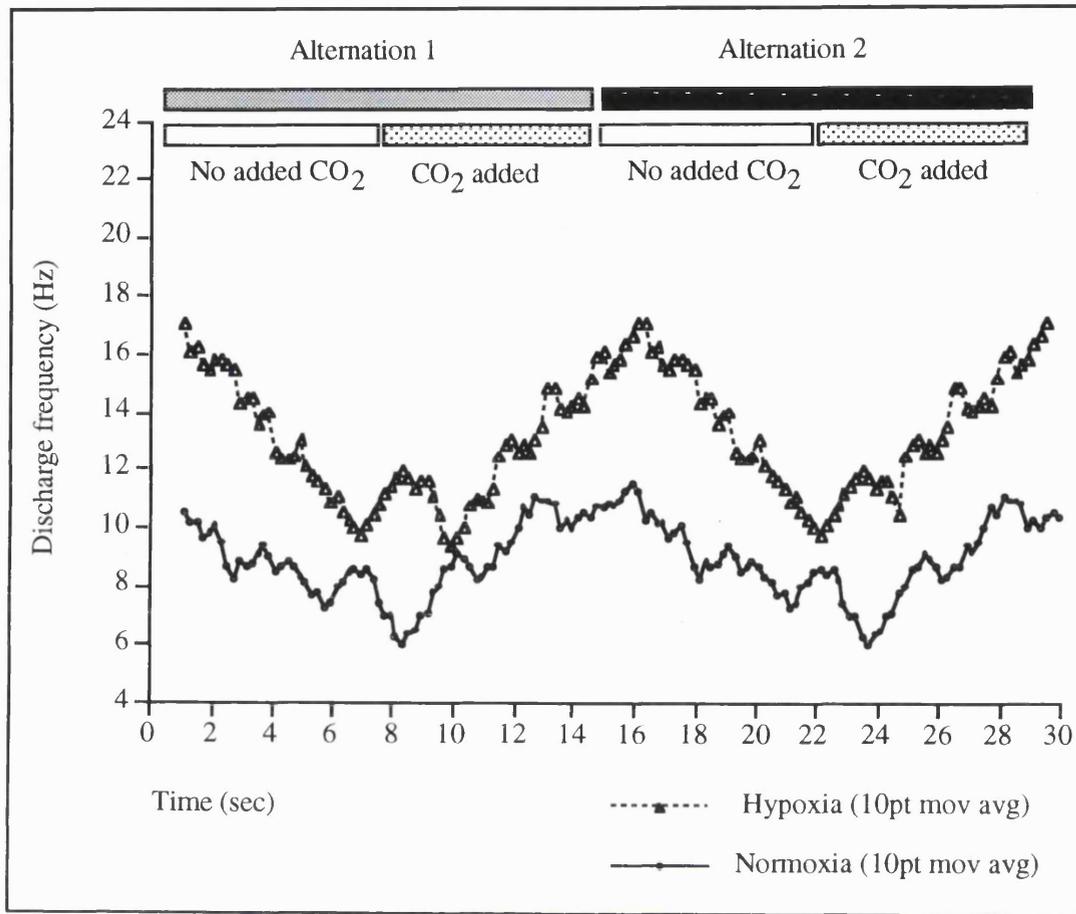


Figure 7.3.2.iv CSN discharge summed over 2 alternation periods for 15 breath alternations in etCO<sub>2</sub> during NX (squares) and MOD HX (triangles).

### 7.3.3 Comparison of oscillations in CSN discharge in response to 1 and 15 breath CO<sub>2</sub> alternations

In some fibres, dynamic responses to CO<sub>2</sub> were measured by both 1 and 15 breath CO<sub>2</sub> alternations. One fibre in NX and 4 fibres in MOD HX at 3-4d, 2 fibres in NX and 4 fibres in MOD HX at 5-9d and 6 fibres in NX and 3 fibres in MOD HX at 10-17d were tested by both CO<sub>2</sub> alternations. I compared CO<sub>2</sub> sensitivity between 15 and 1 breath alternations. Chemoreceptor responses were scored according to which alternation showed a greater CO<sub>2</sub> sensitivity, as determined by the slope between peak and trough discharge when plotted against maximum and minimum etCO<sub>2</sub>. CO<sub>2</sub> sensitivities are given in table 7.3.3.i for both types of alternations. Six of 9 fibres in NX and 5 of 11 fibres in MOD HX showed a greater response to 15 breath alternations compared to 1 breath alternations.

3-4 days				5-9 days				10-17 days			
NX		MOD HX		NX		MOD HX		NX		MOD HX	
15br	1br	15br	1br	15br	1br	15br	1br	15br	1br	15br	1br
9.74	4.35	17.92	11.48	3.91	2.61	6.76	2.60	4.34	2.78	6.44	7.83
		2.48	13.55	9.61	8.90	9.61	9.06	10.82	5.64		
		9.12	5.89			2.05	5.19	5.60	9.33	11.38	17.14
		1.56	2.44			2.94	8.46	2.57	3.09		
								3.47	4.48	12.15	11.52
								4.57	9.98		

Table 7.3.3.i CO<sub>2</sub> sensitivity (% maximal discharge/ % etCO<sub>2</sub>) for 1 (1br) and 15 breath (15br) alternations during NX and MOD HX.

It is difficult to comment on which type of CO<sub>2</sub> alternation was more effective in producing a dynamic chemoreceptor response as I found that both types of CO<sub>2</sub> alternation showed an oscillation in chemoreceptor discharge, and that there was a dynamic chemoreceptor component in the response. Assessment of the dynamic chemoreceptor response to CO<sub>2</sub> is dealt with in further detail in section 7.3.5 when compared to steady state responses.

### 7.3.4 Comparison of steady state and dynamic chemoreceptor response curves to CO<sub>2</sub>

Dynamic responses to CO<sub>2</sub> were compared to steady state CO<sub>2</sub> responses for a total of 23 fibres. This was done in a number of ways. Firstly, the dynamic CO<sub>2</sub> response for each individual fibre was compared against its own steady state CO<sub>2</sub> response. Secondly, dynamic CO<sub>2</sub> responses for 15 breath alternations and 1 breath alternations were compared against steady state CO<sub>2</sub> responses for a group at each particular age.

#### 7.3.4.a Comparison of steady state and dynamic chemoreceptor response curves to CO<sub>2</sub> for each individual fibre

Figure 7.3.4.a.i shows an example of dynamic chemoreceptor responses during NX and MOD HX, and their corresponding steady state responses for one fibre aged 6d. Steady state CO<sub>2</sub> sensitivity is greater in MOD HX compared to NX, and dynamic chemoreceptor responses are greater than steady state. For each individual fibre, there was frequently more than one of each type of CO<sub>2</sub> alternation performed in the experiment. The results for all fibres graphed separated into age groups are summarised in figures 7.3.4.a.ii-iv. If we are to consider the greatest dynamic response attained for a

particular fibre and compare that to steady state responses, 6 of 6 fibres in NX and 6 of 7 fibres in MOD HX showed dynamic responses that were steeper than their steady state for lambs aged 3-4d. For lambs aged 5-9d, 8 of 10 fibres in NX and 4 of 5 fibres in MOD HX, and for lambs aged 10-17d, 5 of 8 fibres in NX and 8 of 9 fibres in MOD HX showed steeper dynamic responses compared to steady state. Thus, the age group which tends to show the most consistent response is 3-4d lambs, and in the older age groups there was a greater proportion of fibres that did not show a steeper dynamic responses to CO<sub>2</sub> compared to steady state.

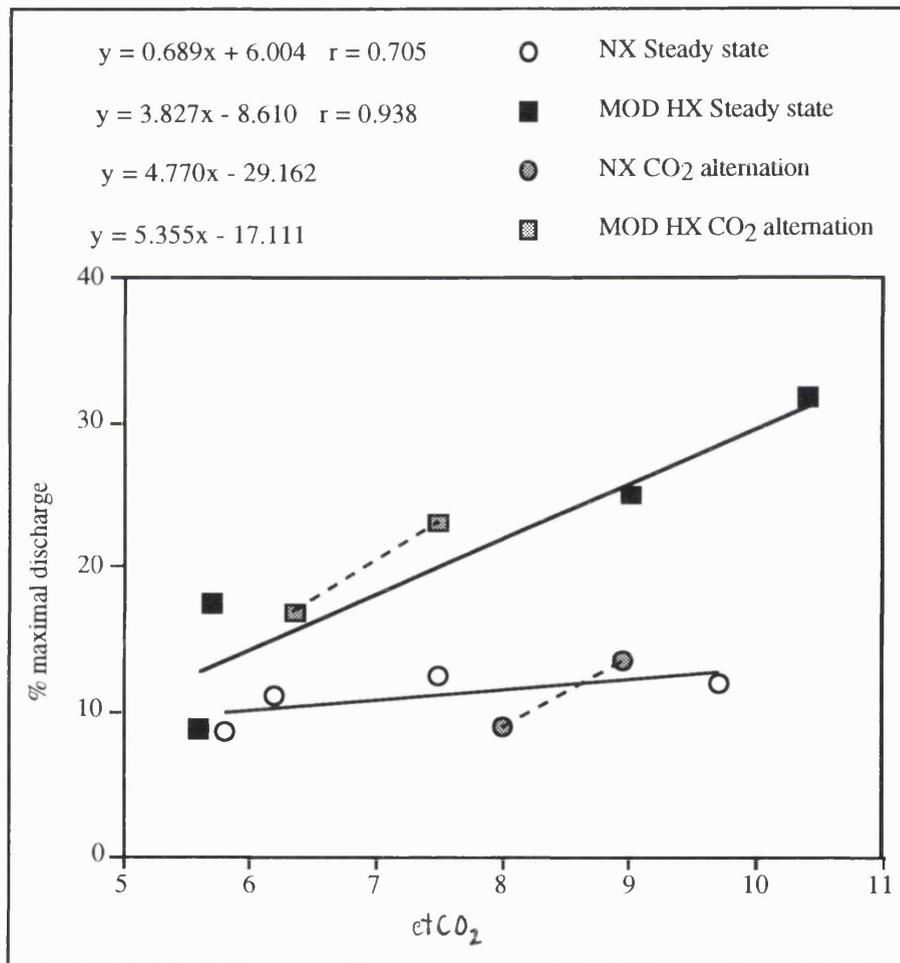


Figure 7.3.4.a.i. Steady state (solid or plain) and dynamic (shaded) CO<sub>2</sub> sensitivities during NX (circles) and MOD HX (squares) for one fibre aged 6d. Discharge as a percentage of maximal is plotted as a function of etCO<sub>2</sub> (%). Linear regression describes the slope (CO<sub>2</sub> sensitivities) of the responses.

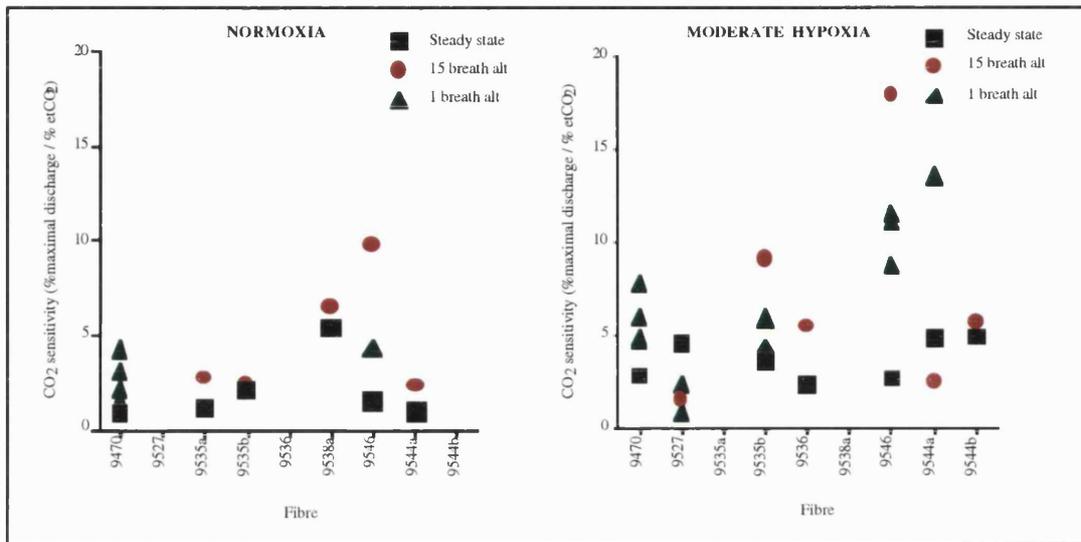


Figure 7.3.4.a.ii Summary of steady state and dynamic chemoreceptor responses to CO<sub>2</sub> in 3-4d lambs.

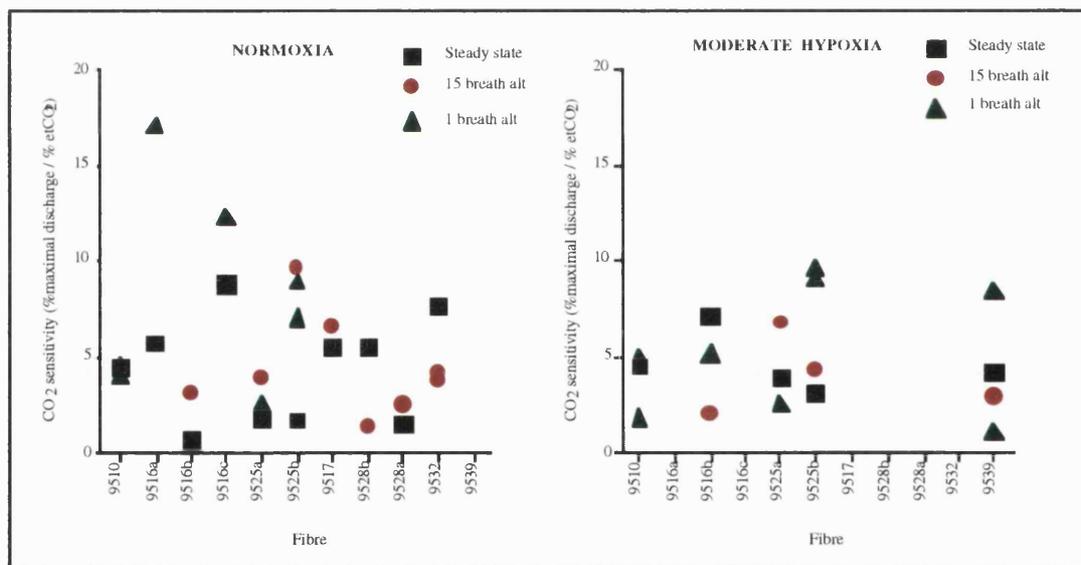


Figure 7.3.4.a.iii Summary of steady state and dynamic chemoreceptor responses to CO<sub>2</sub> in 5-9d lambs.

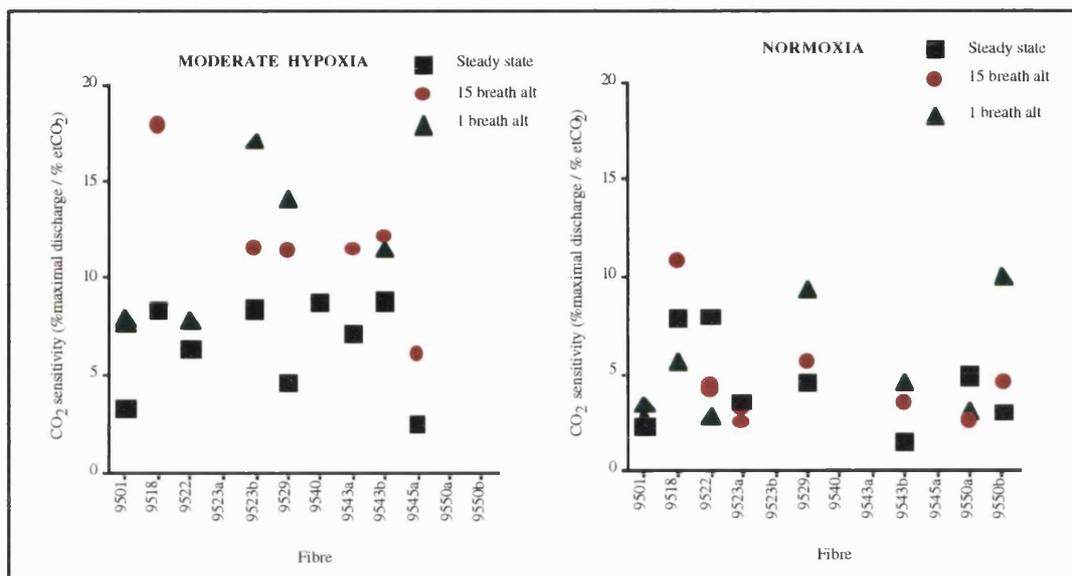


Figure 7.3.4.a.iv Summary of steady state and dynamic chemoreceptor responses to CO<sub>2</sub> in 10-17d lambs.

### 7.3.4.b Comparison of steady state and dynamic chemoreceptor responses between different fibres in the same lamb.

I found in section 7.3.4.a that when dynamic chemoreceptor responses were compared to steady state responses, a proportion of fibres failed to show a greater dynamic sensitivity to CO<sub>2</sub>. More interestingly, I found on occasion that two different responses occurred between separate fibres from the same lamb. To illustrate this point I have shown the responses in separate fibres from two lambs, one aged 6d (figures 7.3.4.b.i-ii) and the other 14d (figures 7.3.4.b.iii-iv).

The 6d lamb shows for two separate fibres in the first instance (figure 7.3.4.b.i) a steeper dynamic response (PaO<sub>2</sub> 100 mmHg) compared to steady state (average PaO<sub>2</sub> 88 mmHg), and in the second fibre (figure 7.3.4.b.ii) a much steeper steady state response (average PaO<sub>2</sub> 108 mmHg) compared to the dynamic response (PaO<sub>2</sub> 109 mmHg). In the 14d lamb, the first fibre (figure 7.3.4.b.iii) shows a steady state response (average PaO<sub>2</sub> 92 mmHg) steeper than the dynamic response (PaO<sub>2</sub> 78 and 99 mmHg), and in the second fibre (figure 7.3.4.b.iv) a steeper dynamic response (PaO<sub>2</sub> 95 and 87mmHg) compared to steady state (average PaO<sub>2</sub> 96 mmHg). The average PaO<sub>2</sub> for steady state and dynamic responses in the same fibre were reasonably closely matched, and also between fibres of the same animal for the 14d lamb. In the 6d lamb there is a larger difference between PaO<sub>2</sub>s in the steady state for the two fibres, however they are still in the normoxic range. Despite the small differences in PaO<sub>2</sub>s, the two different responses in the same animal suggests that variation in responses between fibres occurs.

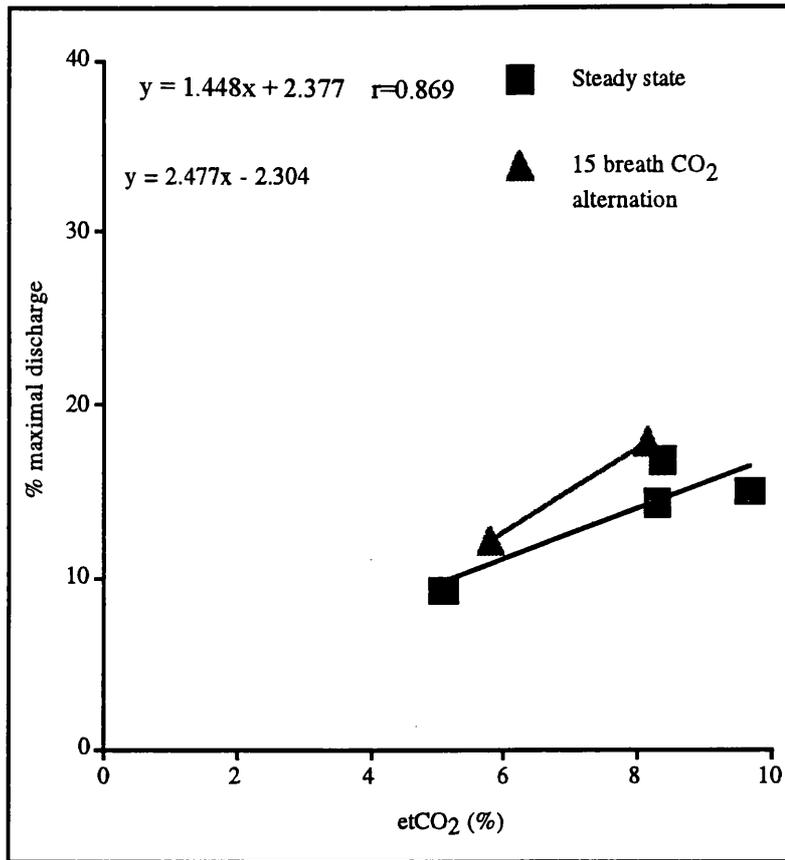


Figure 7.3.4.b.i Steady state and dynamic CO<sub>2</sub> responses in NX for lamb at 6d.

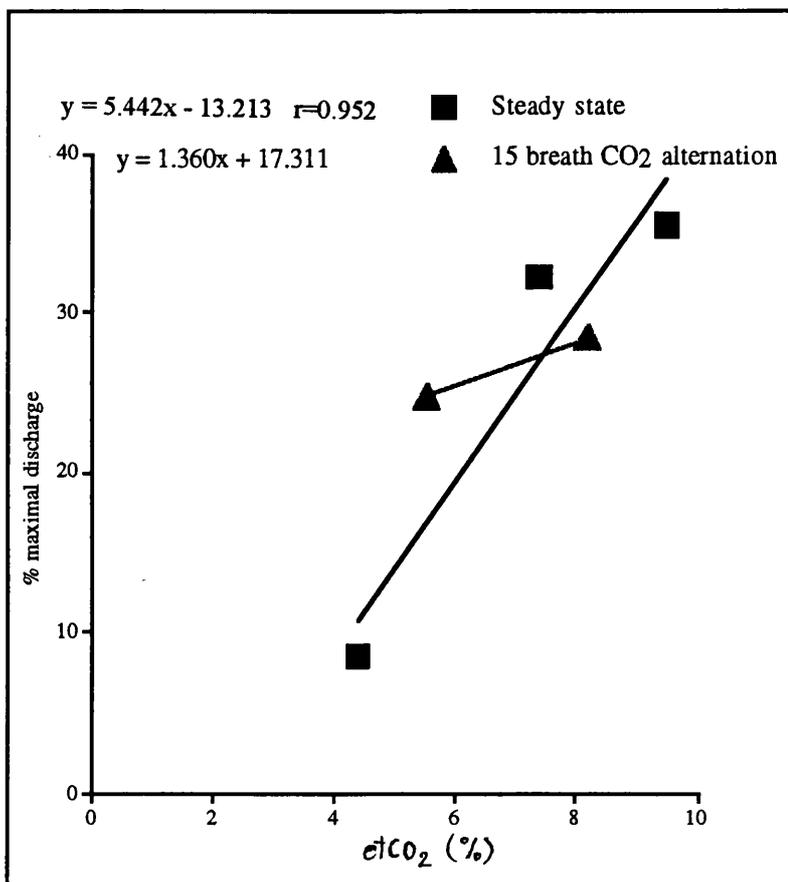


Figure 7.3.4.b.ii Steady state and dynamic CO<sub>2</sub> responses in NX for a different fibre in the same lamb at 6d.

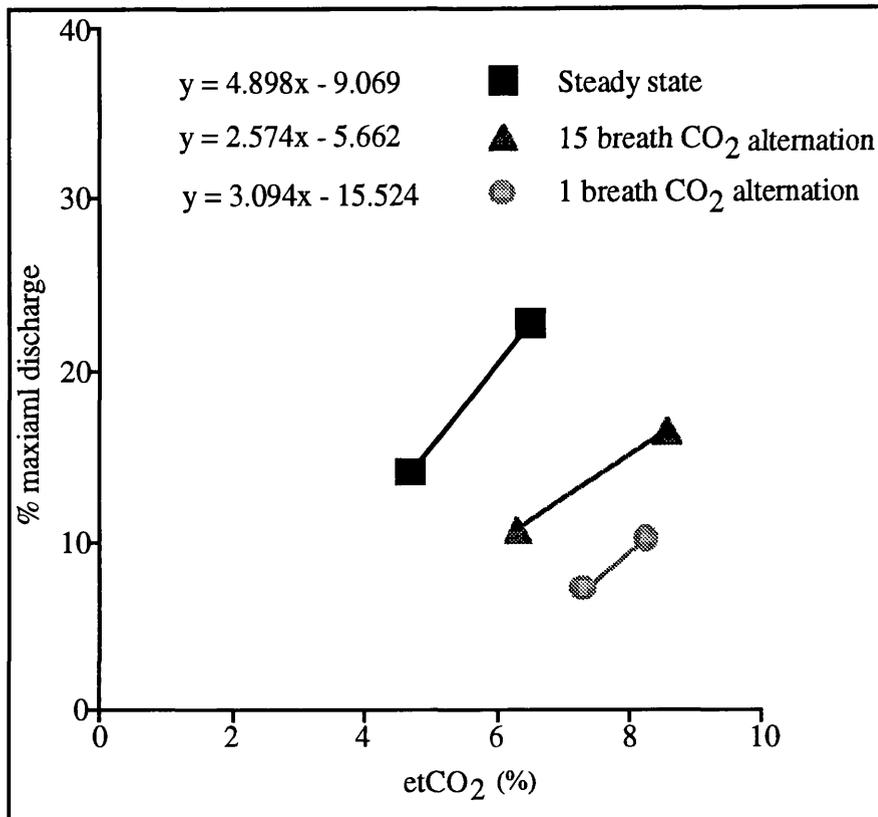


Figure 7.3.4.b.iii Steady state and dynamic CO<sub>2</sub> responses in NX for 14d lamb.

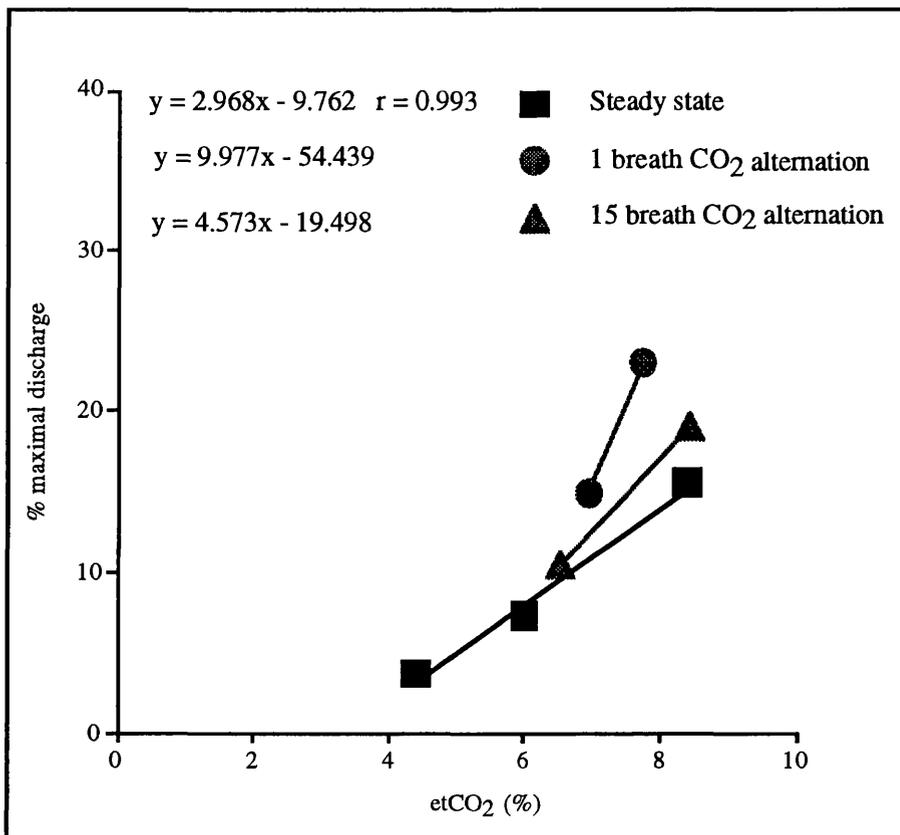


Figure 7.3.4.b.iv Steady state and dynamic CO<sub>2</sub> responses in NX for a different fibre in the same lamb at 14d.

### 7.3.4.c Comparison of steady state and dynamic chemoreceptor response curves to CO<sub>2</sub> within 3 age groups.

Steady state and dynamic chemoreceptor responses for the 23 lambs were grouped according to age and expressed as mean  $\pm$  S.E.M. in table 7.3.4.c.i-ii (when all responses for all fibres were used) and table 7.3.4.c.iii-iv (when only one alternation response per fibre was used in each group). In the case where more than one of a particular type of CO<sub>2</sub> alternation was recorded for a fibre, the largest dynamic response to the CO<sub>2</sub> alternation was used to compare to steady state. This was justified on the basis that I wanted to measure the greatest dynamic response that the fibre was capable of showing.

The steady state chemoreceptor responses curves to CO<sub>2</sub> for the 23 lambs in NX (table 7.3.4.c.iii) and MOD HX (table 7.3.4.c.iv) are similar to those reported for the group as a whole in section 6.3. Mean steady state CO<sub>2</sub> chemoreceptor responses  $\pm$  S.E.M. measured in all fibres (n=56) and the subset of fibres that were also tested for dynamic CO<sub>2</sub> sensitivity (n=23) during NX and MOD are shown in table 7.3.4.c.v. Steady state chemoreceptor responses during NX for 5-9d and 10-24d lambs were slightly less in the subset of fibres that were tested for dynamic CO<sub>2</sub> sensitivity compared to the whole group.

Slope of chemoreceptor response curve (% maximal discharge / % etCO <sub>2</sub> )		NX	NX	NX
		ss	15 breath alt	1 breath alt
3-4d	mean $\pm$ S.E.M.	1.99 $\pm$ 0.71	4.74 $\pm$ 1.47	3.06 $\pm$ 0.56
5-9d	mean $\pm$ S.E.M.	4.30 $\pm$ 0.88	4.39 $\pm$ 0.92	8.08 $\pm$ 1.95
10-17d	mean $\pm$ S.E.M.	4.43 $\pm$ 0.85	4.57 $\pm$ 0.85	5.17 $\pm$ 1.04

Table 7.3.4.c.i Steady state and dynamic (all responses for all fibres) chemoreceptor responses for 3-4d, 5-9d and 10-17d lambs in NX.

Slope of chemoreceptor response curve (% maximal discharge / % etCO <sub>2</sub> )		MOD HX	MOD HX	MOD HX
		ss	15 breath alt	1 breath alt
3-4d	mean $\pm$ S.E.M.	3.74 $\pm$ 0.42	7.06 $\pm$ 2.43	7.01 $\pm$ 1.13
5-9d	mean $\pm$ S.E.M.	4.56 $\pm$ 0.68	5.13 $\pm$ 1.37	4.77 $\pm$ 1.18
10-17d	mean $\pm$ S.E.M.	6.49 $\pm$ 0.82	11.03 $\pm$ 1.50	11.03 $\pm$ 1.61

Table 7.3.4.c.ii Steady state and dynamic (all responses for all fibres) chemoreceptor responses for 3-4d, 5-9d and 10-17d lambs in MOD HX.

Slope of chemoreceptor response curve (% maximal discharge / % etCO <sub>2</sub> )		NX		
		ss	15 breath alt	1 breath alt
3-4d	mean ± S.E.M.	1.99 ± 0.71	4.74 ± 1.47	4.28 ± 0.07
5-9d	mean ± S.E.M.	4.30 ± 0.88	4.45 ± 1.05	9.11 ± 2.62
10-17d	mean ± S.E.M.	4.43 ± 0.85	4.94 ± 1.05	5.53 ± 1.12

Table 7.3.4.c.iii Steady state and dynamic (one response per fibre) chemoreceptor responses for 3-4d, 5-9d and 10-17d lambs in NX.

Slope of chemoreceptor response curve (% maximal discharge / % etCO <sub>2</sub> )		MOD HX		
		ss	15 breath alt	1 breath alt
3-4d	mean ± S.E.M.	3.74 ± 0.42	7.06 ± 2.43	9.47 ± 2.27
5-9d	mean ± S.E.M.	4.56 ± 0.68	5.34 ± 1.75	6.07 ± 1.19
10-17d	mean ± S.E.M.	6.49 ± 0.82	10.43 ± 1.43	11.71 ± 1.79

Table 7.3.4.c.iv Steady state and dynamic (one response per fibre) chemoreceptor responses for 3-4d, 5-9d and 10-17d lambs in MOD HX.

Slope of chemoreceptor response curve (% maximal discharge / % etCO <sub>2</sub> )		Fibres used for comparison between dynamic and steady state chemoreceptor responses (total n=23 fibres)		All fibres measured for steady state chemoreceptor responses (total n=56 fibres)	
		NX	MOD HX	NX	MOD HX
3-4d	mean ± S.E.M.	1.99 ± 0.71	3.74 ± 0.42	1.72 ± 0.50	3.83 ± 1.13
5-9d	mean ± S.E.M.	4.30 ± 0.88	4.56 ± 0.68	5.82 ± 1.21	4.70 ± 1.19
10-24d	mean ± S.E.M.	4.43 ± 0.85	6.49 ± 0.82	5.78 ± 0.79	5.63 ± 0.83

Table 7.3.4.c.v Mean steady state CO<sub>2</sub> chemoreceptor responses ± S.E.M. for fibres compared to dynamic responses and all fibres measured for steady state responses in NX and MOD.

Steady state chemoreceptor responses were compared to dynamic responses for 15 and 1 breath alternations when only one response per fibre was used. This was the maximal response for that fibre as shown in tables 7.3.4.c.iii-iv. Each dynamic response was compared to its own steady state response, hence if different fibres were represented in the alternation groups during NX or MOD HX, the mean steady state may differ slightly between groups as not all fibres were included in all groups. Dynamic responses to 15 breath alternations were greater than steady state responses for all age groups

during NX and MOD HX, particularly for 3-4d lambs during both NX and MOD HX, and 10-17d lambs during MOD HX (see figure 7.3.4.c.i). A one sample *t*-test was used to compare the difference between dynamic and steady state chemoreceptor responses (see section 7.2.4.c). Using a hypothesized difference of zero, chemoreceptor responses to 15 breath alternations were significantly greater than steady state in MOD HX ( $P < 0.02$ ), but not in NX ( $P > 0.20$ ).

Dynamic responses to 1 breath alternations were greater than steady state responses for all age groups, however there was greater variation in the dynamic CO<sub>2</sub> sensitivity for 1 breath compared to 15 breath alternations (see figure 7.3.4.c.ii). During NX lambs at 5-9d showed much larger dynamic responses to the 1 breath alternations compared to steady state, which was seen to a lesser extent in the other 2 age groups. During MOD HX lambs at 3-4d and 10-17d showed much greater dynamic responses to the 1 breath alternations compared to steady state, which was less obvious in lambs aged 5-9d. A one sample *t*-test was used to compare the difference between dynamic and steady state chemoreceptor responses (see section 7.2.4.c). Chemoreceptor responses to 1 breath alternations were significantly greater than steady state in NX ( $P < 0.05$ ) and MOD HX ( $P < 0.01$ ).

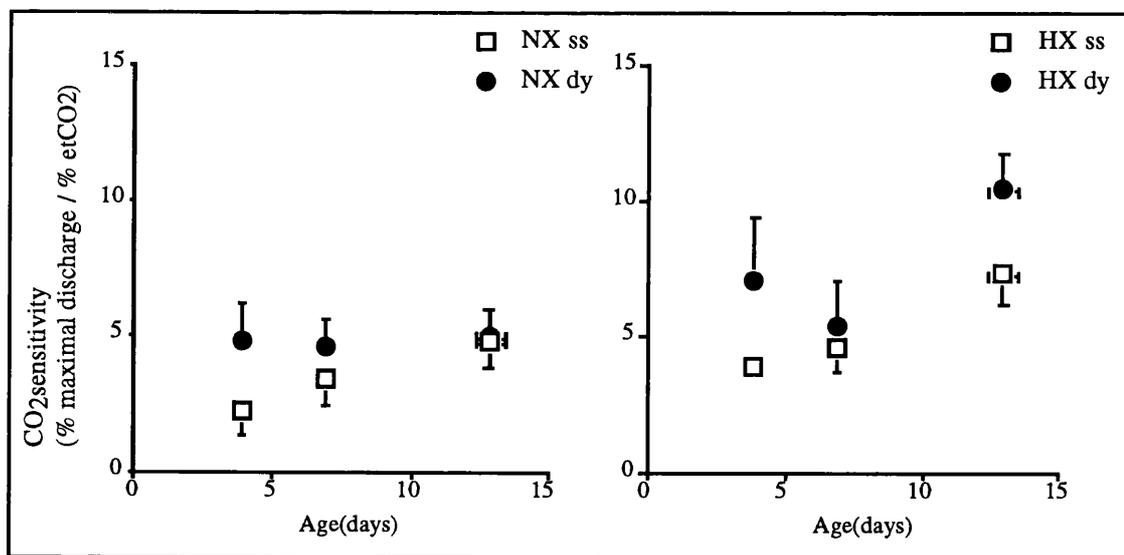


Figure 7.3.4.c.i Steady state and dynamic chemoreceptor responses for 15 breath alternations (mean  $\pm$  S.E.M.).

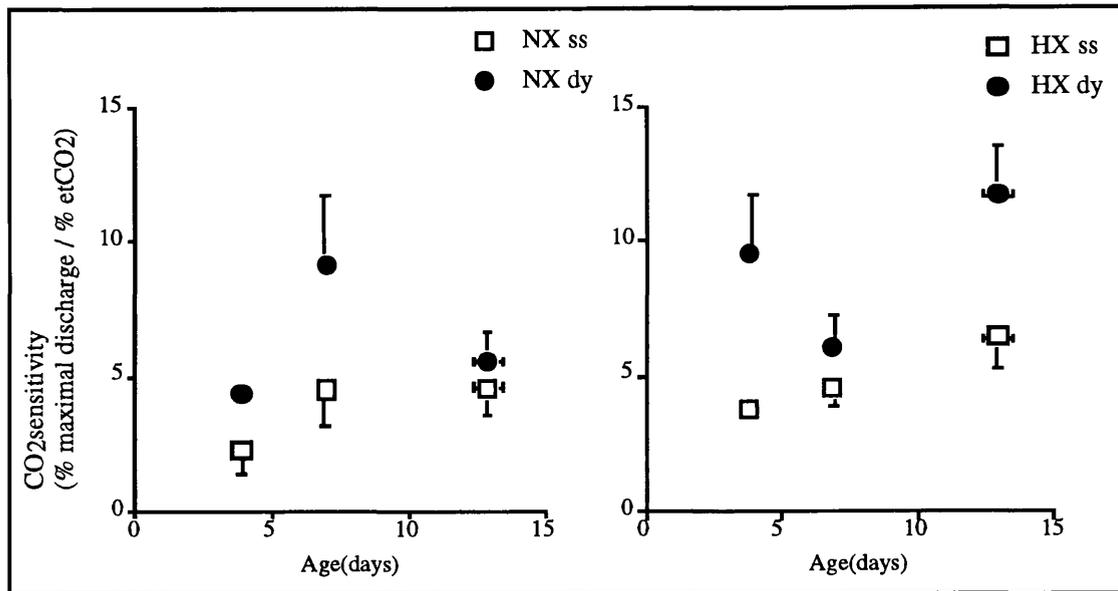


Figure 7.3.4.c.ii Steady state and dynamic chemoreceptor responses for 1 breath alternations (mean  $\pm$  S.E.M.).

### 7.3.5 Effect of age and PaO<sub>2</sub> on dynamic chemoreceptor responses

In section 7.3.4 I showed a dynamic sensitivity to CO<sub>2</sub> that was greater than in the steady state. However, the effect of age and PaO<sub>2</sub> on dynamic chemoreceptor responses were not considered. It can be seen from figures 7.3.4.c.i-ii that there was variation in the chemoreceptor response for a range of age and PaO<sub>2</sub> for both alternation types. To crudely look at the effect of these two factors on CO<sub>2</sub> sensitivity, chemoreceptor responses to CO<sub>2</sub> have been plotted as a function of age and PaO<sub>2</sub> in figure 7.3.5.i for 15 breath alternations, and in figure 7.3.5.ii for 1 breath alternations. In figure 7.3.5.iii chemoreceptor responses to the alternations have been categorized into three age groups and two PaO<sub>2</sub> levels. Chemoreceptor responses to 1 and 15 breath alternations showed no clear relationship with age or the level of PaO<sub>2</sub> when represented in this way.

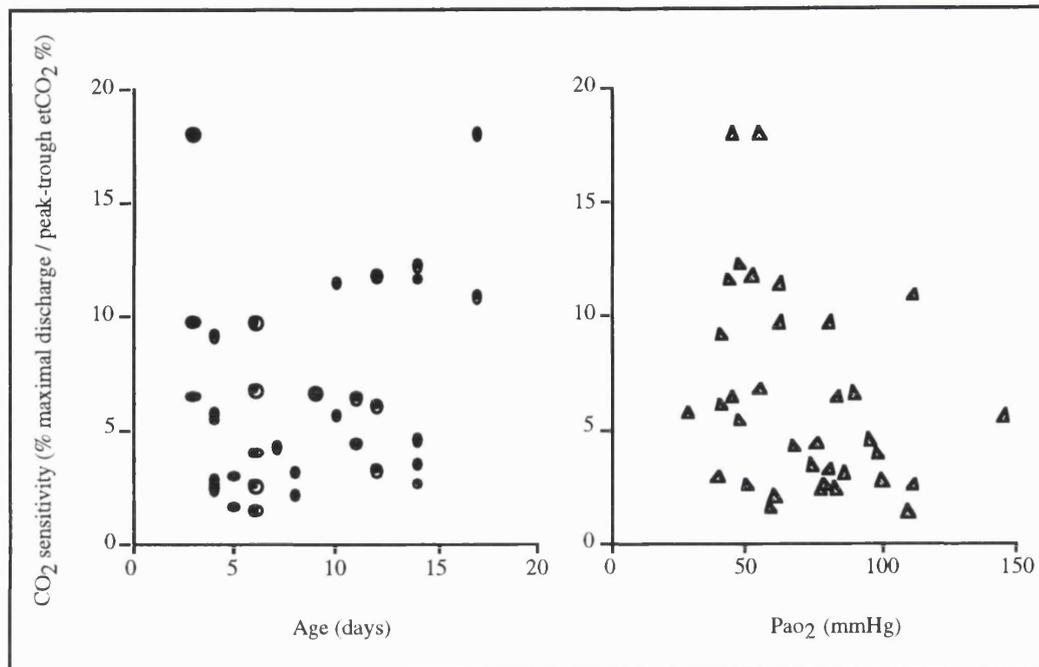


Figure 7.3.5.i Dynamic chemoreceptor responses (CO<sub>2</sub> sensitivity % maximal discharge / peak - trough etCO<sub>2</sub> %) to 15 breath alternations in CO<sub>2</sub> as a function of age (days) and PaO<sub>2</sub> (mmHg).

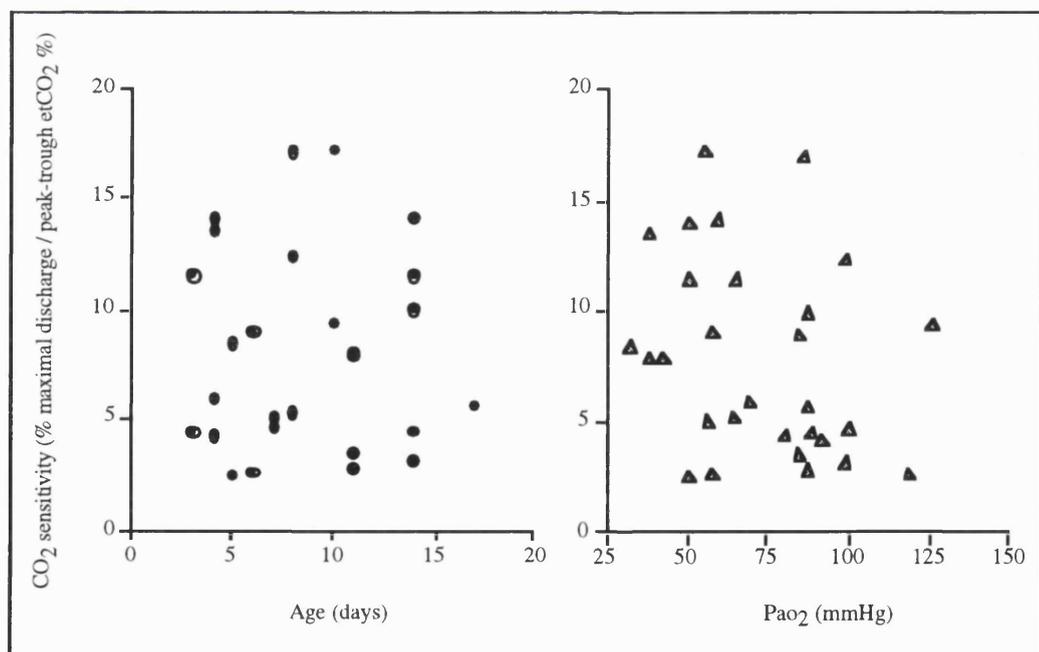


Figure 7.3.5.ii Dynamic chemoreceptor responses (CO<sub>2</sub> sensitivity % maximal discharge / peak - trough etCO<sub>2</sub> %) to 1 breath alternations in CO<sub>2</sub> as a function of age (days) and PaO<sub>2</sub> (mmHg).

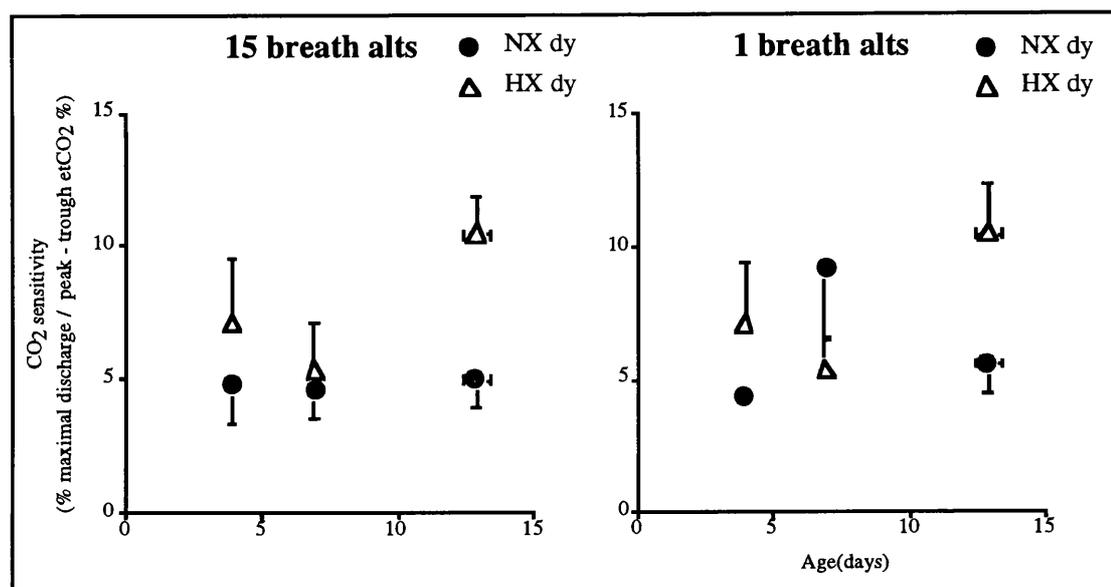


Figure 7.3.5.iii Dynamic chemoreceptor responses (CO<sub>2</sub> sensitivity % maximal discharge / peak - trough etCO<sub>2</sub> %) to 15 and 1 breath alternations when categorized into age (mean  $\pm$  S.E.M.) and PaO<sub>2</sub> groups. When S.E.M. is not shown it is included within the point.

To normalize the distribution of chemoreceptor responses the data was log transformed. In figures 7.3.5.iv-vii I have shown the distribution of chemoreceptor responses before, and after, log transformation for 15 and 1 breath alternations. To determine normality of the data, a Chi<sup>2</sup> test was used to compare between the expected and observed distributions. The null hypothesis predicts no difference between the expected and observed distribution ( $P > 0.05$ ), hence the data is normally distributed. The alternative hypothesis predicts that there is a significant difference between the expected and observed distributions ( $P < 0.05$ ), hence the data is not normally distributed. In figures 7.3.5.iii-vii, the observed responses are shown by the bars and the curve is fitted to the expected normal distribution. Only chemoreceptor responses to 15 breath alternations before log transformation were not normally distributed (figure 7.3.5.iv). However, all analysis was performed on log transformed responses for consistency. Due to the hyperbolic relationship between PaO<sub>2</sub> and CO<sub>2</sub> sensitivity, PaO<sub>2</sub> was also log transformed to linearize the data for the purpose of multiple linear regression.

The relationship between age and log-PaO<sub>2</sub> with log-chemoreceptor responses for 1 and 15 breath alternations are given in a correlation matrix in table 7.3.5.i-ii. There was a significant correlation between age ( $P < 0.04$ ) and log-PaO<sub>2</sub> ( $P < 0.02$ ) with log-chemoreceptor responses to 15 breath alternations. There was no significant correlation between age ( $P > 0.40$ ) and log-PaO<sub>2</sub> ( $P > 0.05$ ) with log-chemoreceptor responses to 1

breath alternations, however the P value for log-PaO<sub>2</sub> was approaching statistical significance.

Multiple linear regression was used to describe the effect of age and log-PaO<sub>2</sub> on chemoreceptor responses. There was a significant effect of log-PaO<sub>2</sub> (P<0.03) but not of age (P>0.05) on log chemoreceptor responses to 15 breath alternations. The multiple linear regression equation is given by:

$$\text{log-CO}_2 \text{ sensitivity (15 br alts)} = (\{0.021 \pm 0.011\} \times \text{Age}) - (\{0.665 \pm 0.287\} \times \text{log-PaO}_2) + (1.743 \pm 0.527)$$

There was no significant effect of log-PaO<sub>2</sub> (P>0.10) nor of age (P>0.62) on log chemoreceptor responses to 1 breath alternations. The multiple linear regression equation is given by:

$$\text{log-CO}_2 \text{ sensitivity (1 br alts)} = (\{0.006 \pm 0.013\} \times \text{Age}) - (\{0.549 \pm 0.324\} \times \text{log-PaO}_2) + (1.780 \pm 0.585).$$

I also compared the effect of age and PaO<sub>2</sub> on dynamic chemoreceptor responses to CO<sub>2</sub> by two-way ANOVA. This alternative method of statistical analysis similarly showed no effect of effect of age (P>0.39) or PaO<sub>2</sub> (P>0.68) on dynamic CO<sub>2</sub> sensitivity for 1 breath alternations. For 15 breath alternations, there was a significant effect of PaO<sub>2</sub> (P<0.005), but no significant effect of age (P>0.06) on dynamic chemoreceptor responses to CO<sub>2</sub>.

In summary, there was no significant effect of age or of PaO<sub>2</sub> on chemoreceptor responses to 1 breath alternations in etCO<sub>2</sub> by multiple linear regression. There was no significant effect of age (however this is borderline at P>0.05), but there was a significant effect of PaO<sub>2</sub> on chemoreceptor responses on chemoreceptor responses to 15 breath alternations in etCO<sub>2</sub> by multiple linear regression.

Correlation coefficients	log-15 breath alt	Age	log-PaO <sub>2</sub>
log-15 breath alt	1.00	0.29, P<0.04	-0.33, P<0.02
Age	0.29, P<0.04	1.00	0.05, P>0.39
log-PaO <sub>2</sub>	-0.34, P<0.02	0.05, P>0.39	1.00

Table 7.3.5.i Correlation matrix (correlation coefficient, one-tailed significance) between age and log-PaO<sub>2</sub> with log-chemoreceptor responses to 15 breath alternations.

Correlation coefficients	log-15 breath alt	Age	log-PaO <sub>2</sub>
log-1 breath alt	1.00	0.03, P>0.44	-0.30, P>0.05
Age	0.03, P>0.43	1.00	0.19, P>0.15
log-PaO <sub>2</sub>	-0.30, P>0.05	0.19, P>0.15	1.00

Table 7.3.5.ii Correlation matrix (correlation coefficient, one-tailed significance) between age and log-PaO<sub>2</sub> with log-chemoreceptor responses to 1 breath alternations.

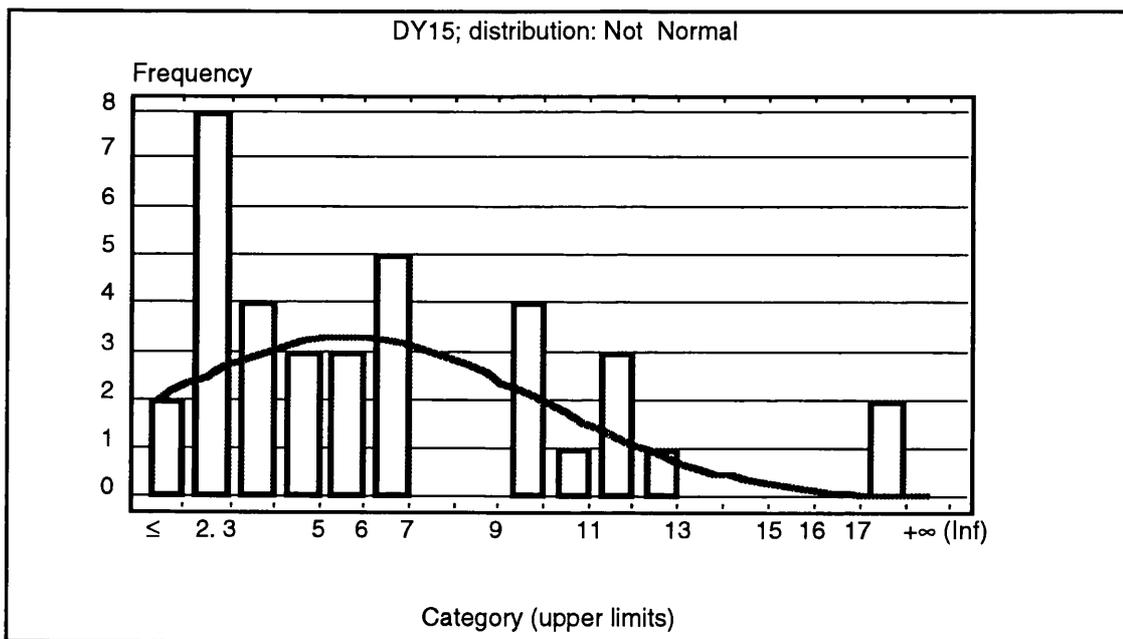


Figure 7.3.5.iv Frequency histogram for chemoreceptor responses to 15 breath alternations. This data was not normally distributed by X<sup>2</sup> test (P<0.0001)

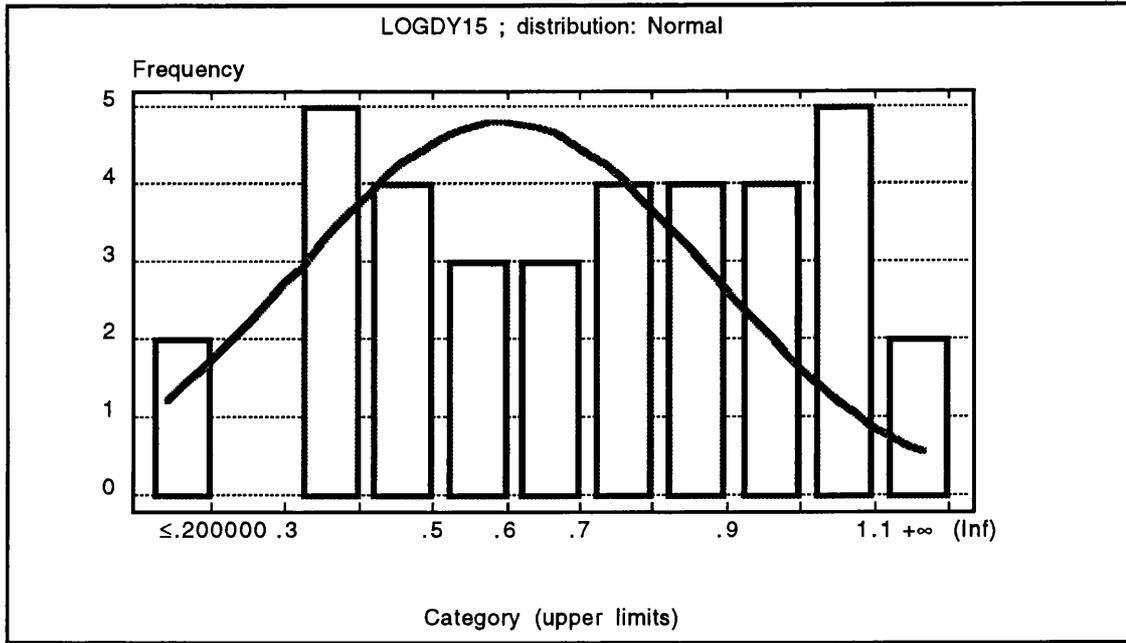


Figure 7.3.5.v Frequency histogram for log transformed chemoreceptor responses to 15 breath alternations. This data was normally distributed by  $\chi^2$  test ( $P>0.60$ )

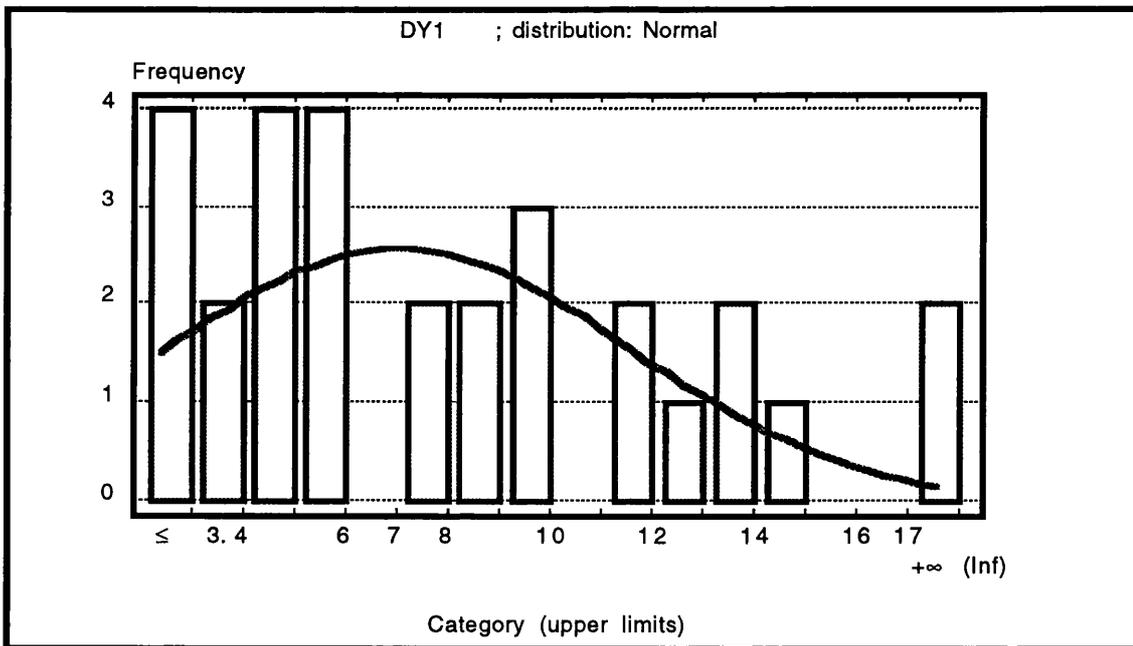


Figure 7.3.5.vi Frequency histogram for chemoreceptor responses to 1 breath alternations. This data was normally distributed by  $\chi^2$  test ( $P>0.60$ )

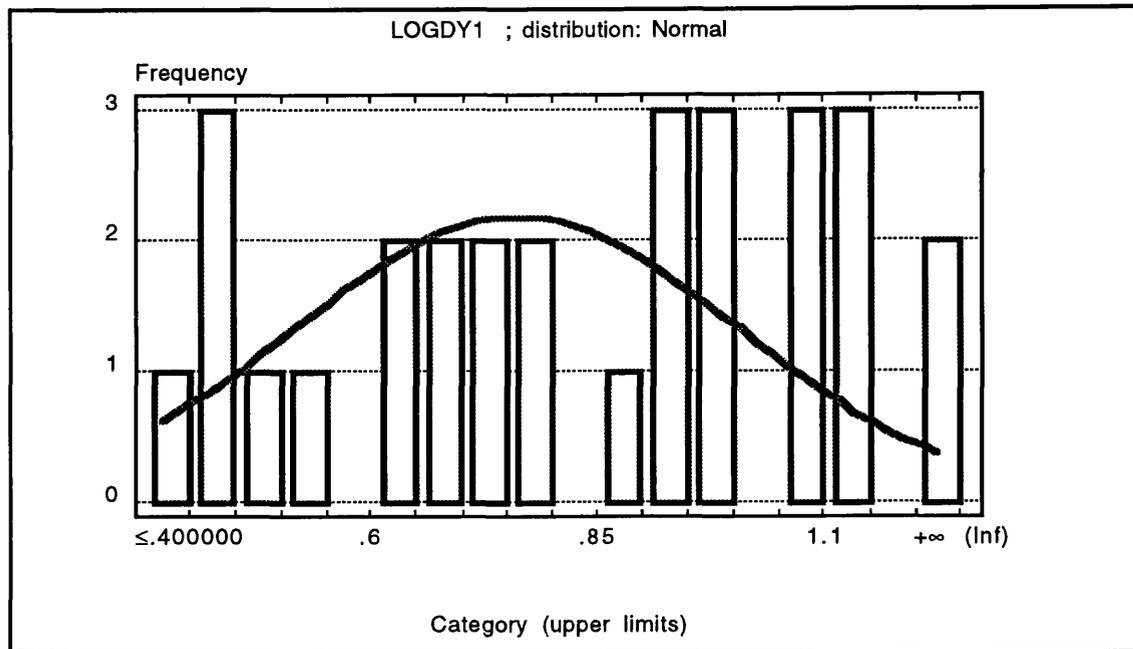


Figure 7.3.5.vii Frequency histogram for log transformed chemoreceptor responses to 1 breath alternations. This data was normally distributed by  $X^2$  test ( $P>0.30$ )

### 7.3.6 Comparison of CSN discharge oscillation amplitude between different steady state chemoreceptor responses to CO<sub>2</sub>

In figures 7.3.6.i-iii I have plotted steady state chemoreceptor responses against etCO<sub>2</sub> for three different fibres. This method has been used by Goodman et al. (1974), Cross et al. (1986) and others. Chemoreceptor responses have been plotted as maximum and minimum discharge frequencies about a mean level of discharge. This was performed so that the amplitude of the oscillation in discharge during the steady state could be analysed as an indication of dynamic CO<sub>2</sub> sensitivity. Figure 7.3.6.i shows the chemoreceptor response to steady state CO<sub>2</sub> in a 4d old lamb. The amplitude of the oscillation during NX was slightly smaller than during MOD HX, however the oscillation was of approximately the same amplitude as etCO<sub>2</sub> increased. The amplitudes in ascending etCO<sub>2</sub> points were 2.23, 2.45 and 3.00 % maximal discharge (3.23, 3.55 and 4.35 Hz). During MOD HX the amplitude of the oscillation was very similar for all four steady state points. The amplitudes in ascending etCO<sub>2</sub> points were 3.05, 3.53, 4.62 and 3.82 % maximal discharge (4.42, 5.11, 6.70 and 5.54 Hz).

Figure 7.3.6.ii shows the steady state chemoreceptor response to CO<sub>2</sub> in an 11d lamb. The amplitude of the oscillation in discharge was constant during NX and MOD HX for an increase in etCO<sub>2</sub>. During NX the amplitude of the oscillation was for increasing

etCO<sub>2</sub> points 4.32 and 4.06 % maximal discharge (3.70 and 3.49 Hz). During MOD HX the amplitude of the oscillation was for increasing etCO<sub>2</sub> points 3.66, 5.47 and 5.29 % maximal discharge (3.13, 4.69 and 4.53 Hz). This fibre shows a good example of the constant oscillation amplitude at different CO<sub>2</sub> and O<sub>2</sub> levels.

Figure 7.3.6.iii shows the steady state chemoreceptor response to CO<sub>2</sub> in an 17d lamb. The amplitude of the oscillation during NX showed considerable variation, however the amplitude was more consistent during MOD HX. During NX the amplitude of the oscillation was for increasing etCO<sub>2</sub> points 6.22, 15.13, 8.17 and 28.43 % maximal discharge (1.57, 3.83, 2.07 and 7.19 Hz). During MOD HX the amplitude of the oscillation was for increasing etCO<sub>2</sub> points 11.49, 17.59, 13.40 and 14.77 % maximal discharge (2.91, 4.45, 3.39 and 3.74 Hz).

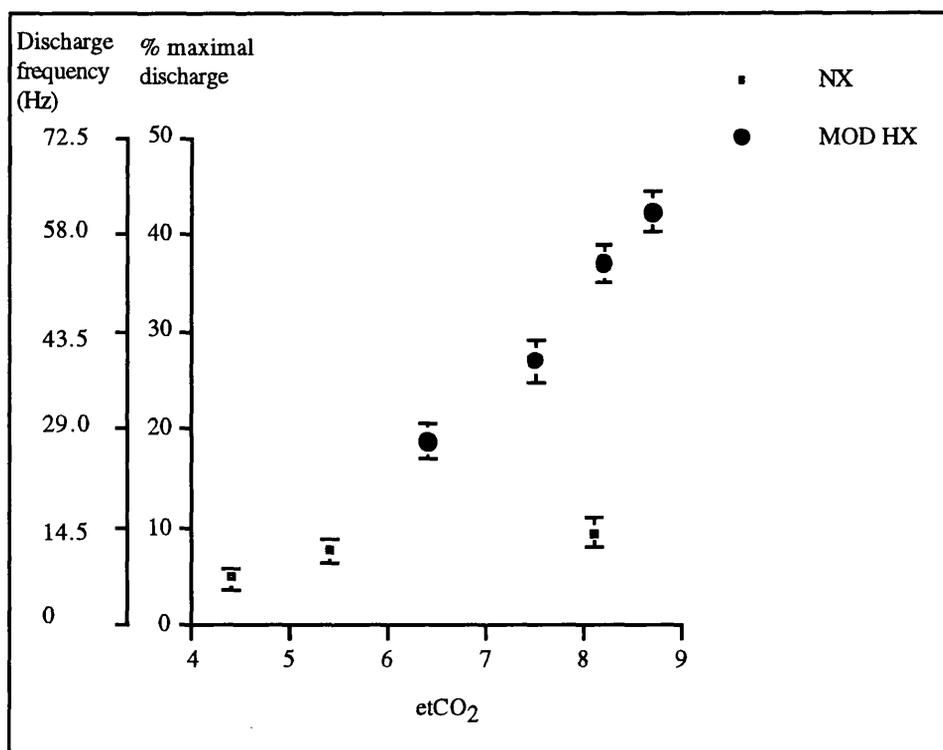


Figure 7.3.6.i Steady state chemoreceptor responses plotted against etCO<sub>2</sub> (%) during NX (open squares) and MOD HX (closed circles) in a lamb aged 4d. Chemoreceptor responses are plotted as maximum and minimum discharge frequencies about a mean level of discharge.

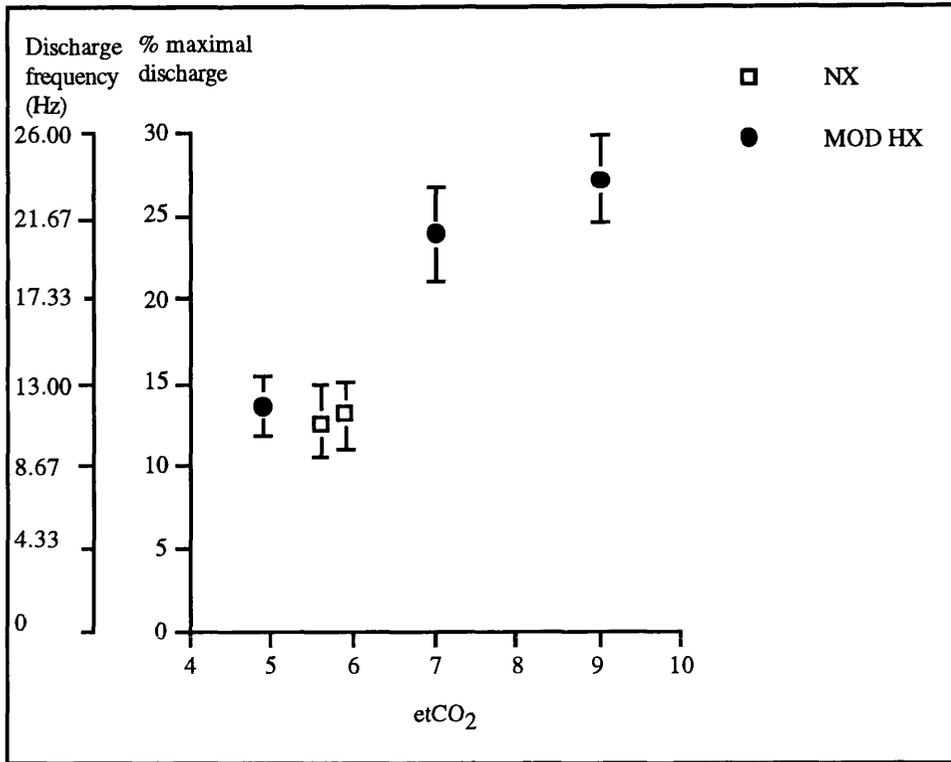


Figure 7.3.6.ii Steady state chemoreceptor responses plotted against etCO<sub>2</sub> (mmHg) during NX (open squares) and MOD HX (closed circles) in a lamb aged 11d. Chemoreceptor responses are plotted as maximum and minimum discharge frequencies about a mean level of discharge.

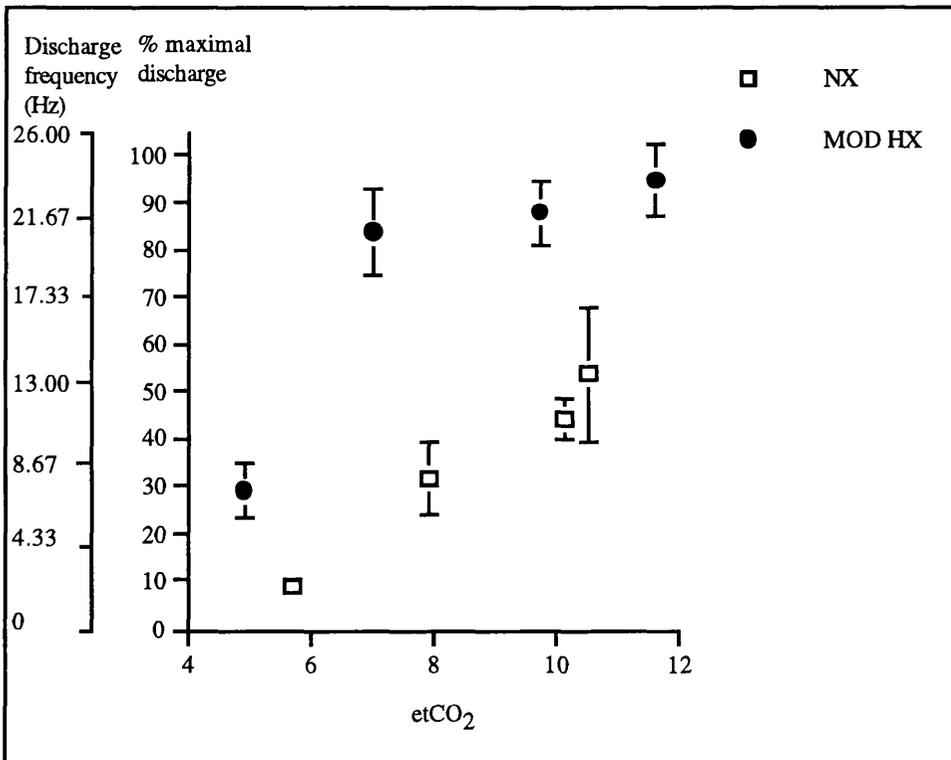


Figure 7.3.6.iii Steady state chemoreceptor responses plotted against etCO<sub>2</sub> (mmHg) during NX (open squares) and MOD HX (closed circles) in a lamb aged 17d. Chemoreceptor responses are plotted as maximum and minimum discharge frequencies about a mean level of discharge.

These fibres were typical of the observations I made on the oscillation amplitude during steady state chemoreceptor responses. Although for any particular fibre the absolute amplitude of the oscillation in chemoreceptor discharge may vary slightly at any PaO<sub>2</sub> level as etCO<sub>2</sub> increased, it remained approximately constant. In addition, the absolute amplitude of the oscillation in chemoreceptor discharge remained approximately constant between different PaO<sub>2</sub> levels i.e. NX and MOD HX. The absolute amplitude of the oscillation also tended to be more constant between etCO<sub>2</sub> points during MOD HX compared to NX in some fibres.

### **7.3.7 Shape of CSN discharge oscillation**

I observed that the shape of the oscillation in CSN discharge was different between fibres. I examined for each fibre the oscillation in CSN discharge and compared it with the rate of change in etCO<sub>2</sub>, and looked for evidence of adaptation in the chemoreceptor response to CO<sub>2</sub>. That is, whether the chemoreceptor response to CO<sub>2</sub> addition to the inspirate showed an initial brisk increase in discharge followed by a fall to a relative plateau. Conversely, chemoreceptor adaptation could also be evident from a rapid fall in discharge frequency upon removal of CO<sub>2</sub> from the inspirate, followed by a more gradual rise to a plateau. I found that it was difficult to see evidence of chemoreceptor adaptation in many of the fibres, as many of the oscillations were more sinusoidal or saw-tooth in shape.

#### **7.3.7.a Adaptation of the chemoreceptor response to CO<sub>2</sub>**

In figures 7.3.7.a.i-ii I have plotted CSN discharge over two 15 breath alternation periods for two different fibres that showed an adaptation in the chemoreceptor response to CO<sub>2</sub>. The chemoreceptor response for one fibre is shown during NX (figure 7.3.7.a.i.a) and MOD HX (figure 7.3.7.a.i.b). The oscillation in chemoreceptor discharge during NX (figure 7.3.7.a.i.a) is noisy and although there is a brisk increase in discharge that appears to be due to the addition of CO<sub>2</sub>, it is more difficult to see a fall in discharge when the CO<sub>2</sub> is removed. In this particular oscillation the fall in discharge is blurred. The chemoreceptor response during MOD HX for the same fibre is better defined although asymmetrical (figure 7.3.7.a.i.b). The fall in discharge that occurs in response to removal of CO<sub>2</sub> is steeper than the response to addition of CO<sub>2</sub>. The second chemoreceptor fibre, aged 12d, is shown in figure 7.3.7.a.ii.a during MOD HX only. This fibre shows adaptation to an increase in CO<sub>2</sub> with a delay of *ca.* 4-5sec. The initial increase in discharge frequency (marked by arrow A) is brisk and steep, and occurs over 1-2sec. Adaptation to the increase in CO<sub>2</sub>, that is the decline in discharge

to a steady state, is slower. I have marked this with the a curve (B), and it could be estimated that adaptation occurs over 6-8sec. The chemoreceptor response to a fall in CO<sub>2</sub> in figure 7.3.7.a.ii.a is steep, however the response is blurred as for figures 7.3.7.a.i.a-b. There is a longer delay to the fall in discharge, and there is no sign of adaptation. Chemoreceptor discharge remains at this low level for only *ca.* 4sec.

Using the definition of adaptation as, the incidence where chemoreceptor discharge either overshoots or undershoots before reaching a steady state, adaptation is only evident in figure 7.3.7.a.i.a by the chemoreceptor response to addition of CO<sub>2</sub>. For each oscillation in CSN discharge I have indicated the initial brisk increase in response to CO<sub>2</sub> by an arrow marked A, and measured the rate of change in discharge frequency as a percentage of maximal discharge over time. The rate of change in chemoreceptor discharge for figure 7.3.7.a.i.a was 3.44 % maximal discharge/sec (1.83 Hz/sec), for figure 7.3.7.a.i.b 1.85 % maximal discharge/sec (0.98 Hz/sec) and for figure 7.3.7.a.ii.a was 9.22 % maximal discharge/sec (2.84 Hz/sec). The fall in discharge frequency to a relative plateau is indicated, and has been described by an exponential curve. In these three examples, it is more difficult to see evidence of adaptation in the chemoreceptor response to CO<sub>2</sub> when it was removed from the inspire. Although there is a fall in discharge corresponding to the removal of CO<sub>2</sub> from the inspire, the chemoreceptor response does not undershoot and then rise to a relative plateau.

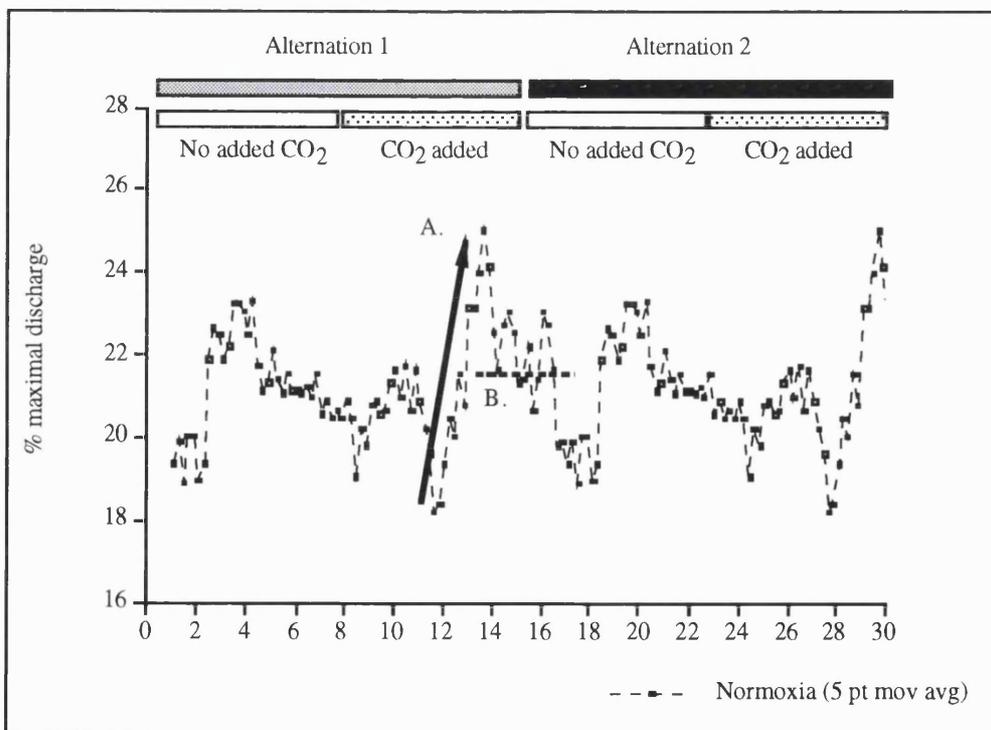


Figure 7.3.7.a.i.a CSN discharge summed over 2 alternation periods for 15 breath alternations in etCO<sub>2</sub> during NX at 4d. See text for description.

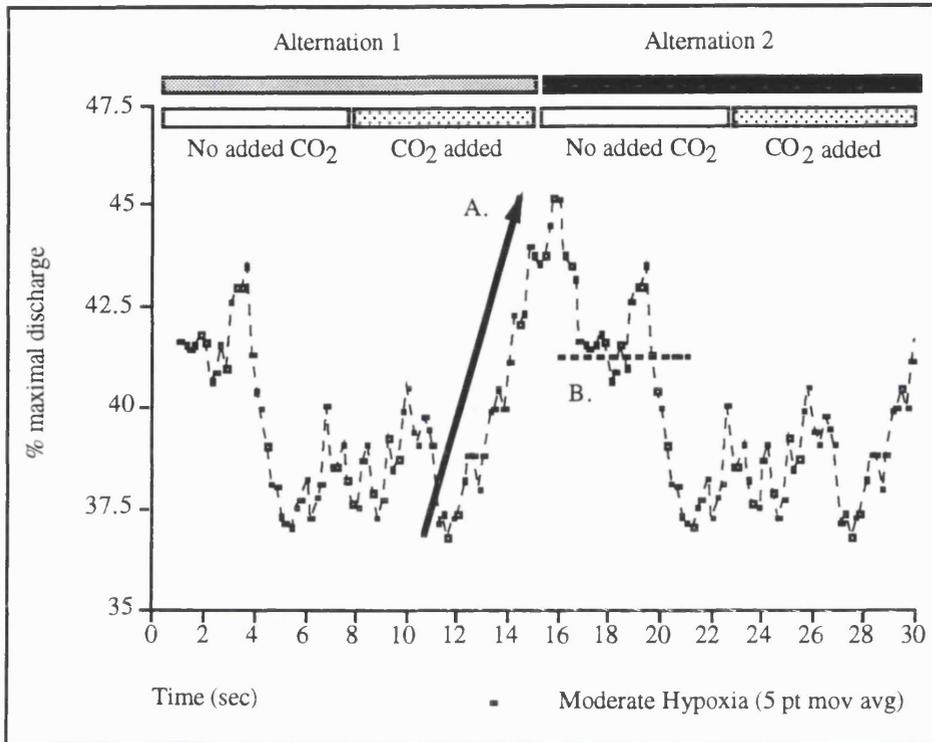


Figure 7.3.7.a.i.b CSN discharge summed over 2 alternation periods for 15 breath alternations in etCO<sub>2</sub> during MOD HX at 4d. See text for description.

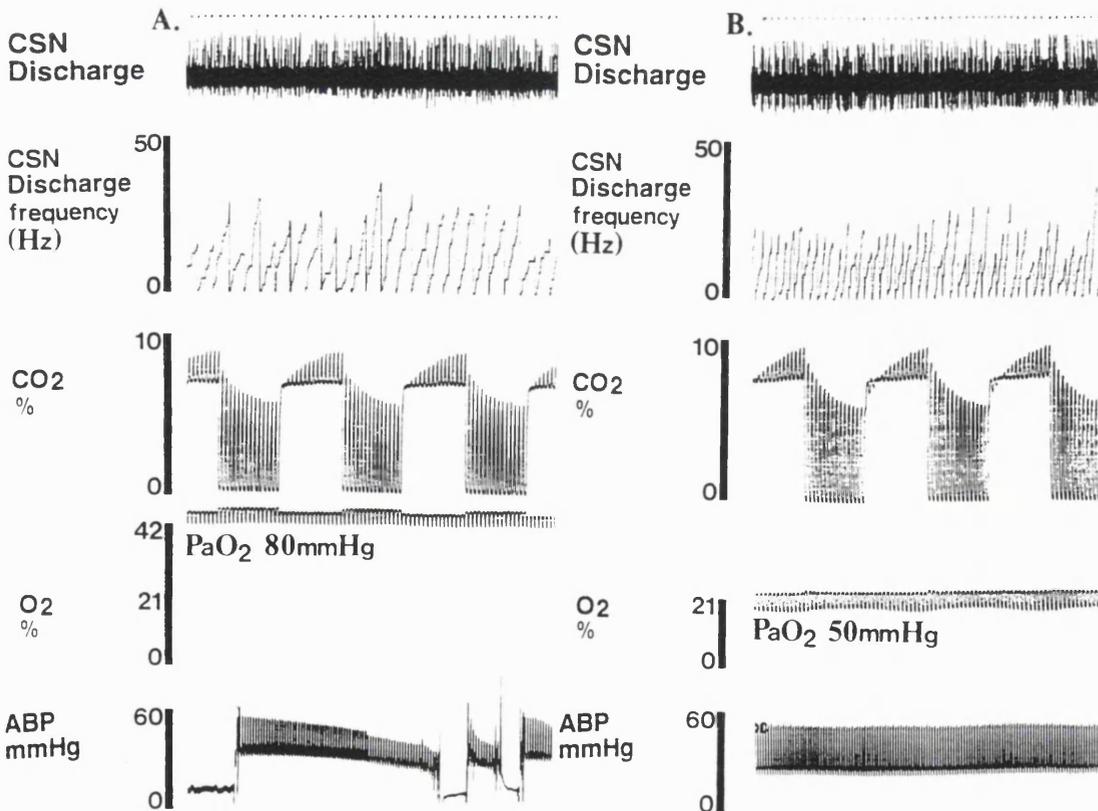


Figure 7.3.7.a.i.c etCO<sub>2</sub> profile for the 15 breath alternation in figure 7.3.7.a.i.a during NX (left) and figure 7.3.7.a.i.b during MOD HX (right) at 4d. CSN discharge ( $\mu$ V), CSN discharge frequency (Hz), CO<sub>2</sub> at the trachea (%), O<sub>2</sub> at the trachea (%), ABP (mm Hg).

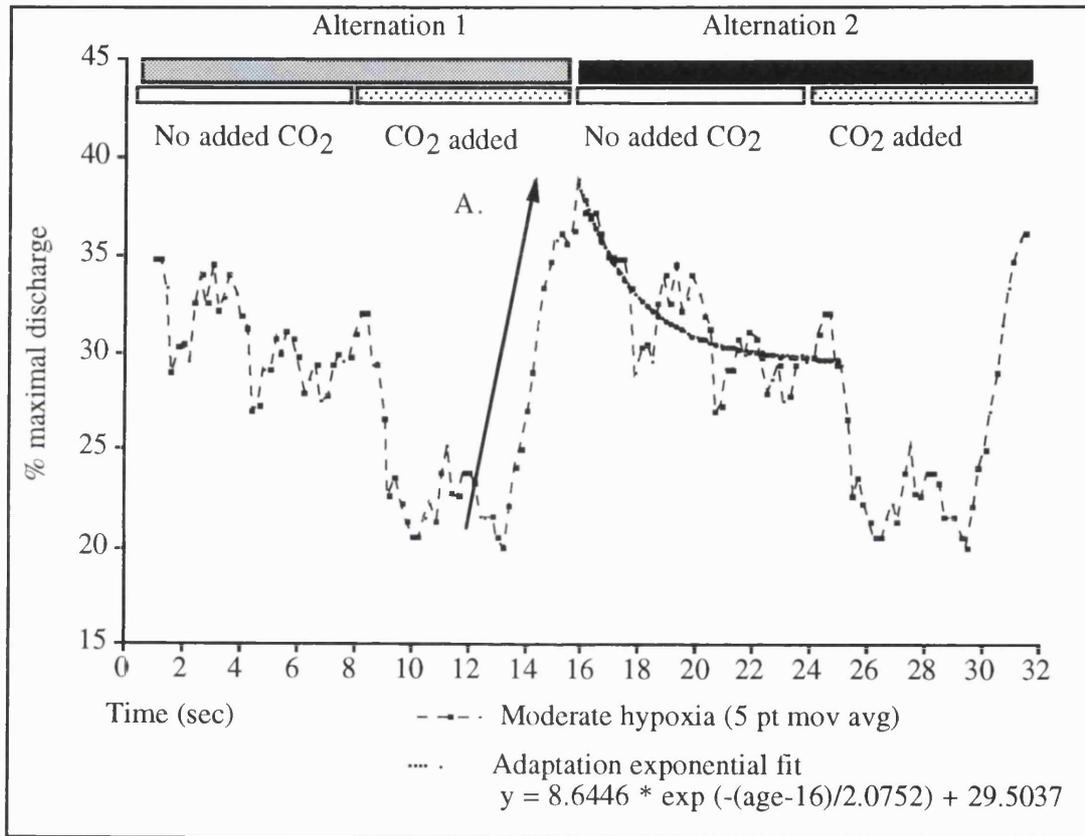


Figure 7.3.7.a.ii.a CSN discharge summed over 2 alternation periods for 15 breath alternations in etCO<sub>2</sub> during MOD HX at 12d. See text for description.

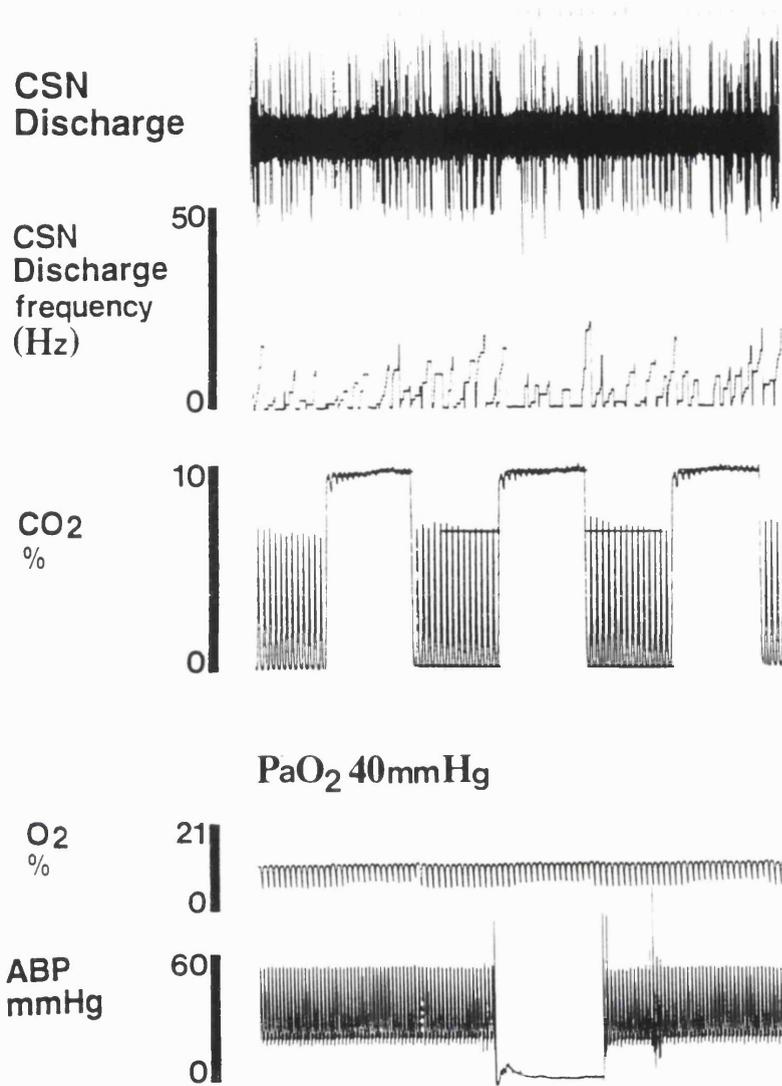


Figure 7.3.7.a.ii.b End-tidal CO<sub>2</sub> profile for the 15 breath alternation shown in figure 7.3.7.a.ii.a during MOD HX at 12d. CSN discharge ( $\mu$ V), CSN discharge frequency (Hz), CO<sub>2</sub> at the trachea (%), O<sub>2</sub> at the trachea (%) and ABP (mm Hg).

Examples of the profile of change in etCO<sub>2</sub> are shown in figures 7.3.7.a.i.c and 7.3.7.a.ii.b. In figure 7.3.7.a.i.c, the etCO<sub>2</sub> signal in A. shows the stimulus for the oscillation in figure 7.3.7.a.i.a, and the etCO<sub>2</sub> signal in B. shows the stimulus for the oscillation in figure 7.3.7.a.i.b. In figure 7.3.7.a.i.c, the profile for the change in etCO<sub>2</sub> signals for both A and B are curvilinear. There is an initial step increase in etCO<sub>2</sub> when CO<sub>2</sub> is added to the inspire followed by a slower increase that approximates to a ramp, and is steeper at the start and less steep towards the end. There is similar profile in etCO<sub>2</sub> change when CO<sub>2</sub> is removed from the inspire, that is an initial step decrease followed by a more gradual rate of decline. The slope of the decreasing ramp was steeper during MOD HX (B.) than NX (A.) for this fibre. In figure 7.3.7.a.ii.b the profile of the etCO<sub>2</sub> signal shows much more of a step change. There is a very small ramp after the initial increase or decrease in etCO<sub>2</sub>, however this is much smaller than in figure 7.3.7.a.i.c. In fact, this was the only experiment in which I was able to produce such a step change in etCO<sub>2</sub>.

I was interested to compare the etCO<sub>2</sub> signal with the shape of the oscillation in CSN discharge. For the oscillation in discharge shown in figure 7.3.7.a.i.a, the brisk increase in discharge is related to the initial step increase in etCO<sub>2</sub> for A. (figure 7.3.7.a.i.c). It is however more difficult to interpret the oscillation in discharge after this initial increase. Irrespective of the continued increase in etCO<sub>2</sub>, there is an adaptation to a lower discharge frequency (marked as the dashed line B in figure 7.3.7.a.i.a), followed by a further fall and then another increase in discharge frequency. In this particular alternation, the blurring of the oscillation may be due to a persistence of breath-by-breath oscillations in PaCO<sub>2</sub>.

For the same fibre during MOD HX (figure 7.3.7.a.i.b), the oscillation in discharge is clearer. The initial increase in discharge frequency, which was less brisk compared to the alternation during NX, also adapts to a relative plateau and is followed by a second small rise in discharge frequency. The etCO<sub>2</sub> signal in B. (figure 7.3.7.a.i.c) shows an initial step increase, and the subsequent ramp is slightly more arc-like in appearance than during NX. This second peak after the initial increase in discharge frequency may be due to the rate at which the etCO<sub>2</sub> continues to change throughout the alternation period. The fall in discharge frequency upon removal of CO<sub>2</sub> is steeper than the increase in frequency, although this is not explicable in terms of the etCO<sub>2</sub> signal. However, it appears that the profile in etCO<sub>2</sub> whilst it is falling is such that it maintains a relative trough in the oscillation discharge frequency.

In figure 7.3.7.a.ii.b the profile of etCO<sub>2</sub> change approximates to a step, more so than in any other 15 breath alternation. The accompanying oscillation in figure 7.3.7.a.ii.a

shows a brisk increase in discharge frequency (9.22 % maximal discharge/sec) which adapts slowly over the next 6-8 sec. The adaptation is marked with a curve (B). Although there is a perturbation in the oscillation following the brisk increase and adaptation, discharge frequency does appear to plateau before falling in response to removal of CO<sub>2</sub> from the inspirate. In response to the step decrease in etCO<sub>2</sub>, discharge frequency falls briskly and does plateau at a trough for approximately 4 sec.

In figures 7.3.7.a.iii-iv I have plotted CSN discharge over two 1 breath alternation periods for two different fibres that show adaptation in the chemoreceptor response to CO<sub>2</sub>. Figure 7.3.7.a.iii.a shows an oscillation in chemoreceptor discharge for one fibre aged 4d during MOD HX, and the profile for etCO<sub>2</sub> change is shown in figure 7.3.7.a.iii.b. Figure 7.3.7.a.iv.a shows an oscillation in chemoreceptor discharge for one fibre aged 14d during NX, and the profile for etCO<sub>2</sub> change is shown in figure 7.3.7.a.iv.b. There is a brisk increase in chemoreceptor discharge when CO<sub>2</sub> is added for both fibres, *ca.* 4sec after the addition of CO<sub>2</sub> and is marked by arrow A. The rate of change in chemoreceptor discharge is 6.60% maximal discharge/sec (9.57 Hz/sec) for the fibre aged 4d (figure 7.3.7.a.iii.a), and 25.99% maximal discharge/sec (10.74 Hz/sec) for the fibre aged 14d (figure 7.3.7.a.iv.a). Chemoreceptor adaptation after the brisk increase in discharge frequency occurs quickly also, over *ca.* 2sec for the fibre aged 4d (figure 7.3.7.a.iii.a) and over *ca.* 1-2sec for the fibre aged 14d (figure 7.3.7.a.iv.a). Adaptation is marked by a curve (B) in both figures, and approximates chemoreceptor adaptation. Chemoreceptor adaptation when CO<sub>2</sub> was removed from the inspirate was less obvious for both the 4d and 14d fibre. During inspiration of the air or hypoxic breath without CO<sub>2</sub>, discharge frequency falls slowly, then rises slowly over the expiration portion of that breath. There is no evidence of an undershoot, and then a more gradual rise to a steady level. This appears to be consistent with my other observations, in that I only see evidence for chemoreceptor adaptation when CO<sub>2</sub> is added to the inspirate. Interestingly, during the phase when chemoreceptor discharge responds to CO<sub>2</sub>, it is possible to see both the increase in discharge frequency due to the expiratory portion of the un-loaded breath, followed by a steeper increase in discharge frequency due to the inhalation of the CO<sub>2</sub>-loaded breath. This is detectable for both fibres aged 4d and 14d, but is particularly well defined in the older animal (figure 7.3.7.a.iv.a).

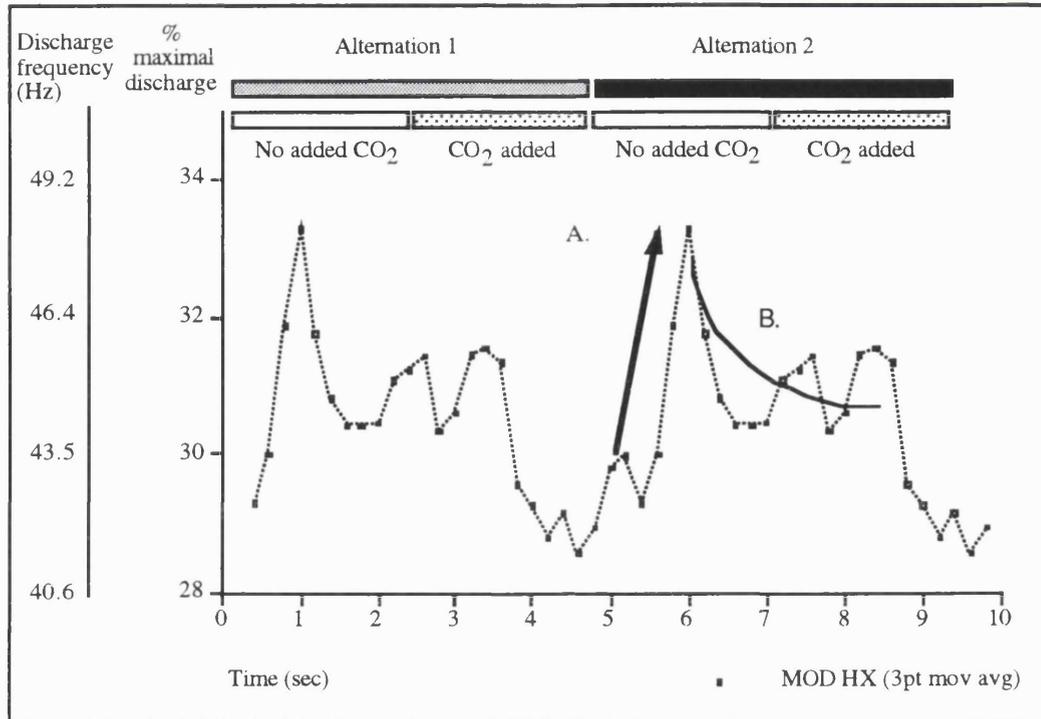


Figure 7.3.7.a.iii.a CSN discharge summed over 2 alternation periods for 1 breath alternations in etCO<sub>2</sub> during MOD HX at 4d. See text for description.

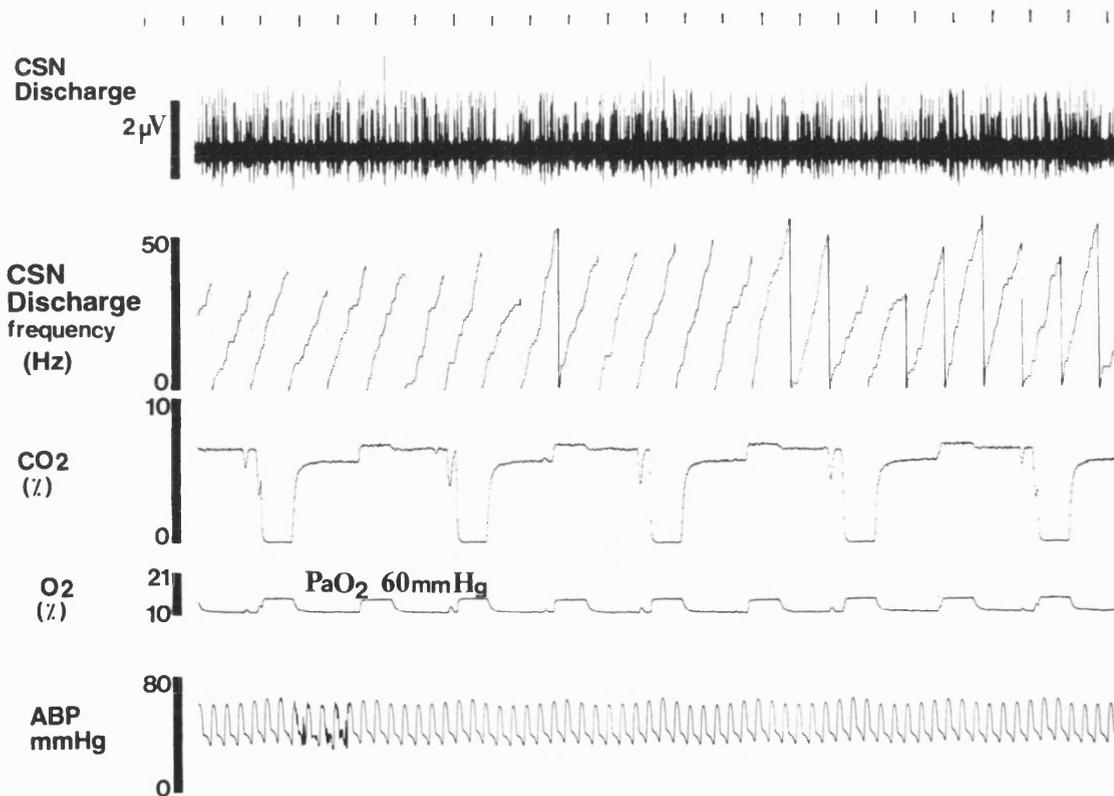


Figure 7.3.7.a.iii.b End-tidal CO<sub>2</sub> profile for the 1 breath alternation shown in figure 7.3.7.a.iii.a during MOD HX at 4d. CSN discharge (µV), CSN discharge frequency (Hz), CO<sub>2</sub> at the trachea (%), O<sub>2</sub> at the trachea (%) and ABP (mm Hg).

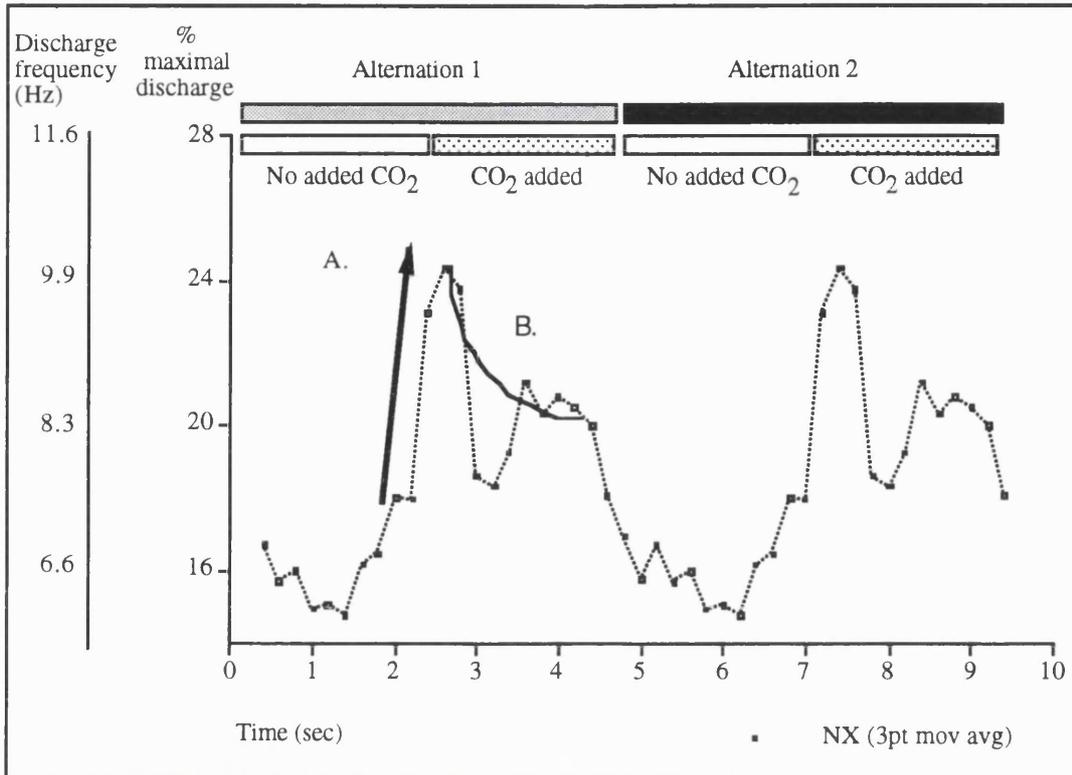


Figure 7.3.7.a.iv.a CSN discharge summed over 2 alternation periods for 1 breath alternations in etCO<sub>2</sub> during NX at 14d. See text for description.

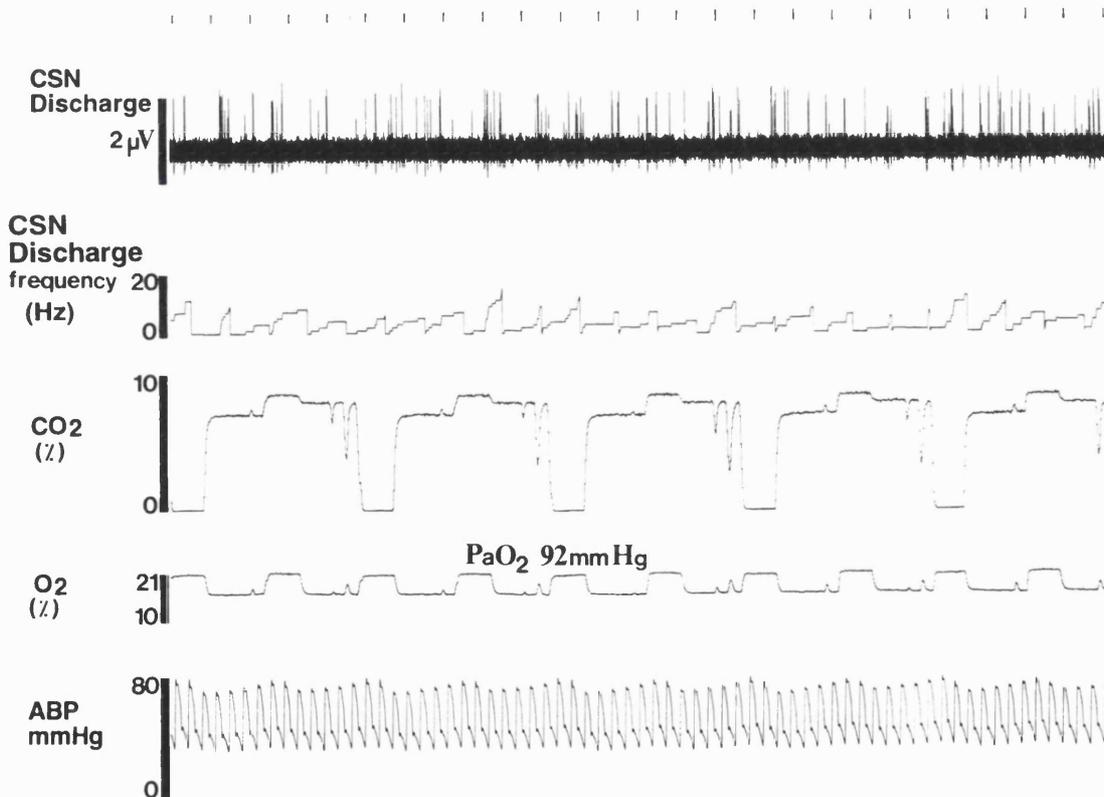


Figure 7.3.7.a.iv.b End-tidal CO<sub>2</sub> profile for the 1 breath alternation shown in figure 7.3.7.a.iv.a during NX at 14d. CSN discharge (μV), CSN discharge frequency (Hz), CO<sub>2</sub> at the trachea (%), O<sub>2</sub> at the trachea (%) and ABP (mm Hg).

From figures 7.3.7.a.iii-iv.b, it is not possible to draw any firm conclusions on the change in CO<sub>2</sub> produced at the carotid body in response to 1 breath alternations. Unlike the change produced in etCO<sub>2</sub> for 15 breath alternations, shown in figures 7.3.7.a.i.c and 7.3.7.a.ii.b, which were designed to eliminate the naturally occurring oscillations in PaCO<sub>2</sub> by increasing respiratory frequency. The 1 breath alternations were performed at a slower respiratory frequency, so the alternating CO<sub>2</sub> stimulus would augment the naturally occurring oscillation in PaCO<sub>2</sub>. During the un-loaded breath, PaCO<sub>2</sub> falls during inspiration and rises during expiration. For the CO<sub>2</sub>-loaded breath, PaCO<sub>2</sub> continues to rise during inspiration and remains elevated during expiration. The carotid chemoreceptors are stimulated by the alternating CO<sub>2</sub> stimulus, and by the underlying oscillation in PaCO<sub>2</sub> produced by respiration. Whilst single breath etCO<sub>2</sub> alternations are unable to produce a step change at the carotid body, figures 7.3.7.a.iii-iv.b provide an indication of the stimulus size.

### 7.3.7.b Step shaped oscillations in CSN discharge

I observed in a some fibres that the oscillation in discharge was stepped in appearance. That is, following an initial brisk increase or decrease in discharge frequency, the oscillation flattened so as to resemble a square wave. There was little or no evidence of adaptation in the chemoreceptor response to CO<sub>2</sub>.

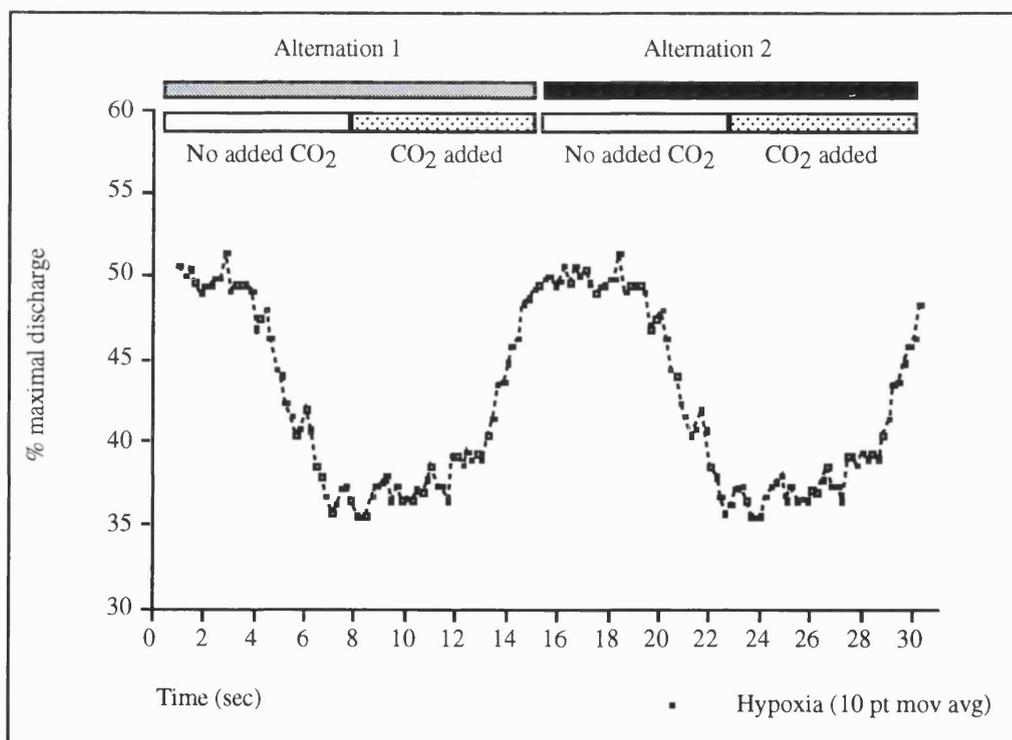


Figure 7.3.7.b.i CSN discharge summed over 2 alternation periods for 15 breath alternations in etCO<sub>2</sub> during MOD HX at 6d.

In figure 7.3.7.b.i the oscillation in discharge is shown during MOD HX for a fibre aged 6d. After the initial increase in discharge frequency, the shape of the oscillation is very flat until CO<sub>2</sub> is removed. When discharge frequency falls to its trough, there is small increase over the next 5-6sec. This suggests that this fibre shows some adaptation after the fall in discharge as the rate of fall is slightly steeper than rate of increase in discharge but there was no evidence of adaptation in terms of an overshoot when CO<sub>2</sub> was added. The delay in response for this fibre was approximately 4-5sec. The profile for the change in etCO<sub>2</sub> is shown in figure 7.3.7.b.ii. When the gases switched there was an initial step increase or decrease in etCO<sub>2</sub>, followed by a less steep ramp that was curvilinear. In this instance, the square top of the oscillation in discharge occurred when etCO<sub>2</sub> was increased slowly in the shape of a curved ramp.

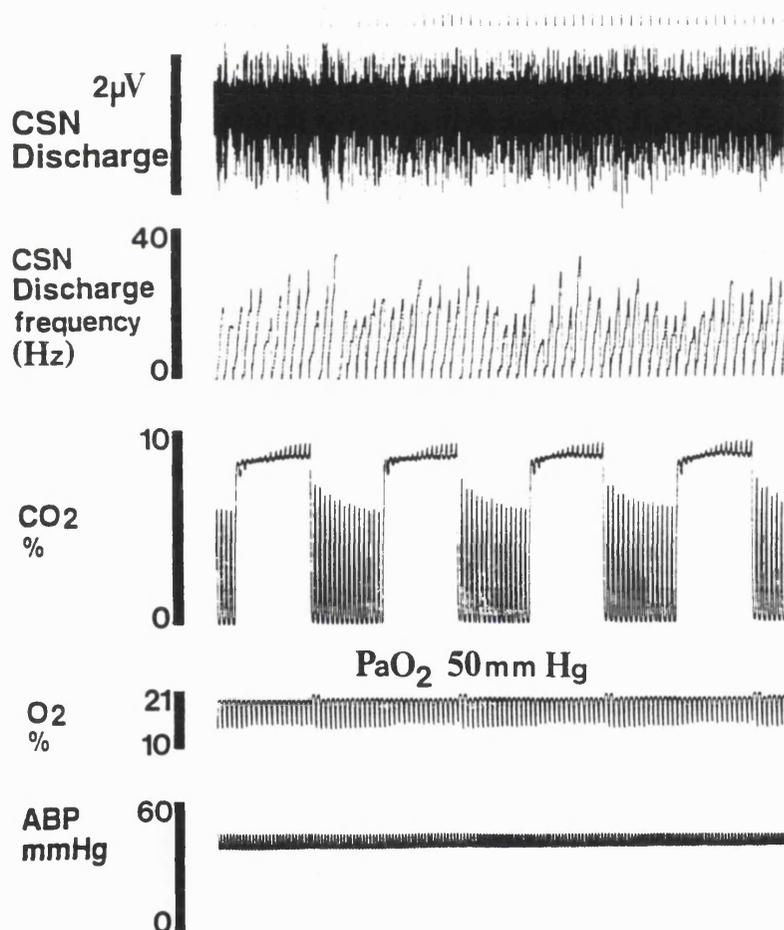


Figure 7.3.7.b.ii End-tidal CO<sub>2</sub> profile for the 15 breath alternation shown in figure 7.3.7.b.i during MOD HX at 6d. CSN discharge ( $\mu$ V), CSN discharge frequency (Hz), CO<sub>2</sub> at the trachea (%), O<sub>2</sub> at the trachea (%) and ABP (mm Hg).

### 7.3.7.c Saw tooth shaped oscillations in CSN discharge

I found in other fibres that the oscillation in discharge was saw-toothed. That is discharge frequency increased linearly for 8sec, then decreased linearly for 8sec. Some fibres showed a slightly more sinusoidal pattern in their oscillation. There was no evidence of adaptation, neither did discharge reach a plateau. Rather, as discharge frequency increased and decreased slowly over the alternation period. In figure 7.3.7.c.i the oscillation in discharge is shown during NX for a fibre aged 7d. Discharge frequency increased over an 8sec period, then fell over an 8sec period and there was no adaptation in the chemoreceptor response to CO<sub>2</sub>. The profile for the change in etCO<sub>2</sub> is shown in figure 7.3.7.c.ii. When the gases switched the initial increase or decrease in etCO<sub>2</sub> was small, and was better described by a steady rate of change in etCO<sub>2</sub> over the alternation period. In this instance, the ramp was almost linear over the 8 sec period.

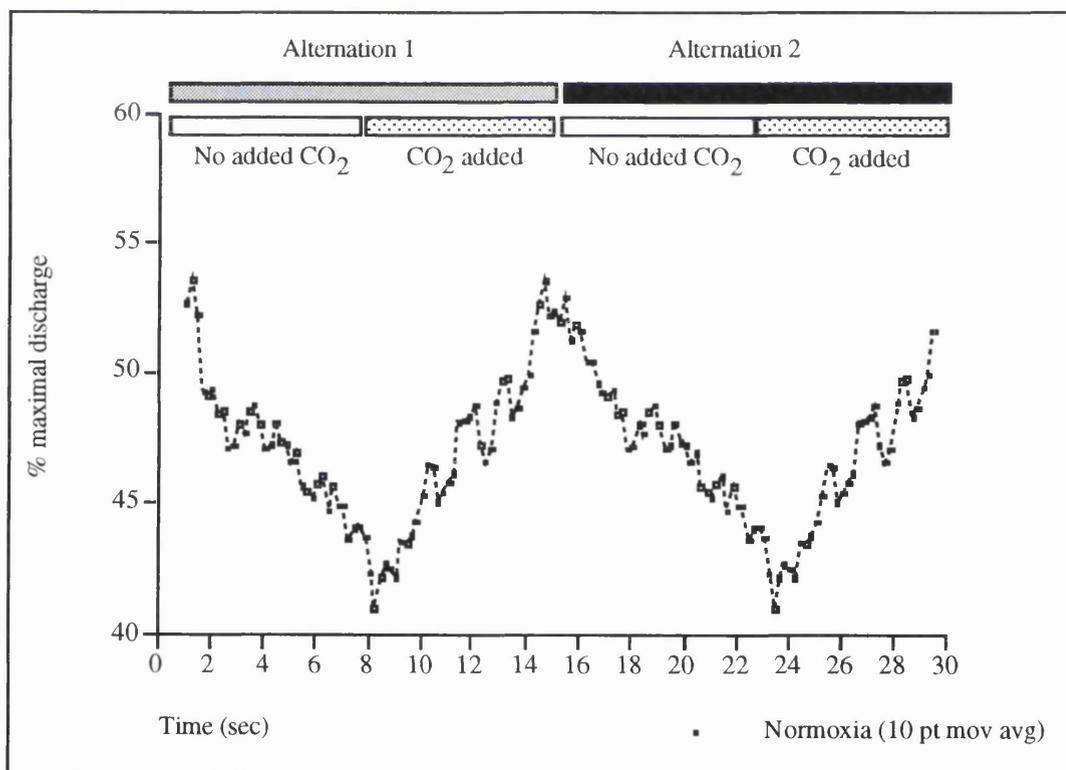


Figure 7.3.7.c.i CSN discharge summed over 2 alternation periods for 15 breath alternations in etCO<sub>2</sub> during NX at 7d.

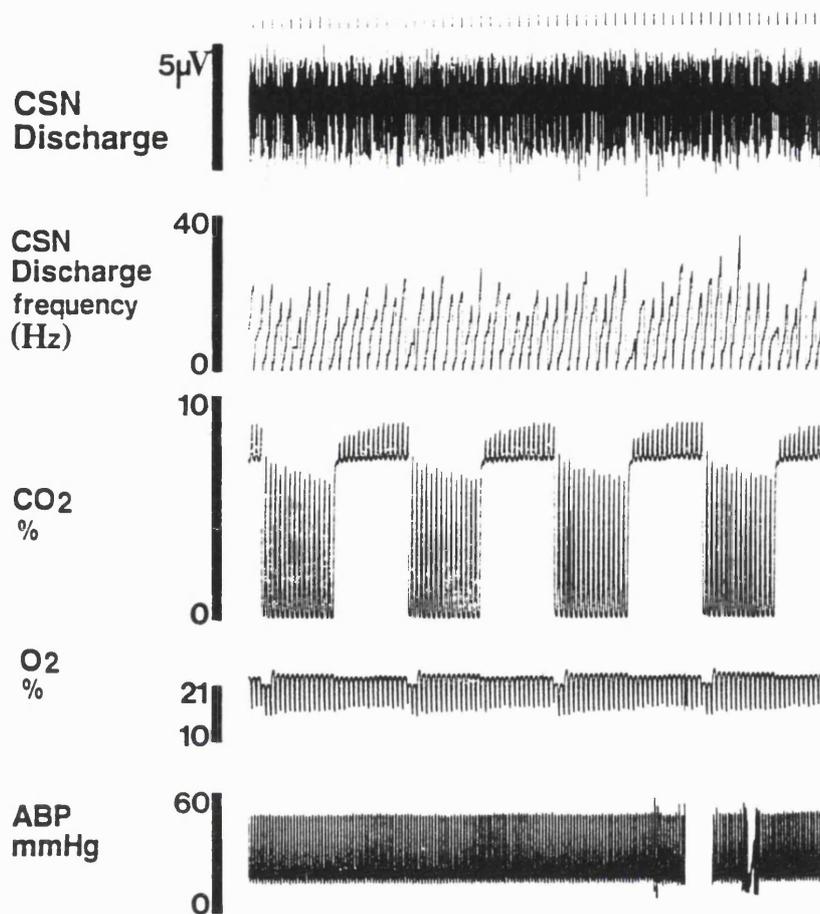


Figure 7.3.7.c.ii End-tidal CO<sub>2</sub> profile for the 15 breath alternation shown in figure 7.3.7.c.i during NX at 7d. CSN discharge (µV), CSN discharge frequency (Hz), CO<sub>2</sub> at the trachea (%), O<sub>2</sub> at the trachea (%) and ABP (mm Hg).

## 7.4 Discussion

### 7.4.1. Overview

I have found a dynamic sensitivity to CO<sub>2</sub> in all lambs aged 3-17d using a 1 breath and 15 breath alternation in etCO<sub>2</sub>. I have shown adaptation in the chemoreceptor response to CO<sub>2</sub> in young lambs and in older lambs. Dynamic CO<sub>2</sub> sensitivity measured from 1 breath alternations in etCO<sub>2</sub> was independent of age and PaO<sub>2</sub>. However there was a relationship between CO<sub>2</sub> sensitivity measured from 15 breath alternations with PaO<sub>2</sub> and age. I speculate that this was in part due to a steady state component measured in the chemoreceptor response to 15 breath alternations in etCO<sub>2</sub>.

## **7.4.2 Comparison of chemoreceptor responses to 1 and 15 breath alternations in etCO<sub>2</sub>.**

I performed two types of alternation in etCO<sub>2</sub>. Single breath alternations were performed at slow respiratory frequencies *ca.* 0.3-0.5 Hz and 15 breath alternations were performed at more rapid respiratory frequencies *ca.* 2.0 Hz. I wanted to measure the dynamic chemoreceptor response to single breath alternations (i.e. at slow respiratory frequencies) in etCO<sub>2</sub> because of the work I had done in newborn babies measuring the respiratory response to single breath alternations in Fio<sub>2</sub> (Chapters 3, 4 and 5). Information on the chemoreceptor response to single breath alternations in etCO<sub>2</sub> at the level of the carotid body would facilitate a better understanding of the respiratory response to this stimulus. At present single breath alternations in Fico<sub>2</sub> has not been performed in the newborn baby.

As I also wanted to eliminate the naturally occurring breath-by-breath oscillation in discharge, I decided to ventilate the animal more rapidly (*ca.* 2Hz) and produce alternations in etCO<sub>2</sub> on a 15 breath basis. In this way I hoped to produce more of a step change in arterial Pco<sub>2</sub> and subsequently more of a step change in the stimulus to the carotid body. I predicted that if it was possible to produce a step change in the stimulus to the carotid body, then I might expect to see adaptation in the chemoreceptor response to CO<sub>2</sub>. However, I found that it was in fact difficult to produce such a stimulus in my experiments.

In section 7.3.7 I showed some examples for 15 breath alternations, the profile of change in etCO<sub>2</sub> that I was able to produce. I found that although I was ventilating the animal rapidly, in almost all instances the change in etCO<sub>2</sub> that I was able to produce was curvilinear (see figures 7.3.7.a.i.c, 7.3.7.b.ii and 7.3.7.c.ii). In one fibre I was able to produce an almost pure step in etCO<sub>2</sub> (figure 7.3.7.a.ii.b). In the instance where I was able to produce a step change in etCO<sub>2</sub>, the resulting chemoreceptor response would be expected to show an initial brisk increase in discharge frequency and possibly followed by adaptation. If the increase in etCO<sub>2</sub> occurred more slowly, then the chemoreceptor response would be a mixture of the initial brisk response and a slower steady state component.

The chemoreceptor responses that I have measured to the 15 breath alternations do in fact support the idea that there is both a dynamic and a steady state component in the response. Multiple linear regression found a significant relationship of PaO<sub>2</sub> ( $P < 0.03$ ), but not of age ( $P > 0.05$ ) on the chemoreceptor response to 15 breath alternations in

etCO<sub>2</sub>. In contrast, there was no significant effect of age ( $P>0.62$ ) or PaO<sub>2</sub> ( $P>0.10$ ) on chemoreceptor responses to 1 breath alternations by multiple linear regression. A significant effect of age or PaO<sub>2</sub> on the response to 15 breath alternations could mean either, that the CO<sub>2</sub> dynamic sensitivity in the neonate was not independent of PaO<sub>2</sub> as in the adult, that CO<sub>2</sub> dynamic sensitivity of the carotid body matured with age, or that the response also contained a steady state component. Black et al. (1971) reported a half time for adaptation to CO<sub>2</sub> of 5-10sec, and so for the 15 breath alternations the stimulus alternation occurred sufficiently slowly so that a steady state component may have been measured. If in fact the dynamic sensitivity of the carotid body to CO<sub>2</sub> matured with age, or was dependent on the background level of PaO<sub>2</sub>, then I would have expected to see a similar relationship between PaO<sub>2</sub> and the chemoreceptor response to 1 breath alternations. This was not the case, however. As the alternation period of the 1 breath alternation was only 6-8sec, there should be less of a steady state component in the chemoreceptor response to CO<sub>2</sub>.

A steady state component of both alternation types could also occur due to the elevation of mean etCO<sub>2</sub> and hence PaCO<sub>2</sub>, as CO<sub>2</sub> was added to the inspire on every other breath, or every 15 breaths for 15 breaths, over a period of time, raising mean PaCO<sub>2</sub> slightly. To minimize such a steady state component in the chemoreceptor response, summation of CSN discharge did not occur until at least 3min after the alternation began. During this time I assumed that a new mean PaCO<sub>2</sub> would have been reached, and so during summation of discharge I only measured the chemoreceptor response to the alternation. I cannot rule out the possibility that mean PaCO<sub>2</sub> continued to increase during the period when I was summing CSN discharge as CO<sub>2</sub> was added to the inspire every 8sec for 15 breath alternations and every 3sec for 1 breath alternations.

I have speculated that the chemoreceptor response to a 1 breath alternation is a better estimate of dynamic CO<sub>2</sub> sensitivity than 15 breath alternation. Firstly, I did not see a relationship between the chemoreceptor response to 1 breath alternations with age or PaO<sub>2</sub>, but I did find a relationship with the chemoreceptor response to 15 breath alternations. This may be due to a steady state component in the latter. Secondly, in section 7.3.4 I analysed CO<sub>2</sub> sensitivity in three age groups and at two PaO<sub>2</sub> levels and found that the magnitude of the response to 1 breath alternations was greater than the response to 15 breath alternations during NX (table 7.3.4.c.iii) and MOD HX (table 7.3.4.c.iv). This is also shown by figures 7.3.4.c.i-ii. The smaller response to 15 breath alternations also suggests that a steady state component may be present and that I have measured the chemoreceptor response after adaptation has occurred.

The 15 breath alternations were used so that the naturally occurring breath-by-breath oscillation in PaCO<sub>2</sub> would be reduced or eliminated. However, this seems to have complicated the interpretation of the results by blurring the response between a pure dynamic chemoreceptor response and a steady state one. Thus, to compare my results of dynamic sensitivity to other studies measuring dynamic CO<sub>2</sub> sensitivity in the newborn and in the adult, I preferentially refer to the chemoreceptor response measured to 1 breath alternations.

### **7.4.3 Comparison of dynamic and steady state chemoreceptor responses**

I showed in section 7.3.4.c that newborn lambs showed a significant dynamic CO<sub>2</sub> sensitivity. Chemoreceptor responses to single breath alternations were significantly greater than steady state responses during NX ( $P < 0.05$ ) and MOD HX ( $P < 0.01$ ), whilst chemoreceptor responses to 15 breath alternations were significantly greater than steady state responses during MOD HX ( $P < 0.02$ ), but not during NX ( $P > 0.20$ ).

Figures 7.3.4.a.ii-iv showed that between fibres of a similar age there was variation in the magnitude of the dynamic chemoreceptor response to CO<sub>2</sub>. Interestingly in figures 7.3.4.b.i-iv, I showed that for two lambs the dynamic chemoreceptor response was different between different fibres in the same animal. So, in some fibres the dynamic response was greater than the steady state response whilst in other fibres in the same lamb the steady state response was greater. This may be in part related to the way I have analysed CO<sub>2</sub> sensitivity. It was clear from the oscillations in chemoreceptor discharge where the maximum and minimum discharge frequencies occurred. However, the assumption had to be made that these points corresponded to the maximum and minimum values in etCO<sub>2</sub> obtained from the chart record. Whilst it is likely that this is a fair assumption, there is no means to guarantee that the maximum and minimum values in etCO<sub>2</sub> were responsible for the maximum and minimum discharge frequencies obtained from the oscillation. Small errors in etCO<sub>2</sub> values could incur a much greater error in CO<sub>2</sub> sensitivity, which was measured as the slope between maximum and minimum discharge frequency plotted against etCO<sub>2</sub>.

It is possible that during some experiments the activity of the fibre showed some deterioration. Steady state chemoreceptor responses were measured immediately after the alternations were completed at the same PaO<sub>2</sub>. Alternations in etCO<sub>2</sub> were then performed at another PaO<sub>2</sub>, and subsequently steady state chemoreceptor responses measured. Due to the time taken to measure these chemoreceptor responses, there was

insufficient time to repeat alternations or steady state chemoreceptor responses in a given fibre. Sometimes this was due to visible deterioration in the signal, the size of action potentials or the amount of discharge. Therefore I was not able to compare chemoreceptor responses measured at the beginning of an experiment to those measured later in the experiment.

It is inevitable that the chemoreceptor responses I have measured will show some biological variation. Analysing chemoreceptor responses as a group means that the relative importance of the difference between fibres is small. There is no doubt that as a group the chemoreceptor response to an alternation in CO<sub>2</sub> was greater than the steady state response to CO<sub>2</sub>, hence there was a dynamic sensitivity. I cannot be certain of the reason for a variation in response between lambs and between fibres in the same animal, however I have based my observations and conclusions on the group as a whole as there will always be some degree of biological variation.

#### **7.4.4 Effect of age on dynamic chemoreceptor responses to CO<sub>2</sub>**

I found no significant effect of age on chemoreceptor responses to 1 breath alternations ( $P > 0.62$ ) nor to 15 breath alternations ( $P > 0.05$ ), however this P value was approaching statistical significance. Statistical analysis by ANOVA showed the same effect, however multiple linear regression is a stronger statistical method as the individual values for age and PaO<sub>2</sub> are used. Both methods suggest that chemoreceptor responses to 15 breath alternations were influenced by age. I have shown in Chapter 6 that steady state CO<sub>2</sub> sensitivity matures with age. Chemoreceptor responses to 1 and 15 breath alternations were plotted as function of age in figures 7.3.5.i-ii. There was a tendency for CO<sub>2</sub> sensitivity to be greater in older animals, and I have speculated that this may be due to a steady state component, particularly measured in the chemoreceptor response to 15 breath alternations.

Only one other study has investigated in the neonate the dynamic sensitivity of the carotid body to CO<sub>2</sub> from direct recordings. Marchal et al. (1992a) report an absence of dynamic sensitivity to CO<sub>2</sub> in the newborn kitten because they did not observe an overshoot in the chemoreceptor response to inhaled 5% or 10% CO<sub>2</sub>. They compare their results to those of Black et al. (1971) yet their methodology is fundamentally different. Marchal et al. (1992a) do not produce a sufficiently rapid stimulus at the carotid body to observe an overshoot in discharge followed by adaptation to the steady state level. In addition they analysed discharge in 10sec periods until a steady state was reached, which is far too slow to measure dynamic sensitivity. To base the presence of

dynamic sensitivity solely on the speed of response to inhaled CO<sub>2</sub> in this way is incorrect. It is not surprising that they were unable to find a dynamic sensitivity to CO<sub>2</sub>, and so they have suggested that it is weak or absent in the newborn.

I have shown that dynamic sensitivity to CO<sub>2</sub> is present in the newborn and is independent of age. This agrees with observations from several ventilatory studies in the newborn lamb and kitten (Canet et al, 1990; Carroll & Bureau, 1987b; Watanabe et al, 1993b). Wolsink et al. (1993) found a maturation in the dynamic ventilatory response to CO<sub>2</sub> using the dynamic end-tidal forcing technique in newborn piglets, however this was only significant when they expressed the peripheral component as a ratio of the total ventilatory response. Perhaps more importantly, Wolsink et al. (1993) compared the ventilatory response of piglets aged 0 to 1.5d to piglets measured in a previous study aged 2 to 11d (Wolsink et al, 1991). In the earlier study they were unable to detect a postnatal increase in dynamic CO<sub>2</sub> sensitivity between 2 and 11d. There is always the possibility from ventilatory studies that the apparent postnatal increase in dynamic sensitivity is due to a change in the mechanical properties of the lungs. Chest wall compliance is high in the newborn period (Gerhardt & Bancalari, 1980; Davis et al, 1988; Colin et al, 1989; Papastamelos et al, 1995) and dynamic lung compliance is low (LaFramboise, Guthrie, Standaert & Woodrum, 1983; LaFramboise, Tuck, Woodrum & Guthrie, 1984). LaFramboise et al. (1983; 1984) found in the newborn monkey during quiet sleep that dynamic lung compliance increased between 2 and 21 days of age, and was also partly attributable (38%) for the postnatal increase in minute ventilation. Upton et al. (1990) also reported a maturation in dynamic sensitivity to tube breathing in sleeping newborn infants. However, the response was measured over a considerable period. The dynamic response was taken as the 63% of the increase in steady state ventilation, however the time taken to reach this point is longer than 30sec in nearly all infants at 72hrs. The dynamic component appears to be blurred with the steady state response.

Thus, direct CSN recordings of chemoreceptor responses to CO<sub>2</sub> are an accurate method to measure dynamic sensitivity. There is always the risk from ventilatory studies that a steady state component may be measured. In addition the postnatal changes in respiratory mechanics cannot be ignored and may very well influence the interpretation of results. My results do not show an age related effect with dynamic CO<sub>2</sub> sensitivity and I speculate that this is because the carotid chemoreceptors do not show a postnatal resetting of dynamic CO<sub>2</sub> sensitivity.

### **7.4.5 Effect of PaO<sub>2</sub> on dynamic chemoreceptor responses to CO<sub>2</sub>.**

In section 7.3.5 I looked at the effect of PaO<sub>2</sub> on dynamic chemoreceptor responses to CO<sub>2</sub>. I found no significant effect of PaO<sub>2</sub> on the chemoreceptor response to 1 breath (P>0.10) alternations in CO<sub>2</sub>, however there was a significant effect on the response to 15 breath (P<0.02) alternations by multiple linear regression. Statistical analysis by ANOVA showed the same effect, however multiple linear regression is a stronger statistical method as the individual values for age and PaO<sub>2</sub> are used. The chemoreceptor response to 1 breath alternations was independent of PaO<sub>2</sub>, unlike 15 breath alternations, and this suggests that a steady state component may have inadvertently been measured in the response to 15 breath alternations. Similarly, I have shown in Chapter 6 that steady state CO<sub>2</sub> sensitivity shows a relationship with the level of oxygen.

That dynamic CO<sub>2</sub> sensitivity is independent of PaO<sub>2</sub> has been reported previously. Band & Wolff (1973; 1978) investigated the role of hypoxia on the amplitude of the chemoreceptor oscillation in anaesthetised cats, and found small variations in the absolute amplitude of the oscillation, but by and large they were unchanged during hypoxia. When the amplitude was expressed in relation to mean discharge, the relative amplitude fell during hypoxia due to the rise in mean discharge. Goodman et al. (1974) similarly observed in anaesthetised cats and dogs that, overall, the absolute amplitude of the oscillation was unchanged during hypoxia. Some fibres showed a small increase, others a small decrease and some were completely unaltered. Kumar et al. (1988) found in the adult cat that the amplitude and shape of the oscillation in chemoreceptor discharge was not significantly altered by a change in P<sub>o<sub>2</sub></sub>, although mean discharge increased in hypoxia as expected. They used a high-frequency high-flow ventilator to investigate the effect of steady state P<sub>o<sub>2</sub></sub> on the chemoreceptor response to alternations in P<sub>co<sub>2</sub></sub>. Whilst mean discharge was increased when P<sub>o<sub>2</sub></sub> was lowered from 98 to 58 torr, neither the amplitude nor the shape of the oscillation in discharge to alternations in P<sub>co<sub>2</sub></sub> between 33 and 47 torr was significantly altered. Kumar et al. (1988) demonstrated that the CO<sub>2</sub> transient responses curves were parallel at different P<sub>o<sub>2</sub></sub>s and mean P<sub>co<sub>2</sub></sub>s. A family of parallel transient CO<sub>2</sub> response curves independent of background P<sub>o<sub>2</sub></sub> were also described by Torrance in terms of a bicarbonate hypothesis (Torrance, 1976; Torrance et al, 1993). This means for an increase in arterial P<sub>co<sub>2</sub></sub>, the carotid chemoreceptors respond along one of the parallel transient response curves for any given P<sub>o<sub>2</sub></sub>, and then adapt down to a steady state level. The slope of the transient CO<sub>2</sub> response curve is always the same, but the degree of adaptation to a steady state level is

dependent on the  $P_{O_2}$ : thus during hypoxia chemoreceptors adapt little and during hyperoxia they adapt considerably.

There is currently no other information available on the effect of  $P_{O_2}$  on dynamic  $CO_2$  sensitivity. My results suggest that dynamic  $CO_2$  sensitivity is  $P_{O_2}$ -independent in the neonatal lamb, and hence in this respect it is similar to adult chemoreceptor responses.

#### **7.4.6 Comparison between neonatal and adult dynamic chemoreceptor responses to $CO_2$**

Kumar et al. (1988) quantified dynamic sensitivity in a similar method employed in this Chapter. Single or few-fibre chemoreceptor activity was plotted as a function of  $P_{CO_2}$  for alternations at two different levels of mean  $P_{CO_2}$ , and dynamic  $CO_2$  sensitivity was *ca.* 2Hz / torr  $P_{CO_2}$  at both mean  $P_{CO_2}$ s. My results were measured as % maximal discharge / %  $etCO_2$ , so it was not possible to directly compare the magnitude of dynamic sensitivity I measured to that measured in the adult. I was not able to record from single chemoreceptor fibres, as I found that I was only able to dissect to the level of few or multi-chemoreceptor fibres. Thus it was necessary to normalize chemoreceptor responses to a percentage of maximal discharge.

Reports of dynamic  $CO_2$  sensitivity in the adult have traditionally focused on the presence of a brisk increase in discharge, possibly characterised by an overshoot in discharge and subsequent adaptation (Black et al, 1966; Leitner & Dejourns, 1968; Black et al, 1971). The stimulus I produced at the carotid body was not sufficiently rapid to observe such an overshoot in discharge. It was therefore difficult to compare dynamic chemoreceptor responses in the neonatal lamb to those in the adult cat on the basis of this criteria. Other workers have compared the amplitude of the naturally occurring oscillation in discharge at different mean  $PaO_2$ s and  $PaCO_2$ s (Leitner & Dejourns, 1968; Goodman et al, 1974; Band & Wolff, 1978; Cross et al, 1986). My results are discussed in relation to these studies in section 7.4.8.

Indisputably, dynamic  $CO_2$  sensitivity is present in newborn lambs. If there was a postnatal resetting of dynamic  $CO_2$  sensitivity after birth, then I would have expected to see a significant effect of age or  $PaO_2$  or both on dynamic chemoreceptor responses. Perhaps of most importance is that I have found dynamic sensitivity to be independent of age and  $PaO_2$  (discussed in section 7.4.4 and 7.4.5). Due to this finding, it is likely that dynamic  $CO_2$  sensitivity in the newborn is in fact similar to that in the adult. Due to the methodological differences between studies, it is very difficult to make any firm

conclusions on how dynamic sensitivity in the neonatal lamb compares to those measured in the adult cat and dog, however I feel that there is sufficient evidence from my results to support the theory that dynamic CO<sub>2</sub> sensitivity is mature at birth.

#### **7.4.7 Differences in the shape of the oscillation in discharge for chemoreceptor responses to 15 breath alternations**

In section 7.3.7 I compared some of the oscillations in chemoreceptor discharge with the profile in etCO<sub>2</sub> produced during 15 breath alternations. I identified that there were three main types of chemoreceptor oscillations: oscillations that showed an adaptation in chemoreceptor discharge, step-shaped oscillations in discharge and sawtooth-shaped oscillations in discharge.

I found that fibres showing an adaptation in chemoreceptor discharge were characterised by relatively fast changes in etCO<sub>2</sub>. In one experiment I was able to produce an almost pure step in etCO<sub>2</sub> (figure 7.3.7.a.ii.b) and I observed some degree of overshoot in the chemoreceptor response to CO<sub>2</sub>. There was however no evidence of undershoot when the CO<sub>2</sub> was removed and this was frequently the case in other experiments. It was not possible to achieve such a pure step in the stimulus at the carotid body in most other experiments and this would be the most appropriate means, for my experiments, to observe an overshoot and adaptation in chemoreceptor discharge to CO<sub>2</sub>. In figures 7.3.7.a.i.a-b, and 7.3.7.a.ii.a I showed oscillations for two other experiments where there was also evidence of adaptation in the chemoreceptor response to CO<sub>2</sub>. The change in etCO<sub>2</sub> in these experiments although not step-shaped was very rapid, and obviously it was the speed at which I was able to deliver the stimulus that would determine the presence of chemoreceptor adaptation.

In figures 7.3.7.a.iii-iv.a I showed the presence of chemoreceptor adaptation to CO<sub>2</sub> in a lamb aged 4d and 14d, when CO<sub>2</sub> was alternated on a single breath basis. These alternations were performed at slower respiratory frequencies compared to 15 breath alternations. Thus, the breath to breath oscillations in PaCO<sub>2</sub> were not eliminated, and the alternations in etCO<sub>2</sub> were superimposed on the underlying oscillation in PaCO<sub>2</sub>. As for the 15 breath alternations I found that chemoreceptor adaptation to CO<sub>2</sub>, in terms of an overshoot, was detectable when CO<sub>2</sub> was added to the inspirate. There was less evidence to suggest the presence of an undershoot when CO<sub>2</sub> was removed. Discharge frequency fell when CO<sub>2</sub> was removed from the inspirate, however a subsequent rise in discharge frequency was small or absent. The changes in CO<sub>2</sub> at the carotid body produced by this method were rapid enough to evoke chemoreceptor

adaptation. Most importantly, these figures show that chemoreceptor adaptation to CO<sub>2</sub> occurred at 4d and at 14d. This once again confirms the presence of dynamic CO<sub>2</sub> sensitivity in neonatal lambs.

That the speed at which the stimulus was delivered determined the presence of chemoreceptor adaptation, was confirmed in two different experiments where I observed a step-shaped (figure 7.3.7.b.i) and sawtooth-shaped oscillation (figure 7.3.7.c.i) in chemoreceptor discharge. The change in etCO<sub>2</sub> for these two experiments (figure 7.3.7.b.ii and 7.3.7.c.ii) were considerably slower than in the experiments where there was evidence of adaptation. For the step-shaped oscillation in chemoreceptor discharge, the change in etCO<sub>2</sub> must have increased at a rate that prevented adaptation, whereas for the sawtooth-shaped oscillation the etCO<sub>2</sub> must have increased at a much slower rate so that it was a constantly increasing ramp.

The shape of the naturally occurring oscillation in discharge is asymmetrical, that is the fall in discharge frequency is steeper than the rise (Goodman et al, 1974). In the three experiments where I have shown evidence for chemoreceptor adaptation there also appears to be some agreement with the observations of Goodman et al. (1974). I similarly observed that the fall in discharge frequency was frequently steeper than the rise.

#### **7.4.8 Oscillation amplitude of steady state chemoreceptor responses as an index of dynamic CO<sub>2</sub> sensitivity**

In section 7.3.6 I looked qualitatively at the amplitude of the oscillation in discharge during steady state increases in etCO<sub>2</sub>. I plotted chemoreceptor responses for one lamb at 4d (figure 7.3.6.i), one lamb at 11d (figure 7.3.6.ii) and one lamb at 17d (figure 7.3.6.iii) as maximum and minimum discharge frequencies about a mean level of discharge against etCO<sub>2</sub>. In figures 7.3.6.i-ii the amplitude of the oscillation in discharge was approximately constant for an increase in etCO<sub>2</sub>, although there was a small variation between steady state levels. The amplitude was also approximately constant between normoxia and moderate hypoxia. The range in amplitude was 2-5 % maximal discharge, or 3-7 Hz. The amplitude of the oscillation in figure 7.3.6.iii was more variable, particularly during normoxia. One of the steady state points during normoxia showed a large amplitude (28.43 % maximal discharge; 7.19 Hz) and another point a very small amplitude (6.22 % maximal discharge; 1.57 Hz). These three examples show the general trend I observed in the amplitude of the oscillation during steady state chemoreceptor responses to CO<sub>2</sub>. That absolute amplitude remained fairly constant for

an increase in  $\text{etCO}_2$  or a fall in  $\text{PaO}_2$ , however there were small variations within a fibre and some fibres showed a larger variation of this than others. This provides additional evidence that dynamic  $\text{CO}_2$  sensitivity is independent of  $\text{Po}_2$ , and does not mature with age. The amplitude of the oscillation in discharge obtained from my experiments agrees with previous reports in the adult (see below). It was not possible to compare the amplitude of the oscillation in Hz between fibres as my recordings were few or multi-chemoreceptor fibres.

Other workers have compared the amplitude of the oscillation in chemoreceptor discharge for a range of steady state  $\text{PaCO}_2$ s and  $\text{PaO}_2$ s, and used this as an index of dynamic  $\text{CO}_2$  sensitivity. Cross et al. (1986) found the amplitude of few or multi-fibre chemoreceptor oscillations to range between 4 and 10 Hz during the steady state for increases in  $\text{PaCO}_2$  in adult cats. The amplitude of the  $\text{PaCO}_2$  oscillation was held constant between *ca.* 1.7-2.7 mmHg at different mean  $\text{PaCO}_2$ s between 30 and 50 mmHg. The discharge frequencies of single chemoreceptor fibres illustrated by Goodman et al. (1974) show an amplitude in the order of 5-7 Hz. The amplitudes of single or few-chemoreceptor oscillations reported by Band & Wolff (1978) in the anaesthetised cat were smaller than those reported by Cross et al. (1986) and Goodman et al. (1974). Band & Wolff (1978) found the mean amplitude to be 1.13 Hz during normoxia (above 80 mmHg) and 1.28 Hz during hypoxia (below 60 mmHg). Leitner & Dejours (1968) found the amplitude of the oscillation in discharge in a anaesthetized paralysed artificially ventilated cat to be *ca.* 3 Hz.

From my experiments, there appeared to be a range in the order of 2-5 % maximal discharge or 3-7 Hz for the amplitude of the oscillation in discharge from steady state chemoreceptor responses. These values are not dissimilar to work previously reported and suggest that dynamic sensitivity to  $\text{CO}_2$  in the neonatal lamb occurs over a similar range to that found from work in the adult cat and dog.

### **7.4.9 Summary**

I have shown that there is a dynamic sensitivity to CO<sub>2</sub> in the newborn period. I have speculated that the chemoreceptor response to 1 breath alternations in CO<sub>2</sub> is a better measure of dynamic sensitivity than the response to 15 breath alternations. I have demonstrated adaptation in the chemoreceptor response to CO<sub>2</sub> in young lambs and in older lambs. These dynamic chemoreceptor responses were age and PaO<sub>2</sub> independent. My results suggest that the neonatal lamb does possess a dynamic CO<sub>2</sub> sensitivity that is similar to that previously reported in adult cats. Most significantly, my results suggest that dynamic CO<sub>2</sub> sensitivity is mature at birth, unlike hypoxia sensitivity and steady state CO<sub>2</sub> sensitivity of the carotid body.

## 8.0 FINAL DISCUSSION

### 8.1 Summary of results

In this thesis I have addressed five main questions directed at the development of respiratory control in the neonate. As I outlined in Chapter 1, the questions I posed considered the peripheral respiratory chemoreflex from two main perspectives.

#### *The respiratory chemoreflex response to hypoxia*

First, there already existed a substantial body of evidence for the carotid chemoreceptor response to hypoxia in the neonate and the postnatal increase in hypoxia sensitivity. This had been obtained from direct recordings of carotid chemoreceptor activity *in vivo*, and more recently *in vitro*. In addition, numerous ventilatory studies in the newborn infant, and in animals, strengthened these observations. They similarly showed a postnatal maturation of the respiratory chemoreflex to hypoxia. However, there was less information on the development of the chemoreflex response after the end of the first postnatal week. Williams et al. (1991) used a method that alternated inspired oxygen concentration on a single breath basis. Their observations suggested that the respiratory chemoreflex was mature at the end of the first postnatal week. Parks et al. (1991) showed in newborn infants that there was no increase in the ventilatory response to a single breath of 100% oxygen between 1 and 3 months of age when corrected for body weight. So to gain further information on the development of the respiratory chemoreflex, I measured the response of newborn infants at two postnatal ages using the method of Williams et al. (1991) (see Chapter 3).

I measured the response to the alternate breath test during the first postnatal week when mothers and newborn babies were still in hospital ( $42.5 \pm 6.8$  hrs), and again at *ca.* 6 weeks ( $46.6 \pm 3.4$  d). Ideally, I would have preferred to study infants towards the end of the first postnatal week, however I found that this was not possible because mothers were frequently discharged on the second postnatal day. My results showed that there was no increase in the hypoxic chemoreflex response between the two age groups.

Further to the method used by Williams et al. (1991), I developed a new method for detecting responders and non-responders. This involved determining a critical value for test alternations, which chemoreflex responses had to exceed to be accepted as significant. The critical value was determined from control responses, approximated to a normal distribution, at the 95th percentile. So, if test responses occurred in the top 5% of the control distribution and in addition were at least twice as great as the largest

control response for that individual, the alternation was deemed significant. A significant chemoreflex response was classified as an individual showing a significant alternation in at least one respiratory variable. I found that all infants showed a significant chemoreflex response at both postnatal ages, although there was a tendency for the magnitude of the test alternation to decrease with age for some individuals. Overall, this decrease with age was not significant for any of the respiratory variables. I concluded that most of carotid chemoreceptor hypoxia resetting occurred over the first 48hrs after birth, and that there was little change in the respiratory chemoreflex between *ca.* the first week and *ca.* the first month of age.

One weakness of this study was that there was no information available on the degree of reflex alternation expected in the adult. Other studies that had measured the degree of reflex alternation in respiratory variables, to an alternating inspired stimulus, focused on the respiratory response to CO<sub>2</sub>. Metias et al. (1981) measured the chemoreflex response to etCO<sub>2</sub> alternations in eucapnia and mild hypercapnia during hypoxia (etO<sub>2</sub> 50-60torr). They observed a reflex alternation, measured on a breath-by-breath basis, of 6.4% for V<sub>t</sub>, 4.7% for t<sub>I</sub> and 4.7% for t<sub>E</sub> when switching of the inspired stimulus occurred during inspiration. Responses were 25% greater when switching occurred at the start of expiration. Although the observations of Metias et al. (1981) provided a useful guideline, they did not allow me to judge the chemoreflex response to single breath alternations between Fio<sub>2</sub> 0.21 and 0.16.

Thus, using the same stimulus I had used in newborn infants, I measured the chemoreflex response in adults (see Chapter 5). Using mass spectrometry to sample end expiratory gases, I was able to confirm that alternations in Fio<sub>2</sub> produced alternations in etO<sub>2</sub>. This can be taken as an indication that alternations occurred in PAO<sub>2</sub> and hence in PaO<sub>2</sub>. I found that 9 of the 14 subjects showed a significant chemoreflex response, and I used the response of these 9 subjects to compare to the infants I had studied in Chapter 3. I observed a large range of control responses between adults, which may have been due to these studies being performed during wakefulness. I found that it was necessary to compare the difference between test and control alternations. This was not necessary in newborn infants when comparing between the two ages, as control alternations occurred over a much smaller range. So, control and test responses were averaged in both adults and infants, and the difference between them used as a index of the hypoxic respiratory chemoreflex response. I found that the range of responses in adults was the same for infants at *ca.* 6wks of age. Furthermore, there was no significant difference between the magnitude of the response for any respiratory variable when compared for age. This provided further evidence that the respiratory chemoreflex was mature at *ca.* 6wks in newborn infants.

To investigate the respiratory chemoreflex further in newborn infants, I was interested to compare the effects of mechanoreflex control on the respiratory response to an imposed hypoxic stimulus (see Chapter 4). There was strong evidence to suggest that the newborn (especially animals and babies), showed a potent HBIR that could be evoked during quiet sleep (Cross et al, 1960; Widdicombe, 1961; Rabbette et al, 1994). Furthermore the strength of this reflex was suggested to play an important role in the control of quiet breathing, and its strength declined over the first year of life (Rabbette et al, 1991, 1994). I had observed in previous studies, as had Williams et al. (1991), that the pattern of the chemoreflex responses, differed between infants. For some chemoreflex responses the pattern of alternation between  $V_{ti}$  and  $t_E$  indicated a role for the HBIR. An increase in  $V_{ti}$  was associated with an increase in  $t_E$  ("in phase"), but this was not the case for all infants. To determine if this pattern of chemoreflex response was in fact produced by HBIR, I used the end-inspiratory occlusion technique to measure the reflex effect on  $t_E$ . I hypothesized that those infants showing an "in phase" pattern of chemoreflex alternation would also show a large HBIR response.

I found that there was a range of mechanoreflex responses to the occlusion test, as there was a range of chemoreflex responses between infants. I did not find any evidence for the strength of the HBIR response affecting the pattern of chemoreflex alternation. Hence, I concluded that the chemoreflex response was not under mechanoreflex control. However, I correlated the strength of the HBIR with the chemoreflex response for different respiratory variables. I Found that there was a significant negative correlation between respiratory variables  $V_{ti}$ ,  $t_E$ ,  $f$ ,  $V_{ti}/t_I$ , and  $V_E$  with the HBIR response to end-inspiratory occlusion. This suggested an interaction between mechanoreflex and chemoreflex control of breathing that had not been previously reported. I have speculated that the newborn maintains a strong HBIR until respiratory chemoreflex responses to hypoxia are fully developed. However, I was unable to study infants over a wide enough range of postnatal ages to determine if this was the case. In the future, a systematic study of the two reflexes over a range of ages will determine if this idea is correct.

#### *Carotid chemoreceptor response to CO<sub>2</sub> in the newborn*

The second part of this thesis involved measuring the response to CO<sub>2</sub> at the level of the carotid body in the newborn. Whilst there was much information on the development of the chemoreceptor response to hypoxia, there was very little information on development of the chemoreceptor response to CO<sub>2</sub>. The two studies on the maturation of CO<sub>2</sub> sensitivity in the newborn kitten had not employed a range of PaO<sub>2</sub>s or a narrow range of ages (Marchal et al, 1992a; Carroll et al, 1993). Thus, I performed two series of experiments to investigate the development of the peripheral

chemoreflex response to CO<sub>2</sub> at the level of the carotid body to provide direct information on the afferent input to the brainstem.

In Chapter 6 I measured the steady state response of few and multi-fibre chemoreceptor preparations to CO<sub>2</sub>. Anaesthetised, paralysed and artificially ventilated lambs were divided into three age groups; 3-4d, 5-9d and 10-24d. Carotid chemoreceptor responses to increases in etCO<sub>2</sub> were measured at four different O<sub>2</sub> levels; 115-150mmHg (HYP), 90-105mmHg (NX), 40-60mmHg (MOD HX) and 20-35mmHg (SVHX). I found that lambs at all ages showed a CO<sub>2</sub> sensitivity, and that the chemoreceptor response to CO<sub>2</sub> was greater during MOD HX. Older lambs (5-9d and 10-24d) were unable to sustain an increase in chemoreceptor discharge during SVHX when etCO<sub>2</sub> was increased. In contrast, younger lambs showed an initial increase in discharge when etCO<sub>2</sub> increased during SVHX, but discharge plateaued and showed no further increase for an increase in etCO<sub>2</sub>. There was a significant effect of age and PaO<sub>2</sub> on chemoreceptor discharge, but only in lambs aged 10-24d was there a significant increase in CO<sub>2</sub> sensitivity at lower PaO<sub>2</sub>s. In younger lambs, increased chemoreceptor discharge at lower PaO<sub>2</sub>s was explicable in terms of an upward shift of the chemoreceptor response curve, and was not due to stimulus interaction between CO<sub>2</sub> and O<sub>2</sub>. This was a new finding, as it had been previously reported in the cat that significant CO<sub>2</sub>-O<sub>2</sub> interaction was not present until 8wks of age (Carroll et al, 1993). This provided strong evidence for the postnatal development of the steady state response to CO<sub>2</sub>, but the question of dynamic CO<sub>2</sub> sensitivity in the newborn remained unanswered.

Preliminary evidence had suggested that carotid chemoreceptors in the newborn kitten did not show a dynamic sensitivity to CO<sub>2</sub> (Marchal et al, 1992a). However in these experiments, the stimulus was not delivered sufficiently quickly to produce the characteristic overshoot and subsequent adaptation to an increase in CO<sub>2</sub>. The bicarbonate hypothesis predicts that the rapid response to CO<sub>2</sub> is independent of the oxygen level and mean CO<sub>2</sub> level (Torrance et al, 1993). So, in the newborn when hypoxia chemosensitivity is increasing with age, there should be a dynamic CO<sub>2</sub> sensitivity present that is age-independent. Blanco et al. (1984a) had shown that the fetal lamb increased chemoreceptor discharge when a bolus injection of CO<sub>2</sub>-equilibrated saline was made into the lingual artery. This qualitative evidence suggested that the fetal carotid body possessed carbonic anhydrase, rendering it capable of responding to rapid changes in Pco<sub>2</sub>. Therefore there was every reason to expect a dynamic response to CO<sub>2</sub> for the neonatal carotid body.

I designed a series of experiments to test the presence of CO<sub>2</sub> dynamic sensitivity in the newborn lamb. The method I used shared similarities with the alternating inspired

stimulus in newborn babies, i.e. CO<sub>2</sub> was added to the inspirate on a single breath basis when the lamb was ventilated at 0.3-0.5Hz, and on a 15 breath basis when the lamb was ventilated at 2.0Hz. The latter was designed to eliminate the naturally occurring oscillations in PaCO<sub>2</sub>, and hence produce more of a step change at the carotid body. Alternations were performed at two PaO<sub>2</sub> levels; 80-100mmHg (NX) and 40-60mmHg (MOD HX).

I found that the single breath alternations gave a better indication of dynamic CO<sub>2</sub> sensitivity, and that the chemoreceptor response to 15 breath alternations was blurred, and incorporated a steady state component. A dynamic chemoreceptor response to single breath alternations in CO<sub>2</sub> was present at all ages. Furthermore, the dynamic chemoreceptor response was independent of age and PaO<sub>2</sub>, unlike the steady CO<sub>2</sub> sensitivity. Thus, it appeared that the bicarbonate hypothesis was also applicable to neonates. The newborn showed a dynamic CO<sub>2</sub> sensitivity at an early age that did not increase with age.

## **8.2 Implications of my findings and future work**

Much of the work in this thesis has been dedicated to understanding the development of respiratory control in the newborn, in the hope that research in this area will highlight a possible cause for SIDS, or explain the events prior to death. In particular, I have focused on the role played by the carotid chemoreceptors in respiratory control. As it is not possible to perform invasive tests of respiratory control in newborn babies, to study certain aspects of ventilatory control in isolation, experimentation on animals is necessary. Thus, the measurement of chemoreceptor responses to CO<sub>2</sub> in newborn lambs is clinically relevant in the context of SIDS and respiratory failure.

Perhaps the most important finding of my experiments is that the newborn lamb shows a dynamic CO<sub>2</sub> sensitivity at 3-4d. I was not able to study lambs from the day of birth but I speculate that they would also show a dynamic chemoreceptor response to CO<sub>2</sub> which was independent of PaO<sub>2</sub> at this age. This provides new information on the control mechanisms that may be important in establishing and maintaining breathing from birth.

There is no doubt that the newborn is relatively insensitive to hypoxia at birth, and so it must play a relatively unimportant role in respiratory control during the first few days of life. I have shown that carotid chemoreceptor steady state CO<sub>2</sub> sensitivity also develops postnatally, which may be due to the increase in hypoxia sensitivity that

occurs at the same time (this is discussed in greater detail in section 6.4.7). Therefore, there is a need for the newborn to possess a functional mechanism from birth which regulates breathing. This mechanism must be able to increase  $V_E$  when there is an increase in metabolism, or when ventilation does not meet metabolic demands. I propose that this mechanism is the dynamic  $CO_2$  sensitivity of the carotid chemoreceptors.

The oscillations in arterial  $PaCO_2$  and pH are very important in terms of respiratory control, and these fluctuations may be more important than the mean  $PaCO_2$  for the determination of respiratory pattern. Band et al. (1978) showed that when pH was reduced by injections of  $CO_2$ -saline into the aortic root during inspiration, tidal volume was increased but mean carotid chemoreceptor discharge was unchanged. Mean chemoreceptor discharge only increased when the fall in pH produced was twice as great as the amplitude of the naturally occurring oscillations in arterial pH. Thus, relatively small changes in arterial  $CO_2$  are able to affect breathing without a change in mean chemoreceptor discharge.

Black & Torrance (1971) commented on the importance of  $PaCO_2$  oscillations determining respiratory pattern during exercise. They showed that the phase of the respiratory cycle in which the carotid chemoreceptors were excited had a marked effect on the respiratory response. Thus, small changes in respiratory frequency would alter the relationship between the oscillation in chemoreceptor discharge arriving at the respiratory centre, and affect respiratory output. Such an effect between respiratory oscillations in arterial pH and the output of phrenic motoneurons has been demonstrated in anaesthetised dogs (Cross, Grant, Guz, Jones, Semple & Stidwell, 1979). The amplitude of the  $PaCO_2$  oscillation is determined by metabolism and respiratory frequency, and during exercise it increases. Black & Torrance (1971) also comment that during exercise, the oscillation is attenuated less in reaching the carotid body due to the increase in cardiac output. Ventilation is increased during exercise to meet the increased demand for oxygen.  $CO_2$  production is also increased, however the mean level of  $PaCO_2$  does not increase. Thus the primary determinant for an increase in  $V_E$  during exercise must be the oscillations in  $PaCO_2$ . Both the amplitude, and the rate of change of the  $PaCO_2$  oscillation (i.e. the slope), is greater when metabolism is increased. During exercise the rate of change of arterial pH is increased, presumably due to an increase in  $CO_2$  production, and it at least partly accounts for the increase in  $V_E$  (Cross, Davey, Guz, Katona, MaClean, Murphy, Semple & Stidwell, 1982). The carotid chemoreceptors are able to respond to a larger  $PaCO_2$  oscillation with a larger oscillation in chemoreceptor discharge. So, the dynamic  $CO_2$  sensitivity of the carotid chemoreceptors is important for matching ventilation with metabolism. In this way, a

stimulus to increase ventilation is provided even when there is no change in mean PaCO<sub>2</sub> and chemoreceptor discharge.

Cross, Corfield, Howells, Stidwell, Newman & Semple (1990) provided further support that the oscillations in PaCO<sub>2</sub> were altered when CO<sub>2</sub> production increased, and that they provided a stimulus for increased V<sub>E</sub> when metabolism increased, e.g. during exercise. In anaesthetised cats, PaCO<sub>2</sub> oscillations were enhanced by CO<sub>2</sub> loading produced via the small intestines. CO<sub>2</sub> loading had minimal effect on mean PaCO<sub>2</sub>, but the oscillation amplitudes for both PaCO<sub>2</sub> and pH were increased, as was the slope of the pH oscillation. As predicted from the dynamic CO<sub>2</sub> sensitivity of the carotid chemoreceptors, the amplitude of the oscillation in chemoreceptor discharge was increased during CO<sub>2</sub> loading. This was accompanied by a much smaller increase in mean discharge.

After birth oscillations in PaCO<sub>2</sub> are produced for the first time. Experiments in which arterial blood was mixed in chambers to eliminate the respiratory induced oscillations in PaCO<sub>2</sub> have not been repeated in the neonate, but in the adult they demonstrate the effect of oscillations on respiration (Purves, 1966f). In anaesthetised cats, oscillations in chemoreceptor discharge were abolished when oscillations in PaO<sub>2</sub> and PaCO<sub>2</sub> were eliminated. In 18 of 37 occasions, this reduced tidal volume by 5-14% without effect on respiratory frequency; on 12 occasions both V<sub>t</sub> and *f* became irregular; and on 7 occasions respiration became periodic (Purves, 1966f). This showed the importance of respiratory related oscillations in chemoreceptor discharge in controlling respiratory pattern. Similar experiments using exteriorized carotid loops in the fetal lamb (Pagtakhan et al, 1971) and monkey (Woodrum et al. 1972) have demonstrated the importance of an increase in PaCO<sub>2</sub> and fall in PaO<sub>2</sub> in the initiation of breathing at birth. A rise in arterial CO<sub>2</sub>, in conjunction with a reduction in body surface temperature have been recognized as the most important stimuli for the onset of breathing at birth (see section 1.6.1).

ECMO provides a method whereby a constant arterial blood gas composition can be delivered to an animal, and has given further evidence that CO<sub>2</sub> is important for respiratory control. In the fetal lamb on ECMO, a reduction in PaCO<sub>2</sub> from 46 to 35mmHg reduced both the incidence and duration of FBM (Kuipers, Maertzdorf, de Jong, Hanson & Blanco, 1994). This effect persisted during low voltage ECoG, and hypocapnia did not alter the incidence of low and high voltage ECoG. Thus in the fetus hypocapnia caused FBM to remain periodic, which provides further evidence for the role of CO<sub>2</sub> in maintaining continuous breathing in the newborn. Neither does fetal breathing become continuous when the cord is clamped during ECMO and the fetus

submerged in warm saline if CO<sub>2</sub> is not allowed to increase (Blanco, 1994). In the context of respiratory control in the newborn, these findings suggest that breathing may become periodic postnatally if CO<sub>2</sub> falls, for instance if the newborn becomes hypothermic or if there is a reduction in metabolism.

Prolonged episodes of periodic breathing could have fatal consequences, as under these conditions the newborn would become hypoxic, and there is evidence to suggest in the adult that hypothermia and hypoxia interact to depress respiration (Maskrey, 1995). In adult rats a model has been developed to set core temperature ( $T_b$ ) by an intra-abdominal heat exchanger (Maskrey, 1995). During hyperthermia ( $T_b$  *ca.* 41°C) the ventilatory response to hypercapnia was enhanced, but during hypothermia ( $T_b$  *ca.* 35°C) breathing was depressed in response to hypoxia. The interaction between hypothermia and hypoxia to depress respiration also reduced, or eliminated, the ventilatory response to CO<sub>2</sub>. This could be explained by a reduction in the slope of the PaCO<sub>2</sub> oscillation during hypothermia due to a fall in metabolism.

In the newborn, respiratory chemoreflexes have been investigated during episodes of hyperthermia, as epidemiological studies have suggested a link between hyperthermia and SIDS (Wigfield, Gilbert & Fleming, 1994). Watanabe et al. (1993a) showed that the peripheral chemoreflex response to both hypoxia and CO<sub>2</sub> in kittens aged 2-7d and 28-39d was less at an ambient temperature of 30°C compared to 25°C. Poor thermoregulation may prove fatal if peripheral chemoreflexes are impaired in newborn infants. Hypoxia also appears to alter the normal febrile response to infection in the newborn, which is of particular note as there is the suggestion from the literature that some SIDS victims suffer mild infection at the time of death. Ricciuti & Fewell (1992) showed in newborn lambs that during hypoxia the rise in body core temperature and O<sub>2</sub> consumption in response to bacterial pyrogen was abolished. These observations suggest a crucial link between body temperature and respiratory control in the newborn. Other studies in newborn infants have investigated the postnatal development of thermoregulatory control, and postulated an effect of heat stress on ventilation (see below).

Measurement of rectal temperature in newborn infants has shown that the diurnal temperature rhythm seen in adults appears in infants between 8 and 16 weeks of age (Lodmore, Petersen & Wailoo, 1991; 1992). This suggests that newborn infants are unable to thermoregulate before the appearance of a diurnal temperature rhythm. The time course for the appearance of the diurnal rhythm may be related to the postnatal increase in metabolic rate, and an infant aged 3 months loses *ca.* 50% more heat per unit surface area than a neonate (Fleming, Azaz & Wigfield, 1992). Heat loss occurs

predominantly via the head in infants, so prone sleeping position is believed to impair the ability of the infant to lose heat and thermoregulate. After the appearance of a diurnal temperature rhythm, body temperature was found to be lower in infants sleeping supine compared to those sleeping prone or lateral (North, Petersen & Wailoo, 1995). This suggests a role for hyperthermia as one of the factors behind the increased incidence of SIDS associated with prone sleeping. It again points towards an intricate link between thermoregulation and metabolism in the newborn, and the importance of matching ventilation with metabolism to maintain a stable respiratory pattern.

One study in preterm infants has demonstrated the effect of warm environmental temperature on respiratory pattern (Berterottière, D'Allest, Dehan & Gaultier, 1990). Respiratory frequency and heart rate was increased in infants at an ambient temperature of 35°C ( $T_b$  ca. 37.7°C) compared to 25°C ( $T_b$  ca. 36.9°C), and the duration and incidence of periodic breathing was also increased during REM sleep. The incidence of central or obstructive apnoea was unaffected by the rise in temperature. The authors concluded that a mild increase in body temperature within the physiological range increased the instability of breathing pattern in preterm infants. Whilst no measurements of metabolic rate were measured, a decrease in CO<sub>2</sub> production at warmer temperatures would be expected. The results suggest a mismatch between the metabolic response and the control of ventilation, perhaps due to the relative insensitivity of the preterm infant to CO<sub>2</sub> (Rigatto et al, 1975b).

Given the reports that peripheral chemoreceptor denervation leads to an instability in respiratory pattern and unexpected death in the newborn (Hofer, 1984; Bureau et al, 1985b; Donnelly & Haddad, 1990), it seems probable that the oscillation in arterial CO<sub>2</sub> is the most important stimulus for the neonate to control breathing. Oscillations in arterial O<sub>2</sub> do contribute to the oscillation in chemoreceptor discharge, but play a less important role than CO<sub>2</sub> (Band et al, 1971; Goodman et al, 1974; Band et al, 1978). Until hypoxia sensitivity of the peripheral chemoreceptors has fully reset to the adult range, the newborn must rely on the information from the peripheral chemoreceptors of the oscillation in PaCO<sub>2</sub> to control respiratory pattern and meet metabolic requirements.

The relationship between metabolism and  $\dot{V}_E$ , and the role for CO<sub>2</sub> in controlling  $\dot{V}_E$ , has important implications for the role of peripheral chemoreceptor function in the aetiology of SIDS. As discussed in sections 1.6.3 and 1.6.4, both abnormalities in the brainstem (Naeye, 1976; Takashima et al, 1978; Takashima & Becker, 1985; Takashima et al, 1985; Naeye et al, 1989; Takashima et al, 1994) and carotid body (Naeye et al, 1976; Cole et al, 1979; Perrin et al, 1984) have been reported in SIDS victims.

Development of respiratory function in the newborn has predominantly focused on the response to hypoxia to provide an answer for the link between SIDS and poor respiratory control. The reason for this may be in part explained by the evidence suggesting a hypoxic episode in SIDS victims prior to death (Rognum & Saugstad, 1991).

Epidemiological studies to date have identified certain risk factors that are associated with an increased incidence of SIDS, and education and promotion of safer health care practices have been successful in reducing the incidence of SIDS in some social groups. They have not however identified any of the mechanisms involved in SIDS. Given the relatively low occurrence of SIDS (*ca.* 2 per 1000; Wigfield et al, 1994), extremely large numbers of infants are needed to establish a causal link between poor respiratory control and SIDS. Nor has it proved helpful for investigators to study respiratory chemoreflexes in near-miss SIDS (those who have suffered an apparent life threatening event, ALTE) or siblings of SIDS, for there is no guarantee that these infants are a representative sample. Clearly it is not feasible to study large enough populations of infants to identify those at risk of SIDS.

Perhaps it is now important to investigate the role of dynamic CO<sub>2</sub> sensitivity in respiratory control in the newborn infant. This may provide more insight into any link between SIDS and poor respiratory control. Assessment of respiratory chemoreflex responses to CO<sub>2</sub> in healthy newborn infants is a step in the right direction, however on the whole measurement of the dynamic ventilatory response to CO<sub>2</sub> have been unsatisfactory. There have been reports of both no change (Carroll & Bureau, 1987b; Canet et al, 1990) or an increase in dynamic CO<sub>2</sub> sensitivity postnatally (Upton et al, 1990; Wolsink et al, 1993). Using the alternating method that I have used, Watanabe et al. (1993b) showed that a reflex alternation was present to CO<sub>2</sub> when the hypoxic response was weak. Most experiments designed to measure the dynamic chemoreflex response to CO<sub>2</sub> have incorporated a steady state component in the respiratory response, or possibly influence from the central chemoreceptors. It is important that these factors are separated if the question of peripheral chemoreceptor failure is to be addressed in the context of SIDS.

Thus, I suggest the area that needs to be addressed for a possible cause in SIDS is the response to CO<sub>2</sub>. The problem could lie either at the level of the carotid chemoreceptor in detecting oscillations in arterial CO<sub>2</sub>, or in the transmission of the afferent signal to the brainstem, or in the ability of the respiratory controller to initiate an appropriate response. It would be desirable to measure simultaneously the carotid chemoreceptor response and the phrenic nerve respiratory response to an oscillation in P<sub>A</sub>CO<sub>2</sub>. If it

were possible to detect a mismatch between the carotid chemoreceptor response and the resultant respiratory response to CO<sub>2</sub> in the newborn, for instance if the oscillation in chemoreceptor discharge was enhanced but no change in respiration was observed when CO<sub>2</sub> production increased due to an increase in metabolism or infection, then it might detect one possible cause of SIDS. Obviously in the newborn infant, it is only possible to measure the respiratory response to a CO<sub>2</sub> stimulus non-invasively. Therefore, there is a need to return to the basic science and animal physiology to answer some of these questions. My experiments have shown the role for such basic science in understanding neonatal respiratory control, and my findings are clinically relevant in the context of SIDS. Further experiments may be able to elucidate if newborns fail to show a respiratory response to PaCO<sub>2</sub> oscillations when carotid chemoreceptor dynamic CO<sub>2</sub> sensitivity is present. Furthermore, there is a need to investigate the inter-relations between body temperature, V<sub>E</sub> and metabolism, so that it can be determined if factors such as hyperthermia are responsible for a mismatch between ventilation and metabolism in SIDS victims.

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**APPENDIX 1:  
INFORMATION SHEET FOR PARENTS**

**STUDIES IN THE CONTROL OF BREATHING IN NEWBORN BABIES**

- M A Hanson
- J A Spencer
- J Wyatt
- N Calder

For the past three years we have been using a test to increase our understanding of the way in which the control of breathing develops in babies. The test is safe and causes minimal disturbance to your baby.

The test is conducted after a feed and when your baby is asleep. No injections or painful procedures are involved. We would let your baby breathe normally while we record their breathing patterns. A small facemask may be used at one or two intervals for a few seconds. This is attached to some recording equipment which measures the baby's breathing whilst they are asleep.

We would also place loose-fitting cloth bands around the chest and tummy. When this has been done, soft tubes will sit just under your baby's nose and he/she will breathe air from these with every second breath containing a slightly smaller amount of oxygen. This will not harm your baby who is likely to sleep throughout the study. Every second breath may be a little larger or a little faster than the previous one, but your baby's average rate of breathing will not change because of the study.

The study takes up to 1 hour and takes place on the postnatal wards.

We stress that the study is voluntary and the treatment and care of your baby will not be affected in any way by your decision whether or not you take part in the study. You may withdraw from the study at any time.

**APPENDIX 2:  
PARENTAL CONSENT FORM**

**STUDIES IN THE CONTROL OF BREATHING IN NEWBORN BABIES**

I have read and understand the information sheet concerning this study. My questions concerning this study have been answered by

.....

I understand that at any time I may withdraw from this study without giving a reason and without affecting the normal treatment and care of my baby.

I agree that my baby should be tested in this way.

Signed.....Date.....

Investigator.....Date.....

**APPENDIX 3:  
INFORMATION SHEET FOR PARENTS**

**STUDIES IN THE CONTROL OF BREATHING IN NEWBORN BABIES**

- M A Hanson
- J A Spencer
- J Wyatt
- N Calder

For the past three years we have been using a test to increase our understanding of the way in which the control of breathing develops in babies. The test is safe and causes minimal disturbance to your baby.

The test is conducted after a feed and when your baby is asleep. No injections or painful procedures are involved. We would simply let you baby breathe through a facemask which is attached to some recording equipment. During this time we will briefly block the apparatus to measure pressures in the lungs. This rarely disturbs the baby, who is likely to sleep throughout the study.

We would also place loose-fitting cloth bands around the chest and tummy. When this has been done, soft tubes will sit just under your baby's nose and he/she will breathe air from these with every second breath containing a slightly smaller amount of oxygen. This will not harm your baby who is likely to sleep throughout the study. Every second breath may be a little larger and a little faster than the previous one, but your baby's average rate of breathing will not change because of the study.

The study takes 1-2 hours. You may also be asked if you are willing to bring your baby back in 6 weeks time.

We stress that the study is voluntary and the treatment and care of you baby will not be affected in any way by your decision whether or not you take part in the study. You may withdraw from the study at any time.

**APPENDIX 4:  
VOLUNTEER INFORMATION SHEET**

**INFORMATION SHEET FOR HEALTHY VOLUNTEERS**

**STUDIES IN THE CONTROL OF BREATHING IN ADULTS**

Prof MA Hanson  
Prof CH Rodeck  
Dr BA Waites  
Ms NA Calder

For the past three years we have been measuring the development of breathing, and the control of breathing in newborn babies. We have collected much data from newborn babies, however studies of this kind have not been performed in the adult. To allow us to compare responses between babies and adults we now need to perform the same study in the adult.

The study involves measuring the breathing response to different types of air mixtures. The air mixtures are changed on a breath-by-breath basis. The first part of the study involves breathing a normal air breath followed by an air mixture with slightly less oxygen than room air. These breath-by-breath changes in air mixtures will be repeated throughout periods of between 2 and 6 minutes. The second part of the study involves breathing a normal air breath followed by an air containing a small amount of carbon dioxide (the concentration of this is less than the carbon dioxide normally exhaled during rest). Again the changes in air mixtures are repeated for several minutes. Such techniques have been used repeatedly previously in newborn babies and there is no risk associated with this technique.

The study is performed seated and during wakefulness in our laboratory. To measure breathing loose-fitting cloth bands are placed around the chest and abdomen. A nose mask is worn on the face and loosely secured by soft straps over the top of the head. The air and air mixtures are delivered to the volunteer via the nose mask. A small monitor is placed on one finger to measure the concentration of oxygen in the blood.

The study takes 1-2hrs. During this time you will be able to read a book and/or listen to music provided to you by the investigators.

The study is voluntary and you may withdraw from the study at any time. We are not able to pay volunteers for their time.

**APPENDIX 5:**  
**VOLUNTEER CONSENT FORM**

**STUDIES IN THE CONTROL OF BREATHING IN ADULTS**

I have read and understand the information sheet concerning this study. My questions concerning this study have been answered by

.....

I understand that at any time I may withdraw from this study without giving a reason.

Signed.....Date.....

Investigator.....Date.....



## APPENDIX 7: Abstract for Physiological Society, Cork Meeting September 1995, Journal of Physiology, 489:162P.

Maturation of carotid chemoreceptor responses to CO<sub>2</sub> in the newborn lamb.

Calder NA, Kumar P\* & Hanson MA.

Dept. Obstetrics & Gynaecology, University College London, \* Dept. Physiology, University of Birmingham.

We measured the maturation of carotid chemoreceptor steady state and dynamic responses to CO<sub>2</sub> in 42 lambs anaesthetised (1.5-2.5% halothane, then a-chloralose 60-70mg/kg), tracheostomized, paralysed and artificially ventilated. The left brachial artery and femoral vein were catheterised. Anaesthesia was judged from stability of heart rate and arterial blood pressure. End tidal CO<sub>2</sub> (etCO<sub>2</sub>) steps measured by a mass spectrometer were produced in hyperoxia (HYP), normoxia (NX) and hypoxia (HX). Few or multi- carotid chemoreceptor fibres were recorded from the left carotid sinus nerve (CSN) and discharge normalised to % maximum was averaged over 20s. To measure dynamic CO<sub>2</sub> sensitivity alternations in etCO<sub>2</sub> were produced over 2-8sec in NX and HX in 15 lambs. Peak and trough values of the oscillation in CSN discharge (summed over 200msec bins) were plotted against max and min etCO<sub>2</sub> for the control and CO<sub>2</sub> loaded breaths. The dynamic response was calculated as the slope between these 2 points.

Significant steady state CO<sub>2</sub> sensitivity (by linear regression analysis  $p < 0.05$ ) was present at 3-4d in HYP and NX, and increased significantly in HX (see Table 1;  $p < 0.05$ , Mann-Whitney U test). At 5-9d CO<sub>2</sub> sensitivity was greater in HX than HYP and NX ( $p < 0.05$ ). In HYP and NX, CO<sub>2</sub> sensitivity increased with age ( $p < 0.05$ ). At 3-4d all fibres in NX (6 fibres; 5 lambs), and 5 of 6 fibres in HX (6 lambs) showed dynamic responses. These were steeper than the steady state responses as expected. For animals 10-17d, 4 of 6 fibres in NX (6 lambs) and all 8 fibres in HX (8 lambs) showed a steeper dynamic response compared to steady state. There was however variation in the size of the dynamic response between NX and HX.

Both dynamic and steady state CO<sub>2</sub> sensitivity are present in newborn lambs. Steady state sensitivity increases with age in NX and HX as the interaction between CO<sub>2</sub> and hypoxia sensitivity increases.

Supported by SPARKS and the Wellcome Trust.

Po <sub>2</sub> mmHg	HYP	NX	HX
	115-150	90-105	40-60
3-4d (16 fibres; 13 lambs)	0.43 n=2	0.24 n=8	0.73 n=10
5-9d (19 fibres; 13 lambs)	0.40 n=5	0.78 n=14	0.81 n=8
10-24d (20 fibres; 16 lambs)	0.79 n=6	1.09 n=11	1.00 n=6

Table 1. Steady state CO<sub>2</sub> chemosensitivity. Median slope (% discharge max/mmHg Pco<sub>2</sub>)

## APPENDIX 8: Abstract for Federation of European Physiological Society September 1995. Pflügers Archives 430 Suppl (4):R117.

### COMPARISON OF ADULT AND INFANT RESPIRATORY CHEMOREFLEXES.

N.Calder, B.Waites, S.J.Wong and M.Hanson

The respiratory chemoreflex response to alternate breaths of  $F_{iO_2}$  0.21 and 0.16 develops in size in the neonate (Calder et al. *Ped Res* 35(3):321-324, 1994), but whether it increases further beyond infancy is unknown. We used the technique used previously in infants to measure the response in 14 awake seated adults breathing through the nose. Respiration was measured by inductance plethysmography and calibrated using a pneumotachometer. A mass spectrometer sampled end-expiratory gases. A computer measured tidal volume ( $V_T$ ), inspiratory ( $T_I$ ) and expiratory time ( $T_E$ ), calculated frequency ( $f$ ),  $V_T/T_I$ ,  $T_I/T_{TOT}$  and  $V_T/f$  for each breath, and switched inspired gas between two lines on a breath-by-breath basis at the start of each expiration. Gas was delivered to the subject at  $25\text{ l min}^{-1}$  via a nose mask. Control runs alternated between  $F_{iO_2}$  0.21 and 0.21, and test runs between  $F_{iO_2}$  0.21 and 0.16; 3 of each were performed in each subject.

9 of the 14 adults showed a significant chemoreflex respiratory response. Their responses were not significantly greater than infant responses (Mann-Whitney U test  $P > 0.05$ ).

	$V_{ti}$	$T_I$	$T_E$	$f$	$V_{ti}/T_I$	$V_{ti} \cdot f$
INF	2.5	0.8	3.0	1.6	1.6	0.8
	-2.8,8.9	-4.3,4.9	-2.5,7.0	-3.9,4.8	-0.1,5.4	-0.8,6.6
AD	1.1	0.3	0.8	1.71	1.6	2.0
	-1.1,9.7	-1.2,13.9	-0.7,6.7	-0.8,8.2	-0.3,4.2	-1.1,4.5

Table 1. Median (range) chemoreflex responses (mean % breath-by-breath alternation) in infants and adults.

Our results suggest that the magnitude of the chemoreflex respiratory response to an alternating hypoxic stimulus is no greater in adults than in infants, however further studies in the adult during quiet sleep are needed to confirm this finding.

Supported by the MRC and Wellcome Trust. Department of Obstetrics and Gynaecology, University College London, 86-96 Chenies Mews, London WC1E 6HX, U.K.

## Absence of Ventilatory Responses to Alternating Breaths of Mild Hypoxia and Air in Infants Who Have Had Bronchopulmonary Dysplasia: Implications for the Risk of Sudden Infant Death

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**ABSTRACT.** Infants who have had bronchopulmonary dysplasia (BPD) are at an increased risk of sudden infant death syndrome. Because failure of the cardiorespiratory response to hypoxia is suggested to play a key role in sudden infant death syndrome, we tested the hypothesis that infants who have had BPD have a reduced respiratory chemoreflex response to hypoxia. We examined the reflex respiratory responses to breath-by-breath alternations in fractional inspired oxygen concentration in eight infants who had had BPD (mean gestation = 27 wk, mean postnatal age = 93 d) who were no longer on supplemental oxygen and compared the responses with those of 12 preterm infants who had not required supplemental oxygen or been mechanically ventilated since birth (mean gestation = 30 wk, mean postnatal age = 38 d). For test runs we alternated fractional inspired oxygen concentration through two gas delivery lines between 0.21 and 0.16 on a breath-by-breath basis, and for control runs we alternated the inspire between the two gas lines with a fractional inspired oxygen concentration of 0.21 in each. Respiration was measured using inductance plethysmography. Infants with BPD showed no significant differences between test and control responses for any respiratory variable. In contrast, all respiratory variables in the preterm infants showed test responses significantly greater than control. We speculate that the "blunted" chemoreflex respiratory response seen in infants with BPD may predispose them to subsequent respiratory failure, but we do not know which component of the chemoreflex is impaired. (*Pediatr Res* 35: 677-681, 1994)

### Abbreviations

BPD, bronchopulmonary dysplasia  
 FIO<sub>2</sub>, fractional inspired oxygen concentration  
 SIDS, sudden infant death syndrome  
 PaO<sub>2</sub>, arterial oxygen partial pressure  
 PaCO<sub>2</sub>, arterial carbon dioxide partial pressure  
 V<sub>T</sub>, tidal volume

T<sub>I</sub>, inspiratory time  
 T<sub>E</sub>, expiratory time

Infants discharged from neonatal intensive care units have an increased risk of postneonatal mortality. BPD is an additional complication in those infants who have been diagnosed with respiratory distress syndrome, and infants who have had BPD are a group in which the incidence of SIDS is reported to be as much as 7 times higher than the population as a whole (1). The reason for this increased risk of SIDS is not known.

Infants with BPD are burdened with decreased dynamic compliance, increased airway resistance, increased functional residual capacity, and airway hyperreactivity for some time after their discharge from intensive care (2, 3). Although considerable, these factors alone may not be sufficient to prevent a ventilatory response to changes in blood gases occurring during respiratory failure, and so it seems possible that infants with BPD may also suffer from a reduced gain of their respiratory chemoreflexes.

It was our hypothesis that infants with BPD are unable to respond to hypoxic conditions because of impaired chemoreflex control. A failure in chemoreflex respiratory control and the response to hypoxia has been suggested for many years to play a role in SIDS (4). We felt that a weak response of the infant with BPD to a mild hypoxic challenge possibly could explain the link between infants with BPD and the high incidence of SIDS.

Infants dying of SIDS must fail to mount an appropriate arousal response to acute hypoxia and hypercapnia experienced before death, regardless of the precipitating factors causing cardiorespiratory deterioration. In newborn lambs, cutting the carotid sinus nerves markedly reduces the arousal response to hypercapnia and hypoxia (5, 6), confirming the role of the carotid chemoreceptors or carotid baroreceptors in initiating the response. Arousal responses in newborn infants occur with low concentrations of steady state fractional inspired carbon dioxide (7), i.e. 0.04, although term and preterm infants tolerate concentrations of 0.05-0.07 fractional inspired carbon dioxide during normoxic rebreathing (8). An FIO<sub>2</sub> of 0.15 elicited arousal responses in 14 of 22 normal infants compared with only one of 11 who had experienced an apparent life-threatening event (9), and more pronounced hypoxia (FIO<sub>2</sub> 0.11) caused nine of nine control infants to arouse in contrast to only 19 of 50 infants who experienced an apparent life-threatening event (10). Arousal responses to an FIO<sub>2</sub> of 0.11 were recorded in 11 of 12 infants with BPD (11); however, they experienced prolonged apnea and

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bradycardia after arousal, with four requiring assisted ventilation to restore spontaneous breathing.

With increasing  $\text{PaCO}_2$  and declining  $\text{PaO}_2$  levels occurring terminally in the infant who dies of SIDS, the question remains as to why no appropriate arousal response occurs. The failure to respond may lie at the level of the arterial chemoreceptor if previous hypoxia and low oxygen saturation have disrupted normal chemoreceptor function, *e.g.* by reducing or delaying the resetting of chemosensitivity that normally occurs postnatally (12–16). It is also possible that the defect lies in the CNS and that an increased chemoreceptor afferent input fails to elicit an adequate increase in respiratory output or to produce arousal.

We have developed a noninvasive test of chemoreflex sensitivity, the alternate breath test, that delivers a rapidly alternating stimulus to the peripheral chemoreceptors in the arterial blood and have used it to measure peripheral chemoreflex sensitivity in healthy term infants, kittens, and lambs. The respiratory response to the alternate breath test is mediated predominantly by the carotid chemoreceptors (13). It is capable of detecting the naturally occurring increase in carotid chemoreceptor sensitivity to hypoxia with increasing postnatal age in kittens (12) and babies (14). In this study, we used the alternate breath test to measure the sensitivity of respiratory chemoreflexes in BPD and preterm control infants.

#### SUBJECTS AND METHODS

Infants were recruited from the neonatal units at University College Hospital, London, and The Royal Berkshire Hospital, Reading. Local ethical committee approval and written parental consent were obtained. Infants were well at the time of the study, were not receiving supplemental oxygen, had oxygen saturations greater than 90% when breathing air, and were due to be discharged from the unit within 1 wk.

Infants with BPD were defined as preterm (gestation <36 wk), having been mechanically ventilated for at least 7 d, and requiring a minimum of 28 d in supplemental oxygen for the treatment of BPD. They had radiographic findings consistent with chronic lung disease. Control infants were preterm but had not required mechanical ventilation or supplemental oxygen after birth.

The infants were studied during quiet sleep as judged behaviorally (17) and 1–2 h after a feeding. Tests were performed in the neonatal unit at an ambient temperature of 23–25°C. Most infants were wearing a one-piece toweling outfit at the time of study and were covered with a single blanket.

The method used has been previously described by Williams *et al.* (14). The experimental setup is shown in Figure 1. Infants were studied in the lateral or supine position. Breathing was measured by inductance plethysmography (Respirace Corp., Ardsley, NY) calibrated by the least squares graphical technique described by Sackner *et al.* (18) to derive scaling factors for the rib cage and abdominal signals. The Respirace plethysmograph was adjusted accordingly. The  $V_T$  signal derived by summation of the rib cage and abdominal signals was passed on-line from the Respirace to a BBC Master 128 Acorn microcomputer (British Broadcasting Corp., Cambridge, UK) and digitized at 100 Hz for off-line analysis. Inspired gas was humidified and supplied to the infant at a rate in excess of minute ventilation, *i.e.* at 2.0–2.5 L/min, via a nasal catheter (no. 1615, Salter Labs, Arvin, CA) attached via a Y-connector to two gas delivery lines. The flow of gas in each delivery line was set using rotameters connected to cylinders of medical grade air ( $\text{FIO}_2$  0.21) and gas with an  $\text{FIO}_2$  of 0.16, with the balance being nitrogen (precalibrated; British Oxygen Co. special gases). Delivery of inspired gas through a pair of three-way solenoid-operated valves was controlled by the computer, which sent a 5-V signal to the solenoid control box at the start of each expiration and switched the gas between an open port to the infant and a diverted port to the atmosphere. During test runs, breath-by-breath alternations of air and an  $\text{FIO}_2$  of 0.16 were delivered for up to 100 breaths. The endpoint occurred prematurely to this if the infant sighed or if a regular breathing pattern was disrupted by the

infant's movements. During control runs, air was delivered in both gas lines. A minimum of two test and two control runs was necessary for the data to be included in the analysis. Oxygen saturation was measured throughout the procedure by a pulse oximeter operating in the beat-to-beat mode (Nellcor N200, Nellcor Inc., Hayward, CA) and never fell below 90%. The saturation monitor was used as safety device and a measure of patient well-being, inasmuch as it was not capable of responding to the very small changes in oxygen saturation produced by breath-by-breath changes in  $\text{FIO}_2$ .

The method of data analysis has been previously described by Williams *et al.* (14). For each breath, the  $V_T$ ,  $T_I$  (s), and  $T_E$  (s) were found and from these were calculated respiratory frequency ( $f$ ) [ $f = 60/(T_I + T_E)$  in breaths/min], mean inspiratory flow ( $V_T/T_I$ ), respiratory timing [ $T_I/(T_I + T_E)$ ], and ventilation ( $V_T \cdot f$ ). For each respiratory variable, the difference between a pair of consecutive breaths was expressed as a percentage of the average of the two breaths. Each breath was compared with the immediately preceding breath, and the percentage alternation was plotted cumulatively with respect to breath number, reversing the sign (+ or -) for every second pair of breaths. Thus, a regular alternation produced a consistent deviation from the baseline. A slope was fitted to the line by regression analysis, and its magnitude indicated the mean breath-by-breath percentage alternation. The magnitudes of the slopes for test and control runs were compared by using the Mann-Whitney U test within each of the study groups. Baseline measurements of frequency,  $T_I$ ,  $T_E$ , and timing were compared between preterm control and infants with BPD using the Mann-Whitney U test. Values were considered significant when  $p$  was <0.05.

#### RESULTS

Patient data are given in Table 1. BPD infant 3 was the same as BPD infant 1 but was studied when older. The exclusion of this repeat study at a greater postnatal age would not change the statistical significance of the results, so it was included in the analysis. The gestational and postnatal ages and the birth weights of the infants with BPD were significantly different from preterm controls using the Mann-Whitney U test.

Baseline measurements of frequency,  $T_I$ ,  $T_E$ , and timing are given as means in Table 2. There was no statistically significant difference in baseline values between the preterm control and BPD groups for the four variables using the Mann-Whitney U test.

Figure 2 is an example of the cumulative alternate breath plot and illustrates responses of a preterm control infant (*top*) and an infant recovered from BPD (*bottom*). The control responses for each of the infants are similar. However, the alternations exhibited for  $T_E$ , frequency,  $V_T$ , and ventilation in the test response of the preterm control infant are not seen in the test response of the infant with BPD.

Figure 3 illustrates the mean control and test responses for each of the seven respiratory variables measured in 12 control infants (from 31 runs) and eight infants with BPD, one of whom was studied at two different postnatal ages (from 21 runs). Infants with BPD showed no significant difference between test and control runs for any of the respiratory variables. In contrast, preterm control infants showed test responses that were significantly different from control responses for  $V_T$ ,  $T_I$ ,  $T_E$ , and ventilation.

#### DISCUSSION

Our results demonstrate a markedly reduced response of infants with BPD to the alternate breath test compared with preterm infants who have not had BPD, even though the infants with BPD were no longer oxygen dependent at the time of the study and were well oxygenated in air.

The infants with BPD were significantly younger by gestational age and significantly older postnatally than the preterm infants.

ABSENT RESPIRATORY CHEMOREFLEXES IN INFANTS WITH BPD

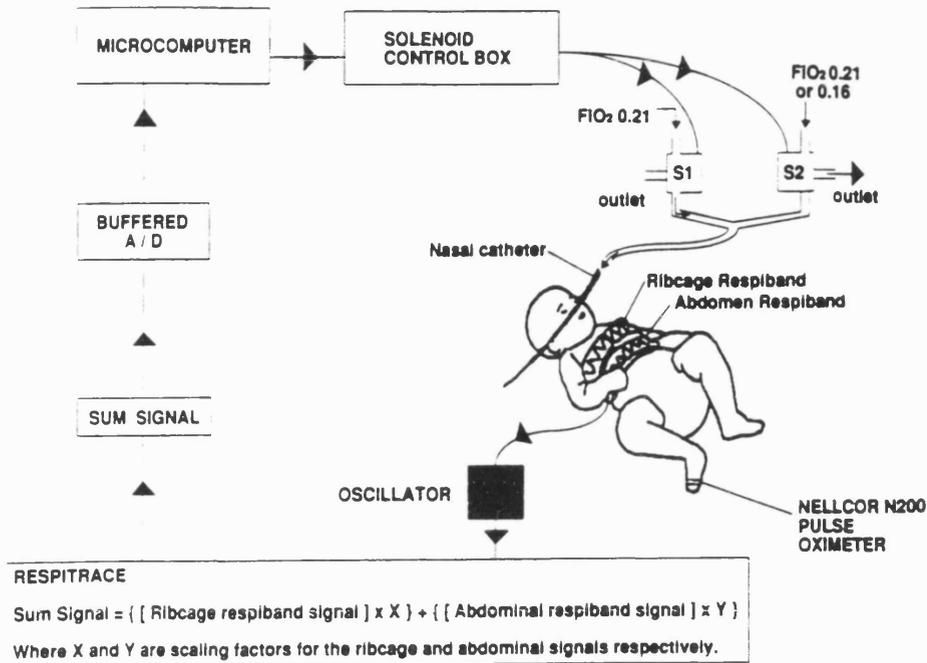


Fig. 1. The experimental setup used for controlling the supply of inspired gas and for measuring and recording the response to alternate breaths of air and test gas (FIO<sub>2</sub> of 0.16). Gas with an FIO<sub>2</sub> of 0.21 is always delivered through solenoid 1 (S1), and a gas with an FIO<sub>2</sub> of either 0.21 or 0.16 is delivered through solenoid 2 (S2).

Table 1. Patient information

Patient no.	Control			BPD		
	Gestation (wk)	Birth weight (g)	Postnatal age (d)	Gestation (wk)	Birth weight (g)	Postnatal age (d)
1	34	1930	13	29	1310	49
2	30	1730	28	25	825	121
3	36	2550	13	29	1310	62
4	28	1700	44	25	760	112
5	28	1240	48	25	960	98
6	31	1630	19	32	1560	42
7	31	1540	19	28	1158	85
8	28	70	42	24	555	183
9	28	70	90	27	957	84
10	29	1540	42			
11	29	1486	63			
12	31	1347	35			
Mean ± SEM	30 ± 0.7	1576 ± 114	38 ± 6	27 ± 0.9	1044 ± 106	93 ± 14

Table 2. Baseline values for respiratory variables (mean ± SEM)

	Frequency (breaths/min)	Timing (T <sub>I</sub> /T <sub>tot</sub> )*	T <sub>I</sub> (s)	T <sub>E</sub> (s)
Control	55.8 ± 3.4	0.38 ± 0.01	0.42 ± 0.02	0.83 ± 0.10
BPD	61.3 ± 3.1	0.40 ± 0.02	0.40 ± 0.02	0.64 ± 0.06

\* T<sub>tot</sub>, total time.

However, in animals and normal infants, greater postnatal age confers an increased chemoreflex (11, 13, 19), so on the basis of age alone the infants with BPD should have exhibited a strong chemoreflex. We found in full-term infants studied in the first few postnatal days (approximately 48 h after birth) and again at 6 wk that no further maturation of the chemoreflex respiratory response to hypoxia occurred during this time (20). Thus, resetting of chemoreceptor hypoxic setting occurs rapidly. Despite the fact that infants with BPD were older postnatally, the difference in the chemoreflex respiratory responses from healthy pre-

term babies cannot be explained by the difference in postnatal ages.

We used a hypoxic stimulus in preference to a hyperoxic stimulus to challenge oxygen chemosensitivity for two reasons. First, a hypoxic stimulus moves up the steeper portion of the arterial chemoreceptor response curve, whereas hyperoxia moves along the flatter portion of the hyperbola. Absent or weak responses to hyperoxia could be equally due to the natural shape of the response curve as to some pathologic process, thus diminishing the value of the results. Second, it is against hypoxia that the infant may have to defend itself, so this is the response that we wanted to assess.

The poor response illustrated by the infants with BPD may indicate impairment at several levels, namely in the transmission of the stimulus from the inspired gas to arterial blood, in peripheral chemosensitivity, in central respiratory control, or in pulmonary mechanics. These will be discussed in turn below.

Histologic examination of the lungs of infants with BPD at autopsy indicates somatic growth retardation, reduced lung volume and abnormal lobar volume proportions, decreased alveolar

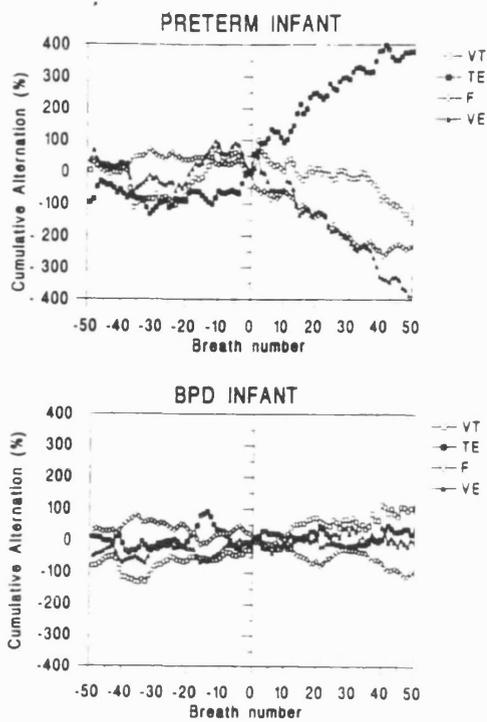


Fig. 2. An example of the cumulative alternate breath plot for a preterm control infant (top) and an infant who had recovered from BPD (bottom). Control responses are plotted to the left and test responses to the right for 50 breaths each. Filled squares represent  $T_E$ , open squares represent  $V_T$ , open diamonds represent frequency, and filled triangles represent instantaneous ventilation. A slope was fitted to each of the cumulative responses by regression analysis to derive the mean breath-by-breath percentage alternation.

number and alveolar hypoplasia, reduced internal surface area, and bronchial and bronchiolar smooth muscle hypertrophy (21). Increased wall thickness, increased muscularization of peripheral arteries, and reduced cross-sectional perfusion area occurred in infants with BPD who had survived past 1 mo of age (22). These factors may reduce the amplitude of the oscillations in  $P_{aO_2}$  that

occur when  $F_{IO_2}$  is made to alternate (23, 24). The smaller oscillations in  $P_{aO_2}$  would correspondingly lead to smaller oscillations in chemoreceptor discharge, and hence the alternation in respiratory variables produced will be expected to be less. This seems unlikely because the infants were no longer oxygen dependent. However, inasmuch as the infants were all due for discharge, none had indwelling arterial lines, so it was not possible to obtain baseline values for  $P_{aO_2}$  or  $P_{aCO_2}$ . In the absence of an indwelling rapidly responding  $P_{aO_2}$  electrode, it was not possible to evaluate the extent to which a reduction in the stimulus accounted for our results. It was not possible to use a transcutaneous  $PO_2$  electrode to quantify the stimulus either, because the intrinsic stabilizing time of the electrode prevents it from responding to a rapidly alternating  $P_{aO_2}$ . Similarly, the small fluctuations in oxygen saturation produced by the test were not detected above random fluctuations by our monitor operating in the beat-to-beat mode. It will be important in future studies to determine baseline oxygenation using, for example, transcutaneous  $PO_2$  measurement. Baseline  $PO_2$  may be important in determining the development of several aspects of respiratory control.

Previous use of the alternate breath test in newborn infants has shown the respiratory response to alternations in  $F_{IO_2}$  to increase in the first week of life, both in terms of the number of variables showing a response and in the magnitude of the response (14). This time course follows the maturation of respiratory chemoreflexes measured in the kitten and lamb (12, 13, 19). Carotid sinus denervation in newborn lambs abolishes the ventilatory response to alternations in  $F_{IO_2}$  and confirms that the carotid chemoreceptors elicit the response (13). Chronically hypoxic kittens exhibit delayed resetting of chemoreceptor hypoxia sensitivity and a blunted chemoreflex response (12). Thus, the blunted response seen in infants with BPD may be caused by chronic hypoxia, inasmuch as infants with BPD are said to be borderline hypoxemic (25). In the absence of the increase in  $P_{aO_2}$  that normally occurs after birth, the time for peripheral chemoreceptor resetting and hence for maturation of respiratory chemoreflexes may be considerably delayed in newborn infants.

Infants recovering from respiratory distress syndrome have lower dynamic compliance than normal preterm infants (3) and increased baseline airway resistance compared with expected control values (2). Thus, a given stimulus may be equally rec-

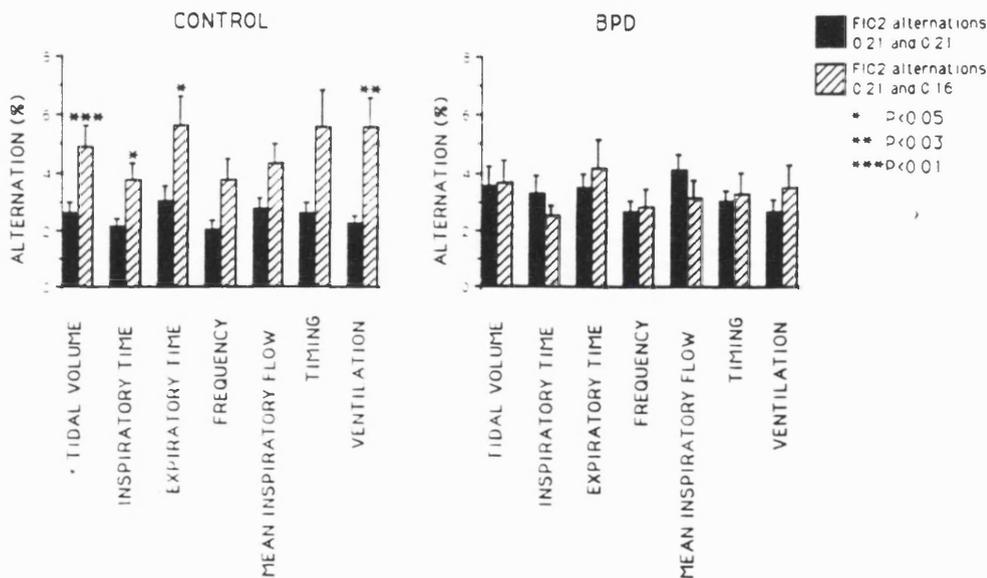


Fig. 3. The mean percentage breath-by-breath alternation for preterm control infants ( $n = 31$  runs from 12 infants) and infants with BPD ( $n = 21$  runs from eight infants). Mean responses  $\pm$  SEM are shown during control runs (solid bars) and test runs (hatched bars) for  $V_T$ ,  $T_I$ ,  $T_E$ , breath frequency, mean inspiratory flow, timing, and ventilation. Asterisks show significant differences between control and test runs: \*,  $p < 0.05$ . \*\*,  $p < 0.03$ . \*\*\*,  $p < 0.01$  by Mann-Whitney U test.

ognized by chemoreceptors in infants with BPD and preterm infants, yet the ventilatory response may be different because of impaired pulmonary mechanics. However, because the magnitude of the mean breath-by-breath response, even in preterm infants, is only approximately 10%, and because the alternation does not take  $V_T$  or respiratory timing outside their normal resting values, it seems unlikely that impaired pulmonary mechanics can account for the lack of a response in infants with BPD. Because pulmonary mechanical function was not measured in the infants with BPD, we do not know the peak inspiratory flow rates achieved to maintain adequate ventilation. If peak inspiratory flow was greater than 2.5 L/min in the infants with BPD, then room air would also be inspired with the hypoxic gas mixture and the size of the stimulus would be subsequently reduced.

Sekar and Duke (26) reported that infants with BPD had lower oxygen saturations and a higher incidence of central apnea at the time of discharge than did healthy preterm infants, although the incidence of obstructive apnea or episodes of periodic breathing did not differ. Subsequent oxygen administration to increase saturation decreased the occurrence of central apnea and periodic breathing in infants with BPD. This evidence suggests that poor respiratory drive is responsible for the origin of apnea and periodic breathing. Our finding that respiratory chemoreflexes are reduced in such infants supports this idea, although it does not allow us to discriminate between two possibilities: 1) that chemoreceptor input to the respiratory controller is reduced and 2) that the sensitivity of the respiratory controller to an adequate stimulus is reduced. Either way, the finding that infants with BPD have reduced chemoreceptor gain may have important consequences to those infants with BPD who die of SIDS.

Compromised infants with BPD, and to a lesser extent preterm infants, do in fact recover in the subsequent years. Mallory *et al.* (27) reported an improvement in forced vital capacity to normal levels by 36 mo in infants with moderate BPD, with little improvement seen in lower airway obstruction and airway hyperreactivity. Other longitudinal studies reported normal total lung capacity and functional residual capacity in infants aged 7 and 10 y, with elevated mean residual volume at both ages (28). An improvement was also seen in the forced expiratory volume during 1 s from y 7 to 10. There is a need to perform longitudinal studies of the alternate breath test in infants who previously had BPD to determine whether the recovery seen in lung function measurements is reflected in improvements in respiratory chemoreflex sensitivity.

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## The Respiratory Response of Healthy Term Infants to Breath-by-Breath Alternations in Inspired Oxygen at Two Postnatal Ages

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**ABSTRACT.** We have studied the reflex respiratory responses to breath-by-breath alternations in fractional inspired oxygen in a group of healthy term infants at two ages,  $43 \pm 7$  h (study 1) and  $47 \pm 3$  d (study 2). Respiration was measured noninvasively using inductance plethysmography. Responses to alternations of fractional inspired oxygen between 0.16 and 0.21 (test runs) were compared with responses to alternating the inspired gas between two lines each containing a fractional inspired oxygen concentration of 0.21 (control runs). The respiratory response was measured as the mean percentage breath-by-breath alternation for inspiratory tidal volume ( $V_{Ti}$ ), expiratory tidal volume ( $V_{TE}$ ), inspiratory time ( $T_i$ ), expiratory time ( $T_E$ ), frequency ( $f$ ), mean inspiratory flow ( $V_{Ti}/T_i$ ), mean expiratory flow ( $V_{TE}/T_E$ ), timing ( $T_i \cdot f$ ), and ventilation. A significant chemoreflex response was present in the infants at the time of study 1, as shown by test runs that were significantly different from control for  $T_i$ ,  $T_E$ ,  $f$ , mean inspiratory flow, mean expiratory flow, timing, and ventilation ( $p < 0.05$ ), and at study 2 for  $V_{Ti}$ ,  $V_{TE}$ ,  $T_E$ ,  $f$ , mean inspiratory flow, mean expiratory flow, timing, and ventilation ( $p < 0.05$ ). When control and test runs were compared separately with respect to age, there were no significant differences for any respiratory variable between study 1 and study 2. Thus, we did not observe significant maturation of respiratory chemoreflex responses to hypoxia after an age at which we could detect an established response, and this suggests that the "resetting" of chemoreceptor responses to hypoxia is essentially complete within approximately 24–48 h of birth in humans. (*Pediatr Res* 35: 321–324, 1994)

### Abbreviations

$FIO_2$ , fractional inspired oxygen concentration  
 $V_{Ti}$ , inspiratory tidal volume  
 $V_{TE}$ , expiratory tidal volume  
 $T_i$ , inspiratory time  
 $T_E$ , expiratory time  
 $f$ , frequency  
 $V_E$ , ventilation  
 SIDS, sudden infant death syndrome

Direct evidence for the process of chemoreceptor "resetting" has been obtained in two species. Blanco *et al.* (1) showed in the fetal lamb that the arterial chemoreceptors are active *in utero* but virtually silenced when arterial  $PO_2$  rises at birth. Resetting of carotid chemoreceptor hypoxia sensitivity occurs in lambs over the next 2–3 d. Similar resetting of aortic chemoreceptor hypoxic sensitivity has been observed in the lamb (2). Marchal *et al.* (3) recorded single carotid chemoreceptor responses to hypoxia in kittens and found that responses were lower in kittens aged less than 10 d than in older kittens. Moreover, the  $PO_2$  response curve of the younger kittens was displaced to the left of the older kittens' response curve.

Support for this concept of resetting of chemosensitivity to hypoxia has been gained from respiratory responses measured noninvasively in the kitten, lamb, and human infant (4). We developed a test of peripheral chemosensitivity, the alternate breath test, that allows the inspired gas to alternate between two mixtures on a breath-by-breath basis. When the chemoreflex respiratory responses to the test were measured in the kitten and lamb (5, 6), we found that the time course of maturation was appropriate for the resetting of peripheral chemosensitivity as determined by the direct recordings from the carotid sinus or aortic nerve (1, 2). We then showed in healthy newborn infants that the respiratory response to breath-by-breath changes in  $FIO_2$  similarly increased over the first 8 postnatal days (7). There was also the suggestion from this work that the most dramatic change occurred over the first postnatal day, with more subtle changes occurring up to the end of the first week. However, the extent to which maturation continued after the most prominent increase in chemoreflex sensitivity was unclear from this work, and because it was a cross-sectional study, intersubject variation may have blurred the results.

We conducted a longitudinal study of the responses of human infants to breath-by-breath alternations in  $FIO_2$  during the first few days after birth and again at approximately 7 wk of age to ascertain whether there was any change in their peripheral chemoreflex sensitivity to hypoxia over this period.

### SUBJECTS AND METHODS

Healthy newborn infants were recruited from the postnatal wards at University College Hospital, London, and The Royal Berkshire Hospital, Reading. Local ethical committee approval and written parental consent were obtained. Infants delivered either vaginally or by cesarean section were studied, inasmuch as it has been shown previously that there is no significant difference in response between the two groups (7). The initial studies were performed on the postnatal wards before mothers and babies were discharged. The study was repeated in the antenatal clinic when mothers and babies returned for their 6-wk postnatal checkup for infants delivered by cesarean section.

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For infants delivered vaginally, the study was repeated at approximately 6 wk in a room on the labor ward.

The infants were settled after being fed, and recording began when they were in quiet sleep as judged behaviorally (8). Ambient temperature ranged between 23° and 28°C on the postnatal wards and 23° and 25°C when the studies were repeated. Infants were wearing a one-piece towelling outfit at the time of study and were covered with a single blanket. They were studied in the lateral or supine position (7). Breathing was measured by inductance plethysmography (Respirace Corp., Ardsley, NY) calibrated by the method of Sackner *et al.* (9) to derive scaling factors for the ribcage and abdominal signals, the Respirace being adjusted accordingly. The "tidal volume" signal derived by summation of the ribcage and abdominal signals was passed on-line to a BBC Master 128 Acorn microcomputer (British Broadcasting Corp., Cambridge, UK) and digitized at 100 Hz for off-line analysis. Inspired gas was humidified and supplied to the infant at a rate in excess of minute ventilation, *i.e.* at 2.0–2.5 L/min, via a nasal catheter (no. 1615, Salter Labs, Arvin, CA) attached via a Y-connector to two gas delivery lines. The composition of gas in each delivery line was set using rotameters connected to cylinders of medical grade air (FIO<sub>2</sub> 0.21) and gas with an FIO<sub>2</sub> of 0.16 and the balance N<sub>2</sub> (precalibrated; British Oxygen Company special gases). Delivery of inspired gas through a pair of three-way solenoid-operated valves was controlled by the computer, which switched between them at the start of each expiration. During test runs, breath-by-breath alternations of air and an FIO<sub>2</sub> of 0.16 were delivered for up to 100 breaths. During control runs, air was delivered in both gas delivery lines. A minimum of two test and two control runs was necessary for the data to be included in the analysis. Oxygen saturation was monitored throughout the procedure by a pulse oximeter operating in the beat-to-beat mode (Nellcor N200, Nellcor Inc., Hayward, CA). Saturation was not recorded but was used as a visual safety check. It never fell below 92%.

**Data analysis.** Data was analyzed as previously described by Williams *et al.* (7). For each breath, the tidal volume ( $V_{TI}$  and  $V_{TE}$ ), inspiratory time ( $T_I$ , s), and expiratory time ( $T_E$ , s) were found and from these were calculated respiratory frequency [ $f = 60/(T_I + T_E)$ , breaths/min], mean inspiratory flow ( $V_{TI}/T_I$ ), mean expiratory flow ( $V_{TE}/T_E$ ), respiratory timing [ $T_I/(T_I + T_E)$ ], and ventilation ( $V_E = \text{tidal volume} \cdot f$ ). For each respiratory variable, the percentage alternation (*i.e.* the difference between a pair of consecutive breaths expressed as a percentage of the mean of the two breaths) was plotted cumulatively with respect to breath number, reversing the sign (+ or -) for every second difference, so that a regular alternation produced a consistent deviation from the baseline. A slope was fitted to the line by regression analysis, the magnitude of which indicated the mean breath-by-breath percentage alternation. The absolute values of the slopes for control and test runs were compared separately between age groups and analyzed by using a Wilcoxon rank sum test. Test runs were also compared with control runs within each of the study age groups with a Wilcoxon rank sum test, using the absolute values of the slopes. In addition, test runs were compared with control runs taking into consideration the sign of the slope. Positive and negative slopes were analyzed separately, and test runs were compared with control runs by paired *t* test. Values were considered significant when *p* was < 0.05.

Each infant had the responses from either two or three control and test runs analyzed. These responses were not averaged for each infant because a particular run may exhibit a stronger response for one variable than in a previous run, and inasmuch as each variable was analyzed separately, we did not wish to reduce the variation within each infant.

## RESULTS

A total of 33 infants were studied in the first few postnatal days of life. Of these, only 13 repeat studies were completed successfully. The most common reason for failure was the inability

to achieve a quiet sleep state in these infants. In addition, some infants were excluded from analysis if the minimum of two test and two control runs were not recorded, and some mothers failed to keep their appointments for repeat studies.

Patient data are given in Table 1. The mean postnatal age of the infants for study 1 was  $43 \pm 7$  h and for study 2 it was  $47 \pm 3$  d. Mean values of  $T_I$ ,  $T_E$ ,  $f$ , and timing during control and test runs are given in Table 2. No significant differences were found in these variables between control and test runs, at either age, or between ages for either control or test runs. There was a small increase in  $T_I$  and  $T_E$  and a small decrease in  $f$  with age for control runs, although this was not significant.

The mean percentage alternations for control and test runs for the two age groups are shown in Figure 1. When control runs were compared with test runs within each age group, all variables exhibited test responses significantly different from control, with the exception of  $V_{TI}$  and  $V_{TE}$  for study 1 and  $T_I$  for study 2. However, when analysis took into account the sign of the slope, and positive and negative values were considered separately, test responses were significantly greater than control for positive values of  $V_{TI}$  and negative values of  $V_{TE}$  for study 1. This method of analysis did not detect a significant difference between test and control for  $T_I$  for study 2. These findings were consistent with previous results.

When control and test runs were compared separately between the two age groups, no significant difference was detected for any of the respiratory variables.

## DISCUSSION

The alternate breath test provides a measure of respiratory peripheral chemoreflex sensitivity. We have previously shown that section of the carotid sinus nerves in newborn lambs within 36 h of birth abolished the response to the test measured when they were 5–6 d old, and the response was still greatly reduced at 10–11 d compared with sham-operated lambs (6). This shows that the carotid chemoreceptors are foremost in producing the

Table 1. Patient information

No.	Sex	Study 1 (h)	Study 2 (d)	Gestation (wk)	Birth weight (g)
1	F	72	31	38	3140
2	M	24	62	38	2900
3	M	48	65	39	3060
4	F	27	43	37	3520
5	M	32	43	38	3100
6	M	54	44	38	3780
7	F	40	59	40	2600
8	M	70	48	42	3860
9	F	15	51	42	3720
10	M	96	44	38	3140
11	F	32	54	42	3220
12	F	17	20	42	3420
13	M	26	42	42	4340
Mean $\pm$ SEM		$43 \pm 7$	$47 \pm 3$	$40 \pm 1$	$3369 \pm 129$

Table 2. Baseline values for respiratory variables (mean  $\pm$  SEM)

	Inspiratory time (s)	Expiratory time (s)	Frequency (breaths/min)	Timing ( $T_I/\text{total time}$ )
Study 1				
Control	$0.50 \pm 0.02$	$0.77 \pm 0.06$	$52.0 \pm 3.0$	$0.41 \pm 0.01$
Test	$0.52 \pm 0.02$	$0.83 \pm 0.07$	$49.8 \pm 3.0$	$0.41 \pm 0.01$
Study 2				
Control	$0.54 \pm 0.02$	$0.79 \pm 0.04$	$48.0 \pm 2.4$	$0.42 \pm 0.01$
Test	$0.53 \pm 0.02$	$0.75 \pm 0.03$	$49.2 \pm 1.8$	$0.42 \pm 0.01$

RESPIRATORY CHEMOREFLEXES IN INFANTS

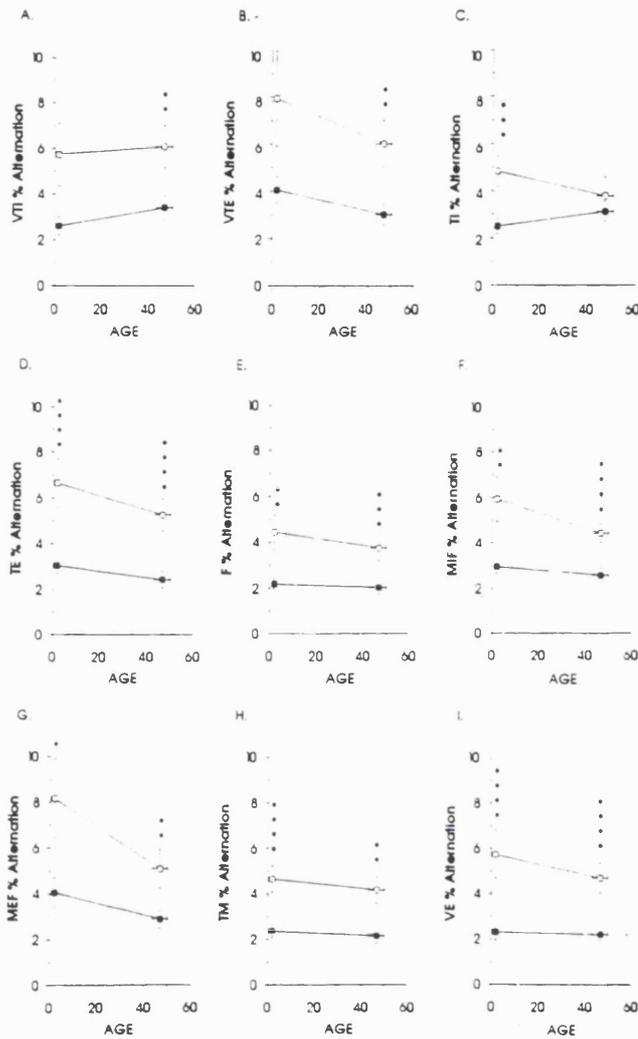


Fig. 1. Mean percentage breath-by-breath alternation between  $FIO_2$  0.21 and 0.21 (control), or 0.21 and 0.16 (test) at two separate postnatal ages. Filled squares are control runs and open squares are test runs. A.  $V_{Ti}$ ; B.  $V_{TE}$ ; C.  $T_i$ ; D.  $T_E$ ; E.  $f$ ; F. MIF, mean inspiratory flow; G. MEF, mean expiratory flow; H. TM, timing; I.  $V_E$ . Asterisks show significant differences between control and test runs: \*,  $p < 0.05$ ; \*\*,  $p < 0.025$ ; \*\*\*,  $p < 0.01$ ; and \*\*\*\*,  $p < 0.005$  by Wilcoxon rank sum test.

response. Moreover, the test detects the postnatal maturation of respiratory chemoreflexes in the kitten and lamb, species in which the neonatal increase in chemoreceptor hypoxia sensitivity has been established by direct recordings (1-3).

Our present results show that the response of the newborn infant to alternate breaths of air and a hypoxic gas remains unchanged from approximately 48 h to approximately 7 wk of age. The response for study 1 is appropriate for the 24- to 48-h age group as measured by Williams *et al.* (7). In their cross-sectional study, the resetting of hypoxic chemosensitivity that had occurred between the 3- to 10-h and 24- to 48-h age groups was evident from the tidal volume, mean inspiratory flow, and  $V_E$  responses. Although the number of variables responding was greater in the babies aged 3-4 d and 5-8 d, and the magnitude of the test responses also increased further, this cannot be taken as firm evidence for a graded maturation of chemosensitivity over this period in a given infant because it was not a longitudinal study. Thus, individual variation between infants may have influenced the results. To investigate specifically the question of whether changes in hypoxic chemosensitivity do occur once the respiratory chemoreflex response has been established, we studied a group of infants at two ages. We did not see a significant

difference in the magnitude of the control or test response between ages, which supports the idea that there is no additional maturation of respiratory chemoreflexes after resetting has occurred. Thus, it appears that resetting of chemoreceptor sensitivity occurs more rapidly in humans than in the lamb and kitten, in which it appears to occur slowly over a few days.

We have used the alternate breath test to measure hypoxic peripheral chemosensitivity in infants because of the advantage of repeating the stimulus over a number of breaths, which allows the response to be averaged. The ventilatory response to hyperoxia has also been used as a test of peripheral chemosensitivity, particularly the Dejours test (10), which substitutes a single breath of 100%  $O_2$  for air during quiet breathing. The response to such transient hyperoxia is variable, and there is no way of identifying the breath responding to the stimulus, making analysis complex. However, results obtained by this method broadly agree with ours. Hertzberg and Lagercrantz (11) used a variation of the Dejours technique and found in term infants that the decrease in  $V_E$  to 30 s of 100%  $O_2$  at 2-6 d was more than that at 2-6 h after birth. Then, in a longitudinal study using the Dejours test, Parks *et al.* (12) reported that the response of infants aged 1, 2, and 3 mo was not significantly different. Although the youngest age group of this study was closer in age to our older age group, it is evident that any maturational changes of hypoxic chemosensitivity have occurred before 1 mo of age.

Poor respiratory control is postulated to be a cause of SIDS. Because peripheral chemoreceptors are predominant in initiating the ventilatory, cardiovascular, and arousal responses to hypoxia or asphyxia, it can be postulated that victims of SIDS had an absent or inadequate input from the peripheral chemoreceptors, and there is evidence for abnormalities of carotid body function in victims of SIDS (13-15). Delayed resetting of peripheral chemosensitivity to hypoxia as reported in chronically hypoxic kittens (5) will prevent an adequate response to acute hypoxia. Alternatively, it may be that SIDS victims were unable to produce an adequate response to an increased chemoreceptor discharge. Elevated environmental temperatures and prone sleeping position, both of which are associated with an increased incidence of SIDS (16), may be involved in such a reduction in the gain of respiratory chemoreflexes, and there is evidence in kittens for a reduced chemoreflex response to hypoxia and  $CO_2$  at warmer temperatures (17).

In summary, we have shown that in healthy newborns there is no additional maturation of respiratory chemoreflexes evident between the first week and the second month of postnatal life. Current evidence suggests that chemoreflex failure may be implicated in victims of SIDS. However, we do not know at present whether this is caused by perturbation of the rapid resetting of hypoxic chemosensitivity we have observed in this study, because other factors may be responsible for reducing the gain of respiratory chemoreflexes in SIDS victims.

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## Announcement

The Stable Isotopes in Nutritional and Metabolic Research 2nd World Conference will be held July 7-8, 1994 at the Erasmus Expo and Conference Centre, Erasmus University Rotterdam, The Netherlands. *For further information, please contact:* Erasmus Forum, Erasmus University Rotterdam, P.O. Box 1738, NL-3000 DR Rotterdam, phone +31.10.408.23.02/10.98, fax +31.10.453.07.84.

## APPENDIX 11: CAROTID CHEMORECEPTOR STEADY STATE CO<sub>2</sub> SENSITIVITY INCREASES WITH POSTNATAL AGE IN THE NEWBORN LAMB.

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We measured the maturation of carotid chemoreceptor responses to steady state CO<sub>2</sub> in 42 lambs anaesthetised (1.5-2.5% halothane, then a-chloralose 60-70mg/kg), tracheostomized, paralysed and artificially ventilated. The femoral vein and left brachial artery were catheterised for the sampling of arterial blood pressure, pH, PaO<sub>2</sub> and PaCO<sub>2</sub>. Anaesthesia was judged from stability of heart rate and arterial blood pressure. Steady state end tidal CO<sub>2</sub> (etCO<sub>2</sub>) were measured by a mass spectrometer and produced in hyperoxia (HYP:PaO<sub>2</sub> 115-150 mmHg), normoxia (NX:PaO<sub>2</sub> 90-105 mmHg), moderate hypoxia (MOD HX:PaO<sub>2</sub> 40-60 mmHg) and severe hypoxia (SV HX:PaO<sub>2</sub> 20-35 mmHg). Discharge frequency of few or multi-carotid chemoreceptor fibres were recorded from the left carotid sinus nerve at each level of etCO<sub>2</sub>. Discharge was normalised to % maximum (measured as the response to 20-30sec inspired nitrogen at constant etCO<sub>2</sub>) and plotted as a function of PaCO<sub>2</sub> (mmHg). Steady state CO<sub>2</sub> sensitivity (% maximal discharge/mmHg PaCO<sub>2</sub>) was described by linear regression.

Chemoreceptor responses are shown in figure 1. At all ages discharge increased with PaCO<sub>2</sub>, and CO<sub>2</sub> sensitivity increased as PaO<sub>2</sub> fell, except in SV HX lambs at 5-9d and 10-24d which showed a fall in discharge with increasing PaCO<sub>2</sub>. Fibres that showed a decrease in discharge during SV HX were excluded from multiple linear regression analysis. Dynamic sensitivity and PaO<sub>2</sub> were log transformed to normalise the data. Both age ( $P < 0.01$ ) and PaO<sub>2</sub> ( $P < 0.03$ ) had a significant effect on CO<sub>2</sub> sensitivity ( $\text{Log-CO}_2 \text{ sensitivity} = (0.027 \pm 0.007 \times \text{Age}) - (0.425 \pm 0.184 \times \log\text{-PaO}_2) + 0.415 \pm 0.341$ ).

In summary, steady state CO<sub>2</sub> sensitivity is present at 3-4d and increases with age. CO<sub>2</sub> sensitivity was also greater at lower PaO<sub>2</sub>s, however older lambs were unable to sustain an increase in chemoreceptor discharge during SV HX. The postnatal increase in CO<sub>2</sub> sensitivity can be explained in terms of the resetting of hypoxia sensitivity that occurs after birth (1).

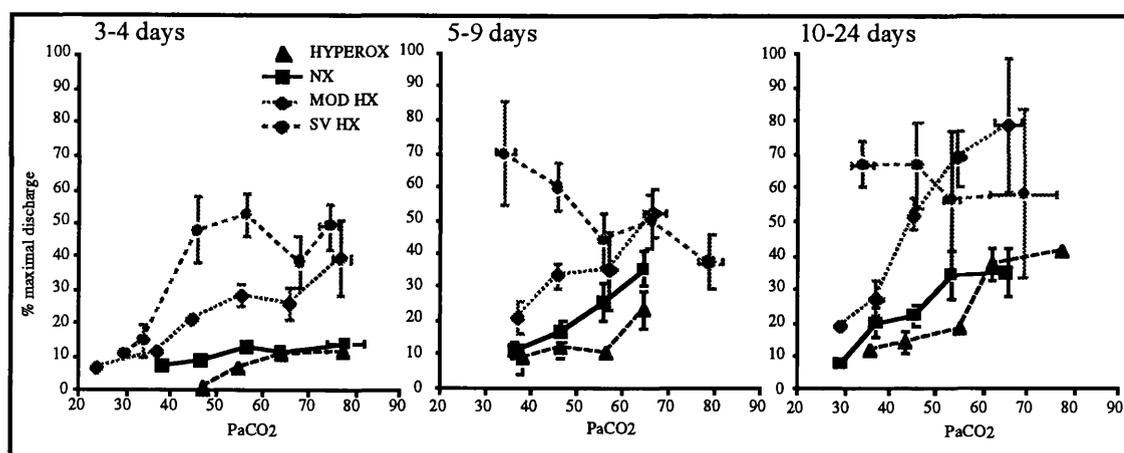


Figure 1. Normalised chemoreceptor responses (mean  $\pm$  S.E.M) to steady state PaCO<sub>2</sub> at 3-4d (squares), 5-9d (circles) and 10-24d (triangles) during HYPEROX, NX, MOD HX and SV HX.

### References

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**APPENDIX 12: DYNAMIC CO<sub>2</sub> SENSITIVITY OF THE CAROTID BODY IS INDEPENDENT OF AGE IN THE NEWBORN LAMB.**

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We have shown that steady state CO<sub>2</sub> chemosensitivity increases with postnatal age (this meeting). This may be due to a maturational increase in the sensitivity of the CO<sub>2</sub>/pH chemotransduction process per se, or to the well-documented increase in hypoxic sensitivity in the neonate. As CO<sub>2</sub> dynamic sensitivity is independent of PO<sub>2</sub> in the adult (1), we tested the hypothesis that CO<sub>2</sub> dynamic sensitivity was independent of postnatal age. We measured dynamic CO<sub>2</sub> sensitivity in anaesthetised (1.5-2.5% halothane, then  $\alpha$ -chloralose 60-70mg/kg), tracheostomized, paralysed and artificially ventilated lambs (n=16) aged 3-17d. Few or multi- carotid chemoreceptor fibres were recorded from the left carotid sinus nerve (CSN) and discharge was summed in 200msec bins. End-tidal CO<sub>2</sub> (etCO<sub>2</sub>) was measured by a mass spectrometer and was made to alternate on a single breath basis with an amplitude of  $1.23 \pm 0.07$  % (mean  $\pm$  S.E.M.) about a mean level of  $7.56 \pm 0.15$  % by adding CO<sub>2</sub> to the inspirate at the start of expiration on every other breath during normoxia (NX:PaO<sub>2</sub> 80-100mmHg; n=14) and moderate hypoxia (HX:PaO<sub>2</sub> 40-60mmHg; n=15). Arterial blood was sampled during alternations to determine mean PaO<sub>2</sub>. Peak and trough discharge frequencies for the oscillation in CSN discharge were normalised to % maximum discharge and plotted against max. and min. etCO<sub>2</sub>. The dynamic chemoreceptor response was calculated as the slope between these 2 points. Steady state CO<sub>2</sub> sensitivity (NX and HX) was determined in each fibre after the CO<sub>2</sub> alternation.

CO<sub>2</sub> sensitivity to single breath etCO<sub>2</sub> alternations was significantly greater than steady state CO<sub>2</sub> sensitivity during NX (P<0.05, paired *t*-test) and HX (P<0.01). Dynamic sensitivity and PaO<sub>2</sub> were log transformed to normalise the data for multiple linear regression. Dynamic CO<sub>2</sub> sensitivity was independent of age (P>0.63) and PaO<sub>2</sub> (P>0.10).

We have found that the carotid body shows a dynamic CO<sub>2</sub> sensitivity in newborn lambs and that this dynamic sensitivity is independent of age and PaO<sub>2</sub>. These findings are important to the understanding of respiratory control in the newborn. Furthermore, in relation to our initial hypothesis, they indicate that the postnatal increase in carotid body steady state CO<sub>2</sub> sensitivity is likely to be due to the accompanying increase in hypoxic sensitivity.

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**References**

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**APPENDIX 13: RESPONSE OF THE CAROTID CHEMORECEPTORS TO A RAMP IN END-TIDAL CO<sub>2</sub> IN THE NEWBORN LAMB.**

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Carotid chemoreceptors in the adult cat respond to a rapid increase in CO<sub>2</sub> with a brisk increase in discharge that adapts. We investigated chemoreceptor responses to alternations of CO<sub>2</sub> in the neonate and analysed the resulting oscillation in discharge with respect to the change in CO<sub>2</sub>. We observed that in some fibres the oscillation in chemoreceptor discharge was step shaped, and so at times discharge frequency was constant despite steadily increasing or decreasing end-tidal (etCO<sub>2</sub>). We reasoned that at such a time the change in discharge due to adaptation was offset by that due to the change in etCO<sub>2</sub>. We have measured the rate at which CO<sub>2</sub> was added or withdrawn to the inspire to prevent any adaptation in discharge frequency.

Lambs aged 4-17d (n=9) were anaesthetised (1.5-2.5% halothane, then  $\alpha$ -chloralose 60-70mg/kg), tracheostomized, paralysed and artificially ventilated. Repeated ramps in etCO<sub>2</sub> were produced by addition of 8-10% CO<sub>2</sub> to one of two inspire lines and measured by a mass spectrometer. A ventilator switched between the two inspires at the start of expiration every 15 breaths (respiratory frequency= 1Hz) during normoxia (NX:PaO<sub>2</sub> 80-100mmHg) and moderate hypoxia (MOD HX:PaO<sub>2</sub> 40-60mmHg). Few or multi- carotid chemoreceptor fibres (n=12) were recorded from the left carotid sinus nerve (CSN) and discharge was summed in 200msec bins. Oscillations in CSN discharge were normalised to % maximum discharge, smoothed by 10 point moving average and viewed for evidence of a plateau. When chemoreceptor discharge was constant, the rate of increase in etCO<sub>2</sub> was  $0.131 \pm 0.013$  % etCO<sub>2</sub>/sec (n=8) and the rate of decrease in etCO<sub>2</sub> was  $-0.149 \pm 0.026$  % etCO<sub>2</sub>/sec (n=6). There was no correlation between age with the rate of increase or decrease in etCO<sub>2</sub>.

In summary, we have found that step shaped oscillations in chemoreceptor discharge can be produced by CO<sub>2</sub> ramps. The rate of change in etCO<sub>2</sub> is related to dynamic sensitivity in that chemoreceptor adaptation to CO<sub>2</sub> is prevented in these oscillations. The rate at which etCO<sub>2</sub> must change to prevent a change in the chemoreceptor response to CO<sub>2</sub> appears to be independent of age. We believe that this provides additional evidence that carotid body dynamic sensitivity to CO<sub>2</sub> does not change with age.

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