Arousal, Sleep and Cardiovascular Responses to Intermittent Hypercapnic Hypoxia in Piglets.

By

Kellie D. Tinworth

A thesis presented for the degree of Master of Science (Medicine)

The University of Sydney, November 2003
Statement of Originality

I declare that all work presented in this thesis is my own, except for the contribution of those acknowledged hereunder. The work was performed whilst the candidate was employed as Research Assistant with Dr Karen Waters in the Department of Medicine, University of Sydney, between July 2000 and November 2003. The methods utilised in these studies are modified from those used routinely in this laboratory and recently published in peer-reviewed journals (Waters and Tinworth 2001, Waters and Tinworth 2003).

General animal husbandry was performed by the Laboratory Animal Services staff at The University of Sydney. Surgical procedures and electrophysiological studies were performed by Dr Karen Waters, with the candidate’s assistance.

No part of this thesis has been previously submitted for any other degree or diploma at any University or Institution. No material in this thesis has been written or published by another individual, except where due credit has been given in the form of a reference.
Acknowledgements

My gratitude to ‘The Boss’, Dr Karen Waters. Karen has acted as my supervisor for this thesis and my employer for the past seven years. Her ideas for research were inspirational, and her editorial input invaluable. The completion of this thesis signals the conclusion of our working relationship, with my imminent departure from this laboratory. Over seven years, we have worked together as a team to achieve great things in respiratory physiology. I am proud of our realizations, and our determination against some almost insurmountable odds to attain those realizations.

My gratitude also to A/Prof. Bob Love and Matthew Van Dijk at the University Farms, Camden. Their help in teaching me about pigs and what piglets regard as ‘treats’ was priceless.

My thanks to Mr Kevin Woodman, anaesthetic technician extraordinaire, who managed to teach me a good portion of all that he knows about anaesthetics and other life matters.

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My gratitude to my friends and family for their patience with my (at times) antisocial behaviour during the writing of this thesis. My most common refrain during these years has been that ‘animals do not observe weekends, public holidays or Christmas’ and how true it is.

The completion of this thesis was one of the most challenging and satisfying aspects of my life yet. My gratitude is held for the animals that contributed to our knowledge of respiratory and developmental physiology. I thoroughly enjoyed my time working with piglets and recommend it as a great and humbling experience.
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Sleep
Proportion of Recovery Asleep
REM Sleep
NREM Sleep
Blood Pressure
Core Temperature
Pco2
Po2
pH
Base Excess
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Discussion

Arousals
Sleep
Blood Pressure
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<td>ABP</td>
<td>arterial blood-gas</td>
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<tr>
<td>ALTE</td>
<td>apparent life-threatening event</td>
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<td>ATP</td>
<td>adenosine triphosphate</td>
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<td>ANOVA</td>
<td>analysis of variance antilogarithm</td>
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<td>BE</td>
<td>base excess</td>
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<td>BL</td>
<td>Baseline</td>
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<td>BP</td>
<td>blood pressure</td>
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<td>CO₂</td>
<td>carbon dioxide</td>
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<td>CPAP</td>
<td>Continuous Positive Airway Pressure</td>
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<td>CSF</td>
<td>cerebrospinal fluid</td>
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<td>EEG</td>
<td>electroencephalogram</td>
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<td>ECG</td>
<td>electrocardiogram</td>
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<td>electromyogram</td>
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<tr>
<td>EOG</td>
<td>electrooculogram</td>
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<td>H⁺</td>
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<td>HCO₃⁻</td>
<td>bicarbonate ion</td>
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<tr>
<td>h</td>
<td>hour</td>
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<td>HH</td>
<td>hypercapnic hypoxia, 8 % O₂ / 7% CO₂ / balance N₂</td>
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<td>HR</td>
<td>heart rate</td>
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<td>IHH</td>
<td>intermittent hypercapnic hypoxia</td>
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<td>min</td>
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<td>N₂</td>
<td>nitrogen</td>
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<td>NREM</td>
<td>non-rapid eye movement sleep</td>
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<td>O₂</td>
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<td>OSA</td>
<td>obstructive sleep apnoea</td>
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<td>PaCO₂</td>
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<td>PaO₂</td>
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<tr>
<td>PCO₂</td>
<td>partial pressure of CO₂</td>
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<td>partial pressure of O₂</td>
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<td>Recovery</td>
<td>recovery, air: 21% O₂ / balance N₂</td>
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<td>Acronym</td>
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<tr>
<td>REM</td>
<td>rapid eye movement sleep</td>
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<td>RR</td>
<td>respiratory rate</td>
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<td>SIDS</td>
<td>Sudden Infant Death Syndrome</td>
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<td>SD</td>
<td>standard deviation</td>
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<td>TST</td>
<td>total sleep time</td>
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Abstract
Clinical studies have demonstrated an arousal deficit in infants suffering Obstructive Sleep Apnoea (OSA), and that treatment to alleviate the symptoms of OSA appears to reverse the deficit in arousability. Some sudden infant deaths are thought to be contingent upon such an arousal deficit. This research utilised young piglets during early postnatal development, and exposed them to intermittent hypercapnic hypoxia (IHH) as a model of clinical respiratory diseases. Arousal responses of control animals were compared to the animals exposed to IHH. Comparisons were also made between successive exposures on the first and the fourth consecutive days of IHH. Time to arouse after the onset of the respiratory stimulus, and frequency of arousals during recovery, demonstrated that arousal deficits arose after successive exposures and that these were further exacerbated on the fourth study day. After an overnight recovery period, the arousal deficit was apparently dormant, and only triggered by HH exposure. These studies confirm that both acute and chronic deficits can be induced on a background of otherwise normal postnatal development, suggesting that deficits observed in the clinical setting may be a secondary phenomenon.
Chapter 1: Literature Review
Overview

This thesis describes studies evaluating the effects of intermittent exposure to hypoxia combined with hypercapnia on arousal responses to hypercapnic hypoxia in young piglets.

Arousal is a vital response to respiratory compromise occurring during sleep. In the context of obstructive apnoea, or other forms of airway occlusion, the objective of arousal is to re-establish patency of the compromised airway. Arousal is therefore an important response to respiratory stimuli that allows the initiation of protective reflexes that are depressed during sleep.

A stimulus threshold is thought to exist, with arousal only occurring once the threshold has been exceeded. Arousal responses invoked by respiratory stimulation are intimately related to the ventilatory responses that the respiratory stimulus invokes. Inspiration of hypoxic and/or hypercapnic gases stimulates the chemoreceptors, which in turn trigger ‘arousal centres’. It has been hypothesized that increased respiratory effort contributes to arousal from Obstructive Sleep Apnoea (OSA). Mediators of this effect may be mechanoreceptors in or near the respiratory tract.

A deficiency in arousal responsiveness could be life threatening, since failure to arouse may render the victim incapable of responding effectively to sleep-related asphyxia, regardless of its cause. It is hypothesized that victims of the Sudden Infant Death Syndrome (SIDS), who typically die quietly in their sleep, must suffer from a defect in arousal as well as, or instead of, a deficit in ventilatory control. A deficit in arousal responsiveness is thus considered a necessary abnormality for SIDS to occur. OSA and prone sleeping both lead to hypercapnic hypoxia, and are correlated to depression of the arousal response, and both have been linked with SIDS. The triple risk hypothesis postulates that an infant's vulnerability to SIDS is dormant until it is combined with a critical period of development and an environmental stressor.

Animal studies have demonstrated habituation of the arousal phenomenon, to both acute and chronic exposure to repetitive hypoxia. Piglets were selected as a model of
early human development, because of their comparable postnatal development in respiratory control and neural processes. The studies described in this thesis will extend earlier findings, presenting hypercapnia with intermittent hypoxic exposures, and will evaluate the effects on sleep and cardiovascular processes as well as arousal.

**Functions of Arousal**

Arousal to wakefulness is a vital response to respiratory compromise occurring during sleep. In the context of obstructive apnoea, or other forms of airway occlusion, the objective of arousal is to re-establish patency of the compromised airway. Arousal is therefore an important response to respiratory stimuli that allows the individual to initiate protective reflexes that are depressed in the sleep state.

Arousal from sleep in response to a threat to respiratory stability is important for two reasons: Firstly, wakefulness in itself is a potent stimulus to breathe. Fink (Fink 1961) was able to show that after a period of hyperventilation resulting in a decrease in the hypercapnic drive to breath, subjects continued to breathe rhythmically while awake but not while asleep. Secondly, arousal permits the initiation of a behavioural as well as a ventilatory response to the respiratory stimulus. Arousal allows the individual to respond to and modify the cause (intrinsic or environmental) of ventilatory compromise, and thus to restore ventilation (Sullivan et al. 1978). An example is that of prone sleeping infants arousing to turn their head to re-establish effective ventilation after rebreathing expired air after facial entrapment (Waters et al. 1996b).

A deficiency in arousal responsiveness could be life threatening, since failure to arouse may render the victim incapable of responding effectively to sleep-related asphyxia, regardless of its cause. It is hypothesized that victims of SIDS, who typically die quietly in their sleep, must suffer from a defect in arousal as well as, or instead of, a deficit in ventilatory control. A deficit in arousal responsiveness is thus considered a necessary abnormality for SIDS to occur.

OSA in infants is correlated to depression of the arousal response to subsequent ventilatory compromise. Treatment to reverse OSA appears to reverse the
depression of arousal responses. It is hypothesized that a brainstem abnormality is responsible for this dysfunction in arousability. It remains unanswered whether the brainstem abnormality is congenital and the result of a delay in the maturation process, or whether it can be induced by postnatal insults.

In animal studies of the arousal phenomenon, acute and chronic exposure to repetitive hypoxia leads to habituation, associated with a decrease in the frequency of arousal responses (Fewell and Konduri 1989, Kimoff et al. 1993, Harding et al. 1997, Johnston et al. 1998).

The Arousal Response

The terminology 'arousal from sleep' is generally used to denote the change from the state of sleep to a state of wakefulness (Phillipson and Sullivan 1978). Arousals are typically scored on electrophysiological sleep studies. The American Sleep Disorders Association in 1992 defined arousals as including a shift in electroencephalogram (EEG) frequency, with changes in muscle tone or electromyogram (EMG) important in Rapid Eye Movement sleep (REM) but not in Non-Rapid Eye Movement sleep (NREM) (ASDA 1992). Since EEG changes are required to meet this definition of an arousal, these events are also recognized as ‘electrocortical arousals’. Because the criteria for scoring electrocortical arousals included the requirement for changes in cortical EEG activity, using this definition can lead to neglect of the potential importance of ‘subcortical’ or brainstem arousals. It has been claimed that this definition of an arousal is unreliable because of contention over the lower time limit of 3 seconds not accounting for physiologically-important arousal events, and interobserver variability when scoring arousals according to EEG criteria regardless of EMG activity (Drinnan et al. 1998). Mograss et al found that the ASDA definition of an arousal was not applicable to children, where the majority of cortical arousals were of a duration of less than the required 3 seconds (Mograss et al. 1994).

In adults, an adverse respiratory event, like an OSA episode, is typically terminated by behavioural arousal from sleep after hypoxic and hypercapnic stimulation of chemoreceptors (Phillipson and Sullivan 1978). Full behavioural arousal is not an important mechanism in the termination of adverse respiratory events in infants.
Infants arouse to wakefulness after only ~20% of events (McCulloch et al. 1982, McNamara et al. 1996), compared to adults who consistently arouse after respiratory stimulation (Issa and Sullivan 1983). The arousal sequence in infants is different from that in adults, being predominantly a brainstem mechanism rather than cortical.

The arousal mechanism in infants was illustrated by McNamara et al, who found that a tactile stimulus elicited an arousal sequence that commenced with a spinal withdrawal reflex, was followed by brainstem responses (respiratory and startle responses), and ended in a cortical arousal. The entire pathway or part of it in the order of spinal to cortical responses could be elicited. REM and NREM sleep responses differed only in the latencies of spinal and subcortical reflexes. These observations suggest that the infant arousal response involves a progression of central nervous system (CNS) activation from the spinal to cortical levels (McNamara et al. 1998).

In response to exposure to a respiratory stimulus (increasing hypercarbia), healthy sleeping infants exhibited airway-defensive behaviours that were predictable and essentially equivalent to those McNamara et al observed. As inspired CO$_2$ increased, the arousal response consisted of four highly stereotyped behaviours: sighs (augmented breaths), startles, thrashing limb movements, and full arousal (eyes open, cry). These behaviours occurred abruptly in self-limited clusters of activity and always in the same sequence. Incomplete sequences, comprised of the initial behaviours only, recurred periodically and with increasing frequency and complexity until the infant either succeeded in restoring ventilation or was completely aroused. Spontaneous arousal sequences, identical to those occurring during hypercarbia, occurred periodically during sleep (Lijowska et al. 1997).

More recently, arousal behaviours have been detected by monitoring markers of the autonomic nervous system. Control of the autonomic nervous system has been linked to the arousal response. During arousal, sympathetic tone changes, seen as increases in heart rate (HR), blood pressure (BP) and breathing efforts, while gross body movements occur to avoid the stimulus (Horne et al. 2002a, Horne et al. 2002b).
Chapter 1: Literature Review

Activation of the sympathetic nervous system is correlated with, and allows for detection of subcortical arousals. Sforza et al showed a link between EEG and HR variation during cortical arousals and the fluctuations in HR during subcortical arousal. This suggested that there is a continuous spectrum in the arousal mechanism, starting at the brainstem level and progressing to cortical activation (Sforza et al. 2000). Others have shown a correlation between finger blood flow and arousal. Finger plethysmography provides a measure of pulse wave amplitude and therefore sympathetic nervous system output (Grote et al. 2003). Pulse transit time (PTT) has also been used as a non-invasive marker of BP and, therefore, subcortical arousal. It was found that PTT was a more sensitive method of detecting arousals than visible EEG records (Katz et al. 2003).

Arousal Stimuli

An arousal response occurs once the arousal stimulus exceeds the arousal threshold. The arousal response that is invoked after respiratory stimulation is intimately related to the ventilatory responses that the respiratory stimulus invokes. After inspiration of hypoxic and/or hypercapnic gases the chemoreceptors are stimulated by the resulting blood-gas disturbances. When the stimulus transmitted from the chemoreceptors to the ‘arousal centers’ exceeds the arousal threshold, arousal occurs. Parslow et al have shown in human infants that arousal occurs at similar levels of desaturation in both REM and NREM but that the rate of oxygen desaturation during hypoxia in REM was faster than in NREM, explaining the longer arousal latency seen in NREM (Parslow et al. 2003). Infants with diminished ventilatory responses to hypercapnia or hypoxia fail to arouse to those stimuli (hypercapnic or hypoxic) (Hunt 1981). McCulloch et al observed that the level of respiratory chemostimulation required to produce an arousal response from sleep was significantly greater in Apparent Life-Threatening Event (ALTE) infants than in normal infants.

Arousal from sleep in response to iso- or hypocapnic hypoxia without airway occlusion is surprisingly inconsistent, in both human adults and infants (McCulloch et al. 1982, Dunne et al. 1992, Berry and Gleeson 1997). In contrast, dogs arouse consistently to hypoxia (Phillipson et al. 1978). Hypercapnia is a much more potent,
and consistent stimulus to arousal (Dunne et al. 1992). The combination of hypercapnia and hypoxaemia is also a potent arousal stimulus (Fewell and Konduri 1988).

Arousal from sleep in response to upper airway narrowing or occlusion, such as occurs in OSA, appears to involve more stimuli than the arterial blood-gas changes alone (Gleeson et al. 1990, Berry and Gleeson 1997). It has been hypothesized that arousal results from the combination of increased respiratory effort that occurs as a result of ventilatory stimulation and primary chemostimulation (Gleeson et al. 1990). The act of inspiring against a narrowed or occluded airway might in itself contribute to the arousal stimulus. Mediators of this effect may be mechanoreceptors in or near the respiratory tract that are activated by increasing respiratory effort (Kimoff et al. 1993). A study of airway occlusion in dogs showed that arousal in response to airway occlusion with no resistance was delayed compared with arousal in response to airway resistive loading (Yasuma et al. 1991). In piglets, arousal in response to rebreathing expired air was delayed when compared to partial or total airway obstruction (Galland et al. 1996). Airway patency relies on a complex balance of upper airway muscle tone to maintain an open airway, and the effects of transluminal pressure and the passive tendency of the airway to collapse (Berry and Gleeson 1997). A failure in any of these systems may contribute to a stimulus to arouse. It is possible that there is an interaction of respiratory or chemical and mechanical stimuli to produce arousal.

Issa et al have shown a clear difference in arousal responses induced by nasal and tracheal occlusions (Issa et al. 1987). It was thought that the marked arousability after nasal occlusion compared to tracheal occlusion in sleeping dogs was due to the stimulation of upper airway mechanoreceptors, and the recruitment of these receptors in inducing an early arousal response to nasal occlusion. It is possible that the upper airway mechanoreceptors would not be subject to stimulation by pressure fluctuations when the obstruction occurs lower in the airway, as in tracheal occlusion tests or some cases of OSA. In studies with cats, tonically active glossopharyngeal receptors increased discharge rates in response to upper airway pressure changes (Hwang et al. 1984).
Sullivan et al studied arousal responses to laryngeal stimulation during sleep in adult dogs. Arousal was induced by fluid deposition in the trachea via an endotracheal tube. They found a marked delay to arouse to this stimulus in REM sleep compared to NREM. When stimuli failed to cause arousal, there was prolonged apnoea and marked bradycardia (Sullivan et al. 1978). To evaluate responses during early development, responses of sleeping piglets were studied after pharyngeal fluid deposition (simulating gastro-oesophageal reflux), and showed that airway protection was primarily achieved by swallowing. Arousal occurred in only 24% of tests. Attenuation of protective mechanisms, and apnoea and bradycardia, were apparent when the infusate carried a more acidic pH or the animals were sedated (Jeffery et al. 1995, Page et al. 1995, McKelvey et al. 1999), suggesting that the laryngeal receptors are not able to stimulate the arousal response when subjected to acidic conditions. Human infants parallel the piglet responses to pharyngeal fluid stimulation, in that the primary airway defence responses are swallowing and to a lesser extent, arousal. Acidic solutions were not applied (Page and Jeffery 1998). It is clear that a delay in the arousal response prolongs the ‘subarousal’ behaviours of apnoea and bradycardia, which are life threatening.

Another possible mechanism for the induction of the arousal response is stimulation of the olfactory system. Berthon-Jones and Sullivan showed arousal results in normal sleeping human adults, that were in sharp contrast with findings in dogs and OSA patients - where asphyxic arousal is delayed in REM sleep compared to NREM. They suggested that the early arousal response in REM in normal humans after inspiration of a hypercapnic gas mix may be related to upper airway sensitivity to carbon dioxide (CO₂), specific to the REM sleep state (Berthon-Jones and Sullivan 1984).

Horne et al measured arousal thresholds in response to air jet stimulation. This tactile stimulus was applied to the nares of human infants, and therefore stimulates the area innervated by the facial nerve (Horne et al. 2002a, Horne et al. 2002b, Horne et al. 2002c). McNamara et al also studied the infant arousal response to a non-respiratory (tactile) stimulus (McNamara et al. 1998).

Other studies of autonomic control have induced arousal with auditory stimuli (Catcheside et al. 2001, Catcheside et al. 2002).
OSA in infants has been correlated with depression of arousal responses to ventilatory compromise. It is also claimed that treatment to alleviate OSA reverses this depression. Extrapolating from animal studies, one explanation for higher arousal thresholds in OSA infants could be habituation to a repetitive arousal stimulus, regardless of its origin, where the repetitive presentation of any stimulus results in a decrease in the response and/or an increase in the threshold of the stimulus magnitude required to elicit a response. McNamara et al also noted that habituation of the infant arousal sequence occurs with repeated tactile stimulation. There was a serial habituation of responses from the cortical to the spinal level, where cortical responses were eliminated first, followed by brainstem responses and finally spinal responses. They also observed that habituation of the arousal mechanisms occur more rapidly during REM sleep (McNamara et al. 1999). Therapy for OSA with Continuous Positive Airway Pressure (CPAP) improves oxygenation and prevents sleep fragmentation, so the impaired arousal response prior to treatment may simply be a result of hypoxaemia or of an increase in the drive to sleep. The authors hypothesized that a brainstem abnormality was responsible for this dysfunction in arousability. The human infant brain is in a period of rapid development and therefore vulnerable to environmental moderators of the development of respiratory and autonomic control. Whether the brainstem abnormality is congenital and the result of a delay in the maturation process, or can be induced by environmental factors, is yet to be determined.

Clinical Abnormalities Associated with Respiratory Compromise in Infants

Obstructive Sleep Apnoea

OSA can cause significant complications in infants. OSA is defined as a cessation of airflow at the mouth and nose during sleep, despite ongoing respiratory efforts, and is characterised by rapid onset blood-gas perturbations in oxygen ($O_2$) and carbon dioxide ($CO_2$) levels (Kahn et al. 1982).
During sleep, infants with OSA commonly snore, with laboured breathing, and profuse sweating (Kahn et al. 1994). In infants suffering this disease, OSA can cause complications as mild as snoring and subtle behavioural changes, to more severe problems such as metabolic alkalosis, delay in growth and development, long-term learning disabilities, chronic respiratory failure, cardiovascular complications, irreversible developmental delay, and respiratory distress which can be life threatening (Brouillette et al. 1982, Leiberman et al. 1988, Singer and Saenger 1990, Freezer et al. 1995, Gislason and Benediktsdottir 1995). Infants with OSA exhibit altered REM sleep architecture, with significantly less REM, compared to infants without OSA. (McNamara and Sullivan 1996, McNamara and Sullivan 2000). Repetitive OSA causes chronic metabolic alkalosis, which shifts the haemoglobin saturation curve and results in chronic hypoxaemia, which may in itself lead to the observed delay in growth and development and long-term learning disabilities (Singer and Saenger 1990). Cor pulmonale is a serious complication of OSA (Hunt and Brouillette 1982). The chronic hypoxaemia resulting from metabolic alkalosis can lead to pulmonary hypertension by stimulating erythropoiesis, which results in increased blood viscosity and ultimately increased pulmonary vascular resistance and cardiac workload.

Obstructive apnoeas are frequently secondary to upper airway anomalies: malformations, soft tissue infiltration, and neurologic lesions impairing muscle function (Kahn et al. 1994, White et al. 1995a, Waters et al. 1998). These anomalies may derive from inherited defects (Redline et al. 1999, Guilleminault et al. 2001). During sleep, the interaction of sleep-related changes in upper airway muscle function and upper airway anatomic abnormalities can combine to lead to narrowing of the oropharyngeal lumen leading to breathing instability and OSA (Bradley and Phillipson 1985). In young infants, craniofacial anomalies predispose to OSA (Ward et al. 1986, Isono et al. 1998). For example, airway obstruction during sleep occurs commonly in micrognathic infants (Roberts et al. 1985). The blood-gas disturbances of OSA can also result when an infant is in a position to rebreathe expired gases by sleeping in the prone position in soft bedding with limited capacity for carbon dioxide dispersal, leading to airway obstruction (Waters et al. 1996b, Campbell et al. 1997, Kemp et al. 1998).
There is a paucity of information regarding the prevalence of OSA during infancy, with most epidemiologic studies concentrating on early childhood (>1 year of age). Ali et al. studied 4-5 year old children via a questionnaire examining breathing disorders during sleep (Ali et al. 1993), and Marcus and colleagues have undertaken extensive studies of OSA in children (Marcus 2000). Gislason and Benediktsdottir undertook a study in Iceland to identify the prevalence of snoring and apnoeic episodes during sleep among children 6 months to 6 years old (Gislason and Benediktsdottir 1995). The lack of epidemiological data regarding the occurrence of OSA in young infants may be because symptoms of OSA are difficult to recognize during infancy, when they are often non-specific or may have been present throughout life.

### OSA Infants and Arousal Responses

OSA in infants depresses the arousal response to a subsequent ventilatory challenge. McNamara et al. found that infants suffering OSA had significantly fewer spontaneous arousals compared to control infants (McNamara et al. 1996). Later, McNamara and Sullivan reported that infants who had their OSA treated with nasal CPAP demonstrated partial reversal of this depressed arousal response (McNamara and Sullivan 1999). Harrington et al. showed that infants suffering OSA (having presented with an ALTE) also displayed a depression in arousability compared to age-matched control infants (Harrington et al. 2002).

It has been shown that infants with a clinical history of OSA display diminished ventilatory responses to hypoxia and hypercapnia (Hunt 1981, McCulloch et al. 1982, Tishler et al. 1996). This suggests that a deficient arousal response may be secondary to dysfunctional respiratory control, in chemoreception and/or the relay of information to the respiratory control centres in the brain. However, it has been demonstrated that a competent respiratory control system does not necessarily equate with an intact arousal response. In 18 healthy term infants younger than 7 months of age, hypoxic arousal challenges (PIO₂: 80 mm Hg compared to a normal of 100 mm Hg) during NREM elicited an appropriate hypoxic ventilatory response in all subjects but failure to arouse in the majority (Ward et al. 1992). Other factors such as mechanoreceptor stimulation in addition to that from the chemoreceptors may have participated in eliciting the arousal response. Animal studies are the only way to
study the contribution of various receptors to arousal, because of the mixture of stimuli present in typical clinical conditions, such as OSA.

It has been shown that REM sleep in infants represents a period of vulnerability. In a large study of normal infants, Kato and others have shown that obstructive apnoeas are rare in healthy infants (McNamara et al. 1996, Kato et al. 2000). In healthy infants, Newman et al found an arousal deficit in NREM from 1 week to 6 months of age and a temporary deficit in REM at 3 months in response to a tactile stimulus (Newman et al. 1989). In addition, in response to auditory stimuli, Trinder et al showed that infants were more responsive during REM sleep than during NREM and gave behavioural responses at lower stimulus intensities than EEG responses. Behavioural responsiveness and EEG responsiveness during REM increased as a function of age, while EEG responsiveness during NREM decreased (Trinder et al. 1990). After nasal obstruction, Newman et al showed that infants were more arousable during REM than NREM (Newman et al. 1986).

Where an arousal deficit appears during REM in the first 6 months of life, in healthy, or affected infants, the majority of obstructive respiratory events also occur in REM sleep (McNamara et al. 1996, Kato et al. 2000). Decreased arousability to obstructive apnoea and chemostimulation in REM sleep can be attributed to different arousal pathways being stimulated. Another potential physiological imbalance during this same period of development, is abnormal HR variability observed in studies of infants that subsequently died of SIDS. This abnormality is thought to reflect sympathovagal imbalance. As such, those infants would also be expected to have altered sympathetic nervous system responsiveness to obstructive apnoeas, compared to healthy infants (Franco et al. 1999).

The Sudden Infant Death Syndrome

SIDS is defined as the sudden death of an infant under 12 months that remains unexplained despite a complete post-mortem investigation, including an autopsy, examination of the death scene and a review of the case history (Bergman et al. 1970).
Currently, the most compelling hypothesis regarding SIDS is a failure in the neuroregulation of cardiorespiratory control because of a brainstem abnormality. Neuropathologic studies support the concept that SIDS victims possess underlying vulnerabilities which put them at risk for sudden death (Filiano and Kinney 1994, Ozawa and Takashima 2002, Sawaguchi et al. 2002a, Sawaguchi et al. 2002c, Sawaguchi et al. 2002b, Sparks and Hunsaker 2002). This concept forms the model for the pathogenesis of SIDS proposed by Filiano and Kinney. Their hypothesis states that an infant's vulnerability lies dormant until he enters the critical period of development and is subject to an environmental stressor (Filiano and Kinney 1994).

It has shown that approximately 70% of SIDS deaths occur between 2-4 months of age, with 90% of deaths occurring before six months of age (Willinger et al. 1991). Due to this peak age, SIDS is thought to be related to mechanisms underlying or associated with the specific stage of development that infants undergo at this age (Nachmanoff et al. 1998). It has not been established whether the hypothesized brainstem abnormality is congenital and the result of a delay in the normal maturation process, or can be induced by environmental factors such as repeated episodes of OSA or prone sleeping (Hunt 1992). Studies with animals allow investigation of this question.

**Risks for SIDS**

**OSA**

Recent studies have suggested that OSA may have a link with SIDS. Kahn and colleagues undertook a retrospective examination of the sleep studies of infants that later died from SIDS. They found that the SIDS infants had significantly more frequent obstructed breathing events compared to age- and sex-matched controls (Kahn et al. 1992, Kahn et al. 2002). A correlation has been made between OSA in adults and sudden unexpected infant death in the same family (Mathur and Douglas 1994, Tishler et al. 1996). Familial factors influencing this association may include the degree of predilection for OSA, liability for respiratory illness or allergy, dimensions of the oral-pharyngeal airway, and ventilatory response to hypoxia. McNamara and Sullivan found that infants of families with multiple histories of SIDS,
ALTE and OSA were more likely to suffer from OSA than infants of families with only one case of SIDS or ALTE (McNamara and Sullivan 2000). However the small numbers (20 multiple-history infants against 105 single-history infants) provide suggestive evidence only, and there is still a need for larger epidemiological studies to confirm this finding.

Pathological findings of petechiae at necropsy of SIDS victims can be explained by negative intrathoracic pressure before death suggesting upper airway obstruction prior to death. OSA has been documented in subsequent SIDS victims after retrospective examination of sleep study data (Kahn et al. 1992). The link between ALTE and SIDS is undefined, however, a proportion of ALTE events appear to be precipitated by upper airway obstruction from which the infant failed to arouse until caregiver intervention. Studies have shown the presence of OSA in ~50% of infants who have presented with an ALTE (Engelberts 1995, Guilleminault et al. 2000, Harrington et al. 2002). OSA causes some cases of ALTE, but there is insufficient data to conclude a definitive relationship to SIDS.

**Prone Sleeping**

The prone sleeping position is a significant risk factor for SIDS (Harper et al. 2000). There is evidence that asphyxia, secondary to rebreathing of expired air, might be causal in some or many SIDS deaths (Kemp et al. 1993, Kemp and Thach 1993, Waters et al. 1996b, Campbell et al. 1997, Kemp et al. 1998, Constantin et al. 1999, Harper et al. 2000). Galland et al, Kahn et al and Horne et al have showed that blunted arousal responses and altered autonomic function are a feature of the prone sleeping position. They hypothesized that abnormal autonomic control may impair normal arousal responses (Galland et al. 1998, Horne et al. 2002b, Kahn et al. 2002).

Studies of HR variability and responses of HR and arousal to head-up tilting in infants sleeping prone and supine have shown that autonomic reflexes are significantly reduced in the prone position for both sleep states (Galland et al. 1998). Harrington et al also found depressed autonomic responses, including BP, to posture changes during sleep (Harrington et al. 2002).
Infants sleeping prone for the first time have a 14-19-fold increased risk for SIDS compared to regular prone-sleepers (Côté et al. 2000). Côté et al found that of 157 SIDS cases studied, 139 were found in the prone position. Of the 139 prone SIDS, 64 of these infants did not usually sleep prone, and for 53% of these infants, deaths occurred shortly after being placed to sleep in the prone position by the parents or another caretaker. Of these, the change in sleep position occurred <1 week before death for 21 infants; for 76% of them, death occurred the first or second time that they slept prone.

Various mechanisms have been postulated to explain the increased risk of SIDS associated with prone sleeping, among these, impairment of arousal from sleep. Interestingly, these ‘risk factors’, such as more time spent sleeping and reduced arousability, also explain why some infants are placed prone to sleep by carers (Côté et al. 2000). Horne et al (Horne et al. 2002b) reviewed the effects of prone sleeping on infant sleep architecture, arousability from sleep and cardiorespiratory control. Sleeping in the prone position has been shown to increase the amount of time spent sleeping, particularly time spent in NREM. Sleeping prone has been demonstrated to be associated with a reduced responsiveness to a variety of arousal stimuli. Such impairment of arousal has been demonstrated to be associated with changes in control of autonomic cardiac function. Normal infants that usually sleep prone frequently adopt the face-straight-down position, arouse (presumably in response to hypoxia and hypercapnia) and return to sleep (Waters et al. 1996b). An intact arousal response to initiate head movement is a vital protective mechanism. It is possible that infants that are unaccustomed to sleeping prone either have a congenital or an acquired defect in the arousal-head-turning response or have encountered insurmountable environmental factors that prevent effective head turning (Waters et al. 1996b).

**Averting SIDS – The Arousal Response**

A deficit in arousal responsiveness is considered a necessary abnormality for SIDS to occur. The ability to arouse from sleep is a very important response to adverse respiratory stimuli. It’s hypothesised that victims of SIDS, who typically die quietly in their sleep, suffer as much or perhaps more from a defect in arousal than in
ventilatory control (Hunt 1992). It was postulated that respiratory pattern abnormalities and diminished ventilatory responses to hypercapnia and hypoxia, as indicators of central respiratory drive, were a major cause of SIDS (Kahn et al. 1992). But respiratory pattern abnormalities, manifesting as apnoea or periodic breathing, and diminished ventilatory responses are not inherently life threatening. A deficiency in the arousal response to hypercapnia and hypoxia however would be life threatening, since they render the infant incapable of responding effectively to sleep-related asphyxia, regardless of its cause. Such a deficit would not be sufficient to cause SIDS alone, unless or until a secondary factor, that required arousal to occur as a defence or protective response, occurred. Apnoea or facial entrapment would constitute such a stimulus, since failure to arouse could facilitate the development of sleep-associated asphyxia.

The Brain and Respiratory Control

As discussed above, respiratory stimuli can provoke arousal. The respiratory control system is, whilst responsive to ventilatory perturbations, also responsible for co-ordinating many different responses, some of which can cause arousal. Responses to a respiratory stimulus are sensed peripherally as well as in the CNS, with responses mediated by several mechanisms within and outside of the CNS, and peripheral motor output. This study focuses on the effects of different patterns of respiratory stimuli on arousal responses. The following discussion reviews the detection of respiratory stimuli, as well as the co-ordination and control of the associated ventilatory responses. The interactions between ventilation, ventilatory responses, and sleep are also discussed.

Survival of an infant suffering from OSA, or other clinical conditions that cause disturbances in ventilation, is contingent on an intact arousal response. As explained above, arousal allows a behavioural as well as a ventilatory response to the resulting hypoxaemia to restore adequate ventilation. A deficit in the arousal response to hypoxia may allow hypoxaemia to continue to life-threatening levels.
Ventilation

Ventilation is normally under autonomic, as opposed to volitional, control, but can be consciously driven via the corticospinal tracts. The automatic process of respiratory efforts originates in impulses from the brainstem. The cortex can override the brainstem centres to acquire voluntary control of breathing. Ventilatory drive and arousal are inter-related. Stimulation of the ventilatory control system can also induce an arousal response, and vice versa. Failure of either of these systems could therefore lead to ventilatory compromise.

Ventilatory Control

The control of ventilation is classically described as a series of feedback loops that work to keep arterial blood CO\textsubscript{2} and O\textsubscript{2} tensions constant. The fundamental reason for breathing is to acquire O\textsubscript{2} and discharge CO\textsubscript{2} between arterial blood and alveolar gas and so maintain physiological levels of P\textsubscript{O\textsubscript{2}} and P\textsubscript{CO\textsubscript{2}} in arterial blood. Despite widely fluctuating demands for O\textsubscript{2} intake and CO\textsubscript{2} output by the body, the arterial blood levels of O\textsubscript{2} (PaO\textsubscript{2}) and CO\textsubscript{2} (PaCO\textsubscript{2}) are kept within extraordinarily tight limits. This remarkable control of gas exchange is attributable to a carefully controlled ventilatory system. The respiratory control system comprises three important elements; sensors which acquire information and transmit to the central controller in the brain which collates and assimilates the information, and in response, sends impulses to the effectors, such as the respiratory muscles, to control ventilation. The increased activity of the effectors decreases the sensory input to the central controller, for example, by decreasing P\textsubscript{CO\textsubscript{2}}, showing an example of negative feedback.

The Central Controller

Normal resting breathing is fundamentally generated and controlled by a brainstem central controller made up of neuronal elements residing in the pons and medulla (Smith et al. 1991) The central respiratory network is comprised of a set of neurones in the medulla and the cervical spinal cord that exhibit rhythmic electrical activity that drive respiratory efforts. Some of these are respiratory motoneurons that control the upper airways (eg. the hypoglossal motoneuron that innervates the tongue) and the
diaphragm (innervated by the phrenic motoneuron). Other components include the medullary and spinal interneurons. The medullary interneurons have been designated the respiratory centres. The respiratory centres form the network that produces the rhythmic central drive received by the respiratory motoneurons (Hilaire and Duron 1999). They regulate timing and shaping generators in various output stations. This neuronal system also integrates peripheral feedback from lung and chest wall mechanoreceptors, peripheral chemoreceptors and higher centres (Smith et al. 1991).

The respiratory network responds to CO$_2$ and pH levels, modulated by cholinergic relays and endogenous serotonin, substance P and catecholamine mechanisms at the medullary motoneuron levels (Hilaire and Duron 1999). Endogenous serotonin is thought to play a crucial role, being one of the first neurotransmitters to be expressed during ontogeny and is involved in nervous maturational processes (Hilaire and Duron 1999).

**Effectors**

Ventilatory efforts are complex motor acts that involve several types of muscles, the effectors. These effectors include the diaphragm, intercostal muscles, abdominal muscles and accessory muscles such as the sternomastoids. To be efficient, the contractions of these muscles are perfectly coordinated by the central controller in the medulla (West 1995, Hilaire and Duron 1999).

**Sensors**

**Chemoreceptors**

Chemoreceptor-generated neural activity is the mechanism for informing the CNS’s respiratory-related nuclei when there is an imbalance in PaO$_2$ and PaCO$_2$.

**Peripheral Chemoreceptors**

P$_o_2$ is sensed peripherally at the carotid and aortic bodies, with impulses transmitted centrally over the 9th and 10th cranial nerves to the CNS for signal processing.
The peripheral chemoreceptors are located in the carotid bodies at the bifurcation of the common carotid arteries, and in the aortic bodies above and below the aortic arch. The carotid bodies contain glomus cells (called Type I cells) of two or more types that contain large amounts of dopamine (Bisgard 2000).

It’s thought that the Type I cells are the sites of chemoreception and that modulation of neurotransmitter release from the Type I cells by physiological and chemical stimuli affects the discharge rate of the carotid body afferent fibres (Bisgard 2000).

The peripheral chemoreceptors respond to decreases in PaO$_2$ and pH, and increases in PaCO$_2$. Changes in PaO$_2$ are detected, beginning at ~500 mm Hg. Little response occurs until PaO$_2$ is below 100 mm Hg but then the rate of stimulation of the respiratory centres rapidly increases. The response time can be very fast. So, an acid load, hypercapnia or hypoxaemia will be counteracted by a rapid ventilatory response (Brensilver and Goldberger 1996).

Central Chemoreceptors
The central chemoreceptors are responsible for sensing and responding to changes in CO$_2$ or H$^+$ concentration, and are located in the medullary reticular formation near the ventrolateral surface of the medulla oblongata in the vicinity of the exit of the 9$^{th}$ and 10$^{th}$ cranial nerves (West 1995). The medullary raphe, within the ventromedial medulla, contains putative central respiratory chemoreceptors. The neurones of the medullary raphe project widely to respiratory and autonomic nuclei and contain co-localized serotonin, thyrotropin-releasing hormone, and substance P, three neurotransmitters known to stimulate ventilation. Some medullary raphe neurones are highly sensitive to pH and CO$_2$ and have been proposed to be the central chemoreceptors (Wang et al. 2001). These serotonergic neurones initiate a homeostatic response to changes in arterial P$_{CO_2}$ that includes increased ventilation and modulation of autonomic function (Wang et al. 2001). The medullary raphe contains two subtypes of chemosensitive neurone: one that is stimulated by acidosis and another that is inhibited. Both types of neurone are putative chemoreceptors, proposed to act in opposite ways to modulate respiratory output and other pH-sensitive brain functions (Richerson et al. 2001). It is further thought that a change in
intracellular pH may be the primary stimulus for chemosensitivity in these putative central respiratory chemoreceptor neurones (Wang et al. 2002).

The central chemoreceptors are surrounded by brain extracellular fluid and respond to changes in the concentration of H\(^+\). An increase in H\(^+\) concentration stimulates ventilation, and a decrease inhibits it. The composition of the extracellular fluid around the receptors is dependent on the cerebrospinal fluid (CSF), local blood flow and local metabolism (West 1995). Central chemoreception is thought to involve cholinergic mechanisms to relay information (Hilaire and Duron 1999).

The CSF is the most important variable influencing the composition of the extracellular fluid in the brain. It is separated from the blood by the blood-brain barrier, which is relatively impermeable to H\(^+\) and HCO\(_3\)\(^-\), although molecular CO\(_2\) diffuses across it easily. Since the CSF is far more permeable to CO\(_2\) than to HCO\(_3\)\(^-\), acute changes in PCO\(_2\) are sensed within minutes, whereas changes in HCO\(_3\)\(^-\) are not sensed for hours. When the blood P\(_{CO2}\) rises, CO\(_2\) diffuses into the CSF from the cerebral blood vessels, liberating H\(^+\) and lowering the pH, which stimulates the chemoreceptors. The cerebral vasodilation that accompanies an increased PaCO\(_2\) enhances diffusion of CO\(_2\) into the CSF. So the PaCO\(_2\) regulates ventilation mainly by its effect on the pH of the CSF. The resulting hyperventilation reduces the PaCO\(_2\) and therefore the H\(^+\) concentration in the CSF (Hilaire and Duron 1999).

O\(_2\) does not normally act as a stimulus for the respiratory centres, as long as normal oxygenation occurs in the alveoli. However, in hypoxic conditions when the concentration of available O\(_2\) is reduced, the decreased PaO\(_2\) acts as a respiratory stimulant, resulting in hyperventilation.

The normal pH of CSF is 7.32, and since CSF contains less protein than blood, it has a lower buffering capacity. This results in a greater change in CSF pH than in blood pH for a given change in P\(_{CO2}\). If the CSF pH is displaced for a prolonged period, a compensatory change in HCO\(_3\)\(^-\) occurs as a result of transport across the blood-brain barrier. The change in CSF pH occurs more promptly than the change in blood pH by renal compensation, over 2-3 days. Because of the rapid response time of CSF pH, CSF pH has a more important effect on the control of ventilation and PaCO\(_2\).
Maturation of Ventilatory Control

Development of the mammalian respiratory control system begins early in gestation and does not achieve mature form until weeks or months after birth (Hilaire and Duron 1999). This period of rapid development is a time of profound vulnerability. The development of the respiratory control system during this time consists of complex pre- and postnatal interactions with the environment, including experiences such as episodic or chronic hypoxia, hyperoxia, and drug or toxin exposures. Long-term or irreversible alterations in respiratory control may be induced by these exposures in critical or vulnerable times of development (Harper et al. 2000, Carroll 2003).

Developmental plasticity in neural respiratory control development can occur at multiple sites during formation of brainstem neuronal networks and chemoafferent pathways, at multiple times during development, by multiple mechanisms (Dobbing 1981, Dempsey and Forster 1982).

Developmental plasticity of the respiratory system can be defined as exposures or insults causing changes in the normal course of maturation of structure or function of the respiratory control neural network occurring in critical windows of vulnerability during ontogeny. A critical period is a time window during development devoted to structural and/or functional shaping of the neural systems responsible for respiratory control. Experience during the critical period can disrupt and alter the developmental trajectory, whereas the same experience before or after has little or no effect.

Normal development sees complex and dynamic interactions between genes, transcriptional factors, growth factors, and other gene products that shape the respiratory control system. Early-life experiences may also lead to maladaptive changes in respiratory control, resulting in pathological conditions as well as normal phenotypic diversity in mature respiratory control (Carroll 2003).

One of the clearest examples of early life experiences resulting in changes in mature respiratory control is the blunting of the adult ventilatory response to acute hypoxia.

A component of the maturation of ventilatory responses in neonatal life is the ‘resetting’ of chemoreceptor sensitivity to hypoxia (Hanson 1998). This resetting is initiated by the rise in PaO$_2$ with the onset of pulmonary ventilation at birth (Hanson 1998). The resetting process confers an increase in the carotid body sensitivity to changes in PaO$_2$, so as the resetting process occurs, the neonatal ventilatory responses to acute hypoxia become larger. This increase in chemoreflex response is a vital component of the body’s defence against hypoxia (Hanson 1998). Hypoxia and CO$_2$ sensitivities interact multiplicatively (in adults) in the carotid body. It is thought that the increase in hypoxia sensitivity will also manifest as an increase in CO$_2$ sensitivity (Hanson 1998).

There is also an age-related increase in cellular chemosensitivity of the central chemoreceptors, that would likely correlate to in vivo developmental changes in respiratory chemoreception (Calder et al. 1997). Disturbances of medullary raphe function during development can alter central chemoreception and normal sleep architecture. The ventilatory response to CO$_2$ was altered when medullary raphe neuronal function was focally and reversibly inhibited in chronically instrumented newborn piglets (Messier et al. 2002).

**Cardiorespiratory Control in Sleep in Neonates**

Sleep induces a global depression in cardiorespiratory control. The rate, depth and regularity of breathing in neonates is closely related to their behavioural state (Read and Henderson-Smart 1984). The variations reflect altered CNS activity, changes in the vigilance of the respiratory centres, and varying metabolic demands.

Central apnoeas, where ventilation and respiratory efforts cease for >2 respiratory cycles, are common in immature neonates. Central apnoeas can be seen as an
indicator of CNS vigilance and their frequency decreases with advancing postnatal age (Read and Henderson-Smart 1984).

It has been shown that chronic repetitive ambient hypoxia, simulating pulmonary gas disturbances observed in apnoea, leads to systemic hypertension in rats (Bakehe et al. 1995). Johnston further showed that there were persisting increases in BP after episodic hypoxia in sleeping neonatal lambs, but when repeated, hypoxia rapidly becomes ineffective in stimulating protective arousal and BP responses (Johnston et al. 1999).

Ventilatory responses to chemostimulation are reduced during NREM and fall further during REM. These reductions are probably due to a combination of decreased basal metabolic rate during sleep, altered neuromuscular function, and increased cerebral blood flow.

During NREM, ventilation decreases and arterial Pco$_2$ increases compared to wakefulness (W). Brain blood flow is enhanced linearly with arterial Pco$_2$, attributable to hypoventilation and hypercapnia. In REM, ventilation is significantly lowered compared to that in both W and NREM, due principally to a decrease in tidal volume. Brain blood flow is globally and substantially increased compared with both the W and NREM states. In addition, cerebral O$_2$ consumption is similar in magnitude in the REM and W states. The high brain blood flow rate of REM is not attributable to an increase of brain metabolic activity (Santiago et al. 1984). It was postulated that this excess of brain blood flow during REM could reduce the central chemoreceptor pH relative to that in NREM. The combination of reduction of sensitivity to CO$_2$ and lower tissue Pco$_2$ during REM makes it likely that the output of the central chemoreceptors during this state is less than that during NREM and W. This may contribute to the low tidal volume, respiratory irregularities and vulnerabilities to arousal deficiency of this sleep state (Santiago et al. 1984).

**Hypoxia**

Hypoxia describes low oxygen content of the blood and is more accurately termed hypoxaemic hypoxia. This may be due to restrictive or obstructive pulmonary
parenchymal disease, upper airway obstruction, or a low inspired oxygen concentration.

The peripheral chemoreceptors are solely responsible for the ventilatory response to hypoxaemia in humans. In the absence of peripheral chemoreceptors, hypoxaemia depresses respiration. There is complete loss of hypoxic ventilatory drive in humans with carotid body resection (West 1995, Hilaire and Duron 1999).

The role of hypoxic stimulation of ventilatory drive in day-to-day control of ventilation is small. When the arterial blood $P_{CO_2}$ level is normal at ~36 mm Hg, the $P_{O_2}$ can drop to ~50 mm Hg before ventilation increases. However, when $P_{CO_2}$ is elevated, there is increased ventilation at any $P_{O_2}$ level (Brensilver and Goldberger 1996).

Normally, hypoxaemia has no effect on central chemoreceptors. However, prolonged hypoxaemia can cause mild cerebral acidosis that in turn can stimulate ventilation by the effect of $H^+$ on the central chemoreceptors (Hilaire and Duron 1999).

The dissociation of oxygen from haemoglobin increases at lower $P_{O_2}$ values, but not linearly. The curve relating arterial oxygen saturation ($SaO_2$) to $PaO_2$ is sigmoid shaped, and can be shifted by factors such as acidosis and increased body temperature, which causes the Bohr Effect where haemoglobin relinquishes oxygen more easily in the tissue (Auer and Benveniste 1997).

The brain compensates for hypoxia in several ways. More oxygen is extracted from the blood. Thus, a lower oxygen content of venous blood is a concomitant of hypoxia. Cerebral blood flow increases, compensating for the decreased oxygen content of arterial blood by increasing flow through the tissue (Berntman et al. 1979a, Darnall et al. 1991). Hypercapnia, which often accompanies hypoxia, increases flow and stimulates oxygen consumption (Berntman et al. 1979b). Together, these mechanisms supplement the hyperventilation that accompanies hypoxia. They act to uphold cerebral oxygenation and cerebral metabolism of oxygen. Cerebral energy levels are maintained in the tissue, although there can be a slight decline in phosphocreatine and adenosine triphosphate (ATP), and some increase in tissue lactate (Berntman et al. 1979a).
The neonatal ventilatory response to an acute hypoxic challenge is recognized as ‘biphasic’, with an initial increase in ventilation being followed after a few minutes by a decline to or below control levels (Hanson 1998). It has been shown that this phenomenon of paradoxical decline in respiratory output is due to powerful descending inhibitory influences on ventilatory output in the brainstem (Lawson and Long 1983, Long and Lawson 1984, Hanson 1998). The structures responsible for this are located in the pons, including the parabrachial nuclear complex and locus coeruleus, rostral to the dorsal and ventral respiratory groups (Hanson 1998). The biphasic ventilatory response to hypoxia represents a balance between synaptically-induced augmentations and reductions of brainstem neuronal activities. The carotid chemoreceptors play a fundamental role in the augmentations, and reductions appear dependent upon actions of hypoxia on brainstem mechanisms (Fung et al. 1996) and seem to be dependent on opioid peptides and adenosine in the early postnatal period (Moss et al. 1987).

Chronic hypoxia in the neonate blunts the ventilatory response to acute hypoxia, because of impairment of carotid body sensitivity (Hanson 1998). There are two general categories of the neuromodulatory agents thought to be responsible for the lowering of the carotid body sensitivity to chronic hypoxia: those thought to be primarily inhibitory to carotid body function: dopamine, noradrenaline, nitric oxide; and those thought to be primarily excitatory: substance P, endothelin. There is evidence that these putative inhibitory agents are up-regulated in the first weeks of chronic hypoxia and that substance P is down-regulated (Bisgard 2000).

Newborn mammals exhibit specific physiological adaptations to chronic hypoxia defined as the phenomenon of neonatal hypoxia tolerance. There is a rapid decrease in hypoxia tolerance with increasing postnatal age (Singer 1999).

As the mammalian foetus is acclimatized to low oxygen partial pressures, the foetal ventilatory system has devised long-term adaptations in defence of limited intrauterine oxygen supply. In chronic hypoxia, the foetus has improved $O_2$ transport by haematological adaptations including a process of polycythaemia where the blood haemoglobin concentration is increased, and the oxygen binding affinity of the blood
is enhanced. There is also reduced oxygen demand by reducing the metabolic rate by deviation from the metabolic size allometry of Kleiber’s rule. Kleiber’s rule follows that the mass-specific basal metabolic rate of mammals increases with decreasing body size, such that the metabolic rate of a mouse is inversely proportional to that of an elephant, and the metabolic rate of the neonate is higher than that of the adult. Metabolic rate undergoes the greatest reduction and the slowest postnatal increase in the smallest and most immature neonates, paralleling the degree and dynamics of neonatal hypoxia tolerance. Immaturity in itself plays an additional role in reducing metabolic demands, with a very low cerebral metabolic rate attributed to the state of immaturity. Another adaptation to chronic hypoxia is diminished cerebral vulnerability, manifested as an attenuated cerebral excitotoxicity, where the liberation of excitatory amino acids is retarded because of the lower energy requirements and a delay in anoxic depolarization of neurones. There is also the phenomenon of metabolic flexibility by optional repartitioning of energy supply from the non-essential processes of growth and reproduction (production metabolism) to the essential processes of maintenance metabolism such as maintenance of basal metabolic rate, thermoregulation and physical work (Singer 1999).

In the case of birth asphyxia or ventilatory compromise early in life, these background mechanisms are complemented by short-term adaptations to acute oxygen lack. These physiological peculiarities of the neonate differ from adult physiological reactions under comparable conditions. Hypoxic neonates will reduce their body temperature. This suppression of thermoregulation means that a fall in body temperature results in a reduction in metabolic rate and exerts protection against hypoxia. The state of electrical silence seen in the hypoxic neonatal brain (Waters et al. 1996a) contributes to a reduced metabolic demand. There is also a reduction of HR and redistribution of circulation from peripheral tissues to central organs. This means then that the most vulnerable and important central organs can continue to function aerobically while the more resistant peripheral tissues are temporarily left to anaerobic metabolism. There is also a reduction of respiration rate (RR) reflecting a protective hypoxic hypometabolism (Fung et al. 1996). A reduction of blood pH contributes to respiratory acidosis and inhibits enzymatic activity, contributing to metabolic slowing. A low pH will also have a vasodilatory effect on cerebral vasculature, contributing to the benefits of a redistributed blood flow to vital organs.
Although anaerobic metabolism is improved in neonatal mammals by increased glycogen stores and sustained washout of acid metabolites, neonatal hypoxia tolerance seems to be primarily based on the ability to maintain tissue aerobiosis as long as possible by reducing metabolic demands.

The combination of long- and short-term mechanisms offers a novel approach to estimation of the newborn's ability to withstand temporary oxygen lack. However, most of these mechanisms are not unlimited in their protective effect and severe prolonged hypoxia cannot be completely compensated for. The protective reflex of thermoregulation suppression in neonates can lead to adverse effects when cold defence reactions are activated, including peripheral vasoconstriction, metabolic increase and RR slowing which may lead to acidosis and impaired lung function and a ‘vicious cycle’ of hypoxia (Singer 1999).

**Hypercapnia**

Hypercapnia describes a high concentration of CO₂ in arterial blood. This may be a result of rebreathing expired alveolar gas, obstructive apnoea or inspiring a hypercapnic gas mix. CO₂ is carried in the blood in three forms: as dissolved, as bicarbonate, or in combination with proteins as carbon compounds (Hilaire and Duron 1999).

Imbalance of PaCO₂ levels is the most important stimulant of the respiratory centres. The main stimulus to increase ventilation when PaCO₂ increases comes from the central chemoreceptors, which respond to the rise in H⁺ of the CSF near the receptors. However, if the P CO₂ of the alveolar air rises above ~65 mm Hg this acts as a CNS depressant and can result in severe respiratory acidosis and CO₂ narcosis. For P CO₂, the peripheral chemoreceptors are less important in detecting changes than the central chemoreceptors, and only contribute ~20% of the ventilatory response to a rise in P CO₂ and a concurrent fall in pH but their response time is faster than the central chemoreceptors so their response is helpful as a ‘first aid’ effect (Hilaire and Duron 1999).
When PaO$_2$ levels are within the normal limits of ~100 mm Hg, ventilation increases by ~2-3 l/min for each 1 mm Hg rise in PaCO$_2$. However, when PaO$_2$ is low, there is a higher ventilation for a given PaCO$_2$ (Hilaire and Duron 1999).

The P$_{CO_2}$ of alveolar air is in equilibrium with the P$_{CO_2}$ of arterial blood, and this is in equilibrium with the carbonic acid content of the blood. So a change in the P$_{CO_2}$ of the alveolar air will cause a corresponding change in the P$_{CO_2}$ and the carbonic acid content of the blood. Alternatively, if metabolic processes cause an increase in carbonic acid or a decrease in bicarbonate in the blood, hyperventilation will result to excrete the resulting CO$_2$.

The lower the saturation of haemoglobin with O$_2$, the larger the CO$_2$ concentration for a given P$_{CO_2}$. This is the Haldane Effect.

In neonatal mammals, there is a developmental increase in the ventilatory response to elevated P$_{CO_2}$. This maturation of central respiratory chemoreception may result from maturation of intrinsic chemosensitivity of brainstem neurones (Wang and Richerson 1999). The incidence and the degree of chemosensitivity of medullary raphe neurones increase with age in brain slices and in culture. This age-related increase in cellular chemosensitivity may underlie the development of respiratory chemoreception in vivo (Calder et al. 1997, Wang and Richerson 1999). Chemosensitivity of raphe neurones increases in the postnatal period in rats, in parallel with development of respiratory chemoreception in vivo. An abnormality of these serotonergic neurones of the ventral medulla has been identified in victims of SIDS (Richerson et al. 2001).

In hypercapnia, brain blood flow increases together with oxygen transport. Cerebral oxygen consumption does not change (Siesjo 1980, Hino et al. 2000).

**pH**

The normal pH range of blood is 7.36-7.44; this means that the blood normally has a faintly basic/alkaline reaction. The extreme range of pH compatible with life is 6.7-7.9. Three important factors help maintain this pH: chemical buffers of the body fluids
and cells, such as the carbonic acid/sodium (or potassium) bicarbonate system which is quantitatively the largest in the body and operates in the extracellular fluid, and the phosphate buffer system in red blood cells and kidney tubule cells, which enables the kidneys to excrete H\(^+\), can neutralize strong acids and bases that are produced in the body. Extracellular chemical buffering occurs instantaneously, intracellular buffering (the diffusion of H\(^+\) into cells and neutralization) takes hours; respiratory compensation can help eliminate and regulate the concentration of carbonic acid (the main acid end product of metabolism). Respiratory compensation occurs in minutes; and metabolic compensation, where the kidneys help eliminate excess acids and bases. The kidneys are the most important mechanism in maintaining physiological pH because they compensate for failures of the buffering salts and the respiratory system. Renal buffering takes hours to days.

A reduction in arterial pH stimulates ventilation and the main site of action of a reduced arterial pH is the peripheral chemoreceptors. However, if there is a large drop in pH, the blood-brain barrier becomes partly permeable to H\(^+\) ions and the central chemoreceptors or the respiratory centre is affected. It is difficult to separate the ventilatory response resulting from a fall in pH from that caused by an accompanying rise in P\(\text{CO}_2\) (Brensilver and Goldberger 1996).

The pH of the blood is dependent on the bicarbonate/carbonic acid ratio in the plasma and extracellular fluid. In physiological conditions, the bicarbonate/carbonic acid ratio is 20/1 and pH is 7.4, where the concentration of bicarbonate is 27 mmol/l and carbonic acid is 1.35 mmol/l. Normal metabolic activities form organic and inorganic acids that are stronger than carbonic acid, causing the production of H\(_2\)O and CO\(_2\). pH can be calculated with the Henderson-Hasselbach equation: 
\[
\text{pH} = 6.1 + \log \frac{\text{HCO}_3^-}{\text{H}_2\text{CO}_3 + \text{CO}_2}
\]
which normally is 
\[
\text{pH} = 6.1 + \log 27 / 1.35.
\]

When bicarbonate rises and carbonic acid falls, the ratio increases and pH rises. This results in alkalosis. When bicarbonate falls and carbonic acid rises, the ratio decreases and pH falls. This results in acidosis (Brensilver and Goldberger 1996).
Respiratory Regulation of pH

Small increments in $P_{CO_2}$ or decrements in pH result in dramatic changes in ventilation triggered by receptors in the CNS. When pH falls to 7.2, there is marked hyperventilation. Ventilation is about maximal at pH of 7.0. When pH falls below 7.0, hyperventilation disappears. The $P_{CO_2}$ of alveolar air is in equilibrium with the $P_{CO_2}$ of arterial blood, and this is in equilibrium with the carbonic acid content of the blood. So, a change in the $P_{CO_2}$ of the alveolar air will cause a corresponding change in the $P_{CO_2}$ and the carbonic acid content of the blood. If metabolic processes cause an increase in carbonic acid or a decrease in bicarbonate in the blood, hyperventilation will result to excrete the resulting $CO_2$.

The buffer and respiratory compensations of pH imbalances are essentially temporary mechanisms. They are often not completely successful at restoring or maintaining the pH of the blood and extracellular fluid. The kidneys make the permanent adjustments (Brensilver and Goldberger 1996).

Acidosis

Acidosis describes a state of pH below 7.36. This increase in $H^+$ concentration may be due to primary respiratory acidosis or primary metabolic disturbance - due to an excess of inorganic or organic acids, or to decreased amount of base in the body.

Alkalosis

Alkalosis describes a state of pH above 7.44. Alkalosis is a very interesting phenomenon, but will not be discussed further as this study focuses on the state of acidosis, in as much as it describes some of the physiological disturbances induced by OSA.

Base Excess

Base excess describes the excess of base or deficit of fixed acid in the blood - alkalosis, or a deficit of base or excess of fixed acid - acidosis. The base excess is
defined as 0 for blood with pH 7.4 and $P_{CO_2}$ of 40 mm Hg. Positive values in a clinical blood-gas profile indicate an excess of base or deficit of fixed acid. Conversely, negative values indicate a deficit of base or an excess of fixed acid. The amount of strong acid or base per litre of blood that has been added as a result of metabolic disturbances represented by the base excess value may be either primary or compensatory.

**Primary Respiratory Acidosis**

Respiratory acidosis is due to an increase in $P_{CO_2}$ in arterial blood, and describes the accumulation of CO$_2$ and carbonic acid in the body and a drop in arterial pH. Primary respiratory acidosis is due to ineffective gas exchange, by either hypoventilation or inspiration of a hypercapnic gas mixture. Plasma $P_{CO_2}$ levels rise and the bicarbonate/carbonic acid ratio of 20:1 decreases, because the carbonic acid concentration rises to 2.70 or higher. In an arterial blood-gas (ABG) profile, the findings would be: low pH, high $P_{CO_2}$, high CO$_2$ content, normal standard bicarbonate, normal base excess, high actual bicarbonate.

**Compensated Respiratory Acidosis**

A mammal with respiratory acidosis will usually develop a compensatory metabolic alkalosis. In a persistent state of respiratory acidosis, compensatory mechanisms are activated. In the kidneys, there is increased secretion and excretion of H$^+$ and ammonium formation is stimulated and ammonium ions are excreted. Bicarbonate is retained and chloride ions are excreted instead; this causes an increased serum bicarbonate concentration and a decreased serum chloride concentration. Interestingly, the increase in serum bicarbonate is virtually the same as the decrease in chloride; therefore, respiratory acidosis does not affect the anion gap. There is also a slight decrease in sodium excretion because of the conservation and retention of bicarbonate, and monohydrogen phosphate is converted to dihydrogen phosphate and excreted (Brensilver and Goldberger 1996).

The bicarbonate/carbonic acid ratio and the pH may rise toward normal to produce a partially compensated respiratory acidosis (even though carbonic acid and
bicarbonate levels are now higher than normal). The blood buffers react with the carbonic acid and form more basic salts. The arterial $\text{PCO}_2$ level remains high.

However, compensation is always incomplete with severe respiratory acidosis. In an ABG profile, the findings would be: low but rising pH, high $\text{PCO}_2$, high $\text{CO}_2$ content, high standard bicarbonate, high base excess, high actual bicarbonate, low $\text{Cl}^-$, high $\text{Na}^+$. This profile suggests that metabolic alkalosis is present, to a degree of overcompensation of the primary respiratory acidosis.

**Animals as Models of Human Disease**

Studies involving humans are restricted ethically and practically to non-traumatic and non-invasive procedures, and in order to enable more detailed examinations, the use of animal models of human disease is important.

Animal studies allow researchers to undertake examinations that are not possible with human subjects, for example studies examining the effect of a treatment on an organ that involve live animal studies of organ function followed by histopathology studies on the organ tissue after harvesting the organ from the euthanased animal.

The commonly used laboratory species of rats, mice, rabbits and pigs breed more frequently and with larger litter sizes than humans, allowing researchers to achieve their statistically optimal sample size more rapidly and with greater control over genetic homogeneity than would be available within a human population. These species mature in parallel but at an accelerated rate compared with humans, so studies assessing developmental aspects are completed more rapidly with an animal rather than human population. The researcher is also in a position to control for exogenous variables in the housing, breeding and execution of experimental procedures for animal subjects. Longitudinal studies that may involve assessment of subjects at intervals over time are easier to complete with an animal population compared to human subjects.

Finding an animal that is a suitable model for a specific human condition is necessary, however there are a number of diseases that occur naturally in animals.
Chapter 1: Literature Review

that parallel human conditions, and with the advent of genetic manipulation, animals suffering diseases of genetic origin can be bred.

Animal Studies of Arousal Responses

The advantage of each animal model is its ability to separate components of the clinical condition that it is designed to mimic. Therefore, by specifying exposures or genetic characteristics, and looking at the response elicited, we can examine the discrete physiological system.

Acute exposure to repetitive hypoxia leads to habituation, associated with a decrease in the frequency of arousal responses. Fewell & Konduri studied neonatal lambs as a model of chronic hypoxaemia, and showed that repeated exposure to rapidly developing hypoxaemia produces an arousal response decrement in sleeping lambs (Fewell and Konduri 1989). They saw that the time to arousal and the decrease in arterial haemoglobin oxygen saturation were significantly increased with repeated exposure to rapidly developing hypoxaemia during both NREM and REM sleep, although there was a delay to arouse in REM compared to NREM. In another study, they demonstrated the influence of carotid denervation on the arousal and cardiopulmonary response to rapidly developing hypoxaemia. Their data provide evidence that the carotid chemoreceptors and/or carotid baroreceptors play a major role in causing arousal from sleep during rapidly developing hypoxaemia in lambs (Fewell and Konduri 1989).

Harding et al and Johnston et al both showed that arousal from sleep is state dependent. They found that in neonatal lambs arousal from REM, but not NREM, in response to the obstruction of respiratory airflow is depressed during early postnatal development and that repeated obstructions and arousals also lead to depressed arousal from REM sleep. Harding et al also observed a postnatal nadir in arousal responses at 7-12 d. Their results suggest that the arousal mechanism is particularly vulnerable to failure during REM after adverse stimulation at a particular period in postnatal development (Harding et al. 1997, Johnston et al. 1998).
Chronic exposure to episodic hypoxia via tracheal occlusion depresses arousal responses to asphyxial gases when rebreathing expired air in dogs (Kimoff et al. 1997). The arousal responses were evaluated prior to ‘treatment’, after 15.5 weeks of tracheal occlusions during every epoch of sleep, and after 1-3 months of recovery with normal ventilation. A study of chronic OSA and sleep fragmentation found that both insults resulted in a delay to arousal in response to acute airway occlusion and in greater arterial oxygen desaturation, and it was concluded that changes in the acute responses to airway occlusion resulting from OSA are primarily the result of the associated sleep fragmentation (Brooks et al. 1997).

Rats have been studied as a model of chronic hypoxaemia to assess the impact of chronic episodic hypoxia on neurocognitive functions. Ando et al exposed rats to 40 min hypoxia (6-4.5% $O_2$/balance $N_2$) 3 h before a one-trial learning passive avoidance task. The animals displayed impaired memory retention 24 h subsequently (Ando et al. 1987). It was concluded that chronic episodic hypoxia impaired performance during acquisition of a cognitive spatial task without affecting sensorimotor function, and that chronic episodic hypoxia is associated with marked cellular changes over time within neural regions associated with cognitive functions. It is thought that such changes may underlie components of the learning and memory impairments seen in OSA sufferers.

Gozal et al (Gozal et al. 2001) exposed rats to hypoxia (10% $O_2$/balance $N_2$) and air (21% $O_2$/balance $N_2$) every 90 s or 30 min for 12 h a day over 14 days. Sleep recordings, Morris water maze experiments, and immunohistochemistry for NMDA NR1 glutamate receptor, c-fos protein, and apoptosis were conducted to assess pathophysiologic consequences of chronic episodic hypoxia. Marked increases in apoptosis occurred in the CA1 hippocampal region (sevenfold) and cortex (eightfold) after 1-2 d of chronic episodic hypoxia. Double labelling for NMDA NR1 and c-fos revealed marked architectural disorganization in CA1 and cortex with increases in c-fos over time. Rats exposed to chronic episodic hypoxia displayed significantly longer escape latencies and swim path lengths to escape a hidden platform during 12 training trials given over 2 d. Differences in the performances of hypoxia-exposed and control rats persisted after 14 d of recovery.
Acute hypoxaemia in neonatal lambs induces hypertension in both REM and NREM. But when repeated, episodic hypoxia rapidly depresses this autonomic function in REM only (Johnston et al. 1999). It has also been shown that chronic repetitive ambient hypoxia, simulating pulmonary gas disturbances observed in apnoea, leads to systemic hypertension in rats (Bakehe et al. 1995). Since Johnston et al saw persisting hypertension in NREM sleep after repeated episodes of hypoxaemia, it is possible that the observations of Bakehe et al were of BP responses to hypoxia in NREM, since they did not distinguish between sleep states.

Studies with dogs simulating OSA episodes were able to show that a competent respiratory control system does not necessarily equate with an intact arousal response. Studies of waking and ventilatory responses to acute hypoxia in dogs during sleep found arousal responses to hypoxia were delayed but ventilatory responses were intact (Phillipson et al. 1978). The importance of an arousal response was demonstrated in dogs where laryngeal stimulation produced apnoea but never coughing during sleep, and coughing but never apnoea during wakefulness (Sullivan et al. 1978).

The Piglet Model of OSA

Pigs, particularly neonatal pigs, are being used increasingly in biomedical research. This increased interest in pigs as animal models of human disease has been based mainly on their remarkable anatomic and physiologic similarity to humans with respect to the cardiovascular system, digestive tract, skin, nutritional requirements, CNS, respiratory physiology, bone development and mineral metabolism. The size and docility of pigs allows repeated collection of biological samples and various surgical interventions. Other advantages are the diverse gene pool, short reproduction cycle, high prolificacy and large litter sizes (Book and Bustad 1974, Swindle et al. 1992).

The chronically instrumented piglet model is described, in light of its suitability as an animal model for study of arousal responses to respiratory stimuli.
Early Development of the Mammalian Brain

Developmental processes throughout the body are generally not influenced by the birth event and much of the course of brain maturation passes imperceptibly from the foetal to the postnatal state. Productive comparisons can thus be made between the developing piglet brain at its peak velocity, which is perinatal, and the human brain at its own perinatal peak (Dobbing and Sands 1979, Dobbing 1981).
The following figure illustrates the different timing of the brain growth spurts of common laboratory animal models, comparative to the human. This figure illustrates why the pig is a more appropriate model of early postnatal human development, when compared to the monkey, sheep and rat.

Figure 1: The brain growth spurts of the pig, the rat, the monkey, the sheep and the human. The units of time for each species: monkey/sheep: 4 days; pig: weeks; human: months; rat: days. (Adapted from (Dobbing and Sands 1979). The figure allows the species to be categorised into prenatal, perinatal and postnatal brain developers and gives a visual impression of the proportion of the brain growth spurt in each case as prenatal or postnatal.

Using brain maturity as an index of developmental age at birth, one can view the newborn pig as equivalent to a newborn human neonate, but with accelerated postnatal development compared to the human infant. Stages or events in development, not ages, are important to interspecies extrapolation between developing mammals (Dobbing and Sands 1979, Dobbing 1981).

The vulnerable period hypothesis depends on an equivalent degree of adversity being imposed for an equivalent proportion of the vulnerable period (Dobbing and Sands 1979). Timing (in relation to birth), severity and proportional duration are
therefore the three aspects of adversity that must be matched in different species before comparisons can be made between them.

**The Piglet Model of Early Brain Development**

*Comparative Brain Growth*

In the pig, the brain growth spurt encompasses the period from about six weeks before birth to about five weeks after birth (Dickerson and Dobbing 1967). Comparatively, in the human, the growth spurt period is relatively skewed more into the postnatal period, beginning at about mid-gestation and continuing to completion at about 3-4 postnatal years. So in effect, the piglet and human newborns are approximately equivalent in neural development at birth, at which point the piglet brain proceeds to develop more rapidly. This developmental progress is then reflected in processes and functions that are controlled by the CNS, such as sleep/wake states, and ventilatory and arousal responses.

*Comparative Sleep/Wake Behaviour and Cardiorespiratory Parameters*

In infants, several sleep/wake states are recognised: a) active sleep (also called rapid-eye-movement sleep or REM); b) quiet sleep (also called non-REM, NREM or slow-wave sleep); c) indeterminate sleep; and d) wakefulness (W, sometimes further separated into crying, active and quiet). These states are defined by behavioural observations and polygraphic recordings of breathing, HR, eye movements, muscle tone and electroencephalogram (Ellingson 1972, Read and Henderson-Smart 1984).

During the first five weeks postnatally, piglets exhibit changes in sleep/wake organisation and cardiorespiratory behaviour that simulate those observed in human infants during early development (Parmelee et al. 1964, Scott et al. 1990).

In the distribution of sleep/wake states, the human infant undergoes postnatal ontogeny (Parmelee et al. 1964, Ellingson 1972, Anders and Keener 1985). In general, the REM state predominates in immature infants and declines during
maturation; sleep onset from wakefulness are to the REM state during the early months of life and to the NREM state later. Diurnal influences on REM/NREM organisation during the night emerge gradually, and poor epoch-by-epoch coordination of physiological systems results in immature sleep state organisation, observed as indeterminate states, in younger infants. It is seen also that a circadian (24 h) rhythm of sleep/wake states gradually develops and the infant’s night-time sleep becomes consolidated into longer intervals with age.

Using non-invasive methods to investigate the maturation of sleep state and cardiorespiratory parameters, Galland et al studied neonatal piglets at 2, 4, 7 and 10 days after birth (Galland et al. 1993). The piglets paralleled the changes seen in human infants in development of sleep/wake states, displaying however a much more rapid development of the characteristic patterns from 2 to 10 postnatal days. Piglets, like human infants, are also alike behaviourally, in that with age, there is an apparent increase of time spent in the awake state, and increasing proportions of sleep time spent in NREM and decreasing proportions in REM (Anders and Keener 1985, Scott et al. 1990, Galland et al. 1993, Post et al. 1995). Scott et al also noted a parallel increase in BP accompanied by a decrease in HR and RR in piglets and human infants with age (Ruckebusch 1972, Scott et al. 1990). The electrocorticographic patterns seen in pigs are similar to those seen in other mammals – high-frequency low-voltage waves in the alert animal and in REM sleep, and low-frequency high-voltage waves in NREM sleep. The pig also resembles the human in exhibiting alpha waves when awake in the dark (Stromberg et al. 1962, Usenik et al. 1962, Ellingson 1972, Ruckebusch 1972, Pond and Houpt 1978).

**Comparative Respiratory Control – Hypoxic Response**

In newborn infants less than three weeks of age, hypoxia (when \( P_{\text{ao2}} \) is less than 80 mmHg) induces hyperventilation, a resulting decrease in \( P_{\text{aco2}} \) and tachycardia during the first 30 s of exposure. After 5 min, while the decreased \( P_{\text{aco2}} \) and tachycardia persist, ventilation falls to below baseline levels (Brady and Tooley 1966, Rigotto 1977b, Douglas 1985). After 18 days of age, there is no depression of ventilation during hypoxia, and inspiring 12% \( \text{O}_2 \) causes sustained hyperventilation.
The biphasic and paradoxic response to hypoxia seen in the human infant is paralleled by the neonatal piglet (Lawson and Long 1983, Douglas 1985, Moss et al. 1987, Darnall et al. 1991). In piglets less than 5 days of age, hypoxia induces hyperventilation during the first 30 s of exposure. After 1-2 min, ventilation falls to below baseline levels (Lawson and Long 1983, Moss et al. 1987, Darnall et al. 1991). In older piglets, there is no depression of ventilation during hypoxia, and inspiring 12% O\textsubscript{2} causes sustained hyperventilation (Moss et al. 1987, Waters and Tinworth 2001).

**Comparative Respiratory Control – Hypercapnic Response**

The newborn human responds to hypercapnia by increasing its ventilation, as do adults (Brady and Tooley 1966, Rigatto 1977a). However, in sleeping term infants and in premature infants, the response is diminished (Rigatto et al. 1975, Rigatto 1977a, Rigatto 1984). The ventilatory response to CO\textsubscript{2} is dependent on the developmental stage of the CNS. Therefore, the more immature the infant, the more immature the CNS, the more time the infant will be spending asleep, and the more depressed the ventilatory response to CO\textsubscript{2} will be (Rigatto 1977a, Rigatto 1984).

The newborn piglet responds to hypercapnia similarly to the infant, by increasing its ventilation (Brady and Tooley 1966, Rigatto 1977a, Waters and Tinworth 2001).

**Comparative Period of Vulnerability**

As described above, the transient period defined as the brain growth spurt represents a period of increased vulnerability to intrinsic and extrinsic factors (Dickerson and Dobbing 1967, Dobbing and Sands 1979, Dobbing 1981). The brain growth spurt and period of increased vulnerability to experiences that may modify normal developmental processes starts in mid to late gestation for both the human and the pig and continues for a relatively short time after birth. Since the pig and human are approximately equivalent in neural development at birth, and following the doctrine that timing in relation to birth, severity and proportional duration are the three aspects of adversity which must be matched in different species before
comparisons can be made, we can say that early postnatal experiences by the pig, intermittent hypercapnic hypoxia, for example, that causes similar physiological perturbations as OSA in human infants which one can examine, such as blood-gas profiles, can be assumed to induce comparable disturbances in physiological systems that can not be examined in humans.

Our Piglet Model of OSA

Our laboratory utilises piglets as an animal model of human infants suffering OSA. The piglets are exposed to intermittent hypercapnic hypoxia (IHH), mimicking apnoeic episodes that occur in infants suffering OSA on a recurring basis.

Studies in unsedated piglets breathing a mild HH gas mixture (10 % O$_2$ / 6% CO$_2$ / balance N$_2$) achieve disturbances in arterial O$_2$ and CO$_2$ levels characteristic of OSA (Waters and Tinworth 2001, Waters and Tinworth 2003). Studies included exposure to both acute and chronic HH gas mixtures, delivered in an intermittent manner. A cycle time of 6 min was selected as appropriate after earlier studies demonstrated that this cycle time, compared to 2, 4, 8 or 24 min, produced features of poor compensation (Waters and Gozal 2003, Waters and Tinworth 2003).

Piglets were selected as the animal model for this study on the basis that their early postnatal development most closely approximates that of the human infant (Dickerson and Dobbing 1967, Book and Bustad 1974, Pond and Houpt 1978, Dobbing and Sands 1979, Dodds 1980, Scott et al. 1990, Swindle et al. 1992). The age range of 9-13 days over the period of study was carefully selected as a developmental stage equivalent to 2-6 months in the human infant, an age of peak vulnerability to SIDS (Kahn et al. 2002), and an age of increased vulnerability to respiratory compromise (Côté et al. 1996).

Summary

The arousal response involves a complex interaction between various functions of the brainstem, reflexes associated with the respiratory tract and ventilatory control. The arousal response that is invoked by respiratory stimuli is intimately related to the
ventilatory responses that the respiratory stimulus invokes. In clinical settings, OSA induces hypoxia and hypercapnia. The combination of respiratory and arousal responses is important in averting SIDS – where failure to arouse is thought to be fatal. However, it may also be important in clinical events from which the infant survives, such as ALTE or OSA. In both of these conditions, studies have found arousal responses are correlated to depression of the arousal response to subsequent ventilatory compromise. In addition, treatment of OSA has been associated with improvement in the arousal deficit.

It is vital to examine whether a deficit in the arousal response can be induced by repeated episodes of a respiratory stimulus, such as that induced by OSA or sleeping prone. These conditions are characterised by rapid onset blood-gas disturbances in both oxygen and carbon dioxide levels (hypercapnic hypoxia), whereas studies to date have focussed on acute effects, often to an isolated hypoxic stimulus.

This study aims to evaluate the effects of intermittent exposure to hypoxia combined with hypercapnia on arousal responses to hypercapnic hypoxia in young piglets. Our hypothesis was that depression of arousal responses, with concurrent changes in sleep architecture and cardiorespiratory control, could be induced by exposure to hypercapnic hypoxia.

A pattern of intermittent hypercapnic hypoxia (IHH) is used, mimicking common clinical conditions, such as OSA. Episodes of OSA are simulated by the unsedated piglets breathing 6 min HH gas mixture (8% O$_2$ / 7% CO$_2$ / balance N$_2$) alternating with 6 min air (21% O$_2$ / balance N$_2$) for a total time of 48 minutes daily for 4 days. Sleep, cardiovascular and arousal responses will be monitored, with acute responses measured on the first day of exposure, and chronic effects measured on the 4$^{th}$ consecutive day.
Chapter 2: General Methods
Summary

Data for this study were obtained from piglets aged 9-13 days, which is approximately equivalent to the 2-6 month old human infant. It is expected that any ventilatory perturbations during this time that the piglets suffer will reflect the clinical scenario of infant OSA. Côté et al showed that between the ages of 12-15 days, piglets are most vulnerable to ventilatory disturbances (Côté et al. 1996). Treatment comprised exposure to intermittent hypercapnic hypoxia (IHH), and the IHH-Treated and Control groups differed only in their treatment protocols. Piglets were selected as the animal model for this study on the basis that their early postnatal development most closely approximates that of the human infant (Book and Bustad 1974, Dobbing and Sands 1979, Dodds 1980, Scott et al. 1990). Animals were chronically instrumented, and studied at least 48 hours after surgery. Leads for electrophysiological monitoring were implanted under general anaesthetic using aseptic technique. All piglets were treated identically throughout the period of study. The techniques and scoring criteria described in this chapter were common to all studies.

Animals and Housing

Mixed-breed miniature piglets were transported from the University of Sydney farms soon after birth, and housed in McMaster Annex animal facility with light exposure between 12 midday and 12 midnight. The piglets were housed in groups of two or three, with raised rubber flooring, heat lamps, soft bedding and toys for enrichment. The pens were cleaned and disinfected daily. The piglets were fed warm pig milk replacer (Wombaroo Pig Milk Replacer, Cooinda Downs Pty Limited, Unanderra, NSW 2526) until 10 days of age, then weaned onto cereal. All piglets were reviewed daily regarding their general health, feeding habits, and weight gain.
Acute Effects of IHH on Arousal, Sleep and Cardiovascular Responses in Piglets

We studied 23 mixed-breed miniature piglets aged 9.91 ± 0.6 days to assess the acute effects of IHH on sleep, cardiovascular and arousal responses. The responses of the 11 IHH-Treated animals were compared with 12 age- and sex-matched controls, the ‘Control’ group.

Chronic Effects of IHH on Arousal, Sleep and Cardiovascular Responses in Piglets

The piglets were 9.91 ± 0.6 days of age on their first study day (Day 1). They were studied again exactly four days later to assess the chronic effects of IHH on sleep, cardiovascular and arousal responses. The responses of the 11 IHH-Treated animals were compared with 12 age- and sex-matched controls.

Animal Preparation

Aseptic surgery was performed under general anaesthesia. The piglets were pre-medicated with atropine (0.1 mg kg\(^{-1}\)). Inhalational anaesthesia with 1-3% isoflurane in 30-50% nitrous oxide in \(\text{O}_2\) was induced using a facemask and continued throughout surgery via an endotracheal tube. The piglets were mechanically ventilated throughout the anaesthetic, and HR was monitored continuously, using surface electrodes. Anaesthesia was adjusted according to the level of spontaneous respiratory efforts and HR.

Electrodes were implanted to monitor sleep/wake state and cardiorespiratory variables, including: an arterial catheter placed in the descending aorta via the right femoral artery, tunnelled subcutaneously to exit on the flank surface, for BP measurements and ABG sampling; Grass gold cup electrodes (Grass model E6GH, Grass Instrument Div., Warwick, RI, USA) glued to the skull for electroencephalogram (EEG) measurements; Grass gold cup electrodes sutured into the neck muscle for nuchal EMG recording (EMG); and teflon-coated stainless steel
wire electrodes (A-M Systems, Inc., Carlsborg, WA, USA) sutured into the outer canthus of each eye for electrooculogram (EOG) and sutured transcutaneously for electrocardiogram (ECG) recording. All leads were protected in the pockets of jackets that were worn from the time of surgery. Antibiotic therapy with intramuscular cephazolin ($30 \text{ mg kg}^{-1}$) and analgesia with paracetamol rectal suppository ($100 \text{ mg kg}^{-1}$) commenced at anaesthetic induction and continued postoperatively with intraarterial cephazolin ($100 \text{ mg kg}^{-1} \text{ d}^{-1}$) and oral paracetamol ($100 \text{ mg kg}^{-1} \text{ d}^{-1}$) for 2 days or as required (Reyes et al. 2002). Studies commenced a minimum of 48 h after surgery to permit full recovery from the anaesthetic. The piglets were unsedated at the time of study, and had returned to normal feeding and activity. We aimed to perform the studies in a normally dark or ‘sleep’ time for the piglets, which with their adjusted night/day cycle, was before midday. After the final study, all animals were killed painlessly with an overdose of pentobarbitone ($200 \text{ mg kg}^{-1}$).

**Ethical Approval**

Ethical approval for the study was obtained from the Animal Ethics Committee of the University of Sydney. Approval: K14/1-2000/3/3075.

**The Study Environment**

The study environment comprised a transparent temperature-regulated perspex box. Box temperature was maintained within a thermoneutral range for the piglets, with a servo-controlled incubator, modified to suit the experimental set-up (Thermoline, RI 250, Smithfield, NSW Australia). Piglets were suspended in ventral recumbency in a vinyl hammock within the box, to maintain their head position relative to the respiratory-monitoring devices, while still allowing free movement of the limbs. Airflow was recorded via a calibrated, heated pneumotachograph (Hans Rudolph, 4500A, Kansas City, MO, USA) attached to a full facemask. The mask was sealed against the snout by a layer of thixotropic gel under soft rubber (from a party balloon) inside the firm rubber seal of an anaesthetic mask designed for animals (1583, Lyppard, NSW, Australia). The inspiratory limb provided fresh gas flow, and incorporated a gas-tight 3-way tap to permit rapid switching between reservoir bags containing the
required gas mix. The mean time for stabilisation at the new gas level was 13.20 \( \pm \) 5.7 s, and was not different amongst the gas types or studies. A one-way valve was incorporated into the expiratory limb of the circuit to prevent side streaming of air into the gas mix, and \( \text{O}_2 \) and \( \text{CO}_2 \) concentrations were measured on the distal side of the pneumotachograph with a gas analyser (Datex AS3 capnograph). BP was measured continuously via the central arterial catheter, with a differential pressure transducer (Validyne, Northridge, CA).

Signals were amplified on a Grass Model 8 polygraph and then digitised using a commercially available 8-channel data acquisition program (Labdat, RHT-InfoDat, Montreal, Canada). The sampling frequency was 100 Hz. Recordings included continuous measurement of \( \text{O}_2 \) and \( \text{CO}_2 \), EEG, EMG, EOG, BP and calibrated airflow from the pneumotachograph. Figure 2.1 depicts the study environment.
Figure 2.1: The study environment comprised a temperature-regulated perspex box. Piglets were suspended in a vinyl hammock within the box. Flow was recorded via a pneumotachograph attached to a full facemask. The inspiratory limb provided fresh gas flow, and incorporated a gas-tight 3-way tap to permit rapid switching between reservoir bags containing air or the required gas mix. \( \text{O}_2 \) and \( \text{CO}_2 \) concentrations were measured on the distal side of the pneumotachograph. Signals were amplified on a Grass Model 8 polygraph and then digitised using a commercially available 8-channel data acquisition program, Labdat. Recordings included continuous measurement of \( \text{O}_2 \) and \( \text{CO}_2 \), EEG, EMG, EOG, BP and calibrated airflow from the pneumotachograph.


Study Protocol

Cardiorespiratory and sleep/wake variables were recorded for 53 min, including a 5 min Baseline (BL) recording breathing air (21% $\text{O}_2$ / balance $\text{N}_2$) prior to the first HH exposure. The study protocol was as depicted in Figure 2.2 below. After 5 min breathing air (BL), the inspired gas mixture was changed in a step-wise fashion by manually switching a gas-tight three way tap to hypercapnic hypoxia (HH) for 6 min, then returned to air (Recovery) for 6 min. This pattern of HH exposure and Recovery was repeated for a total of 48 min, completing 4 x 6 min cycles of HH and 4 x 6 min cycles of Recovery. Data were analysed for sleep, cardiovascular and arousal responses. Immediately prior to each gas change, blood was sampled via the catheter for ABG analysis.

<table>
<thead>
<tr>
<th></th>
<th>6 Min</th>
<th>12 Min</th>
<th>18 Min</th>
<th>24 Min</th>
<th>30 Min</th>
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<td></td>
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<tr>
<td>HH: 8%$\text{O}_2$/7%$\text{CO}_2$/N$_2$</td>
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<td>Recovery: 21%$\text{O}_2$/N$_2$</td>
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Figure 2.2: Study Protocol. Cardiorespiratory and sleep/wake variables were recorded for 53 min; after 5 min BL, the inspired gas mixture was changed to HH (8% $\text{O}_2$/ 7% $\text{CO}_2$/balance $\text{N}_2$) then returned to Recovery for 6 min. This pattern of HH exposure and Recovery was repeated for a total of 48 min, completing 4 x 6 min cycles of HH and 4 x 6 min cycles of Recovery. Control animals were also exposed to 9 cycles with the gas-tap turned between each, but both reservoirs contained air (not HH).
Identification of Arousals

The raw data was reviewed visually using a commercially available digital data acquisition and analysis program (Labdat and Anadat, RHT-InfoDat, Montreal, Canada). The raw signals derived from EEG, EMG, Airflow, O$_2$, CO$_2$ and BP were reviewed in 60 s epochs and inspected for arousals. An arousal was scored if a change in frequency and amplitude occurred in two or more signals, including EEG. We did not count arousals that were due to technical interference of the BP line or arousals occurring within 10 s of a gas changeover. All arousals adhering to these criteria were counted, regardless of lower limit in time, since an EEG disruption reflected a disturbance at the cortical level. Figure 2.3 depicts an annotated example as generated by Anadat. When examining the data for the arousal latency the start of the cycle was taken to be the point when the inspired levels of O$_2$ and CO$_2$ had both plateaued at the new levels. The time to the first arousal was then measured as the start time of the arousal from the start time of the cycle (in seconds). If the piglet failed to arouse during the cycle, a time of 360 s (as the total time of the cycle) was assigned as the time to arouse for that cycle.
Chapter 2: Methods

<table>
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<th>Unit</th>
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<th>-3.4</th>
<th>-4.3</th>
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<th>8.2</th>
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<td></td>
<td></td>
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</tr>
</tbody>
</table>

- EEG: Electroencephalogram
- EMG_{Neck}: Electromyogram of the neck
- AIRFLOW: Airflow
- O₂: Oxygen concentration
- CO₂: Carbon dioxide concentration
- BP: Blood pressure

**Gas changeover**

**Arousal**

60 s
Figure 2.3: Arousals were identified by a visual review of the raw data with Anadat, derived from Labdat. The raw signals of EEG, EMG, Airflow, $O_2$, $CO_2$ and BP were reviewed in 60 s epochs. An arousal was scored if a change in frequency and amplitude occurred in two or more signals, including EEG. We did not count arousals that were due to technical interference of the BP line or arousals occurring within 10 s of a gas changeover.

Scoring Sleep State

The raw data was reviewed visually with Anadat. The raw signals derived from EEG, EMG, Airflow and BP comprised the montage. Each epoch was 15 s in duration. Sleep and wakefulness were scored according to criteria set forth by Anders et al (Anders et al. 1971). Briefly, three states are defined: Active Sleep (also called REM), Quiet Sleep (also called Non REM or NREM) and Wakefulness. These states are characterised as below:

REM sleep: Low amplitude / high frequency EEG, low voltage EMG, irregular Airflow, and irregular BP.

NREM sleep: High amplitude / low frequency EEG, high voltage EMG, regular Airflow, and regular BP.

Wakefulness: low amplitude / high frequency EEG, high voltage EMG, regular or irregular Airflow, and regular or irregular BP. Figure 2.4 A and B depict annotated examples as generated by Anadat.
Chapter 2: Methods

A

NREM  NREM  NREM  REM

EEG \( (\mu V) \)

EMG_{Neck} \( (\mu V) \)

AIRFLOW \( (l/min) \)

BP \( (mm \, Hg) \)

60 s
Chapter 2: Methods

B

EEG (µV)

EMG_{Neck} (µV)

AIRFLOW (l/min)

BP (mm Hg)

15 s epochs

60 s

NREM  Wake  Wake  NREM
Figure 2.4 A and B: Annotated examples of electrophysiological sleep studies, as generated by Anadat. Sleep stage was scored in 15 s epochs. These states are characterised as below:
REM sleep: Low amplitude / high frequency EEG, low voltage EMG, irregular Airflow, and irregular BP.
NREM sleep: High amplitude / low frequency EEG, high voltage EMG, regular Airflow, and regular BP.
Wakefulness: low amplitude / high frequency EEG, high voltage EMG, regular or irregular Airflow, and regular or irregular BP.

Arterial Blood-gas Measurements

Arterial blood samples were collected for ABG analysis at the end of each cycle, including BL. Approximately 0.4 ml arterial blood was sampled into a heparinized syringe, and the samples stored on ice for a maximum time of 2 h until analysed. Arterial gas tensions, pH, base excess and bicarbonate were measured by an automated blood-gas analyser (Radiometer, ABL, Model 520, Copenhagen). All values were corrected to the rectal temperature of the animal, which was recorded along with box (ambient) temperature at the time each blood sample was taken (ESO-1, and Thermalert TH-8, Physitemp Instruments, Clifton NJ USA).

Blood Pressure Analysis

BP was monitored continuously throughout the studies. The BP signal in the first and last 300 s of each study was reviewed visually using Anadat. BP was analysed in 15 s epochs to yield mean values for those periods.

Data Analysis

Data are presented as mean ± standard deviation (SD), unless otherwise stated. A p-value of ≤ 0.05 was considered statistically significant. T-tests and ANOVAs were performed with Analyse-it for Microsoft Excel (Version 1.62, UK). Statistical analysis for each specific measure will be described in detail in the relevant chapters.
Chapter 3: Acute Effects of IHH on Arousal, Sleep and Cardiovascular Responses in Piglets
Introduction

This study examined whether arousal deficits can be induced by exposure to an intermittent stimulus of hypercapnic hypoxia.

Arousal is a vital response to respiratory compromise during sleep. In the context of a threat to ventilation, the objective of arousal is to re-establish a compromised airway. Arousal is an important response to respiratory stimuli and allows the individual to initiate protective reflexes that are depressed in the sleep state (Fink 1961, Phillipson and Sullivan 1978).

A deficit in arousal is considered a necessary abnormality for an infant to succumb to SIDS. It’s hypothesised that victims of SIDS, who typically die quietly in their sleep, suffer as much or perhaps more from a defect in arousal than in ventilatory control (McCulloch et al. 1982, Hunt 1992, Kahn et al. 2002, Sawaguchi et al. 2002c). Since it is possible that alterations in the arousal response to respiratory stimuli play a role in SIDS, studies to investigate the mechanism of the arousal response decrement following repeated exposure to rapidly developing hypercapnic hypoxaemia are warranted.

There is evidence that asphyxia, secondary to rebreathing of expired air in the prone sleeping position, with hypercapnic and hypoxic alterations blood-gas profiles, mimicking those of obstructive sleep apnoea (OSA), might be causal in some or many SIDS deaths (Kemp et al. 1993, Kemp and Thach 1993, Waters et al. 1996b, Campbell et al. 1997, Kemp et al. 1998, Constantin et al. 1999, Harper et al. 2000). Impairment of arousal has been demonstrated to be associated with changes in control of autonomic cardiac function (Horne et al. 2002a, Horne et al. 2002b). Galland et al and Kahn et al showed that blunted arousal responses and altered autonomic function are a feature of the prone sleeping position (Galland et al. 1998, Horne et al. 2002b, Kahn et al. 2002). It has also been shown that OSA in infants induces changes in sleep architecture (McNamara et al. 1996, McNamara and Sullivan 1996). A canine model of OSA exhibited changes in sleep architecture, similar to that seen in OSA infants. The REM rebound seen during recovery was not the result of an overall REM sleep deficit per se. Rather, it was thought that repeated
sleep disruption due to the effects of repetitive apnoea and hypoxia may lead to an increased REM sleep drive that manifests itself as a REM sleep rebound during recovery sleep after OSA (Horner et al. 1998).

Prone sleeping for the first time represents a 14-19-fold increased risk for SIDS (Côté et al. 2000). Côté et al found that 53% of SIDS victims, who had not slept prone before, died shortly after being placed to sleep in the prone sleep position. For a number of these infants, death occurred the first or second time that they slept prone.

Various mechanisms have been postulated to explain the increased risk of SIDS associated with prone sleeping, among these, impairment of arousal from sleep. Horne et al (Horne et al. 2002b) reviewed the effects of prone sleeping on infant sleep architecture, arousability from sleep and cardiorespiratory control. Sleeping in the prone position has been shown to increase the amount of time spent sleeping, particularly time spent in NREM. Sleeping prone has been demonstrated to be associated with a reduced responsiveness to a variety of arousal stimuli. Such impairment of arousal has been demonstrated to be associated with changes in control of autonomic cardiac function. During arousal, HR, BP and breathing movements increase, while gross body movements occur to avoid the stimulus (Horne et al. 2002a, Horne et al. 2002b). It is thought that abnormal autonomic control may impair these normal arousal responses (Galland et al. 1998, Horne et al. 2002b, Kahn et al. 2002). Normal infants that usually sleep prone frequently adopt the face-straight-down position, arouse (presumably in response to hypoxia and hypercapnia) and return to sleep (Waters et al. 1996b). An intact arousal response to initiate head movement is a vital protective mechanism. It is possible that infants that are unaccustomed to sleeping prone either have a congenital or an acquired defect in the arousal-head turning response or have encountered insurmountable environmental factors that prevent effective head turning (Waters et al. 1996b).

In animal studies of the arousal phenomenon, acute exposure to repetitive hypoxia leads to habituation, associated with a decrease in the frequency of arousal responses. Fewell & Konduri studied neonatal lambs as a model of OSA, and showed that repeated exposure to rapidly developing hypoxaemia produces an arousal response decrement during sleep (Fewell and Konduri 1989). Harding et al
found that repeated episodes of airflow obstruction led to reduced arousability in REM and concluded that arousal in response to the obstruction of respiratory airflow from REM, but not NREM sleep, is transiently depressed during early postnatal development. Repeated obstructions and arousals also led to depressed arousal responses (Harding et al. 1997). Johnston et al also showed that exposure to repetitive hypoxia rapidly becomes ineffective in stimulating protective arousal responses in REM in neonatal lambs (Johnston et al. 1999).

Acute hypoxia leads to stimulation of the peripheral chemoreceptors, which in turn increases sympathetic outflow, acutely increasing BP (Fletcher 2001). Johnston et al also showed an acute rise in BP after hypoxia in sleeping lambs (Johnston et al. 1999).

In the case of ventilatory compromise early in life, foetal compensatory mechanisms to hypoxia are complemented by short-term adaptations to acute oxygen lack. These physiological peculiarities of the neonate differ from adult physiological reactions under comparable conditions. The hypoxic neonate exhibits suppression of thermoregulation, manifesting as a fall in body temperature, and resulting in a reduction in metabolic rate (Singer 1999). The state of electrical silence seen in the hypoxic neonatal brain (Waters et al. 1996a) also contributes to a reduced metabolic demand, as does a reduction of HR and redistribution of circulation from peripheral tissues to central organs (Singer 1999). This means then that the most vulnerable and important central organs can continue to function aerobically while the more resistant peripheral tissues are temporarily left to anaerobic metabolism. There is also a reduction of RR reflecting a protective hypoxic hypometabolism (Fung et al. 1996). A reduction of blood pH secondary to respiratory acidosis inhibits enzymatic activity and contributes further to metabolic slowing. A low pH will also have a vasodilatory effect on cerebral vasculature, contributing to the benefits of a redistributed blood flow to vital organs.

This study evaluated the effects of intermittent exposure to hypoxia combined with hypercapnia on arousal responses in young piglets. Our hypothesis was that depression of arousal responses, with concurrent changes in sleep architecture and cardiorespiratory control could be induced by exposure to hypercapnic hypoxia.
A pattern of IHH was used, mimicking common clinical conditions, such as chronic OSA. Classically, OSA is characterised by rapid onset hypercapnic hypoxia, occurring in a cyclical pattern. In children, OSA occurs predominantly in REM sleep with episodes of cyclical HH lasting ~30 min (McNamara et al. 1996, McNamara and Sullivan 1999, Harrington et al. 2002).

Episodes of OSA were simulated by the unsedated piglets breathing 6 min HH gas mixture (8% O₂ / 7% CO₂ / balance N₂) alternating with 6 min air (21% O₂ / balance N₂) for a total time of 48 minutes. Sleep, cardiovascular and arousal responses were monitored throughout.

**Methods**

**The Piglets**

We studied 23 mixed-breed miniature piglets aged 9.91 ± 0.6 days to assess the acute effects of IHH on sleep, cardiovascular and arousal responses. The responses of 11 ‘IHH-Treated’ animals were compared with 12 age- and sex-matched controls, the ‘Control’ group. The animals were transported from the University of Sydney farms at age 2.35 ± 1.5 days, and housed in McMaster Annex animal facility as described in Chapter 2. Aseptic surgery for chronic instrumentation to monitor sleep state and cardiorespiratory variables was undertaken under general anaesthesia at age 7.83 ± 0.7 days, when piglets weighed 1.68 ± 0.3 kg. Physiological studies (Day 1) were undertaken when the piglets were aged 10.0 ± 0.6 days. Table 3.1 summarises the physical characteristics of the piglets. Average body weight at Day 1 was 1.89 ± 0.3 kg.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>NUMBER OF PIGLETS</th>
<th>SEX (F:M)</th>
<th>AGE AT ARRIVAL (DAYS)</th>
<th>AGE AT SURGERY (DAYS)</th>
<th>AGE AT STUDY (DAYS)</th>
<th>LITTERS REPRESENTED</th>
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<td>IHH-Treated</td>
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<td>5:6</td>
<td>3.27 ± 0.9</td>
<td>7.55 ± 0.5</td>
<td>9.55 ± 0.5</td>
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<tr>
<td>Control</td>
<td>12</td>
<td>7:5</td>
<td>1.50 ± 1.4</td>
<td>8.08 ± 0.7</td>
<td>10.25 ± 0.5</td>
<td>5</td>
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</table>
Table 3.1: Physical characteristics of the IHH-Treated and the Control group of piglets.

Study Protocol

The study environment and protocol was as described in Chapter 2.

Scoring Sleep State

The raw data were reviewed visually with Anadat. The raw signals derived from EEG, EMG, Airflow, and BP comprised the montage. Each scoring epoch was 15 s in duration. Sleep and wakefulness were scored according to the criteria set forth by Anders et al (Anders et al. 1971). Briefly, three states are defined: REM sleep, NREM sleep and W. The criteria for characterising the states is described in Chapter 2.

Identification of Arousals

The raw data were reviewed visually with Anadat. The raw signals derived from EEG, EMG, Airflow, O₂, CO₂, and BP were reviewed in 60 s epochs and inspected for arousals. The criteria for characterising an arousal is described in Chapter 2.

Arterial Blood-Gas Measurements

Arterial blood samples were taken for gas analysis at the end of each 6 min epoch, including the BL recording period. Core temperature, arterial blood-gas tensions, pH, base excess, and bicarbonate were measured by an automated blood-gas analyser (Radiometer, ABL, Model 520, Copenhagen). The sampling technique was as described in Chapter 2.

Blood Pressure Measurements

BP was monitored continuously throughout the studies. After excluding any artefact due to line sampling, the BP signal in the first and last 300 s of each study was analysed in 15 s epochs to yield mean values for those periods.
Data Analysis

In the IHH-Treated group, 1 piglet was excluded from the analysis; after completing the protocol, because the digital data was lost.

All analyses were performed on the group of 12 Control piglets except where specified otherwise.

Each piglet yielded information on sleep architecture. Sleep states were only reviewed during periods in air (Recovery cycles), including BL. Each piglet yielded a score for W, REM and NREM in each cycle. Data were normalised to yield a proportion of the Recovery cycle when examining total sleep time (TST) or a proportion of TST when we examined REM and NREM. Comparisons between IHH-Treated and Control scores were made using one-way analysis of variance antilogarithm (ANOVA) with repeated measures. Animals in which the one of the signals was not satisfactory for accurate sleep scoring were excluded. The analyses were therefore performed on 10 IHH-Treated piglets against 8 Controls.

The time to the first arousal was examined. Each piglet in the IHH-Treated group yielded a score for arousal latency after presentation of the noxious stimulus of HH in each cycle of HH. Differences in responses between each cycle were analysed with one-way ANOVA.

Each piglet yielded a score for the number of spontaneous arousals in BL and Recovery for the IHH-Treated group, and for the entire study for the Controls. Data were normalised to a frequency of spontaneous arousals min\(^{-1}\). Comparisons of spontaneous arousal frequency between the BL and the Recovery cycles of the IHH-Treated piglets were made using two-tailed paired samples t-tests. A comparison of the arousability at BL in the IHH-Treated piglets and the Control piglets was made with an independent samples t-test.

The BP signal in the first 300 s (BL) and last 300 s (Final Recovery) was analysed in 15 s epochs to yield mean values for those periods. Differences in mean BP in the
BL and the Final Recovery within the IHH-Treated and the Control groups were tested for with two-tailed paired samples t-tests; independent samples t-tests were used to test for differences between the groups. BP data were complete for 10 IHH-Treated piglets and 10 Controls.

At each gas change, arterial blood was sampled as described in Chapter 2. The resulting data yielded information on core temperature, $P_O_2$ and $P_C_o_2$, pH, base excess and bicarbonate levels. The changes over the 53 min study between the IHH-Treated and the Control groups were compared with two-way ANOVA. Complete temperature data were available for 11 IHH-Treated piglets and 11 Controls, with $P_O_2$ and $P_C_o_2$, pH, base excess and bicarbonate levels available for 10 IHH-Treated piglets and 10 Controls.

**Results**

**Arousal Latency**

The time to arouse (s) after presentation of the HH stimulus increased across cycles: 16.87 ± 7.1 (mean ± SD), 30.10 ± 17.1, 33.78 ± 23.0 and 41.69 ± 28.6 (p=0.004 between cycles; one-way ANOVA). Again, individual variability in the responses was significant. Figure 3.1 depicts the results.

**Spontaneous Arousals from Sleep**

Spontaneous arousability was depressed after IHH exposure. There was no difference in the rate of spontaneous arousals in the Control group when compared with the IHH-Treated group at BL: 0.27 ± 0.09 vs 0.35 ± 0.28 min$^{-1}$ (NS). Figure 3.2. The frequency of spontaneous arousals from sleep in the Recovery cycles fell in the IHH group, and was significantly reduced from the BL: 0.35 ± 0.28 to 0.13 ± 0.13 min$^{-1}$ in Recovery (p=0.01).
**Sleep**

The IHH-Treated piglets spent an increasing proportion of each Recovery cycle asleep, and an increasing proportion of their TST in NREM. The Control piglets showed a reduction in the time they spent asleep, over successive cycles, with no change in the proportion of TST in NREM. Figure 3.3

**Proportion of Recovery Asleep**

The IHH-Treated piglets as a group spent an increasing proportion of their Recovery time asleep. Sleep was assessed in a total of four Recovery cycles. The proportion of TST (%) increased in each Recovery cycle: 81.11 ± 12.7, 91.97 ± 6.9, 95.49 ± 4.0, 95.86 ± 3.4 and 96.50 ± 3.1 (p=0.004 between cycles; one-way ANOVA). The individual variability was also significant. The control piglets showed a reduction in the time they spent asleep, over successive cycles, with mean values: 91.82 ± 12.3, 83.74 ± 22.4, 85.83 ± 17.9, 76.85 ± 24.3 and 75.68 ± 24.8 (p<0.001 between cycles with one-way ANOVA). Figure 3.3A.

**REM Sleep**

The IHH-Treated piglets spent a decreasing proportion of their TST in REM sleep: 23.58 ± 19.1%, 6.93 ± 13.7%, 11.28 ± 13.4%, 16.48 ± 24.5% and 12.10 ± 16.8% (p=0.01 between cycles, one-way ANOVA). There was no difference in the proportion of REM sleep in the Controls (%): 17.14 ± 31.5, 8.65 ± 12.0, 6.89 ± 9.2, 12.47 ± 21.3 and 7.97 ± 11.1 (NS, one-way ANOVA). Figure 3.3B.

**NREM Sleep**

The IHH-Treated piglets spent an increasing proportion of their TST in NREM sleep (%): 76.42 ± 19.1, 93.07 ± 13.7, 88.72 ± 13.4, 83.52 ± 24.5 and 87.90 ± 16.8 (p=0.01 between cycles; one-way ANOVA). The controls showed no difference in the proportion of their TST in NREM: 82.87 ± 31.5%, 91.35 ± 12.0%, 93.11 ± 9.2%, 87.53 ± 21.3% and 92.03 ± 11.1% (NS, one-way ANOVA). Figure 3.3C.
Chapter 3: Acute Effects of IHH

**Blood Pressure**

BP rose from BL in the Recovery periods following IHH exposure (p<0.001). Figure 3.4.

**Core Temperature**

Temperature data were normalised to reflect the change from BL (Δ Core Temperature). All piglets dropped their core body temperature over time significantly (p<0.0001 between cycles) with no difference between the IHH-Treated and the Control groups. The individual variation was significant in both groups. Figure 3.5.

**Pco₂**

Pco₂ was elevated during the HH exposures but recovered to BL levels during the intervening Recovery periods. Figure 3.6. In the HH cycles Pco₂ rose from a BL value of 36.39 ± 4.3 mm Hg to a peak level of 59.58 ± 5.2 mm Hg in the second HH cycle. In the Recovery cycles Pco₂ fell from a BL value of 36.39 ± 4.3 mm Hg to the lowest level of 33.06 ± 6.8 mm Hg in the third Recovery cycle. Two-way ANOVA showed a difference in the responses in HH compared to Recovery, but no difference over time in either exposure. There was no change in the Pco₂ levels of the Control group over time.

**Po₂**

Po₂ fell during the HH exposures but recovered to BL levels during the intervening Recovery periods. Figure 3.7. In the HH cycles Po₂ fell from a BL value of 112.30 ± 11.0 mm Hg to it’s lowest level of 42.00 ± 3.4 mm Hg in the final HH epoch. Two-way ANOVA showed a difference in the responses in HH compared to Recovery, but no difference over time in either exposure. There was no change in the Po₂ levels of the Control group over time.
Chapter 3: Acute Effects of IHH

**pH**

pH fell over the course of the 53 min study in the IHH-Treated group. Figure 3.8. In the HH cycles pH fell from a BL value of $7.36 \pm 0.0$ (mean ± SD) to $7.05 \pm 0.1$. In the Recovery cycles pH fell from a BL value of $7.36 \pm 0.0$ to $7.21 \pm 0.1$. One-way ANOVA showed a difference between cycles ($p<0.0001$), with significant variation between individuals. Two-way ANOVA showed a difference in the responses in HH compared to Recovery ($p<0.0001$), and a non-significant trend over time ($p=0.07$). There was no change in the pH levels of the Control group over time.

**Base Excess**

Base Excess (BE) fell during the HH exposures and did not recover to BL levels during the intervening Recovery periods. Figure 3.9. In the HH cycles BE fell from a BL value of $-4.47 \pm 3.1$ mmol/l (mean ± SD) to the lowest level of $-14.69 \pm 3.6$ mmol/l in the third HH cycle. In the Recovery cycles BE fell from a BL value of $-4.47 \pm 3.1$ mmol/l to the lowest level of $-13.29 \pm 4.4$ mmol/l in the third Recovery cycle. Two-way ANOVA showed a difference over time in both the HH and the Recovery cycles ($p=0.04$), but not in the responses in HH compared to Recovery. There was no change in the BE levels of the Control group over time.

**Bicarbonate**

Bicarbonate ($\text{HCO}_3^-$) fell after the first HH cycle and fell further in the first Recovery cycle. From then on, the levels actually recovered towards BL levels in HH, and fell further in Recovery. Figure 3.10. In the HH cycles $\text{HCO}_3^-$ fell from a BL value of $19.24 \pm 3.0$ mmol/l to the lowest level of $14.82 \pm 2.8$ mmol/l in the third HH cycle. In the Recovery cycles $\text{HCO}_3^-$ fell from a BL value of $19.24 \pm 3.0$ mmol/l to the lowest level of $12.83 \pm 3.6$ mmol/l in the third Recovery cycle. Two-way ANOVA showed a difference in the responses in HH compared to Recovery ($p=0.015$), but no difference over time in either exposure. There was no change in the $\text{HCO}_3^-$ levels of the Control group over time.
Discussion

The most important finding in this study was that acute exposure to intermittent hypercapnic hypoxia induced an arousal deficit.

All piglets were healthy and normal, and all underwent the same surgical and experimental procedures including gas switches, except that the gas changes included exposure to HH in the IHH-Treated group. Therefore, an arousal deficit was induced on the background of otherwise normal early postnatal development. The age of piglets in this study (9-13 days) places them in a developmental stage equivalent to 2-6 months in the human infant (Dobb ing and Sands 1979, Scott et al. 1990, Galland et al. 1993), an age of peak vulnerability to the Sudden Infant Death Syndrome (Kahn et al. 2002), and an age of increased vulnerability to respiratory compromise (Côté et al. 1996).

Arousals

In each cycle of HH, the first arousal was provoked by the noxious respiratory stimulus of HH. Therefore we looked at the latency to the first arousal in each HH cycle. We saw an increase in the time to arouse in each successive cycle of HH. Our results suggest habituation of the chemoreceptors in their response to asphyxial changes in gas tensions and pH, and imply that a higher level of chemostimulation of the chemoreceptors was required to elicit the arousal response. This finding correlates with other animal studies where it was seen that the time to arouse and the level of arterial desaturation prior to arousal increased with repeated episodes of rapidly developing hypoxaemia in neonatal lambs (Fewell and Konduri 1989, Harding et al. 1997, Johnston et al. 1999).

The finding of a depression in spontaneous arousals after IHH treatment correlates with the finding by McNamara et al that infants suffering OSA had significantly fewer spontaneous arousals compared to control infants (McNamara et al. 1996).

Sleep

Possible explanations for the increase in proportion of time spent asleep by the IHH-Treated piglets across successive Recovery cycles, include sleep-deprivation during
the HH cycles, increasing the homeostatic drive to sleep in the Recovery cycles. Hypercapnia is a potent stimulus to arouse (Dunne et al. 1992), so it is probable that the piglets did not sleep once aroused in the HH cycles and had to ‘catch up’ once the noxious stimulus of HH was removed. Sleep/wake regulation has been proposed to be under the combined influence of an intrinsic circadian pacemaker and a homeostatic process, influenced by endogenous somnogens, such as cytokines, melatonin, hormones and prostaglandins that accumulate during prolonged waking. The homeostatic process can increase the pressure to sleep, regardless of the circadian cycle phase (Hobson and Pace-Schott 2002). The increasing proportion of sleep time in NREM provides further support for the hypothesis that homeostatic sleep drive was increased (Hobson and Pace-Schott 2002).

It is also possible that the increased drive to sleep is a reflection of compromised neurocognitive function as an effect of exposure to IHH. Sleep is deeply involved in the processes of brain plasticity for memory consolidation (Peigneux et al. 2001) and the increased pressure to sleep may be a compensatory mechanism. It has been shown in rats that chronic episodic hypoxia impaired performance during acquisition of a cognitive spatial task without affecting sensorimotor function, and that chronic episodic hypoxia is associated with marked cellular changes over time within neural regions associated with cognitive functions (Ando et al. 1987, Gozal et al. 2001, Hobson and Pace-Schott 2002). The increased TST occurred despite the fact that over the course of the study period, the normal night or ‘sleep time’ of the piglets was drawing to a close, and their day or ‘active’ time beginning. An increase in the homeostatic sleep drive can predominate or override the circadian rhythm (Hobson and Pace-Schott 2002). Control piglets showed a reduction in the time they spent asleep in successive Recovery cycles, suggesting that they had more or ‘sufficient’ sleep over the study, coupled with their activity levels increasing as their night was ending.

**Cardiovascular Changes**

There was acute hypertension after IHH, as the mean BP rose over time in the IHH-Treated piglets compared to the Controls. Our finding agrees with that of Bakehe et al who found cardiovascular changes in rats exposed to episodic hypoxia and hypercapnic hypoxia (Bakehe et al. 1995), and Johnston et al who showed a rise in
BP after exposure to hypoxia in neonatal lambs (Johnston et al. 1999). There is also an association of sleep breathing disorders and elevated BP in humans (Kraiczi et al. 2001).

**Arterial Blood-Gases**

The changes in arterial Po$_2$ and Pco$_2$ reflected the composition of the inspired gases throughout the study. The peripheral chemoreceptors are solely responsible for the ventilatory response to hypoxaemia in humans (West 1995, Hilaire and Duron 1999). When Pco$_2$ is elevated, there is increased hypoxic stimulation of ventilatory drive at any Po$_2$ level. Hypercapnia or hypoxaemia will be counteracted by a rapid ventilatory response in an attempt to restore Po$_2$ and Pco$_2$ to physiological levels (Brensilver and Goldberger 1996).

There was a progressive respiratory acidosis, as pH fell over time. The pH of the blood is dependent on the bicarbonate/carbonic acid ratio in the plasma and extracellular fluid. In hypercapnia, bicarbonate falls and carbonic acid rises, decreasing the ratio and pH falls (Brensilver and Goldberger 1996). When pH falls, there is marked hyperventilation mediated by the peripheral chemoreceptors. However, with a large drop in pH, the blood-brain barrier becomes partly permeable to H$^+$ ions and the central chemoreceptors or the respiratory centre itself is affected (Brensilver and Goldberger 1996). When pH falls below 7.0, the chemoreceptors fail to respond and therefore fail to stimulate ventilation. It is possible that the continued fall in pH is attributable to this lack of response of the chemoreceptors. It is also possible that the compensatory efforts of the respiratory system were incomplete. Alternatively, it is possible that primitive compensatory mechanisms to hypoxia were activated. The hypoxic neonate actively reduces its blood pH to contribute to respiratory acidosis and inhibit enzymatic activity and contribute to metabolic slowing. A low pH will also have a vasodilatory effect on cerebral vasculature, contributing to the benefits of a redistributed blood flow to vital organs. Brain blood flow during NREM is directly correlated with arterial Pco$_2$ (Santiago et al. 1984).

**Metabolic Changes**

We saw that all piglets dropped their core body temperature over time. Possibly this hypometabolism is mediated by primitive compensatory mechanisms to hypoxia.
Classically, the hypoxic neonate suppresses thermoregulation (Singer 1999). However, since the Control piglets’ core temperature fell at an equivalent rate, it is possible that this is a symptom simply of immature thermoregulation, that the box temperature was not high enough (not thermoneutral). Other possible explanations include the saline flushes to the arterial catheter, since these were at room, rather than body, temperature.

In summary, this study demonstrates that a subtle insult to the cardiorespiratory system can induce acute changes in the arousal responses of piglets to noxious respiratory stimulus, coupled with changes in spontaneous arousability. There were also changes in autonomic function and sleep architecture.
Figure 3.1 Arousal latency. Shows comparisons of the time to arouse in successive cycles of HH after presentation of HH. Results are presented as mean ± SD. The time to arouse in successive cycles increased (p=0.004).

Figure 3.2: Frequency of spontaneous arousals from sleep, comparing spontaneous arousals (min⁻¹) during the entire study for the Controls and the BL and Recovery cycles for the IHH-Treated piglets (mean ± SD). The rate of spontaneous arousals during the Recovery period was significantly decreased compared to the BL period (p = 0.01).
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Over time: $p=0.004$

**Latency to Arousal (s)**

- Cycle 1
- Cycle 2
- Cycle 3
- Cycle 4

**Arousal Frequency (min$^{-1}$)**

- Controls
- IHH-Treated Baseline
- IHH-Treated Recovery

$p=0.01$
Figure 3.3: Sleep architecture on Day 1. Shows the mean ± SD for A: the proportion of TST of the Recovery cycle or a proportion of TST when we examined B: REM and C: NREM. The IHH-Treated piglets as a group spent an increasing proportion of each Recovery cycle asleep, with an increasing proportion of their TST in NREM. Legend: ■ = IHH-Treated, □ = Control.
Chapter 3: Acute Effects of IHH

A

Sleep in Recovery

B

REM

C

NREM

Proportion (%)

BL  Cycle 1  Cycle 2  Cycle 3  Cycle 4
Figure 3.4: Blood pressure. Shows comparisons of the BP at the BL and Final Recovery periods in the IHH-Treated and the Control groups (mean ± SD). Legend: ■ =IHH-Treated, □ =Control.

Figure 3.5: ∆ Core Temperature. The change in core temperature over the 53 min study from BL (mean ± SD). Legend: ● =IHH-Treated, ○=Control.
Chapter 3: Acute Effects of IHH

![Graph showing blood pressure and core temperature changes over time.]

**Blood Pressure (mm Hg)**

- **IHH-Treated**
- **Control**

**Core Temperature (°C)**

- **Over time: p<0.0001 for both groups**
Figure 3.6: $P_{CO_2}$ (mean ± SD). A: $P_{CO_2}$ over the study, comparing the IHH-Treated group with the Control group. Legend: ◦ = Control, ● = IHH-Treated. B: $P_{CO_2}$ of the IHH-Treated group presented as $P_{CO_2}$ after time in minutes into HH or Recovery. Legend: ◇ = HH, □ = Recovery.
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![Graph A: IHH-Treated vs Control](image1)

![Graph B: HH vs Recovery](image2)
Figure 3.7 $P_{O_2}$ (mean ± SD). A: $P_{O_2}$ over the study, comparing IHH-Treated and Control groups. Legend: $\bigcirc = $ Control, $\bullet = $ IHH-Treated. B: $P_{O_2}$ of the IHH-Treated group presented as $P_{O_2}$ after time in minutes into HH or Recovery. Legend: $\bigcirc = $ HH, $\square = $ Recovery.
Figure 3.8: pH (mean ± SD). A: pH over the study, comparing the IHH-Treated group with the Control group. Legend: ○ = Control, ● = IHH-Treated. B: pH of the IHH-Treated group presented as pH after time in minutes into the exposure or Recovery. Legend: ○ = HH, □ = Recovery. See text for statistical analyses.
Figure 3.9: Base Excess (BE) (mean ± SD). A: BE over the study, comparing the IHH-Treated with Controls. Legend: ○ = Control, ● = IHH-Treated. B: BE of the IHH-Treated group presented as Po2 after time in minutes into the exposure or Recovery. Legend: ○ = HH, □ = Recovery. See text for statistical analysis.
Chapter 3: Acute Effects of IHH

![Graph showing the acute effects of IHH](image-url)

**A**
- Control
- IHH-Treated

**B**
- Recovery
- HH

Base Excess (mmol/l)

Time into Study (min)

Time into HH or Recovery (min)
Figure 3.10: Bicarbonate (HCO$_3^-$) (mean ± SD). A: HCO$_3^-$ over the study, comparing the IHH-Treated and Control groups. Legend: ○ = Control, ● = IHH-Treated. B: HCO$_3^-$ of the IHH-Treated group presented as Po$_2$ after time in minutes into the exposure or Recovery. Legend: ○ = HH, □ = Recovery. See text for statistical analyses.
Chapter 3: Acute Effects of IHH

A

Bicarbonate (mmol/l)

5 10 15 20 25 30

Control

IHH-Treated

Time into Study (min)

0 6 12 18 24 30 36 42 48

B

Bicarbonate (mmol/l)

5 10 15 20 25

HH

Recovery

Time into HH or Recovery (min)

0 6 12 18 24

89
Chapter 4: Chronic Effects of IHH on Arousal, Sleep and Cardiovascular Responses in Piglets
Introduction

This study assessed the effects of four consecutive days of IHH on arousal responses of piglets. The hypothesis of this study was that the depression of arousal responses, with concurrent changes in sleep architecture and cardiovascular control that were seen after acute IHH, would persist and would be more severe after chronic IHH. The exposure of young piglets to chronic IHH was designed to mimic OSA during early postnatal development.

In Chapter 3, it was demonstrated that four cycles of IHH over 48 minutes induced habituation of the arousal response to a noxious respiratory stimulus. Spontaneous arousability was depressed. Sleep states were redistributed, and BP was elevated after acute IHH.

OSA can cause significant complications in infants. OSA is defined as a cessation of airflow at the mouth and nose during sleep, despite ongoing respiratory efforts, and is characterised by rapid onset blood-gas perturbations in O\textsubscript{2} and CO\textsubscript{2} levels (Kahn et al. 1982).

During sleep, infants with OSA commonly snore, with laboured breathing, and profuse sweating (Kahn et al. 1994). In infants suffering this disease, OSA can cause complications as mild as snoring and subtle behavioural changes, to more severe problems such as metabolic alkalosis, delay in growth and development, long-term learning disabilities, chronic respiratory failure, cardiovascular complications, irreversible developmental delay and respiratory distress which can be life threatening, (Brouillette et al. 1982, Leiberman et al. 1988, Singer and Saenger 1990, Freezer et al. 1995, Gislason and Benediktsdottir 1995). Infants with OSA exhibit altered REM sleep architecture, with significantly less REM, compared to infants without OSA. (McNamara and Sullivan 1996, McNamara and Sullivan 2000). Repetitive OSA causes chronic metabolic alkalosis which shifts the haemoglobin saturation curve and results in chronic hypoxaemia. This may in itself lead to the observed delay in growth and development and long-term learning disabilities (Singer and Saenger 1990). Cor pulmonale is a serious complication of OSA (Hunt and Brouillette 1982). The chronic hypoxaemia resulting from metabolic alkalosis can
lead to pulmonary hypertension by stimulating erythropoiesis, which results in increased blood viscosity and ultimately increased pulmonary vascular resistance and cardiac workload.

OSA in infants is correlated with depression of the arousal response to subsequent ventilatory compromise (McNamara et al. 1996, Harrington et al. 2002). It is also claimed that treatment to alleviate OSA reverses this depression (McNamara and Sullivan 1999).

Recent studies have suggested that OSA may have a link with SIDS (Engelberts 1995, Tishler et al. 1996, Kahn et al. 2002).

A deficit in arousal responsiveness is considered a necessary abnormality for an infant to succumb to SIDS. It’s hypothesised that victims of SIDS, who typically die quietly in their sleep, suffer as much or perhaps more from a defect in arousal than in ventilatory control (McCulloch et al. 1982, Hunt 1992, Kahn et al. 2002, Sawaguchi et al. 2002c). It is hypothesized that a brainstem abnormality is responsible for this dysfunction in arousability (Sawaguchi et al. 2002b, Sparks and Hunsaker 2002).

Currently, the most compelling hypothesis regarding SIDS is a failure in the neuroregulation of cardiorespiratory control because of a brainstem abnormality. Neuropathologic studies support the concept that SIDS victims possess underlying vulnerabilities which put them at risk for sudden death (Filiano and Kinney 1994, Ozawa and Takashima 2002, Sawaguchi et al. 2002a, Sawaguchi et al. 2002c, Sawaguchi et al. 2002b, Sparks and Hunsaker 2002). This concept forms the model for the pathogenesis of SIDS proposed by Filiano and Kinney. Their hypothesis states that an infant’s vulnerability lies latent until he enters the critical period of development and is subject to an environmental stressor (Filiano and Kinney 1994). It has not been established whether the hypothesized brainstem abnormality is congenital and the result of a delay in the normal maturation process, or can be induced by environmental factors (Hunt 1992).

It has been shown that REM sleep in infants represents a period of vulnerability. In a large study of normal infants, Kato and others have shown that obstructive apnoeas
are rare in healthy infants, and the majority of those occur in REM (McNamara et al. 1996, Kato et al. 2000).

In studies of infants that subsequently died, future SIDS infants displayed abnormal HR variability translating as abnormal sympathovagal balance, and altered sympathetic nervous system responsiveness to obstructive apnoeas, compared to healthy infants (Franco et al. 1999).

Chronic exposure to episodic hypoxia via tracheal occlusion depresses arousal responses to asphyxial gases when rebreathing expired air in dogs (Kimoff et al. 1997). The arousal responses were evaluated prior to ‘treatment’, after 15.5 weeks of tracheal occlusions during every epoch of sleep, and after 1-3 months of recovery with normal ventilation. Chronic hypoxia in the neonate blunts the ventilatory response to acute hypoxia, presumably because of impairment of carotid body sensitivity (Hanson 1998). In cats and rats, it was found that there is an important role for dopamine and sympathetic innervation in the carotid body response to hypoxia, and that chronic hypoxia alters sympathetic control (Bisgard 2000). A study of chronic OSA and sleep fragmentation in dogs found that both insults resulted in a delay to arousal in response to acute airway occlusion and in greater arterial oxygen desaturation, and it was concluded that changes in the acute responses to airway occlusion resulting from OSA are primarily the result of the associated sleep fragmentation (Brooks et al. 1997).

Animal studies have shown that hypoxia leads to stimulation of the peripheral chemoreceptors, which in turn increases sympathetic outflow, increasing BP (Fletcher 2001). It has been shown that chronic repetitive ambient hypoxia, simulating pulmonary gas disturbances observed in apnoea, leads to systemic hypertension in rats (Bakehe et al. 1995). In studies of chronic episodic hypoxia, where rats were exposed to 15 s of hypoxia alternating with 15 s air for 7 h a day for 35 consecutive days, Fletcher et al found that systemic BP was elevated and sustained for several weeks after the hypoxic exposures ceased (Fletcher 2001). Bao et al further showed that the chronic episodic hypoxia-mediated increase in diurnal BP is facilitated by the adrenergic and renin-angiotensin systems. Hypercapnia, combined with episodic hypoxia, induces a profound increase in sympathetic activity,
and the adrenal glands as well as renal sympathetic nerves, participate in the chronic diurnal BP elevation (Prabhakar et al. 2001).

To examine how chronic IHH exposure affects arousal, sleep architecture, and cardiovascular control, unsedated piglets were exposed to 6 min cycles of an HH gas mixture (8 % O$_2$ / 7% CO$_2$ / balance N$_2$) alternating with 6 min air (21% O$_2$ / balance N$_2$) for a total time of 48 minutes, daily for 4 days. Sleep, cardiovascular and arousal responses were monitored on Day 4 and compared with those on Day 1.

**Methods**

**The Piglets**

A total of 23 mixed-breed miniature piglets were studied, with their first study at age 9.91 ± 0.6 days. The animals were transported from the University of Sydney farms at age 2.35 ± 1.5 days, and housed in McMaster Annex animal facility as described in Chapter 2. Aseptic surgery for chronic instrumentation to monitor sleep state and cardiorespiratory variables was undertaken under general anaesthesia at age 7.83 ± 0.7 days, when piglets weighed 1.68 ± 0.3 kg. Responses to IHH were monitored on Day 1 and compared with the same parameters on Day 4. Average weight gain was 70 ± 60 g per day during the period of the study with no difference between the study groups. Table 4.1 summarises their physical characteristics.
### Table 4.1: Physical characteristics of the IHH-Treated and Control groups of piglets.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>NUMBER OF PIGLETS</th>
<th>SEX (F:M)</th>
<th>LITTERS REPRESENTED</th>
<th>AGE AT ARRIVAL (DAYS)</th>
<th>AGE AT SURGERY (DAYS)</th>
<th>AGE AT DAY 1 (DAYS)</th>
<th>DAILY WEIGHT GAIN (G)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHH-Treated</td>
<td>11</td>
<td>5:6</td>
<td>4</td>
<td>3.27 ± 0.9</td>
<td>7.55 ± 0.5</td>
<td>9.55 ± 0.5</td>
<td>60 ± 40</td>
</tr>
<tr>
<td>Control</td>
<td>12</td>
<td>7:5</td>
<td>5</td>
<td>1.50 ± 1.4</td>
<td>8.08 ± 0.7</td>
<td>10.25 ± 0.5</td>
<td>80 ± 80</td>
</tr>
</tbody>
</table>

**Study Protocol**

The unsedated piglets were presented with IHH for a total time of 48 minutes each day for 4 days. Physiological data were recorded on Day 1 and Day 4, and analysed for sleep, BP and arousal responses. Results were also compared to those of a group of age- and sex-matched control piglets that underwent all features of the same study protocol except that gas exposures always consisted of fresh air. The study environment was as described in Chapter 2. The same techniques and protocols were used on Day 1 and Day 4 for all parameters.

Briefly, cardiorespiratory and sleep/wake variables were recorded for 53 min, including a 5 min baseline (BL) recording breathing air (21% O₂ / balance N₂). The first (Day 1) and final (Day 4) days were recorded for analysis of sleep, cardiovascular and arousal responses. In general, the studies were at the same time of day for each animal and in a normally dark or 'sleep' time. Immediately prior to each gas change, blood was sampled via the catheter for arterial blood-gas (ABG) analysis.

**Data Analysis**

In the IHH-Treated group, 1 piglet was excluded from the analysis; after completing the protocol, because the raw signals were corrupted. All analyses were performed on the remaining 11 piglets. Unless specified otherwise, all analyses were performed on the group of 12 Control piglets.
Each piglet yielded a score for W, REM and NREM in each cycle. Data were normalised to yield a proportion of the Recovery cycle when examining total sleep time (TST) or a proportion of TST when we examined REM and NREM. Comparisons between IHH-Treated and Control scores were made using one-way ANOVA with repeated measures. The analyses were performed on the group of 10 IHH-Treated piglets against 8 Controls. Animals in which the one of the signals was not satisfactory for accurate sleep scoring were excluded. Differences in responses between study days, and in each cycle on Day 1 compared to each cycle on Day 4 in each group were analysed with two-way ANOVA.

Each piglet yielded a score on Day 1 and on Day 4 for arousal latency after presentation of the noxious stimulus of HH in each cycle of HH. Differences in responses between study days, and in each cycle on Day 1 compared to each cycle on Day 4 in HH were analysed with two-way ANOVA.

Each piglet yielded a score for the number of spontaneous arousals in BL and in Recovery for the IHH-Treated group and the entire study for the Control group. Data were normalised to a frequency of spontaneous arousals min\(^{-1}\). Comparisons of spontaneous arousal frequency between the BL and the Recovery cycles on each study day of the IHH-Treated piglets were made using two-way ANOVA. A comparison of the arousability of the Controls on each study day was made with a paired-sample two-tailed t-test.

The BP signal in the first 300 s (BL) and last 300 s (Final Recovery) of each study was analysed in 15 s epochs to yield mean values for those periods. Differences in mean BP in the BL and the Final Recovery periods on each study day, and for the same cycle between study days within each group were tested for with two-way ANOVA. There were complete data for 10 IHH-Treated piglets and 10 Controls only.

Core temperature, arterial gases, pH, base excess and bicarbonate levels were analysed for changes over the 53 min study on each study day, and compared using two-way ANOVA. Temperature data were complete for 11 IHH-Treated piglets and
11 Controls, with the remaining variables available in 10 IHH-Treated piglets and 10 Controls.

**Results**

**Arousal Latency**

The time to arouse in successive cycles on each study day increased (p<0.001) and the time to arouse in each cycle was greater on Day 4 compared to the same cycle on Day 1 (p<0.001, two-way ANOVA). Table 4.2. Figure 4.1.

<table>
<thead>
<tr>
<th>HH Cycle</th>
<th>STUDY DAY</th>
<th>CYCLE 1</th>
<th>CYCLE 2</th>
<th>CYCLE 3</th>
<th>CYCLE 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>16.87 ± 6.7</td>
<td>30.10 ± 16.3</td>
<td>33.78 ± 22.0</td>
<td>41.69 ± 27.3</td>
<td></td>
</tr>
<tr>
<td>Day 4</td>
<td>14.36 ± 8.5</td>
<td>52.80 ± 48.2</td>
<td>89.38 ± 66.1</td>
<td>84.09 ± 57.5</td>
<td></td>
</tr>
</tbody>
</table>

*Table 4.2: Arousal latency times in IHH-Treated group. Data is presented as mean ± SD s*

**Spontaneous Arousals from Sleep**

There were significantly more spontaneous arousals on Day 4 compared to Day 1, in the Controls (p=0.03), and during baseline for the IHH-Treated group. However, in the Recovery periods after IHH, there were 90% fewer arousals on Day 4, whereas on Day 1 there were 64% fewer arousals in the Recovery periods after HH compared to BL (p=0.05 by Study Day, p<0.001 by Cycle). Table 4.3 presents the data. Figure 4.2.

<table>
<thead>
<tr>
<th>STUDY DAY</th>
<th>CONTROLS</th>
<th>IHH-TREATED BASELINE</th>
<th>IHH-TREATED RECOVERY</th>
<th>DIFFERENCE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>0.26 ± 0.1</td>
<td>0.35 ± 0.3</td>
<td>0.13 ± 0.1</td>
<td>64</td>
</tr>
<tr>
<td>Day 4</td>
<td>0.50 ± 0.3</td>
<td>0.65 ± 0.3</td>
<td>0.07 ± 0.2</td>
<td>90</td>
</tr>
</tbody>
</table>

*Table 4.3: Spontaneous arousal rates. Data presented as mean ± SD min⁻¹.*
Sleep

There was no difference between the TST on Day 4 compared to Day 1 in either the IHH-Treated or the Control group. There was a non-significant trend for the IHH-Treated piglets to spend more of their TST in each successive cycle in NREM on Day 4 compared to Day 1. Figures 4.3 and 4.4.

Proportion of Recovery Asleep

On Day 4, the IHH-Treated piglets spent an increasing proportion of each successive Recovery cycle asleep, with the proportion of TST (%) increasing in each cycle: 75.85 ± 8.8 (mean ± SD), 93.52 ± 5.7, 93.58 ± 6.3, 95.26 ± 3.0 and 97.83 ± 5.6 (p=0.02 between cycles; one-way ANOVA). The individual variability was significant. The proportion of TST (%) over successive cycles for control piglets was: 85.63 ± 20.8, 87.34 ± 12.2, 84.13 ± 20.8, 89.94 ± 13.3 and 88.00 ± 12.2 (p=0.004 between cycles with one-way ANOVA). Figure 4.3A. When we compared TST on Day 4 with Day 1, there was no difference between the TST on Day 4 compared to Day 1 in either the IHH-Treated or the Control group. Figure 4.4A.

REM Sleep

There was no difference in the proportion of TST in each cycle spent in REM by the IHH-Treated piglets. However, the Controls spent an increasing proportion of their TST in REM (%): 2.39 ± 3.5, 4.40 ± 7.8, 5.42 ± 11.8, 13.59 ± 18.7 and 8.30 ± 14.7 (p=0.0001, one-way ANOVA). Figure 4.3B. There was a non-significant trend for the IHH-Treated piglets to spend less of their TST in each successive cycle in REM on Day 4 compared to Day 1 (p=0.07). Figure 4.4B.

NREM Sleep

There was no difference in the proportion of TST in each cycle spent in NREM by the IHH-Treated piglets. However, the Controls spent a decreasing proportion of their
TST in NREM (%): 97.61 ± 3.5, 95.60 ± 7.8, 94.58 ± 11.8, 86.41 ± 18.71 and 91.71 ± 14.71 (p=0.0001, one-way ANOVA). Figure 4.3C. There was a non-significant trend towards the IHH-Treated piglets spending more of their TST in each successive cycle in NREM on Day 4 compared to Day 1 (p=0.07). Figure 4.4C.

**Blood Pressure**

For IHH-exposed piglets, mean BP on Day 4 was lower than the BP on Day 1 (p=0.03). Exposure to IHH appeared to induce acute hypertension, with mean BP in Recovery elevated compared to the BP at BL on both study days (p=0.001). There was no change in BP either between days or cycles in the Control group. Figure 4.5.

**Core Temperature**

All piglets dropped their core body temperature over time significantly (p<0.001 between cycles). However, there was no difference between study days in the IHH-Treated group. In contrast, body temperature did not drop as much on Day 4 compared to Day 1 for the Control group (p<0.001). Figure 4.6.

**Pco₂**

Pco₂ was elevated during the HH cycles but recovered to BL levels during the intervening Recovery cycles. Figure 4.7. The increase in Pco₂ from BL 37.08 ± 5.6 mm Hg was equivalent on Day 4 to Day 1 (peak level 59.36 ± 7.1 mm Hg in the first HH cycle). Two-way ANOVA showed no difference over time on each study day or between Day 1 and Day 4. In the Recovery cycles, Pco₂ values were lower on Day 4, and fell from 37.08 ± 5.6 mm Hg to 29.55 ± 4.2 mm Hg in the third Recovery cycle. Two-way ANOVA showed a difference over time on each study day (p=0.006) and a difference between Day 1 and Day 4 for the IHH exposed animals (p=0.003). The controls showed no change in Pco₂ over time or between days.
Chapter 4: Chronic Effects of IHH

**Po$_2$**

$Po_2$ fell during the HH cycles but recovered to BL levels during the intervening Recovery cycles. Figure 4.8. In the HH cycles, on Day 4, $Po_2$ fell from 114.90 ± 16.2 mm Hg to its lowest level of 42.61 ± 3.8 mm Hg in the final HH cycle. Two-way ANOVA showed no difference over time. In the Recovery cycles, on Day 4, $Po_2$ at BL was 114.90 ± 16.2 mm Hg. Two-way ANOVA showed no difference over time on each study day or between Day 1 and Day 4 for the Controls or for the IHH exposed piglets.

**pH**

$pH$ fell over the course of the 53 min study on both study days. Figure 4.9. In the HH cycles, on Day 4, $pH$ was lower and fell from 7.37 ± 0.0 to 7.00 ± 0.1. Two-way ANOVA showed a difference by study day ($p=0.01$) and a difference over time ($p=0.04$). In the Recovery cycles, on Day 4, $pH$ fell from 7.37 ± 0.0 to 7.17 ± 0.1. Two-way ANOVA showed no difference by study day but a difference over time for the IHH-exposed piglets ($p<0.001$). There was no change over time or between study days for the Controls.

**Base Excess**

Base Excess (BE) fell during the HH cycles and did not recover to BL levels during the intervening Recovery cycles. Figure 4.10. In the HH cycles, on Day 4, BE was lower than Day 1, and fell from −3.28 ± 1.5 mmol/l to the lowest level of −17.27 ± 3.6 mmol/l in the final HH cycle. Two-way ANOVA showed a difference over time on each study day ($p=0.01$) and between Day 1 and Day 4 ($p=0.006$). In the Recovery cycles, on Day 4, BE remained lower than Day 1 and fell from −3.28 ± 1.5 mmol/l to −16.21 ± 4.0 mmol/l in the final cycle. Two-way ANOVA showed a difference over time on each study day ($p<0.001$) and between Day 1 and Day 4 ($p=0.01$). There was no change in BE in the Controls either over time or between study days.
Bicarbonate

Bicarbonate (HCO₃⁻) fell after the first HH cycle and fell further in the first Recovery cycle. From then on, the levels actually recovered towards the BL levels in HH and fell further in Recovery. Figure 4.11. In the HH cycles, on Day 4, HCO₃⁻ was lower than for Day 1, and fell from 20.22 ± 1.8 mmol/l to the lowest level of 12.89 ± 2.4 mmol/l in the final HH cycle. Two-way ANOVA showed a difference over time on each study day (p=0.02) and between Day 1 and Day 4 (p=0.004). In the Recovery cycles, on Day 4, HCO₃⁻ was lower than for Day 1, and fell from 20.22 ± 1.8 mmol/l to 10.57 ± 2.7 mmol/l in the final cycle. Two-way ANOVA showed a difference over time on each study day (p<0.001) and between Day 1 and Day 4 (p<0.01).
Discussion

This study demonstrated that when exposure to a HH stimulus is repeated intermittently, arousal deficits become more severe. In addition, a cumulative arousal deficit was induced by daily exposure to IHH, despite some maturation in physiological functions between study days.

**Arousals**

In successive cycles of HH, the time to arouse on each study day increased significantly, and the time to arouse in each cycle, after the first, on Day 4 compared to the same cycle on Day 1 also increased. The arousal latency in Cycle 1 on Days 4 and 1 were equivalent, but a cumulative deficit was subsequently apparent, suggesting that overnight recovery provides only partial recovery of arousal responses. It is probable that the level of chemoreceptor stimulation increased as time into the exposure elapses, so the level of respiratory chemostimulation required to produce an arousal response from sleep also appeared to be significantly greater.

There were significantly more spontaneous arousals on Day 4 compared to Day 1, in the Controls, as a model of normal development. The IHH-Treated piglets parallel this, having more spontaneous arousals during the BL period on Day 4 compared to Day 1. However, in the Recovery periods after IHH, there was a 90% reduction in arousability on Day 4 compared to Day 1 (64%). These data suggest, as in the arousal latency data, an overnight recovery period permits only partial recovery of the deficient arousal response.

**Sleep**

The changes in sleep parameters between Day 4 and Day 1 were best explained by the change in time of day when the studies were performed for both the IHH-Treated and the Control piglets. The IHH-Treated piglets spent an increasing proportion of each successive Recovery cycle asleep, but there was no difference in TST between the study days. Since Control piglets also showed an increase in the time they spent asleep over time on Day 4, this suggests that the sleep deficit induced by IHH was not cumulative over days. This may suggest that chronic exposure to IHH interrupted
the normal developmental process or that a change in their TST was not detected because of some change in the time of study between days. Although we attempted to maintain consistency of all the variables between the study groups except for their HH exposure, there was a significant variation for study time both within groups and between study days. See Table 4.4 below.

<table>
<thead>
<tr>
<th>STUDY DAY</th>
<th>CONTROLS</th>
<th>IHH-TREATED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>5:86 ± 2:4</td>
<td>8:00 ± 2:4</td>
</tr>
<tr>
<td>Day 4</td>
<td>4:19 ± 1:2</td>
<td>6:02 ± 1:6</td>
</tr>
</tbody>
</table>

Table 4.4: The study times. Data are presented as mean ± SD in h:min. The times have been adjusted to allow for the shifted day/night cycle that the piglets were acclimatized to. Two-way ANOVA showed variation by group (p<0.01) and between study days (p=0.001).

On Day 4, the Control piglets were studied at the point of their circadian cycle equivalent to 4 am, which is a normal period of sleep so it is not surprising to see their tendency to sleep was pronounced compared to Day 1, where their normal night was drawing to an end and wakefulness becoming prominent. On Day 4 compared to Day 1, there was no difference in the proportion of TST in each cycle spent in NREM and REM by the IHH-Treated piglets. However, the Controls spent an increasing proportion of their TST in REM and a corresponding decreasing proportion of their TST in NREM. These results suggest that the variations in study time contributed significantly to the changes in sleep architecture observed, and make it more difficult to determine which changes were due to IHH exposure.

It remains possible that the IHH-Treated piglets adapted their sleep architecture to compensate for the induced sleep-deprivation of HH, such that the redistribution of states that were seen on Day 1 was no longer apparent. Different forms and stages of development and memory formation might benefit from different stages of sleep and be subserved by different forebrain regions. Peigneux et al have presented data to support the idea that sleep is deeply involved in neuroplasticity in humans, and that this is dependent upon both REM and NREM sleep processes (Krueger et al. 1995, Peigneux et al. 2001).
Blood Pressure

The fall in BP was unexpected, particularly because of the acute increase in BP after exposure to HH. Mean BP was elevated after IHH, in the Final Recovery cycle compared to BL on both study days. However, the mean BP at BL on Day 4 was lower than the BP at BL on Day 1. There was also no change in BP either between days or amongst cycles for the Control group. As models of normal development, we would have expected the Control blood pressure to rise from Day 1 to 4 as the piglets matured. As with the data pertaining to arousability and normal maturation, we expected the blood pressure at BL in the IHH-Treated piglets to also rise from Day 1 to 4. Again, the variations in study times may have superimposed normal circadian variations in BP over the effects of the HH exposures.

BP exhibits a circadian rhythm composed of a diurnal and a nocturnal period (Guo and Stein 2003, Portman 2003). The rhythms are influenced by the 24 hour biological clock, as reflected by the periods of wake and sleep. During sleep, BP falls progressively, to its lowest values during NREM. BP tends to rise during REM, but still remains lower than wake values (Guo and Stein 2003, Portman 2003). It is possible that the well-established ‘early morning surge’ in BP is apparent in the Control group at BL on Day 1 but not in the IHH-Treated group who were studied, on average, later in the day, and possibly explaining the difference in their BP results. Although during infancy and childhood, BP is strongly influenced by gender and age (Wuhl et al. 2002), subgroup analyses showed no differences according to sex in this study.

It is possible that the lack of change in systemic BP was due to an effect of the IHH treatment. It is possible that in parallel with the lack of maturing thermoregulation that was apparently due to the treatment, chronic IHH interrupted normal developmental processes that would have seen a rise in BP. Alternatively, it is possible that the interval of four days was not long enough to track developmental changes in BP.

Arterial Blood-Gases

The changes in Po$_2$ and Pco$_2$ reflect the inspired levels of O$_2$ and CO$_2$. On Day 4, Po$_2$ fell during the HH exposures, and recovered to BL levels during the intervening
Recovery periods on both days, but with no statistical difference over time on either study day or between Day 1 and Day 4. Pco₂ was elevated during the HH exposures, and in Recovery fell below BL levels. In hypercapnia, bicarbonate falls and carbonic acid rises, lowering the pH (Brensilver and Goldberger 1996). When pH falls, there is marked hyperventilation mediated by the peripheral chemoreceptors. In a large drop in pH, the blood-brain barrier becomes partly permeable to H⁺ ions and the central chemoreceptors or the respiratory centre itself is affected (Brensilver and Goldberger 1996). The excessive hyperventilation in Recovery suggests that the respiratory system was overcompensating for the impaired gas exchange represented by the HH cycles, and that IHH had induced some overactive compensatory mechanism where the chemostimulation of HH after chronic IHH induced excessive hyperventilation.

Respiratory acidosis became progressively more severe on each day. Respiratory acidosis is due to an increase in Pco₂ in arterial blood, and describes the accumulation of CO₂ and carbonic acid in the body and a drop in arterial pH. Primary respiratory acidosis is due to ineffective gas exchange, by either hypoventilation or inspiration of a hypercapnic gas mixture. The respiratory acidosis over time was only statistically significant on Day 4, when the fall in pH was more marked. With each successive cycle of HH and Recovery, the pH levels fell further and recovered less adequately. This suggests incomplete compensation for the H⁺ imbalance with repeated exposure to HH, and a further impairment of this compensatory mechanism after chronic exposure to IHH.

Base Excess (BE) fell progressively over time on both study days, and the fall in BE levels on Day 4 was more pronounced than that on Day 1. Bicarbonate (HCO₃⁻) levels also failed to recover, and HCO₃⁻ levels fell further in subsequent Recovery cycles, with the pattern further exaggerated on Day 4. These biochemical profiles suggest that only partial compensation of the primary respiratory acidosis was possible. Persistent respiratory acidosis in mammals triggers compensatory mechanisms. In the kidneys, there is increased secretion and excretion of H⁺ and ammonium formation is stimulated and ammonium ions are excreted. Bicarbonate is retained and chloride ions are excreted instead; this causes an increased serum bicarbonate concentration and a decreased serum chloride concentration. The blood buffers react with the carbonic acid and form more basic salts. The
bicarbonate/carbonic acid ratio and the pH may rise toward normal to produce a partially compensated respiratory acidosis. The arterial P$_{CO_2}$ level remains high (West 1995, Brensilver and Goldberger 1996). In this study, there was an overall fall in bicarbonate levels and progressive fall in pH levels. It is possible that at the intervals used in this study, repeated ventilatory insults were associated with less adequate compensation, rather than habituation, during this period of early development (Waters and Gozal 2003).

In summary, after four days of IHH, mimicking the recurrent physiological perturbations of chronic OSA, we have seen more severe changes in the chemostimulation threshold, especially at the central level, since pH changes were more severe over the time course where central chemoresponses would be activated, at the same time as arousal responses were suppressed.
Figure 4.1: Arousal latency. Comparisons of the time to arouse on Day 1 and Day 4 after presentation of IHH (mean ± SD). The time to arouse in successive cycles on each study day increased and the time to arouse in each cycle on Day 4 compared to the same cycle on Day 1 was increased. Legend: □=Day 1, ■=Day 4.

Figure 4.2: Frequency of spontaneous arousals from sleep. Comparisons between Day 1 and Day 4 scores during the BL and Recovery for the IHH-Treated group and the entire study period for the Control group (mean ± SD). In the Recovery periods after IHH, there were less arousals on Day 4 (90%) compared to Day 1 (64%). Legend: □=Day 1, ■=Day 4.
Figure 4.3: Sleep architecture on Day 4. Shows the mean ± SD for A: the proportion of TST of the Recovery cycle or a proportion of TST when we examined B: REM and C: NREM. Legend: ■ = IHH-Treated, □ = Control. See text for statistical analyses.
Figure 4.4: Sleep architecture of IHH-Treated group on Day 1 vs Day 4. Shows the mean ± SD for A: the proportion of total sleep time (TST) of the Recovery cycle or a proportion of TST when we examined B: REM and C: NREM. Legend: □=Day 1, ■=Day 4. See text for statistical analyses.
Chapter 4: Chronic Effects of IHH

A  
Sleep in Recovery

B
REM

C
NREM

Proportion (%)
Figure 4.5: Blood pressure. Comparisons of the BP at the BL and final Recovery periods on Day 1 and Day 4 (mean ± SD). After IHH, BP in Recovery was elevated when compared to the BP at BL on both study days. Legend: □ = BL in IHH-Treated piglets, ■ = Recovery in IHH-Treated piglets, ▣ = BL in Control piglets, ▦ = Recovery in Control piglets.

Figure 4.6: ∆ Core Temperature. The change in core temperature over the 53 min study from BL. Results are presented as mean. Error bars have been omitted for clarity. Legend: ○ = IHH-Treated, Day 1, ● = IHH-Treated, Day 4, □ = Control, Day 1, ■ = Control, Day 4.
Chapter 4: Chronic Effects of IHH

After IHH: Over time: $p=0.001$
Between days: $p=0.03$

Over time: $p<0.0001$ for both groups
Between study days: $p<0.0001$ in Controls
Figure 4.7: $P_{CO_2}$. A: $P_{CO_2}$ over the study, comparing the IHH-Treated group with the Control group (mean). Error bars have been omitted for clarity. Legend: $\bigcirc = \text{IHH-Treated, Day 1}$, $\bullet = \text{IHH-Treated, Day 4}$, $\square = \text{Control, Day 1}$, $\blacksquare = \text{Control, Day 4}$. B: $P_{CO_2}$ of the IHH-Treated group presented as $P_{CO_2}$ after time in minutes into HH or Recovery (mean ± SD). Legend: $\bigcirc = \text{HH, Day 1}$, $\bullet = \text{HH, Day 4}$, $\square = \text{Recovery, Day 1}$, $\blacksquare = \text{Recovery, Day 4}$. 
Figure 4.8: Po$_2$. A: Po$_2$ over the study, comparing the IHH-Treated group with the Control group (mean). Error bars have been omitted for clarity. Legend: ○ = IHH-Treated, Day 1, ● = IHH-Treated, Day 4, □ = Control, Day 1, ■ = Control, Day 4. B: Po$_2$ of the IHH-Treated group presented as Po$_2$ after time in minutes into HH or Recovery (mean ± SD). Legend: ○ = HH, Day 1, ● = HH, Day 4, □ = Recovery, Day 1, ■ = Recovery, Day 4.
Figure 4.9: pH. A: pH over the study, comparing the IHH-Treated group with the Control group (mean). Error bars have been omitted for clarity. Legend: ○ = IHH-Treated, Day 1, ● = IHH-Treated, Day 4, □ = Control, Day 1, ■ = Control, Day 4. B: pH of the IHH-Treated group presented as pH after time in minutes into HH or Recovery (mean ± SD). Legend: ○ = HH, Day 1, ● = HH, Day 4, □ = Recovery, Day 1, ■ = Recovery, Day 4. See text for statistical analyses.
Chapter 4: Chronic Effects of IHH

A

Control

IHH-Treated

pH

Time into Study (min)

7.45
7.40
7.35
7.30
7.25
7.20
7.15
7.10
7.05
7.00
6.95

0 6 12 18 24 30 36 42 48

B

Recovery

HH

Time into HH or Recovery (min)

7.50
7.40
7.30
7.20
7.10
7.00
6.90

0 6 12 18 24
Figure 4.10: Base Excess (BE). A: BE over the study, comparing the IHH-Treated group with the Control group (mean). Error bars have been omitted for clarity. Legend: ○ = IHH-Treated, Day 1, ● = IHH-Treated, Day 4, □ = Control, Day 1, ■ = Control, Day 4. B: BE of the IHH-Treated group presented as BE after time in minutes into HH or Recovery (mean ± SD). Legend: ○ = HH, Day 1, ● = HH, Day 4, □ = Recovery, Day 1, ■ = Recovery, Day 4. See text for statistical analyses.
Figure 4.11: Bicarbonate (HCO₃⁻). A: HCO₃⁻ over the study, comparing the IHH-Treated group with the Control group (mean). Error bars have been omitted for clarity. Legend: ○ = IHH-Treated, Day 1, ● = IHH-Treated, Day 4, □ = Control, Day 1, ■ = Control, Day 4. B: HCO₃⁻ of the IHH-Treated group presented as HCO₃⁻ after time in minutes into HH or Recovery (mean ± SD). Legend: ○ = HH, Day 1, ● = HH, Day 4, □ = Recovery, Day 1, ■ = Recovery, Day 4. See text for statistical analyses.
Chapter 4: Chronic Effects of IHH

![Graph A: Bicarbonate Levels over Time]
- **Control** line shows relatively constant bicarbonate levels.
- **IHH-Treated** line shows a decrease in bicarbonate levels over time.

![Graph B: Bicarbonate Levels during HH and Recovery]
- **HH** phase shows a decrease in bicarbonate levels with variability.
- **Recovery** phase shows stabilization and slight increase in bicarbonate levels.

Time into Study (min):
- 0, 6, 12, 18, 24, 30, 36, 42, 48

Bicarbonate (mmol/l):
- 10 to 22

Time into HH or Recovery (min):
- 0, 6, 12, 18, 24
Chapter 5: General Discussion
The most important finding in this study was that a cumulative arousal deficit was induced by daily repetitions of an intermittent respiratory stimulus during early development of the respiratory control system, whether this was measured after acute or chronic exposure.

An acute response to successive cycles of HH saw the time to arouse in each cycle increased. This arousal latency also displayed a chronic deficit, in that the time to arouse was increased on Day 4, after four consecutive days of IHH exposure, compared to the same cycle on Day 1.

Possible mechanisms to explain the arousal deficit have been elaborated earlier in this thesis and will be discussed in the framework of:
1. Habituation of the arousal response in both acute and chronic timeframes
2. Plasticity of respiratory sensitivity, causing changes in the chemostimulation threshold required to induce arousal
3. Depression of spontaneous arousability following return to normal inspired gases, in both acute and chronic settings, and
4. Dormancy of the arousal deficit in the chronic setting, since the above deficits were not apparent in animals exposed to chronic IHH, until the stimulus had been repeated.

**Habituation of the Arousal Response**

The arousal response decrement observed in this study may be due to habituation of the arousal process. Features of habituation that we observed included a decreased arousal response to the repeated respiratory stimulus, functional recovery of the arousal response when the stimulus presentations ceased, and a repeat of the habituation process when the stimulus was presented again later.

Acute habituation of the arousal response was seen in the study described in Chapter 3. We saw a cumulative depression in arousability after four episodes of HH interrupted with recovery periods of normal ventilation. It is likely that HH provoked arousal through stimulation of the chemoreceptors responsive to hypoxia and hypercapnia. Repeated exposures to HH lengthened the time to arouse. In Chapter
4, after three consecutive days of exposure to IHH, the arousal response seemed to have recovered, since the time to arouse after the first HH exposure on Day 4 was equivalent to that on Day 1. However, habituation was again initiated, since a cumulative depression was seen when HH exposures were repeated. However, the times to arouse in each cycle on Day 4 were longer than those in the same cycle on Day 1, so an underlying cumulative deficit was revealed, apparently dormant until provoked by the next exposure. This phenomenon may be attributable to plasticity of the CNS at this stage of development.

McNamara et al showed that there was serial habituation of arousal responses from the cortical to the spinal level during sleep (McNamara et al. 1999). In earlier studies McNamara et al replicated findings by Lijowska et al, characterizing the infant arousal response. The arousal response consists of stereotypical behaviours involving a progression of CNS activation from the spinal to cortical levels. The sequence of brainstem responses progressing through to functional awakening was thought to be endogenously regulated. Although the studies used different stimuli to provoke arousal: one respiratory and the other tactile and non-respiratory, the arousal sequences elicited were equivalent (Lijowska et al. 1997, McNamara et al. 1998). Our study was designed to evaluate cortical arousals only, but it is fair to assume that having seen habituation of the cortical processes, habituation of subcortical processes could also occur.

The fundamental properties of a behaviour undergoing habituation are reviewed by McSweeney and Murphy (McSweeney and Murphy 2000), and McSweeney and Swindell (McSweeney and Swindell 2002). Features of our data that include accepted characteristics of habituation include: increases in a response decrement with repetition of the stimulus; recovery of native behaviour after removal of the stimulus; long-term habituation where habituated behaviour does not fully recover to native levels of responsiveness and the habituated behaviour dominates when the stimulus is presented again later. This also implies that as long-term habituation accumulates, the recovery of the native response is cumulatively depressed, with the response decrement following a negative exponential equation. Typically, for a dataset to qualify for this criteria, it needs to include 8 points, however since our data does not, the equation cannot be applied, but the trend is apparent. Specificity of the
stimulus is another factor, where despite timely responses to other irregular stimuli (such as noise and light) the response decrement to HH was apparent.

One feature against the habituation hypothesis is that the habituation phenomenon would predict that the presence of stimuli from another modality, such as light and noise, should produce sensitization to HH. Our study environment clearly allowed irregular stimulation of the piglets by light and noise in the course of the IHH exposure, however the response decrement to HH persisted.

To test this hypothesis further, studies would test whether a change in context (such as removal from the study environment) would lead to renewal of the native response to HH exposure, and whether reinstatement in the study environment would see rehabituation. Dishabituation might also be demonstrable, where simultaneous delivery of another stimulus with HH would allow for reassertion of the native response to HH.

An alternative explanation for the arousal response decrement may be effector fatigue. Ventilatory effort was not measured in this study, so we are unable to assess fatigue of the respiratory muscles.

**Plasticity of Respiratory Sensitivity**

Another possible explanation for the response decrement to HH is plasticity of respiratory control, where respiratory plasticity is defined as a persistent change in the neural control system based on prior experience (Mitchell and Johnson 2003). The only variation between normal early postnatal development of the control piglets and our study group was exposure to HH to simulate respiratory compromise. Past concepts of respiratory control system maturation as rigidly predetermined by a genetic blueprint have now yielded to a different view in which extremely complex interactions between genes, transcriptional factors, growth factors, and other gene products shape the respiratory control system, and experience plays a key role in guiding normal respiratory control development (Carroll 2003).
Plasticity in neural respiratory control development can be seen as changes in the normal course of maturation of structure or function of the respiratory control neural network occurring in critical windows of vulnerability during ontogeny, caused by exposures such as IHH. Long-term or irreversible alterations in mature respiratory control may be induced by these exposures in critical or vulnerable times of development (Harper et al. 2000, Carroll 2003). Respiratory plasticity is made more likely by the fact that the piglets in this study were in such a crucial stage of neural development (Dickerson and Dobbing 1967, Dobbing and Sands 1979, Dobbing 1981). Development of the mammalian respiratory control system begins early in gestation and does not achieve mature form until weeks or months after birth (Hilaire and Duron 1999). This period of rapid development is a time of profound vulnerability to pre- and postnatal interactions with the environment, including experiences such as episodic or chronic hypoxia. The relative follow-up of the “chronic” exposure in these piglets is short compared to those studies, and longer follow-up or repeated studies after more than 24 hours recovery would be required to determine whether this is true plasticity.

The clearest indication of the impact of daily HH on normal maturation is that the Control piglets gained an ability to attenuate the fall in their core body temperature on Day 4 compared to Day 1. In contrast, the IHH-Treated piglets dropped their core body temperature to the same extent on both study days, suggesting that chronic IHH abolished control over normal metabolic processes or induced some maturational delay that caused their response to hypoxia to parallel their immature ‘neonatal’ response seen on Day 1. Whilst this is not a direct ‘respiratory’ response, it does support a change in the fundamental responses of the study piglets to the IHH stimulus itself. Mortola hypothesises that the normal neonatal hyperventilation response to hypoxia is achieved by a reduction in metabolic rate rather than by an increase in ventilation (Mortola 1999). This response is a regulated phenomenon largely based on inhibition of thermogenesis. Our findings suggest that the IHH treatment interrupted normal maturation and caused the IHH-Treated piglets to display a neonatal response to HH on Day 4.
Chapter 5: General Discussion

The Chemostimulation Threshold

It is possible that the IHH exposures during this vulnerable window impacted on the development of the respiratory control system and increased the chemoreceptor thresholds.

The combination of hypercapnia and hypoxaemia is a potent stimulus to arouse (Fewell and Konduri 1988). Parslow et al have shown in human infants that arousal to hypoxia occurs at similar levels of desaturation in both REM and NREM but that the rate of oxygen desaturation during hypoxia in REM was faster than in NREM, explaining the longer arousal latency seen in NREM (Parslow et al. 2003). Hypercapnia is a potent arousal stimulus (Dunne et al. 1992). Therefore, the longer latency to arousal in this study may reflect a similar change.

Hypercapnia stimulates arousal though the brainstem reticular activating system, resulting functionally in cortical arousal (Siesjo 1980). The arousal response invoked by respiratory stimuli is therefore intimately related to the ventilatory responses that the respiratory stimulus invokes.

Since the same levels and rate of change in inspired gases failed to induce arousal in the later exposures (acute or chronic), it is possible that the chemoreceptors themselves adapted to the hypoxic and hypercapnic levels of O$_2$ and CO$_2$. Evidence against this hypothesis is provided by Blanco et al, who showed that changes in the detection of O$_2$ levels by the peripheral chemoreceptors occur over 24-31 hours of continuous exposure to a new O$_2$ concentration (Blanco et al. 1988). The exposure of HH in this study was 24 min in total but not continuous, and so not directly comparable with Blanco’s protocol. Since sensory adaptation after presentation of an intermittent respiratory stimulus has not been evaluated, this mechanism is a possible explanation for the response decrement.

Other compensatory mechanisms to hypoxia are also activated, and may be influenced by the gas exposures used in this study. Whether they also influence arousal is less clear than for the chemoreceptors. However, the hypoxic neonate exhibits suppression of thermoregulation, manifesting as a fall in body temperature,
Chapter 5: General Discussion

and resulting in a reduction in metabolic rate (Singer 1999). There is also a reduction of blood pH to contribute to respiratory acidosis, inhibit enzymatic activity and contribute to metabolic slowing. A low pH will also have a vasodilatory effect on cerebral vasculature, contributing to the benefits of a redistributed blood flow to vital organs. Brain blood flow during NREM is directly correlated with PaCO$_2$ (Santiago et al. 1984). Since this predictable cascade of compensatory responses may be thrown awry when the hypoxic exposure is presented intermittently rather than as a sustained stimulus (Waters and Gozal 2003), it is possible that the normal metabolic responses to hypoxia are necessary for an intact arousal response, and the disruption of these processes also disrupted the initiation of arousal.

After reviewing this data, the question of whether the increased time to arouse was an induced abnormality or simply a result of habituation to the study environment arose. We studied spontaneous arousability to address this question.

**Depression of Spontaneous Arousability**

There were more spontaneous arousals on Day 4 compared to Day 1, in the Controls, and this was taken as our model of normal maturation for piglets. Piglets, and human infants, spend an increasing proportion of time in the awake state with age (Anders and Keener 1985, Scott et al. 1990, Galland et al. 1993). The IHH-Treated piglets parallel this, having more spontaneous arousals during the BL period on Day 4 compared to Day 1. However, in the Recovery periods after IHH, there was a reduction in arousability. On Day 1, the reduction in arousability was 64% from Baseline, on Day 4 habituation or development plasticity meant that the deficit was exaggerated (90%). As in the arousal latency data, after an overnight recovery period, the deficient arousal response only partially recovers to its baseline state, with the deficit apparently dormant until provoked by the next HH exposure.

**Dormancy of the Arousal Deficit**

The time to arouse in HH Cycle 1 on Day 4 was the same as that on Day 1, but thereafter, the cumulative deficit was exaggerated. Since an overnight recovery
period of normal ventilation leads to apparent recovery of arousability, the true deficit was not apparent until the next HH cycle.

Such a hidden defect fits with the hypothesis of vulnerable infants as a cause for SIDS. Disturbances of medullary raphe function during development can alter central chemoreception and normal sleep architecture, although it is not clear how they would be revealed in a physiological setting. If the medullary raphe were affected by the HH insult, this would influence arousability to respiratory stimuli, because stimulation by acidosis only occurs in the specific phenotypic subset of neurones within the medullary raphe that are serotonergic (Richerson et al. 2001).

A proposed mechanism for this association is through the dependence of the respiratory network on chemoreception of CO$_2$ and pH levels, via cholinergic relays. This is modulated by neurones in the rostral ventrolateral medulla and motoneurones mediated by endogenous serotonin, substance P, and catecholamines (Hilaire and Duron 1999). Serotonin is thought to play a crucial role, as one of the first neurochemicals to be expressed during ontogeny (Hilaire and Duron 1999, Ozawa and Takashima 2002) and is important in initiating and maintaining respiratory plasticity following intermittent hypoxia (Mitchell and Johnson 2003). The medullary raphe, within the ventromedial medulla, contains putative central respiratory chemoreceptors. The neurones of the medullary raphe project widely to respiratory and autonomic nuclei and contain three neurotransmitters known to stimulate ventilation, serotonin, thyrotropin-releasing hormone, and substance P (Wang et al. 2001). These serotonergic neurones initiate a homeostatic response to changes in PaCO$_2$ and intracellular pH that includes increased ventilation and modulation of autonomic function (Wang et al. 2001, Wang et al. 2002). Chemosensitivity of medullary raphe neurones increases in the postnatal period in rats, in parallel with development of respiratory chemoreception in vivo. An abnormality of serotonergic neurones of the ventral medulla has been identified in victims of sudden infant death syndrome (SIDS) (Sawaguchi et al. 2002b). The ventilatory response to CO$_2$ was altered when medullary raphe neuronal function was focally and reversibly inhibited in chronically instrumented newborn piglets (Messier et al. 2002).
The intermittent nature of the stimulus appears to have disrupted the predictable cascade of responses normally elicited by sustained hypoxaemia. Ventilatory, neurochemical and metabolic responses are possibly interrupted before they can fully compensate for the physiological disturbances (Waters and Gozal 2003). Physiological markers, such as pH, base excess and bicarbonate levels, suggest that at the intervals used in this study, the repeated insults were associated with less adequate ventilatory compensation. Neonatal hypoxia tolerance is also marked by adaptations, such as thermosuppression, reduction in neural energy consumption, lowering of HR and RR, the redistribution of metabolic demands from production to maintenance functions, the lowering of blood pH, all of which contribute to acute defence reactions to hypoxaemia (Singer 1999). However, these adaptive measures may not be adequate to fully compensate for prolonged or chronic intermittent hypoxic insults, and may eventually be a cause of, rather than protection from, hypoxic injury.

In the brain, the rates of synthesis of catecholamines and serotonin are increased in hypercapnia (Siesjo 1980). Chronic intermittent hypoxia (with or without hypercapnia) augments carotid body sensitivity to hypoxia and causes long-lasting activation of sensory discharge.

**Possible Implications for Infants**

The study described in Chapter 3 was designed to mimic first-time prone sleeping with associated ventilatory compromise during early development. It was demonstrated that four cycles of IHH over 48 minutes induced habituation of the arousal response to a noxious respiratory stimulus. Spontaneous arousability was depressed. Sleep states were redistributed, with an increase in sleep time and a tendency to spend more of that in NREM, and BP was elevated after acute IHH.

The prone sleeping position is a significant risk factor for SIDS (Harper et al. 2000). There is evidence that asphyxia, secondary to rebreathing of expired air in the prone sleeping position, with hypercapnic hypoxic alterations in blood-gas profiles that mimic those induced by OSA, might be causal in some or many SIDS deaths (Kemp et al. 1993, Kemp and Thach 1993, Waters et al. 1996b, Campbell et al. 1997, Kemp...
et al. 1998, Constantin et al. 1999, Harper et al. 2000). Galland et al and Kahn et al have shown that blunted arousal responses, together with altered sleep architecture and autonomic function are a feature of the prone sleeping position (Galland et al. 1998, Kahn et al. 2002). These findings were confirmed by Horne et al (Horne et al. 2002b).

Prone sleeping for the first time represents a 14-19-fold increased risk for SIDS (Côté et al. 2000). Côté et al found that 53% of SIDS victims who had not slept prone before died shortly after being placed to sleep in the prone sleep position. For a number of these infants, death occurred the first or second time that they slept prone.

Various mechanisms have been postulated to explain the increased risk of SIDS associated with prone sleeping, with impairment of arousal from sleep being a crucial component of many of these mechanisms. Horne et al (Horne et al. 2002b) reviewed the effects of prone sleeping on infant sleep architecture, arousability from sleep and cardiorespiratory control. Sleeping in the prone position has been shown to increase the amount of time spent sleeping, particularly time spent in NREM. Sleeping prone has been demonstrated to be associated with a reduced responsiveness to a variety of arousal stimuli. Such impairment of arousal has been demonstrated to be associated with changes in control of autonomic cardiac function. Normal infants that usually sleep prone frequently adopt the face-straight-down position, arouse (presumably in response to hypoxia and hypercapnia) and return to sleep (Waters et al. 1996b). An intact arousal response to initiate head movement is a vital protective mechanism. It is possible that infants that are unaccustomed to sleeping prone have a relative deficit in the arousal-head turning response or have encountered insurmountable environmental factors that prevent effective head turning (Waters et al. 1996b). Arousal is associated with increases in HR, BP and RR, while gross body movements occur to avoid the stimulus (Horne et al. 2002a, Horne et al. 2002b). Abnormal autonomic control may impair these normal arousal responses (Galland et al. 1998, Horne et al. 2002b, Kahn et al. 2002).

Chapter 4 confirmed the hypothesis that the depression of arousal responses, apparent after acute IHH, and would still be apparent after chronic IHH, and may in
fact be exaggerated. The presentation of IHH over four successive days models clinical situations such as OSA during early postnatal development.

A timely and intact arousal response is vital when one is confronted with respiratory compromise during sleep. In the context of obstructive apnoea, or other forms of airway occlusion, the objective of arousal is to re-establish patency of the compromised airway and reinstate effective ventilation. Arousal is therefore an important response to respiratory stimuli that allows the individual to initiate protective reflexes that are depressed in the sleep state (Fink 1961, Phillipson and Sullivan 1978). OSA has been documented in subsequent SIDS victims after retrospective examination of sleep study data (Kahn et al. 1992, Kahn et al. 2002, Sawaguchi et al. 2002b). In characterizing sleep/wake behaviour, there is a strong association of OSA with SIDS, and neuropathology on the same infants found apoptosis and synaptic plasticity of midbrain structures that are important in the control of respiration and arousal (Sawaguchi et al. 2002c), findings of chronic tissue hypoxia that were attributed to hypoxaemia resulting from repetitive airway obstruction. The link between ALTE and SIDS is undefined, however, a proportion of ALTE events appear to be precipitated by upper airway obstruction from which the infant failed to arouse until caregiver intervention.

A deficit in arousal responsiveness is considered a necessary abnormality for an infant to succumb to SIDS. It's hypothesised that victims of SIDS, who typically die quietly in their sleep, suffer as much or perhaps more from a defect in arousal than in ventilatory control (McCulloch et al. 1982, Hunt 1992, Kahn et al. 2002, Sawaguchi et al. 2002c). A defect in ventilatory control in itself would not be life threatening, but an arousal deficit would be, since a victim would be unable to respond appropriately to ventilatory compromise.

It has been shown that infants with diminished ventilatory responses to hypercapnia or hypoxia fail to arouse to hypercapnic or hypoxic stimuli (Hunt 1981). McCulloch et al observed that the level of respiratory chemostimulation required to produce an arousal response from sleep was significantly greater in ALTE infants than in normal infants (McCulloch et al. 1982). It appears that OSA underlies some cases of ALTE; studies have shown the presence of OSA in ~50% of infants who have presented
with an ALTE (Engelberts 1995, Guilleminault et al. 2000, Harrington et al. 2002). OSA in infants is correlated with depression of the arousability to subsequent ventilatory compromise (McNamara et al. 1996, Harrington et al. 2002). It is also claimed that treatment to alleviate OSA reverses this depression (McNamara and Sullivan 1999), suggesting that the deficit in the arousability response can be induced by repeated episodes of OSA. Other studies have corroborated this hypothesis, showing habituation of the arousability response after postnatal exposure to a noxious respiratory stimulus (Fewell and Konduri 1989, Ward et al. 1992, Harding et al. 1997, Johnston et al. 1998).

It is hypothesized that a brainstem abnormality is responsible for this dysfunction in arousability (Sawaguchi et al. 2002b, Sparks and Hunsaker 2002). It remains unanswered whether the brainstem abnormality is congenital and the result of a delay in the maturation process, or can be induced by environmental factors. Pathological studies of SIDS victims brain tissue have illustrated neuronal changes in the arousability pathway (Sawaguchi et al. 2002c). They further found that the duration, rather than the frequency, of OSA episodes were correlated with SIDS and adverse neuropathological findings (Sawaguchi et al. 2002b). The results of this study suggest that an environmental influence, such as prone sleeping, can induce arousability deficits in otherwise normal infants, and that no congenital defect needs to be invoked, to explain vulnerability to severe respiratory insults.

**Limitations of the Study**

The data is limited to a brief stimulus, delivered over a period of four days. Therefore, it is not possible to answer the question of ‘how severe’ such an arousability deficit might become. In addition, we did not study the sequence or time course over which the deficit develops or ‘recovers’, for example if IHH is subsequently avoided.

Habituation to the study environment may have contributed to our observations, but we have attempted to eliminate this element by studying a Control group of piglets undergoing exactly the same study procedures without HH exposure.
The study environment permitted other arousal stimuli (in addition to blood-gas disturbances). For example, auditory stimuli were produced by solenoid valves within the incubator, and visual stimulation was produced by the investigator’s hand entering the box to turn the three-way tap. Bao et al were able to show that chronic noise stimulation did not induce any sustained changes in the cardiovascular system (Bao et al. 1999) so we can assume that the auditory stimulation of the switching solenoid valves did not affect the outcome measures of this work.

The study is isolated to investigating the effects of blood-gas abnormalities, and not to all features associated with OSA or prone sleeping. Whilst this was a deliberate component of the study design, it limits the applicability to clinical settings. The episodic negative intrathoracic pressure changes of obstructed breathing were not replicated, except to the extent that the increase in ventilation would induce larger pressure swings. Observations by White et al suggest that any alterations in autonomic control after IHH are elicited by chemoreception of hypercapnic hypoxia and are independent of mechanoreceptor stimulation by variations in intrathoracic pressure (White et al. 1995b).

Arousal from sleep in response to upper airway obstruction, such as occurs in OSA, appears to involve more stimuli than the arterial blood-gas changes alone (Gleeson et al. 1990, Berry and Gleeson 1997), where the increasing respiratory effort of inspiring against an obstructed airway could stimulate mechanoreceptors that may also contribute to the arousal response (Kimoff et al. 1993). Mechanoreceptors in the upper-airway have been postulated to participate in the arousal response to OSA. Issa et al showed that arousal responses differed depending on whether they were induced by nasal or tracheal occlusions (Issa et al. 1987). It was concluded that the marked arousability after nasal occlusion compared to tracheal occlusion in sleeping dogs was due to the stimulation of glossopharyngeal mechanoreceptors (Hwang et al. 1984). In contrast, if stimulation of the olfactory system is thought to participate in the generation of arousal, then this would have been present in the animals included in this study (Berthon-Jones and Sullivan 1984).

Arousal definitions vary amongst studies. For the purposes of this study, an arousal was defined as a full cortical disturbance, with respiratory and movement
involvement, with no lower limit for duration. This method of scoring may underestimate the frequency of arousal compared to other studies. For example, infants arouse in a sequential manner, beginning with presumptive brainstem responses before cortical arousal, with subcortical arousals sometimes occurring without cortical arousal (Lijowska et al. 1997, McNamara et al. 1998).

Arousability may vary between sleep states, particularly REM and NREM, but sleep stages were not controlled at the onset of the stimulus used in these studies. In human infants arousal appears to be delayed in NREM compared to REM sleep. Parslow et al have shown in human infants that arousal to hypoxia was delayed in NREM, despite arousal occurring at similar levels of desaturation in both REM and NREM (Parslow et al. 2003). Another study in human infants showed an arousal deficit in NREM from 1 week to 6 months of age and a temporary arousal deficit in REM at 3 months of age (Newman et al. 1986, Newman et al. 1989). Studies are not all consistent in this finding however, and McNamara et al found a reduction in the number of spontaneous arousals during REM in infants (McNamara et al. 1996). It’s also been shown that obstructive apnoeas occur more commonly during REM (Kahn et al. 2002), and that infants with OSA had significantly fewer spontaneous arousals than did control infants in REM (McNamara et al. 1996). In animal studies, Harding et al and Johnston et al have also shown that arousal from sleep is state dependent (Harding et al. 1997, Johnston et al. 1998). They found that arousal from REM, but not NREM, sleep in response to the obstruction of respiratory airflow was transiently depressed during early postnatal development and that repeated obstructions and arousals also led to depressed arousal from REM sleep in neonatal lambs. Berthon-Jones and Sullivan showed in dogs that asphyxic arousal is delayed in REM compared to NREM (Berthon-Jones and Sullivan 1984). The study design required to examine this phenomenon is quite different from that used in the studies described within this thesis, since examination of this phenomenon would require that blood-gas disturbances be induced in different sleep states, and it seems likely that they would have to be of shorter duration.
Conclusion

These studies have demonstrated that cumulative arousal deficits can be induced after acute and chronic exposure to a noxious respiratory stimulus.

The arousal deficits that were demonstrated were complex, and included changes in the chemostimulation threshold, habituation, depression of spontaneous arousability, and dormancy, so that the deficit was not apparent until a subsequent HH exposure occurred.

Extrapolating these results to the clinical situation provides data consistent with studies indicating that repeated episodes of OSA induce arousal deficits in infants. The additional information from this thesis suggests that not all of the deficits would be demonstrable, without examining such infants during exposure to further (appropriate) arousal stimuli.


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