THE EFFECTS OF MILD HYPOXAEMIA ON HYPOGLOSSAL MOTONEURONE ACTIVITY IN NEONATES

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by

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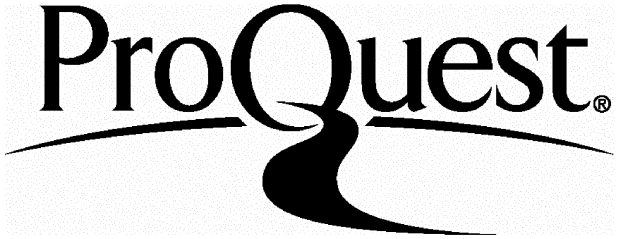

To

My husband Shiyi Zhou

and

daughter Liye Zhou

Dedicated

to

My Father Z.B. Li

and

My Mother Y.P. Shen

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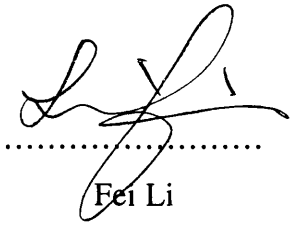
PERSONAL STATEMENT

The work in this thesis was performed solely by the candidate and is original.



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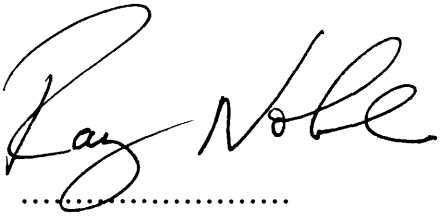

Dr. Ray Noble

(Supervisor)

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ABSTRACT

Introduction:

Apneic episodes and consequent hypoxaemia are common features of breathing in high-risk neonates. Apneas of central origin (no respiratory effort) usually terminate with an obstructive component due to collapse of the upper airway. The genioglossus muscle, the main protruder muscle of the tongue, plays a crucial role in maintaining upper airway patency by opposing the negative intra-airway pressure generated during contraction of the diaphragm and by preventing the tongue blocking the oropharyngeal opening.

In adults, the respiratory-related activity of the hypoglossal nerve (the motoneurone of the genioglossus) increases during hypoxaemia in order to maintain upper airway patency. However, in neonates it has been shown that the genioglossus muscle during hypoxia is age-related and this increased activity is not sustained.

In neonates, little is known about how the hypoglossal motoneurones respond to hypoxaemia and the role of hypoglossal motoneurones during hypoxia in the maintenance of upper airway patency.

Aim:

The aim of this study was to determine the effects of hypoxaemia on hypoglossal motoneurones in neonates.

Methods:

Extracellular and intracellular recordings were made from hypoglossal motoneurones

in vagotomized and vagi-intact neonatal kittens during normoxia and hypoxia.

Results:

The results showed: (1) the majority of hypoglossal motoneurones either decreased their discharge frequency or had only a transient increase during hypoxia. (2) During intracellular recordings, the membrane potential showed a sustained depolarisation during hypoxaemia in most cases and respiratory-related rhythmic EPSP activity was reduced in amplitude. The membrane impedance of these motoneurones increased and the excitability was reduced. (3) During upper airway stimulation, the amplitude of the laryngeal-evoked potentials was reduced during hypoxia.

Conclusions:

My results demonstrate that, in neonates, hypoglossal motoneurone activity is inhibited during hypoxia and the hypoglossal-upper airway reflexes are also inhibited.

The probable consequence of such inhibition, for the newborn human infant, would be the failure of the maintenance of upper airway patency, thus leading to obstructive apnea.

The mechanisms mediating the inhibition of hypoglossal motoneurones during hypoxia remain to be determined.

CHAPTER 1
INTRODUCTION

General Introduction

Upper airway stability is dependent upon the co-ordination of the upper airway muscles with the major muscles of breathing during respiration. It is thought that episodes of hypoxia compromise this co-ordination and increases the likelihood of obstructive apneic episodes in newborn babies. However, little is known about the effects of hypoxia, and the processes mediating the effects of hypoxia on the motoneurones controlling upper airway musculature.

Apnea (cessation of breathing ≥ 20 secs) is probably the most common respiratory event occurring in high-risk neonates (Marchal et al., 1987; Mathew et al., 1982a,b). Pauses in breathing of 2-10 sec are usual in preterm babies and often seen in term infants. These normal pauses are more common in active sleep and their frequency decreases with advancing postnatal age (Flores-Guevara, 1982; Hodgman & Hoppenbrouwers, 1983). Prolonged apnea has been defined as absent respiratory airflow for 20 sec or more in newborn babies (Rigatto, 1982; Schulte et al., 1977). Polygraphic studies have shown that central apneic episodes (no respiratory effort) usually terminate with an obstructive component due to collapse of the upper airway on resumption of respiratory effort. One possibility is that the consequent hypoxia of an apneic episode compromises airway stability.

The ability of certain respiratory-related muscles, such as the genioglossus, to dilate the airways is crucial for the maintenance of airway patency (Miki et al., 1989; Van Luteren & Strohl, 1986). Factors affecting their function would have profound effects on the air flow. (1) An imbalance of activity between the upper airway and chest wall muscles is involved in the genesis of obstructive apneas (Bruce et al., 1982; Carlo et al., 1985; Gauda et al., 1987). (2) Decreased upper airway muscle activity during sleep and infant immaturity leads to pharyngeal narrowing. (3) The increase in upper airway resistance increases transmission of intrathoracic pressure to the upper airway during inspiration. (4) The increase in intraluminal pressure promotes further pharyngeal narrowing. (5) A reduction of upper airway muscle activity relative to respiratory

pump muscle activity (such as diaphragmatic muscle), in response to the increased resistive load and chemical stimulation, results in pharyngeal closure. (6) Progressive hypoxia and hypercapnia and changing of pressure in the larynx during the ensuing apnea increases motor output to the respiratory pump muscles and upper airway muscles. (7) Upper airway re-opening occurs with arousal and a large increase in upper airway muscle activity.

The genioglossus muscle, the main protruder muscle of the tongue plays an important role in keeping the airway open. Respiratory-related activity of this muscle and its motor nerve, the hypoglossal, braces the airway against the negative pressure generated in the airway during inspiration. In this thesis I have examined this issue by investigating the effects of hypoxia on hypoglossal motoneurone discharge and membrane potential, and also the effects of hypoxia on laryngeal evoked potentials recorded in these motoneurones.

In neonates, hypoglossal motoneurones (HMNs) do not sustain the response to hypoxia. Studies in rat brain slices *in vitro* show that hypoxia alters the membrane excitability of hypoglossal motoneurones. The membranes are depolarized and there is an increased input resistance. This thesis investigated the hypothesis that the hypoglossal-genioglossus transient response to hypoxaemia in neonates is due to decreased hypoglossal membrane excitability.

The following questions will be addressed:

1) What are the effects of mild hypoxaemia on hypoglossal motoneurone output and discharge pattern?
2) Does hypoxaemia affect both inspiratory- and expiratory-related activity in these motoneurones?
3) What are the effects of systemic hypoxia on hypoglossal motoneurone membrane potentials and excitability?
4) How do these effects of hypoxia compare to the effects previously studied in

hypoglossal motoneurones recorded in *in vitro* brain slices?

5) What are the effects of hypoxaemia on laryngeal-evoked potentials recorded in hypoglossal motoneurones?

Overview of the introduction

This chapter is divided into four sections:

Section 1. Apnea in the newborn and respiratory function in response to hypoxia in neonates

Section 2. The role of the genioglossus muscle in maintaining the patency of the upper airway.

Section 3. Control of the genioglossus muscle-hypoglossal motoneuron and hypoglossal nerve.

Section 4. Laryngeal function in neonates and the response of hypoglossal motoneurones to upper airway stimulation in the neonate.

1. INTRODUCTION (SECTION 1.):
APNEA IN THE NEWBORN AND RESPIRATORY FUNCTION IN RESPONSE TO HYPOXIA IN NEONATES

1.1.1 General background

Sudden infant death syndrome (SIDS) is the leading cause of postnatal mortality in non-third world countries (Poets & Southall, 1994), there were about 1500 deaths per year in the U.K. and 5500 in the United States during the late 1980s. It is a common cause of death between the age of 1 month and 1 year with a peak at 2-4 months of postnatal age, 74% of deaths occur between these ages. Pathological apnea during sleep has been suggested as a mechanism for SIDS (Baker & McGinty, 1979). Most SIDS victims die during the night while asleep (Kahn et al., 1984). Both short ($\geq$2 sec) and more prolonged ($>$15 sec) sleep apneas have been observed in infants that subsequently died of SIDS. In human babies, brief apneas during sleep are observed in virtually all normal infants. Prolonged apneas are most commonly observed in preterm infants (Rigatto, 1982; Schulte et al., 1977).

Apnea has been classified according to whether both nasal airflow and breathing efforts cease (central apnea), breathing efforts continue but with no airflow (obstructive apnea), or both of the above occur during an episode (mixed apnea). Evidence has shown that obstructive and mixed apneas were more common than central apneas (Thach, 1983). Several studies have shown a history of hypoxemia in SIDS victims (Marchal et al., 1987; Mathew et al., 1982a). A crucial question is whether the hypoxaemia causes the apnea, or is itself caused by the apneic episodes? Hypoxia may interact with respiratory control systems to cause apnea (Baker & McGinty, 1979). Prolonged cessation of respiratory efforts may cause hypoxaemia; this mechanism may be of key importance in the majority of SIDS cases. The respiratory response to hypoxia and hypercapnia will be discussed below when brain stem inhibitory mechanisms are considered.

1.1.2 The behavioural states of the newborn

Apneic episodes during sleep may form part of the aetiology of SIDS. Clinical results show that most SIDS victims die during the night (Kahn et al., 1984). Furthermore, both short (≥2 sec) and more prolonged (> 15 sec) sleep apnea were observed in infants that subsequently died of SIDS (Kahn et al., 1984). The relationship to sleep state is controversial, perhaps due to difficulties in sleep-state determination in preterm infants. One group emphasizes the occurrence in active sleep, particularly during periods of maximal spinal inhibition (Schulte, 1977); another claims it is more common in quiet sleep (Kraus et al., 1977). In infants, several sleep states are usually recognized: 1) active sleep (called rapid eye-movement sleep, REM); 2) quiet sleep (called non-REM, NREM, or slow-wave sleep); 3) indeterminate sleep; and 4) waking (sometimes separated into crying, active, and quiet). In animals, the distribution of sleep-wakefulness in a given species depends on the degree of maturity of its central nervous system (CNS). The more immature the animal is at birth, the greater is the time spent in REM sleep in early life (Bonora & Boule, 1994). In human babies, especially in mature infants, standard criteria have been recommended (Acher et al., 1980). Use of behavioural criteria have been advocated to overcome the variability of physiological recordings in immature infants and animals (Blanco et al., 1982; Blanco et al., 1983; Henderson-Smart, 1981). Whether apnea is dependent on sleep state will be discussed further in section 1.1.5.

1.1.3 Breathing patterns in the newborn

In the newborn, breathing is clearly related to behavioral state. The various breathing patterns may be related to altered central nervous system activity, changes in the performance of the respiratory pump, as well as the different metabolic demand in each state (Schulze et al., 1981). It is generally agreed that breathing is regular in quiet sleep and irregular in REM. In quiet sleep, the rate and depth cycles are out of phase, and the minute ventilation is stable. In active sleep, the rate and depth cycles are of larger amplitude and are in-phase, leading to marked swings in minute ventilation (Bolton &

Herman, 1974). It has been suggested that fluctuating chemoreceptor drives may be responsible for these oscillations (Bolton & Herman, 1974).

Newborn babies have small oxygen stores in the lungs, in relation to metabolic oxygen consumption, and this is a factor producing instability of arterial oxygen tension. Further instability would be expected in active sleep since lung volume is reduced (Henderson-Smart & Read, 1979) and metabolic rate is increased (Stothers & Warner, 1978). These may be some of the reasons for neonates being more at risk during hypoxia.

1.1.4 The ventilatory responses to isocapnic hypoxia

1.1.4.a The ventilatory response to isocapnic hypoxia in neonates

During the first week of life for the full-term human infant, breathing a hypoxic gas mixture causes a transient increase in minute ventilation in the first minute, followed by a decrease to, or below, the pre-hypoxic levels in the second and third minutes (Brady & Ceruti, 1966; Lahiri et al., 1983; Rigatto et al., 1975). Qualitatively similar biphasic ventilatory responses have been reported in the newborns of most species, although the magnitude of the late ventilatory decrease varies between different levels of O_2.

The biphasic response to hypoxia has also been found in neonatal animals. For example, ventilation increased after 1 minute of 12% or 14% hypoxia, and returned to pre-hypoxic ventilatory levels between 3 and 5 minutes after the onset of the hypoxic stimulus in newborn monkeys (Woodrum et al., 1981). The increase in ventilation becomes more sustained with postnatal maturation (Woodrum et al., 1981). This characteristic biphasic response of the neonate has also been observed in anaesthetized kittens (Schweiler, 1968) and rabbits (Grunstein et al., 1981; Williams & Hanson, 1989).

1.1.4.b The ventilatory responses to isocapnic hypoxia in adult

The ventilatory response of the more mature human differs from the newborn in that there is a sustained increase in ventilation during a period of low O_2 (Sankaran et al., 1979). However, even adult humans show a late decrease in ventilation during isocapnic hypoxia although this does not occur until about 10 minutes of hypoxia (Kagawa et al., 1982). There are two differences between the biphasic response of infant and adults: 1) ventilation starts to decline at a much earlier time in hypoxia in infants than in adults; 2) the ventilation decreases to below the pre-hypoxic level in the neonates, but the fall is much less marked in adults (Kagawa et al., 1982; Rigatto, 1979).

There are obviously different ventilatory responses to hypoxia in adult and neonates. The next section will discuss whether there is a difference in the response of the GG muscle between adults and neonates.

1.1.5 Ventilatory responses to hypoxia in sleep state

Apneas are longer and more frequent during active than during quiet sleep (Gabriel et al., 1976; Rigatto, 1982, 1984). In the preterm infant, there was a sustained increase in ventilation in response to mild hypoxia during quiet sleep; a transient increase followed by a reduction during wakefulness; and predominantly a decrease during active sleep (Rigatto, 1984). Also, in animal research, responses to hypoxia differ between sleep state. Calves (Jeffrey & Read, 1980), lambs (Henderson-Smart & Read, 1979), and puppies (Henderson-Smart & Read, 1979) each have a progressive ventilatory increment during hypoxia in quiet sleep. In contrast, during active sleep, calves showed no ventilatory response to hypoxia; lambs have a rate increment but little increase in ventilation, and the puppies responded as in quiet sleep.

1.1.6 Arousal response to hypoxia

Arousal from sleep may be important in the pathogenesis of SIDS. Adult animals and humans respond to hypoxia during sleep by decreasing ventilation and cardiac output, presumably to preserve O_2 delivery to tissues; they also respond by arousing from sleep (Berthon-Jones & Sullivan, 1982; Bowes et al., 1981; Neubauer et al., 1981; Phillipson & Sullivan, 1978). However, arousal responses were absent in babies with apnea or with low ventilatory responses (Hunt et al., 1981). About 91% of babies with apnea failed to arouse from quiet sleep in response to hypoxia, but 30% of control infants also failed to arouse (McCulloch et al., 1982). Babies with abnormal hypoxic arousal responses had more severe subsequent apneas than those with a normal arousal response (Van Der Hal et al., 1985). In contrast, the majority of adult human subjects are not aroused by hypoxia even when O_2 saturation falls to 70-75% (Berthon-Jones & Sullivan, 1982), a saturation level that can compromise cerebral structure and function.

Arousal threshold is another important feature of the response to hypoxia. In calves and lambs, arousal occurred at much lower arterial oxygen saturation in active compared with quiet sleep (Henderson-Smart & Read, 1979; Jeffery & Read, 1980). This is consistent with observations in tracheotomized sleeping dogs, where the arousal was shown to relate to carotid body chemoreceptor activity (Pillipson & Bowes, 1983; Sullivan, 1980). Infants believed to be at high risk for SIDS demonstrate decreased waking episodes during the course of 24 hours (Harper et al., 1981) and decreased arousal response to respiratory stimuli when compared with age-matched normal infants (Hunt et al., 1981).

1.1.7 The lung compliance response to hypoxia

Previous studies have shown that the control of the respiratory function of the lung develops well before birth (Boddy et al., 1974; Boddy & Dawes, 1975; Boddy & Robinson, 1981; Bowes et al., 1981; Dawes et al., 1972).

Mechanoreceptor activity in the lung is important in maintaining lung volume and in promoting inflation (Olinsky et al., 1974; Henderson-Smart & Read, 1979). For example, the lung inflation reflex (Hering-Breuer) is an important vagally mediated mechanism for regulating the rate and depth of respiration in newborn mammals during early life, and vagotomy modifies the respiratory pattern in newborn animals (Fedorko et al., 1988; Grunstein et al., 1973). Indeed, it has been suggested that the fall in ventilation during hypoxia in early life may be related to a change in pulmonary mechanics (Woodrum et al., 1981). For example, newborn monkeys show a reduction in dynamic lung compliance during hypoxia in the first week of life but not after three weeks of age when the response to hypoxia in sustained throughout the hypoxic challenge (Woodrum et al., 1981). More recently, observations suggest that in the pulmonary vasculature of the neonatal pig, hypoxia results in arterial constriction as evidenced by a large increase in upstream resistance (Hasebe et al., 1992; Inscore et al., 1991; Nelin et al., 1994a, b) and a decrease in total pulmonary blood volume and a small but significant venous constriction (Nelin et al., 1994a, b). The close relationship between mechanoreceptor reflexes and airway resistance raises the question of whether changes in lung compliance during hypoxia influence the activity of upper airway muscles? This will be discussed in the next section 1.2.10.

1.1.8 Depression of central respiratory output

The ventrolateral medulla influences the control of breathing through intracranial chemoreception (Arita et al., 1988; Coates et al., 1993; Cozing & Ngai, 1967; Fukuda et al., 1980; Issa & Remmers, 1992; Okada et al., 1993), through chemoreceptor integration (Budzinska et al., 1985; Cherniack et al., 1979; Schlaefke et al., 1979), through facilitation or inhibition of dorsal and ventral respiratory group neurones (Brice et al., 1991; Fedorko & Merrill, 1984; Lipski & Merrill, 1980; Nattie et al., 1992; Nattie & Li, 1990; Nattie et al., 1991; Nattie et al., 1988) and/or as the site of respiratory rhythmogenesis (Connelly et al., 1990; Nattie et al., 1992; Nattie & Li, 1990; Nattie et al., 1991; Nattie et al., 1988; Onimaru et al., 1988; Smith et al., 1991). Experiments have shown that there is an increase in ventilation in response to

hypoxia after birth in newborn. However, this increase is only maintained for 2-3 minutes and then falls to, or to below the control level, despite carotid sinus nerve activity being sustained throughout the hypoxic period (Blanco et al., 1984).

Several lines of evidence suggest that the secondary fall in ventilation during hypoxia could be due to the operation of an inhibitory mechanism in the CNS (Kumar et al., 1992; Moore & Hanson, 1994, Ackland et al., 1995). To study this hypothesis, several techniques have been used. For example, cooling of the intermediate area of the ventral medullary surface has been shown to inhibit central neural pathways mediating CO_2 sensitivity in adult animals (Schläfke & Loeschcke, 1967; Cherniack et al., 1979; Millhorn & Eldridge, 1986). Also in the newborn, the secondary fall in ventilation during hypoxia can be abolished by unilaterally cooling the rostral pons in newborn lambs (Moore et al., 1991). This fall in ventilation returned after rewarming the area. In piglets, ventral medullary surface cooling decreased central chemosensitivity and increased the inhibitory effect on diaphragmatic activity in response to laryngeal stimulation (Litmanovitz et al., 1994). A further experiment found that ventral medullary surface cooling increased a non-chemoreceptor-related ventilatory mechanism in adult goats (Forster et al., 1995). Further, direct evidence for an inhibitory process mediating the fall in ventilation during hypoxia in neonate has been found by localized lesions in the brain stem. Thus, bilateral lesions of the red nuclei abolish the secondary fall in ventilation in hypoxia (Ackland et al., 1995). Furthermore, both electrical and chemical stimulation in this area inhibits respiratory output (Ackland et al., Waites et al., 1995)

1.1.9 Carotid chemoreceptors in the newborn

1.1.9.a Activity of carotid chemoreceptors in normoxia

The differences in ventilatory and upper airway responses to hypoxia in the newborn compared to adult raises a number of questions relating to chemoreceptor function and reflexes. Chemoreceptor and chemoreceptor mediated reflexes are known be present

in the fetus and the newborn. However, of necessity, they operate within substantially different ranges of PaO_2. It is known that the chemosensitivity is subject to a process of resetting to the increased level of PaO_2 in the neonate (Hanson et al., 1993; Hanson & Kumar, 1994).

The carotid chemoreceptors are active in normoxia soon after birth in several species, and inhalation of 50% or 100% O_2 is associated with a prompt decrease of ventilation (Bureau & Begin, 1982; Fagenholz et al., 1976; Sankaran et al., 1979). As in adults, this response is attributed to cessation of carotid chemoreceptor firing, when the PaO_2 exceeds the upper level of chemosensitivity. However, maintenance of hyperoxia eventually increased ventilation in both infants and adult man (Sankaran et al., 1979). This is attributed to cerebral vaso-constriction and retention of metabolic CO_2. Using jugular venous occlusion to estimate cerebral blood flow by cranial plethysmography in preterm infants, it has been found that inhalation of 100% O_2 for approximately 6 mins was associated with a 15% decrease of flow (Leahy et al., 1980).

1.1.9.b The response of carotid chemoreceptors in hypoxia

Afferent activity of the carotid chemoreceptors plays a key role in reversing central inhibition by hypoxaemia after birth (Blanco, 1994). The neonatal response to hypoxia is biphasic. As described in a previous section, in the kitten (Blanco et al., 1984; Sankaran et al., 1979), monkey (Leahy et al., 1980; Woodrum et al., 1981), rabbit (Grunstein et al., 1981; Schweiler, 1968), and human infant (Brady & Ceruti, 1966; Brady & Dunn, 1970; Cotton & Grunstein, 1980; Rigatto, 1977), a sudden decrease of inspired O_2 is associated with a transient increase of ventilation which, after 30-90 seconds, falls back to or to below the control level of ventilation. The initial hyperventilation is due to peripheral chemoreceptor stimulation. This "biphasic" response occurs in infants from at least 33 weeks gestational age (Rigatto, 1977). It occurs whether the alveolar PCO_2 is allowed to fall, is maintained, or increases during the hypoxia (Brady & Dunn 1970). This response pattern persists for about 1 week in term infants and up to 3 weeks in those born prematurely (Rigatto, 1977). This

secondary fall in ventilation may be mediated by an inhibitory mechanism involving the rostral pons and /or the midbrain (Hanson & Williams, 1989; Kumar et al., 1992; Ackland et al., 1995).

In contrast to the effect of a rapid change in FiO_2, progressive isocapnic hypoxia is associated with a progressive increase of ventilation in lambs (Henderson-Smart & Sohnson, 1979), calves (Jeffrey & Read, 1980), and puppies (Henderson-Smart & Read, 1979). The increase of ventilation during hypoxia, whether transient or sustained, has been attributed to peripheral chemoreceptor stimulation. The ventilatory responses to hypoxia or hyperoxia are weak just after birth and take weeks to reach adult levels (Carroll et al., 1993). The increased carotid sinus nerve activity to hypoxia is smaller in 1 week old kittens compared with that in older kittens (Carroll et al., 1993). The hypoxic response was biphasic in most one week old kittens, but by 4 weeks of age the magnitude of the response was similar to that of adult. Carotid chemoreceptor discharge increased with decrease of PaO_2 in newborn lambs < 1 day old (Jansen et al., 1980). Newborn lambs with carotid denervation showed little or no increase of breathing during hypoxia (Blanco et al., 1983); at rest they hypoventilated and had a lower PaO_2 and higher $PaCO_2$ compared to intact lambs.

1.1.9.c The response of carotid chemoreceptors to hyperoxia

The interaction between CO_2 and O_2, in relation to chemoreceptor function, appears to be different in neonates and adults. In human infants, a fall of ventilation on inhalation of 100% O_2 demonstrates that the peripheral chemoreceptor drive was present throughout that period of time (Cotton & Grunstein, 1980; Rigatto, 1977). In 1 to 25 day old kittens, the activity of carotid chemoreceptors decreases significantly during hyperoxia (Bairam et al., 1993). Increased inspired CO_2 was found to increase the level chemoreceptor discharge in hyperoxia in neonates (Landauer et al., 1995). However, this is the opposite of the response in the adult subject, where the response to CO_2 is greatest at lowest O_2 concentrations (Rigatto, 1984). For example, the experiment showed that chronic hyperoxia ($100\%O_2$) for 60-67 hours eliminated the

O_2-sensitive response of the carotid chemoreceptors in adult cats (Lahiri et al., 1983).

1.1.9.d The response of carotid chemoreceptors to CO_2

The carotid chemoreceptor CO_2 response is weak at birth and matures with age, but more slowly than the hypoxia response (Carroll et al., 1993; Guthrie et al., 1980). Newborn animals and both premature and mature human infants increase ventilation when breathing low concentrations of CO_2 (Guthrie et al., 1980; Haddad et al., 1982; Rigtto et al., 1981; Rigatto et al., 1980). In human babies, in which measurement of occlusion pressure was used to estimate central respiratory drive, there was no difference in the relationship between occlusion pressure and alveolar PCO_2 in premature and mature infants (Haddad et al., 1982), normal and "respiratory distressed" infants (Haddad et al, 1982), and neonates 7-15 days old compared with older children and adults (Cosgrove et al., 1975).

Sensitivity of carotid chemoreceptors to CO_2 increase with age (Calder et al., 1995; Carroll et al., 1993). For example, carotid chemoreceptor sensitivity to CO_2 was weakest in 1 week and matured with age until 8 weeks in kittens (Carroll et al., 1993). In infant monkeys studied using 2-5% CO_2 showed an increase of the CO_2 response between 2 and 21 days of age (Guthrie et al., 1980). Also, increasing levels of hypoxia increase CO_2 chemosensitivity in the adult but not in the neonate (Pepper et al., 1995).

1.1.10 Summary

(1) Prolonged apnea is a common feature in early postnatal life particularly in immature babies. The cause of these apneic episodes is poorly understood. However, there is growing evidence from recent pathological and epidemiological studies and from investigations into hypoxaemic episodes in infancy that the onset of hypoxaemia may play an important role.

(2) In adults, there is a sustained increase in ventilation in response to hypoxia, but in

neonates the increase is only transient (biphasic ventilatory response). This fall in ventilation during hypoxia can not be explained by a absence of chemoreceptor activity as chemoreceptor have been shown to be active after birth and chemoreceptors activity is sustained in response to hypoxia.

(3) Central ventilatory inhibitory mechanisms have been implicated in the biphasic response. Electrical stimulation in the brain stem ventrolateral pons causes normoxic newborn animals to become apneic and cooling or lesion in the rostral brain stem abolished the secondary fall in ventilation.

(4) The lung inflation reflex is an important vagally mediated mechanism for regulating the rate and depth of respiration in the newborn during the early life. Lung compliance is reduced during hypoxia and this may alter the respiratory pattern.

(5) Arousal is a very important response to hypoxia and hypercapnia in animals and humans, but this response is reduced in newborns. Failure of arousal during hypoxia may be involved in SIDS.

1. INTRODUCTION (SECTION 2.)

THE GENIOGLOSSUS MUSCLE IS INVOLVED IN KEEPING THE UPPER AIRWAY OPEN

1.2.1 General background

The genioglossus (GG) muscle, the main protruder muscle of the tongue, has been found to play an important role in maintaining upper airway patency. A respiratory-related rhythmicity is present at rest in many upper airway muscles. This is also the case for the GG muscle. This activity is believed to be important for maintaining airway patency by bracing the airway against the negative pressure generated by the inspiratory muscles of the chest wall. Increased tone of the GG muscle also prevents it from falling back and blocking the upper airway.

Hypoxaemia is a common feature in the newborn, particularly in those born prematurely (Marchal et al., 1987; Mathew et al., 1982a,b). Hypoxic episodes would be associated with central apnea. It is important, therefore, to understand the influence of hypoxia on upper airway muscle function as this may impair the maintenance of an open airway. In this chapter, the function of the GG muscle and its response to hypoxia and hypercapnia will be discussed.

1.2.2 Anatomy and function of the tongue

The mammalian tongue has been described as being composed of two groups of muscles: intrinsic and extrinsic (Lowe, 1981). The intrinsic muscles have no bony attachment and are usually divided into three groups: longitudinal, transverse and vertical. The extrinsic muscles have one bony attachment and the free end decussate with the other muscle fibres within the body of the tongue. The extrinsic muscles are comprised of genioglossus, hypoglossus and styloglossus. All tongue movements result from various combination of extrinsic and intrinsic muscle activity. Genioglossus is the main protruder of the tongue, whereas the other extrinsic muscles primarily act as

retractors.

1.2.3 Patency of the upper airway

Lack of coordination between upper airway and chest wall muscle activation can compromise upper airway patency. To prevent airway obstruction, a correct balance is important between excitation of the GG muscle and the diaphragm (DIA) muscle.

In studies of awake adult humans, progressive excitation of these two components is balanced (Kuna and Sant'Ambrogio, 1991; Sant'Ambrogio & Mathew, 1988). During hypoxia-induced periodic breathing in non-rapid eye movement sleep, as respiratory motor output wanes, upper airway muscle activity decreases at a faster rate than chest wall activity (Hudgel et al., 1987). A fall in the ratio of upper airway to chest wall activity below a critical level is associated with an increase in upper airway inspiratory resistance (Önal et al., 1986). This shift in the balance of forces affecting upper airway patency may explain the upper airway obstruction reported with induction of periodic breathing in normal human subjects during sleep (Önal et al., 1986).

In sleeping subjects, Remmers and colleagues found a very reproducible pattern of phasic genioglossus activation during obstructive apneas: a nadir at the beginning of the apneic episode, a progressive increase throughout the apnea, and a large increase in activity associated with arousal at the termination of the apnea (Remmers et al., 1978). Subsequent studies have identified other skeletal muscles surrounding the upper airway that are phasically active on inspiration and display a pattern of activation similar to the GG muscle during obstructive apneas (Anch et al., 1981; Jeffris et al., 1984; Hollowell et al., 1987).

1.2.4 Effects of increased activity of genioglossus muscle on upper airway patency

Evidence for the role of the GG muscle in maintenance of upper airway patency comes from both human and animal studies (Miki et al., 1989; Van Luteren & Strohl, 1986).

The anatomic arrangement of the muscle suggests that its contraction must dilate the pharyngeal airway (Remmers et al., 1978; Van Lunteren & Strohl, 1986; Van Lunteren et al., 1990). Thus, applying increased tension to the GG muscle reopened the collapsed airway by enlargement of the oropharynx in infant cadavers studied shortly after death (Kuna and Sant'Ambrogio, 1991; Reed et al., 1985). Electrical stimulation of the GG muscle reduces upper airway resistance, increases pharyngeal stability and improves tidal volume in anaesthetized animals (Miki et al., 1989; Van Lunteren et al., 1990). Furthermore, airway rigidity was increased when the activity of genioglossus was increased in rabbits (Brouillette & Thach, 1980).

1.2.5 Genioglossus activity associated with respiration

Genioglossus activity in relation to respiratory function has been investigated in both animal and human experiments. Records of genioglossus electrical activity show inspiratory bursts of phasic activity against a variable background of tonic, expiratory discharge in humans and animals (Kuna & Smickley, 1988; Sauerland & Harper, 1976; Doble et al., 1985). The rise in activity at the onset of inspiration slightly precedes the start of diaphragmatic activity (Haxhiu et al., 1984) a pattern well suited to the avoidance of inspiratory obstruction. Lowe and Sessle (Lowe & Sessle, 1973) have found the GG muscle activity in phase with inspiration in cat and monkey when the animal is in the resting state. A sustained activity could also be elicited by compression of the chest wall in cat and monkey. Brouillette & Thach (Brouillette & Thach, 1980) confirmed that spontaneous augmented deep inspiration showed a biphasic pattern in both GG and DIA EMGs. Hyperventilation abolished phasic inspiratory activity in the GG before the DIA (Brouillette & Thach, 1980). The inspiratory augmentation was continuous until the onset of apneic inhibition during asphyxia in anaesthetized rabbits (Davis et al., 1986). Increased respiratory activity of the GG causes protrusion of the tongue rhythmically in phase with respiration, enlarging the volume and increasing conductance in the oropharynx, against upper airway collapse.

1.2.6 The activity of genioglossus in the newborn

The regulation of upper airway activity and stability is important in newborn babies particular those born prematurely. It is quite common for such babies to have episodes of spontaneous airway obstruction during sleep (Henderson-Smart et al., 1986; Mathew et al., 1982a,b). The response of preterm babies to brief airway occlusions showed a significant progressive increase in the rhythmic inspiratory activity of the GG muscle (Cohen & Henderson-Smart, 1989; Sant'Ambrogio & Mathew, 1988), a response similar to that observed in full-term babies (Roberts et al., 1986) and in animals (Brouillette & Thach, 1979; Issa et al., 1987). This excitatory response has been confirmed in piglets where the GG muscle showed a significant sustained increase above pre-hypoxic levels after 10 minutes of 12% O_2 hypoxia (Martin et al., 1990). In contrast to the excitatory effect of GG muscle, evidence has shown that infants with mixed and obstructive apnea have decreased activation of their GG in response to occlusion which may reflect their inability to dilate the upper airway during airway obstruction (Gauda et al., 1987). Also a small group of preterm infants showed a reduction of GG muscle in response to airway occlusion (Cohen & Henderson-Smart, 1989).

An immaturity in GG muscle control exists during the neonatal period. For example, phasic inspiratory and expiratory GG activity is frequently recruited in anaesthetized kittens during acute hypoxia and hypercapnia and this recruitment is more frequent with increasing postnatal age (Watchko et al., 1989). However, in contrast to the response to hypoxia and/or hypercapnia in newborn, the absence of phasic GG muscle activity during normoxia has also been recorded in full-term and preterm infants (Cohen & Henderson-Smart, 1989; Roberts et al., 1986). This absence of GG muscle activity may reflect the relative immaturity of the central nervous system of the newborn.

1.2.7 The activity of genioglossus and diaphragm muscle during hypoxia and hypercapnia

Three kinds of responses to hypoxia have been observed. Firstly, upper airway muscles are generally activated in a parallel pattern to the diaphragm during hypoxia in humans (Onal et al., 1981a,1981b; Patrick et al., 1982), whereas increases in the inspiratory activity of the hypoglossal nerve and the genioglossus muscle were relatively greater than the increases in the respiratory activity of the phrenic nerve and diaphragm in anaesthetized animals (Haxhiu et al., 1984; Weiner et al., 1982). In anaesthetized adult cats, there was an increase in GG activity in response to airway occlusion during hypoxia (Gauda et al., 1991). A similar response was found during post-occlusion in response to normoxia, hypoxia and hyperoxia. They also found that respiratory-related EPSP amplitude increased during hypoxia and was abolished during hyperoxia. Secondly, Okabe and co-workers demonstrated that during spontaneous tidal breathing, responses of GG activity to sustained hypoxia showed a biphasic response that initially increased and then subsequently declined in both normal and obstructive sleep apnea patients (Okabe et al., 1993). The biphasic response of the GG muscle during hypoxia (for 10 minutes) was paralleled by phrenic activity in the anaesthetized cat (Van Lunteren et al., 1989). However, other have shown that a depression in GG EMG activity was found in OSA patients, but not observed in normal humans during sustained hypoxia (Kimura et al., 1993). Moreover, a sustained inspiratory depression in response to hypoxia was also recorded in GG muscle in rat (Megirian et al., 1985).

Differences in CO_2 threshold may cause an imbalance in GG and DIA muscle activation with changes in chemical drive leading to upper airway instability and obstructive apnea (Carlo & DiFiore, 1990). With hypercapnia, activity of the GG muscle increased linearly with increasing CO_2 in humans and animals (Haxhiu et al., 1984; Hollowell et al., 1991; Önal et al., 1981b). Furthermore, the central chemoreceptors influence the level of phasic inspiratory GG activity during hypercapnia and hyperventilation abolishes phasic inspiratory activity before DIA activity (Brouillett & Thach, 1980; Bruce et al., 1982). Equally, hyperoxia decreased

GG more than DIA EMG activity. Such differential effects have also been reported for hypoglossal activity which increased more than phrenic nerve activity in anaesthetized dogs in severe hypoxia and hypercapnia (Weiner et al., 1982).

The recruitment of GG activity in hypercapnia is more profound with increasing postnatal age. During the neonatal period, the GG muscle has a higher threshold to CO_2, compared to adults (Carlo & DiFiore, 1990; Carlo et al., 1988). In the kitten, genioglossus recruitment during acute hypoxia or hypercapnia is different in one and two months of age (Watchko et al., 1989). The recruitment to hypoxia in two month old animals was sustained whereas it was transient in the one month old kittens. In comparison the DIA EMG responses of 2-7 days old piglets remained significantly above pre-hypoxic levels after 10 min of 12% O_2 (Martin, et al., 1990).

1.2.8 Influence of superior laryngeal stimulation and upper airway pressure changes on genioglossus muscle

Afferent activity originating from the upper airway has been shown to play an important role in the regulation of breathing patterns as well as the regulation of upper airway muscle activity. A major mechanical influence derives from receptors located somewhere in the walls of the upper airway. The GG muscle is a pharyngeal dilator; an increase in pharyngeal dimensions corresponding to the increased muscle tension could reduce upper airway resistance and lead to reduction of the negative pressure load. The effects of changes in pharyngeal airway pressure on GG EMG activity has been studied in anaesthetized animals where negative pressure increased GG activity; positive pressure decreased it (Mathew et al., 1982a.b; Sant'Ambrogio et al., 1985; Van Lunteren & Strohl, 1986). These results have been partly confirmed in normal awake humans (Leiter & Daubenspeck, 1990), where negative pressure tends to collapse the airway associated with enhanced phasic GG EMG activity. In addition, there is an increase in GG EMG activity during nasal occlusion in dogs during both wakefulness and sleep (Issa et al., 1988). Nasal occlusion caused a negative airway pressure and these findings are in agreement with the human data. Similarly, the

depressed response of positive airway pressure on GG muscle was also confirmed in other experiments in anaesthetized rabbits (Woodall et al., 1989). Negative airway pressure evokes changes in upper airway nerve or muscle activity which exceed any changes in phrenic or diaphragmatic activation (Leiter & Daubenspeck, 1990; Widdicombe et al., 1988). Thus, negative pressure produces an inhibitory effect in the upper airway and an increased DIA activity (Leiter & Daubenspeck, 1990; Widdicombe et al., 1988). This afferent feedback to the central pattern generator in the brain stem exerts an excitatory influence on the motoneurones of the upper airway dilating muscles. Furthermore, a distension produced by the inflations of a balloon in the upper airway, inhibited phrenic output but increased GG muscle activity in anaesthetized spontaneously breathing dogs (Cherniack et al., 1984). This reflex system appears to play a role in regulating GG activity during breathing and could be important in ensuring pharyngeal airway patency.

1.2.9 The influence of blood pressure on GG muscle

Hypertension is thought a common feature during apnea in infants (Sharpart Jr., 1989). At the onset of the apnea, blood pressure is reduced, but as the apnea progresses, blood pressure begins to rise before the nadir of O_2 saturation is reached and before the apnea terminates and continues to rise after restoration of airway patency (Sharpart Jr., 1989). It was been hypothesized that stimulation of the arterial baroreceptors in humans and animals inhibits GG muscle activity. Acute increases in arterial blood pressure have been shown to inhibit the activity of GG muscle (Van Lunteren & Strohl, 1986). Even small elevations in blood pressure (15-20mmHg above baseline), significantly reduced GG activity in normal awake humans (Garpestad et al., 1990) and a similar response has been observed in animal experiments (Wasicko et al., 1993). In anaesthetized cats, increased blood pressure leads to an inhibition of the respiratory activity of the hypoglossal nerve compared with the phrenic nerve activity (Salamone et al., 1983; Wasicko et al., 1993). In contrast to increased baroreceptor stimulation, reduced baroreceptor stimulation in humans increased the level of arousal, manifested by an increase in upper airway activity (Coles et al., 1989).

1.2.10 Influence of vagotomy in GG muscle

If upper airway function is coordinated with respiratory dynamics. we might expect that airway muscles are influenced by changes in lung volume. Indeed, a decrease in volume feedback from the lung augments the response of GG activity to upper airway pressure change (Zhang & Mathew, 1992). Vagotomy would abolish the Hering-Breuer inflation-inhibition reflex in both GG and DIA muscles. Furthermore, phasic feedback from pulmonary stretch receptors was found to be a potent inhibitor of reflex activation of the GG in response to negative pressure applied to the upper airway in cats (Gauda et al., 1994).

1.2.11 Influence of anaesthesia on GG muscle

Airway function can be compromised by many agents used clinically and experimentally. Many anaesthetic agents, even when administered in sub-anaesthetic doses, reduce genioglossus activity more than they reduce diaphragmatic activity during awake and sleep states (Broullette and Thach, 1980; Bruce et al., 1982; Hwang et al., 1983a,b). The anaesthetic agent halothane (1.0-3.0% in O_2) has been shown to reduce respiratory-related GG muscle activity in the adult cat (Ochiai et al., 1989). Halothane anaesthesia significantly decreased phasic inspiratory activity of the inspiratory muscles in a dose-dependent fashion. GG activity was completely abolished at 1.5% halothane (Ochiai et al., 1992). Furthermore, chloralose is also known to have a depressive action on the respiratory-related activity of the control nerve of GG muscle, hypoglossal nerve, even when sub-anaesthetic doses are used (Hwang et al., 1983b; Bonora et al., 1985). However, respiratory time and inspiratory cycle did not change significantly with increasing concentrations of halothane. Other agents, such as alcohol (Krol et al., 1984; Bonora et al., 1984) and diazepam (Leiter et al., 1985; Bonora et al., 1985) produce this same effect.

1.2.12 Influence of sleep on GG muscle activity

It is possible that apnea occurs during sleep when GG activity is reduced in newborn babies. The effect of sleep-wakefulness cycles on GG muscle is well recognized. Both tonic and phasic activity of GG muscle decrease with the onset of sleep (Sullivan et al., 1978). It has been proposed by several investigators that an imbalance in the activity of upper airway dilating muscle (like the GG muscle) and the diaphragm might lead to airway obstruction in humans, particularly during sleep (Broullette & Thach, 1979; Broullette & Thach, 1980; Remmers et al., 1978). During sleep respiratory activation of upper airway muscles, particularly the GG muscle, is ineffective (Decker et al., 1993; Mathew & Remmers, 1984; Tangel et al., 1991; Hudgel, 1992). Inspiratory GG muscle activity was significantly decreased during sleep, especially during active sleep in normal humans (Wiegand et al., 1991). Indeed the GG lost its respiratory-related activity during quiet sleep in rats (Megirian et al., 1985). The ability of the GG muscle to respond to respiratory stimuli during sleep was significantly reduced in normal humans (Tangel et al., 1991). In goats, the response to central and peripheral stimulation in GG activity were reduced during sleep compared to wakefulness (Parisi et al., 1988). Sleep not only reduces the response of GG to inspiratory and central or peripheral stimulation but also reduces the response to upper airway pressure (Horner et al., 1994).

1.2.13 Summary

(1) An open upper airway is required for the normal function of respiration. A combination of morphologic and functional features safeguard against upper airway closure during normal inspiration. Respiratory performance is effectively maintained when an appropriate coordination, both in magnitude and timing, is present between the activation on inspiration of chest wall muscles and upper airway muscles. For example, contraction of the genioglossus muscle protrudes the tongue and dilates the pharyngeal airway to maintain upper airway patency.

(2) A major mechanical influence derives from receptors located in the walls of the larynx. Negative pressure in this region elicits increased genioglossus activity and positive pressure has a opposite effect. A number of factors have been found to reduce genioglossus activity. For example, sleep, anaesthetics and blood pressure changes.

(3) During apneic episodes a major site of airway closure is the pharynx. In infants, the activity of the GG muscle is compromised during hypoxia. It is therefore important to know which factors modulate and influence its activity.

Several questions are raised: Can the difference between adult and neonate be explained by differential effects of hypoxia on the motoneurones which control the GG muscle? If so, is the difference due to changes in inhibitory influences in development?

1. INTRODUCTION (SECTION 3.)

CONTROL OF THE GENIOGLOSSUS MUSCLE, HYPOGLOSSAL MOTONEURON AND HYPOGLOSSAL NERVE

1.3.1 General background

As outlined in the previous section, a substantial body of evidence now exists showing that the genioglossus muscle is involved in keeping the airway open. Respiratory-related activity of genioglossus muscle increases with increasing levels of hypercapnia or hypoxia in adults. However, in neonates this response is transient and is similar to the biphasic ventilatory response in the newborn.

The activity of the GG muscle is controlled by the hypoglossal nerve. In this section, afferent inputs which are known to influence the activity of hypoglossal motoneurones will be discussed. In the particular, the following questions will be considered:

1) What is the pattern of hypoglossal motoneuron or nerve activity in adults and neonates?
2) What is the response of hypoglossal motoneurones or nerve activity to hypoxia?
3) Is this response due to the effect on the motoneurone directly or through interneural influences?
4) How do changes in cardio-pulmonary dynamics, mediated via the vagus, influence hypoglossal activity?

1.3.2 Anatomical studies

In cats, the hypoglossal nuclei are strips of cell somata (Hayashimoto, 1960). The rostral three-fifths of the nucleus is located anterior to the level of the obex. The nucleus is within an area 2.0 mm lateral from the midline (Smider & Neimer, 1961). The dorsoventral extent of the nucleus is from 0.5 to 2.5 mm deep from the surface of the medulla at the middle of the nucleus (Berman et al., 1968; Smider & Neimer,

1961). The hypoglossal nerve passes between the mylohyoid and hypoglossal muscle and divides into the medial and the lateral branches. The fibres of the medial branch supply the tongue protruder muscles (genioglossus, geniohyoid, transverse and vertical intrinsic muscles) and the smaller lateral branch supplies the tongue retractor muscles (hypoglossus, styloglossus, infrahyoid and longitudinal intrinsic muscles) (Lewis et al., 1971).

Since the last century, several anatomical studies have been done to understand the topographical organization of the hypoglossal nucleus. In 1843, Stilling first found the hypoglossal nucleus as the origin of the hypoglossal nerve when producing movements of the tongue in response to stimulation of the nucleus (Lowe, 1981). The histological characteristics of the hypoglossal nucleus in cats also has been described by Kappers et al. (Kappers et al., 1960), Green and Negishi (Green & Negishi, 1963) and Taber (Taber, 1961). Two types of motoneurones in the hypoglossal nucleus have been described in cat by Cajal (Lowe, 1981). The majority of motoneurones have only short dendritic branches within the anatomical boundary of the nucleus (Lowe, 1981). The other type of motoneurone is located chiefly in the ventrolateral region of the nucleus and have, in addition to short dendrites within the nucleus, longer dendrites which extend for considerable distances beyond the boundary of the nucleus in a ventrolateral direction. A few of these longer dendrites run ventral ward and are intermingled with the motor axons. The right and left hypoglossal nuclei have interwoven dendritic plexuses in their caudal halves (Lowe, 1981). A short length of the intra-medullary segment of the axon next to the soma is unmyelinated, whereas the rest of the motor axon is myelinated.

1.3.3 Hypoglossal characteristics

Most units with axons running in the hypoglossal nerve are motoneurones. But this is not true of all such fibres. The activity which is synaptically activated is characterized by a burst of action potentials in response to the stimulus. Sumi reported that 149 of 158 neurons recorded could be excited antidromically by stimulation of the hypoglossal

nerve; the rest could be synaptically activated by such stimulation (Sumi, 1969b). In the other group, the cells had a latency of less than 1 msec, and Sumi suggested that these cells were analogous to the Renshaw cells described in the spinal ventral horn. However, Sumi also postulated a second group of cells which could be activated by afferent fibres found in the hypoglossal nerve.

Results of further studies also suggest that afferent fibres run in the hypoglossal nerve. For example, spontaneous discharges of HMNs are inhibited by hypoglossal nerve stimulation (Morimoto & Kawamura, 1972). During the period of this inhibition, inhibitory postsynaptic potentials (IPSPs) with a latency of 14 msec could be recorded intracellularly. When the hypoglossal nerve was ligated or sectioned proximal to the stimulating electrode, the inhibition of the spontaneous discharge disappeared. The threshold of IPSPs in response to hypoglossal stimulation was higher than that of antidromic action potentials. On this basis, the authors suggested that this effect may be due to activation of high-threshold afferent fibres in the hypoglossal nerve.

1.3.4 Respiratory-related hypoglossal activity

The primary mechanical effect of HMN activation by the respiratory pattern generator is to protract the tongue and thereby reduce airway resistance during inspiration. Expiratory activation of these motoneurones might be related to either tongue protraction or retraction, although expiratory activity has been recorded in muscles that dilate the pharynx (Kuna, 1986; Van Lunteren et al., 1984). In both anaesthetized or decerebrate animals, activity of the hypoglossal nerve is present in both inspiration and expiration (Hwang et al., 1983a,b; Mitra & Cherniack, 1983; Sica et al., 1984; Van Lunteren & Dick, 1992). The motor units that protract the tongue are located in the ventromedial portion the hypoglossal nucleus.

Even when only those motor units recruited during breathing are considered, the firing patterns are diverse (Mitra & Cherniack, 1983; Van Lunteren & Dick, 1992; Withington-Wray et al., 1988). Recordings from the hypoglossal nerve and various

tongue muscles reveal that activity in phase with respiration does occur in these sites. Sumi showed that single HMNs fired in phase with respiration (Sumi, 1969a,b). This suggested that the activity of HMNs is under the control of the respiratory rhythmic generator. Hwang and co-workers examined the characterization of respiratory-modulated activities of HMNs in decerebrate, vagotomized, ventilated cats (Hwang et al., 1983a). The great majority of HMN activities were inspiratory-related, discharging during a period approximating that of phrenic discharge.

Not all HMNs appear to have activity modulated by the central respiratory pattern generator. In α-chloralose anaesthetized vagotomized cats, extracellular recordings were made by Mitra and Cherniack (Mitra & Cherniack, 1983) from single fibres in the medial branch of the hypoglossal nerve which innervates the GG muscle. In the all 42 fibres recorded, 33% of the population had inspiratory activity, 45% had inspiratory-expiratory activity, and the rest had activity unrelated to respiration even under high respiratory drive due to hypercapnia or hypoxia. In pentobarbital sodium anaesthetized vagi-intact cats, 34 out of 72 motoneurones recorded had respiratory-related excitatory postsynaptic potentials (EPSPs) (Withington-Wray et al., 1988).

In infants, HMNs are poorly modulated by central inspiratory activity. A lack of spontaneous hypoglossal inspiratory discharges have been reported in studies of unanesthetized fetal sheep (Johnston et al., 1986); unanesthetized awake lambs and ewes (Harding et al., 1987; Haxhiu et al., 1984). Furthermore, lack of hypoglossal activity has been reported in response to airway pressure change in EMG studies of GG muscle discharges in preterm infants (Carlo et al., 1985) and micrognathic infants (Roberts et al., 1986).

1.3.5 Excitatory and inhibitory influences of hypoglossal motoneurones

1.3.5.a The hypoglossal activity during hypoxia

1) The response in the adult

In unanesthetized humans exposed to hypoxia and hypercapnia, GG and DIA muscle activities increased proportionally (Önal et al., 1981a, b). In decerebrate cats, hypoglossal and phrenic nerve activity increases in hypoxia or hypercapnia (Hwang et al., 1983b). Furthermore, activities of phrenic and hypoglossal nerves increase or decrease in parallel in response to hypoxia and hypercapnia in decerebrate, vagotomized cats (St. John, 1986). However, following small doses of anaesthetics the increases of hypoglossal and phrenic nerve activity during hypoxia and hypercapnia are disproportionate (Hwang et al., 1983a).

The decrease in the resistance to airflow in the upper airway reported in adults with hypoxia and hypercapnia has been suggested to reflect the responses of lower cranial nerves to chemical stimulation (McCaffrey & Kern, 1979; Sherrey & Megirian, 1975). This is no less true for the hypoglossal nerve, the activity of which increased during hypoxia in anaesthetized cats (Brouillette & Thach, 1980; Bruce et al., 1982). Equally, in adult dogs, hypoxia increased the inspiratory activity of both the hypoglossal and phrenic nerves (Weiner et al., 1982). Linear increases occurred in phrenic nerve activity with hypoxia, whilst the relationship between hypoglossal and phrenic nerve activity was curvilinear. At lower levels of chemical drive, changes in hypoglossal nerve were less than those of the phrenic, but the reverse was true at higher levels of chemical stimulation. This indicates a differential effect of chemical stimulation on hypoglossal versus phrenic activity.

In a study of single hypoglossal nerve fibres respiratory related modulation of hypoglossal nerve activity occurs under hypoxic conditions (Mitra & Cherniack, 1983). Three different kinds of discharge patterns were observed: (a) type I fibres had activity

which occurred in the inspiratory phase, (b) type II fibres had activity during both inspiration and expiration, (c) type III had continuous random activity with no respiratory modulation. They suggested that the Type I and II fibres may cause contraction of the genioglossus muscle during inspiration which protrudes the tongue rhythmically in phase with breathing. The expiratory activity of Type II fibre and the continuous activity of Type III fibre may activate the GG muscle to advance the base of the tongue and enlarge the volume of the oropharynx throughout the breathing cycle.

2) The response in the neonate

In contrast to the adults, little is known about neonatal hypoglossal activity during hypoxia; what is known suggests that there are substantial differences. For example, in newborn pigs, no respiratory-related activity was observed during hyperoxia, normoxia or hypoxia (Sica et al., 1988).

However, in contrast to the sustained increase in response to hypoxia in adults, in 6-70 days old kittens there is only a transient increase in hypoglossal and phrenic discharge during hypoxia (Bruce, 1986). The effect of hypoxia on the individual motoneurones in the neonate *in vivo* have not been recorded. One possibility is that there are excitatory or inhibitory mechanisms or that for some reason the motoneurones are not able to sustain the response to increased chemoreceptor input.

Studies of rat brain stem slices *in vitro* show that hypoxia alters hypoglossal membrane excitability. The membranes are depolarized and there is an increased input resistance. Change in excitability could explain the transient hypoglossal response to hypoxia in neonates. But it was not known whether these changes occur in hypoxaemia in an *in vivo* preparation.

The present study was designed therefore to investigate the effects of hypoxia on the activity of HMNs in neonatal kittens and to investigate whether these effects are mediated by changes in membrane potential and/or excitability.

1.3.5.b The activity of hypoglossal motoneurones during hypercapnia

Respiratory-related hypoglossal activity increases during hypercapnia in adults (Weiner et al., 1980, 1982; Hwang et al., 1983a; Bruce et al., 1982). However, the response is more dependent on peripheral (carotid) chemoreceptor input than is the phrenic response. Mifflin (Mifflin, 1990) recorded intracellularly responses of HMNs during selective stimulation of the carotid body chemoreceptors. In pentobarbital anaesthetized, vagotomized, paralysed, and artificially ventilated cats, chemoreceptor activation increased the inspiratory depolarization of membrane potential. This was shown as an increase in both amplitude and duration of the inspiratory membrane potential depolarization. In addition to changes in inspiration, similar changes were observed in expiratory HMNs after chemoreceptor activation.

As with the response to hypoxia there are substantial differences in neonatal hypoglossal activity during hypercapnia. As described above, in adults hypoglossal nerves respond more to peripheral chemoreceptor stimulation than central chemoreceptor stimulation (Bruce, 1986). In anaesthetized kittens the hypoglossal responds more to central chemoreceptor inputs whereas phrenic was qualitatively similar to adults. The response to CO_2 of the different units that make up the HMNs pool may vary (Mitra et al, 1986). In anaesthetized adult cats examination of single fibre activity patterns suggests that the fibres making up the hypoglossal nerve differ from each other in their sensitivity to CO_2 and in the CO_2 threshold to initiate respiratory-related activity (Mitra et al., 1986).

1.3.5.c Influence of central and peripheral chemoreceptors on hypoglossal motoneurones

Cooling of the intermediate area of the ventral medullary surface is an experimental technique that has been shown to inhibit central neural pathways mediating CO_2 sensitivity in adult animal models, and has been found to cause hypoventilation (Schläfke & Loescheke, 1967; Cherniack et al., 1979; Millhorn & Eldridge, 1986).

During early postnatal maturation, ventral medullary surface cooling inhibited central neural pathways mediating CO_2 activity in anaesthetized, paralysed, ventilated piglets aged 14-20 days old (Martin et al., 1994). Cooling central chemoreceptor structures also reduced the activity of hypoglossal nerve in anaesthetized kittens (Bruce, 1986).

Intracellular recording of inspiratory-related hypoglossal activity shows that the increased activity of the carotid body chemoreceptors, by both increases in end-tidal CO_2 and electrical stimulation of the carotid sinus nerve, provides a powerful excitatory input to respiratory HMNs (Mifflin, 1990). This suggests that hypercapnia increases ventilatory activity by action upon the central chemoreceptors whereas peripheral chemoreceptor mechanisms mediate the responses to normocapnic hypoxia (St. John & Wang, 1976). In contrast, section of the carotid sinus nerve and central chemoreceptor cooling shows that inspiratory activity during hypercapnia depends more on carotid body chemoreceptor input than does phrenic nerve inspiratory activity (Bruce, 1982).

1.3.5.d Stimulation of the brain stem

The existence of a projection to the hypoglossal nucleus from the lateral reticular formation was established from anterograde studies in the cat (Holstege & Kuypers, 1977; Holstege et al., 1977). Further experiments have supported this. Axons from the dorsolateral reticular formation terminate on hypoglossal dendrites outside the borders of the nucleus (Cooper et al., 1981). It was also found that neurons of the caudal raphe nuclei and adjacent reticular formation that project to the hypoglossal nucleus also project to the cervical and lumbar spinal cord (Manaker et al., 1992). Augmentations of activity within the pontile and medullary reticular formation cause increases in the activity of the hypoglossal nerve (St.John, 1986). Stimulation at many loci within the reticular formation, by micro-injections of glutamate for example or by electrical stimulation, caused augmentation in hypoglossal activity (Goodchild et al., 1982; St.John, 1986). Stimulation of many brain stem structures can alter respiratory-modulated neural activities (Bassal & Bianchi, 1982). In vitro studies have suggested

that electrical stimulation of the caudal raphe nuclei or application to the neonatal rat brain stem had a depressant effects upon hypoglossal motoneuronal activity (Monteau et al., 1990; Morin et al., 1990a,b). Typical results of electrical stimulation and L-glutamate pressure micro-injection of the midline pontine raphe nuclei, increased respiratory frequency discharge recorded from the hypoglossal root.

1.3.5.e The influence of lung volume and vagal afferent activity

The pattern of hypoglossal response to lung inflation suggests that the relief of upper airway obstruction may be a major role of the Hering-Breuer reflex. In an early study in intact anaesthetized kittens, Sumi and colleagues reported that only a few hypoglossal fibres exhibited a respiratory rhythm during normal breathing (Sumi, 1969a,b). A numbers of studies have confirmed that when lung inflation is withheld during neural inspiration, hypoglossal activity is markedly augmented. Following vagotomy, these manoeuvres had no effect on nerve discharge (Sica et al., 1984; Kuna, 1986; Bartlett & St.John, 1988). The recruitment of hypoglossal inspiratory activity with a decrementing pattern in many animals was surprising since, in adult cats, hypoglossal decrementing patterns became augmenting patterns when pulmonary stretch receptor afferents were removed by withholding inflation (Sica et al., 1984). With intact vagi, spontaneous hypoglossal inspiratory activity was not observed. But, respiratory-related hypoglossal activity appeared after bilateral vagotomy in 4-6 day old newborn pigs (Sica et al., 1988).

1.3.6 Membrane properties of hypoglossal motoneurones

Electrical properties of HMNs appear to change with development from neonate to adult. Electrophysiological membrane properties of HMNs were examined in brain stem slices from adult rats (Haddad et al., 1990; Jiang & Haddad, 1991; Viana et al., 1993) and guinea pig (Viana et al., 1990). Haddad and colleagues examined the properties of HMNs from both adult and neonatal ($\leq$12 days old) rat brain stem slices (Haddad et al., 1990). In the adults, the HMN resting membrane potential was -80$\pm$2

mV. Rheobase was 2.1±0.4 nA, and the input resistance was 20.8±1.5 MΩ. In neonates, HMNs had significantly lower resting potentials (Vm=-73±2 mV), lower rheobase (0.7±0.2 nA) with higher membrane resistance (27.6±3.9 MΩ). As with other motoneurones in the mammalian CNS, action potentials generated by HMNs are based on the presence of a Na^+ current that is tetrodotoxin (TTX) sensitive (Haddad et al., 1990).

Hypoxia induces a depolarization of HMNs recorded in vitro (Haddad & Donnelly, 1990). To compare the response to hypoxia with maturation, the study was performed in both adult and neonatal rats. All the neurons, in both groups, were depolarized in hypoxia and there was no evidence of hyperpolarization at any time during the hypoxic period (15-20 Torr for 5 minutes). The results showed that adult neurons initially increased peak and steady-state discharge frequency to current injections. Later, both spike frequencies decreased and in half of the adult neurons, there was a depolarization block. This was not observed in the neonate. Most adult neurons increased input resistance during hypoxia but the input resistance of the neonate did not change.

It has been shown that when the brain of adult mammals is deprived of O_2, potassium (K^+) accumulates in the extracellular compartment (Astrup et al., 1981; Blank & Kirschiner., 1977; Hansen, 1985; Kawasaki et al., 1990; Sick et al., 1982). Jiang and colleagues measured both intra- and extracellular K^+ activities by the use of ion-selective microelectrodes during normoxia and anoxia in hypoglossal neurones in rat brain slices (Jiang & Haddad, 1991). Extracellular recordings showed that even a short period of anoxia (4 minutes) induced an increase in extracellular K^+ and intracellular recording showed a substantial decrease in K^+ during anoxia. This result suggests that intracellular K^+ is released into the extracellular space during hypoxia. Furthermore, two specific ATP-sensitive K^+ channel blockers (glibenclamide and tolbutamide), prevented a substantial amount of K^+ loss during anoxia with a dose-dependent effect. Apamin (a Ca^{2+} dependent channel blocker) had no effect. These results suggest that anoxia-induced K^+ loss from HMNs is related to the activation of ATP-sensitive but not Ca^{2+} dependent K^+ channels. Also, intracellular recordings of membrane potential

showed that the anoxia-induced depolarization in HMNs was significantly increased when the perfusate contained glibenclamide, demonstrating that activation of ATP-sensitive K^+ channels during anoxia can limit the depolarization in HMNs. ATP-sensitive K^+ channels are present in a number of cell types, such as cardiac muscles (Norma, 1983), cardiac myocytes (Escande et al., 1989; Weiss et al., 1987), skeletal muscle (Spruce et al., 1987). There are also some reports about the ATP-sensitive K^+ channel in the CNS (Amoroso et al., 1990; Ashford et al., 1988; Ben Ari, 1989; Mourre et al., 1983). These studies showed that the activity of these channels is determined by intracellular ATP and other nucleotide concentrations (Ashcroft, 1988). Thus, reduction in cytosolic ATP can alter channel activity and regulate neuronal activity. With a reduction in O_2 there is a decrease in the production of ATP and the ATP dependent pumps which maintain membrane integrity begin to fail thus leading to alterations in intra- and extra- cellular ion levels which in turn alter neuronal activity.

The studies above used very severe levels of hypoxia or anoxia *in vitro*. However, such evidence suggests that there is a direct effect of hypoxia on HMNs. Several questions remain. 1) Do these effects occur in *in vivo* preparations? 2) Are there comparable changes in membrane potential, excitability and impedance during even mild levels of systemic hypoxia? These questions are addressed in the experimental approach section of the present study.

1.3.7 Factors which may influence the activity of the HMNs and their response to hypoxia

1.3.7.a Influence of anaesthetics and drugs on HMNs and hypoglossal nerve

The respiratory phasic activities of HMNs and/or hypoglossal nerve are more sensitive to anaesthesia than is phrenic respiratory discharge (Cohen, 1979; Murakami & Kirchner, 1974; Sherrey & Megirian, 1977). The respiratory neural activities have been recorded from the efferent vagal, hypoglossal and phrenic nerves in spontaneously

breathing rats anaesthetized with halothane (Fukuda & Honda, 1982). When the level of anaesthesia was increased by the concentration of halothane or by injection of pentobarbital, inspiratory discharges in the three nerves were well maintained although there was a progressive decrease in respiratory frequency and prolongation of the delay from the start of hypoglossal and superior laryngeal nerve activity compared to phrenic nerve activity. It has been reported that in the anaesthetized dogs, no phasic respiratory-related activity could be recorded in 50% of the study in the hypoglossal nerve (Weiner et al., 1980, 1982). In rabbits, the mechanism responsible for activation of HMNs is much more sensitive to increases of anaesthesia (Brouillette & Thach, 1979) and this is also the case in cats (Bruce et al., 1982). In adult cats, hypoglossal activity was typically decreased following sub-anaesthetic doses of halothane, pentobarbital, chloralose and ketamine, and hypoglossal activity was depressed more than phrenic discharge (Hwang et al., 1983b).

1.3.7.b Influence of blood pressure on activity of hypoglossal motoneurones

Increased blood pressure results in inhibition of hypoglossal nerve activity (Salamone et al, 1983). The inhibition of GG muscle in response to an increase in blood pressure is not influenced by bilateral vagotomy, which suggests that the inhibition is mediated by the carotid baroreceptors and not by aortic receptors (Salamone et al., 1983). Increased carotid sinus pressure immediately inhibits inspiratory phasic hypoglossal nerve activity and there is a direct inverse relationship between phasic hypoglossal activity and carotid sinus pressure up to a carotid pressure of 285 mmHg (Wasicko et al., 1993). However, increased carotid sinus pressure had no effect to tonic hypoglossal nerve activity. This response is abolished by cutting the carotid sinus nerve. It has been suggested that a complex circuit exists that involves an inhibitory feedback from the respiratory centres onto the pathways governing baroreceptor-mediated inhibition of hypoglossal activity (Wasicko et al., 1993).

Stimulation of the proximal end of the cut hypoglossal nerve in dogs has been reported to evoke a reflex rise in blood pressure (Tarkhan, 1936; Downman, 1939). Also

impulses in the efferent sympathetic nerves could be evoked by stimulation of afferent fibres in hypoglossal nerve in dogs (Whitwam et al., 1969). In contrast, in rats, no evidence was found for the presence of afferent fibres in the hypoglossal nerve, where there were no effects on blood pressure following stimulation of the hypoglossal nerve except when the stimulus intensities were 50-100 times threshold (Lodge et al., 1973). Moreover, phasic hypoglossal activity tended to increase when carotid pressures were lower than the control level (Brunner et al., 1982; Grunstein et al., 1975).

Given this inhibitory effect on HMNs cause by changes in blood pressure it is possible that the inhibitory effect of hypoxia may be caused a consequent rise in blood pressure. This will be discussed in section 4.

1.3.7.c The effects of vagotomy on hypoglossal activity during hypoxia and hypercapnia

Pulmonary stretch receptor feedback also influences the output of upper airway motor nerves (Weiner et al., 1982). Studies of adult animals have shown that HMNs were more markedly affected than phrenic motoneurones by removal of pulmonary stretch receptor afferents (Fukuda & Honda, 1982; Sica et al., 1984; Weiner et al., 1982). With cervical vagotomy, the hypoglossal activity increased markedly during normoxia, hypoxia and hypercapnia. It was found that after bilateral vagotomy, some newborn pigs had episodes of spontaneous hypoglossal inspiratory activity which could not be found before vagotomy (Sica et al., 1984). Hypoglossal inspiratory discharges were also elicited by hypoxic stimulation and usually had a decrementing discharge pattern after vagotomy. Bruce reported the there is a similar lack of inspiratory modulation of hypoglossal nerve discharge in kittens, but spontaneous hypoglossal inspiratory activity appeared after bilateral vagotomy (Bruce, 1986). Section of the vagus nerve in kittens impairs the phrenic response to hypoxia more than the hypoglossal response (Bruce, 1986). Comparing the different changes in response to eucapnic hypoxia, bilateral vagotomy has qualitatively similar effects on phrenic and hypoglossal nerve activity, which showed a transient increase during hypoxia (Bruce, 1986). Therefore, lung

compliance and vagal afferent input play a role in decreased hypoglossal activity.

In this thesis therefore, one crucial question to be addressed is whether the effects of hypoxia on hypoglossal motoneurones are mediated through changes in vagal afferent input? I have addressed this by looking at the effects of hypoxia in vagotomized kittens compared with result obtained in vagi-intact kittens. In this case the mid-cervical vagotomy preserved the baroreceptor inputs from the carotid sinus but removed pulmonary afferent information

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1.3.8 Summary

(1) Hypoglossal motor units protract or retract the tongue and may receive input from central respiratory drive. Some hypoglossal motoneurones, particularly those innervating the genioglossus muscle, discharge action potentials during inspiration, which results in an inspiratory phasic contraction of the genioglossus muscle and protrusion of the tongue. This protrusion can prevent airway collapse with the increasing negative intrathoracic pressure generated by contraction of the diaphragm during inspiration. Thus, the respiratory rhythmic activity of hypoglossal motoneurones assists in keeping the airway open during inspiration.

(2) Recordings of hypoglossal motoneurones and nerve electrical activity show inspiratory bursts of phasic activity against a variable background of tonic, expiratory discharge, but this inspiratory activity is reduced or absent in newborn. Respiratory-related activity of hypoglossal nerve augments with increasing levels of hypercapnia or hypoxia. During hypoxia in adults the nerve sustains this increased activity. In neonates, the response is age-related. In newborns, there may be no increase at all during hypoxia.

(3) Hypoglossal activity is strongly inhibited by lung inflation in animals with intact vagus nerves.

(4) Hypoglossal activity is decreased in sleep and by anaesthetic agents and alcohol.

(5) HMNs are depolarized in hypoxia in brain slices *in vitro*. The effect of hypoxia may be a direct effect on HMNs. There is a depolarization blockade in the adult rat brain stem in response to anoxia. Experiments have also shown K^+ loss from the hypoglossal neurons during anoxia due to decreased activity of ATP-sensitive but not Ca^{2+} dependent K^+ channels. Potassium produce an inhibition in expiratory neurones in the brain stem in *in vivo* studies. This inhibition is calcium-dependent and $GABA_B$ receptor-controlled.

The obstructive apneic episodes in the newborn are typically of sufficient duration to result in hypoxia and hypercapnia. Thus, an important question arises regarding the behaviour of HMNs under these conditions. However, the response of HMNs to hypoxia is not clear, especially in neonates. The purpose of this study was to address whether the HMNs of the newborn respond differently to hypoxia compared to the adult.

Studies of rat brain stem slices *in vitro* shows that hypoxia affects hypoglossal membrane excitability. The membranes are depolarized and there is an increased input resistance. Changes in excitability could explain the transient hypoglossal response to hypoxia in neonates.

The key questions are:
1) Do mild levels of hypoxia produce changes in HMNs?
2) Does hypoxia have a direct effect on HMNs?
3) Are the effects on HMNs independent of or in addition to the effects of hypoxia on respiratory discharge?
4) Are there changes in membrane impedance and excitability of the HMNs.

1. INTRODUCTION (SECTION 4.)

LARYNGEAL FUNCTION IN NEONATES AND RESPONSE OF HYPOGLOSSAL MOTONEURONES TO UPPER AIRWAY STABILITY IN NEONATES

1.4.1 General background

Obstructive apnea in infants usually occurs in the upper airway (Mathew, et al, 1982a.b). Muscles of the oropharynx and the larynx have phasic activity coincident with respiration (Andrew, 1956; Broullette Jr. & Thach, 1980). This activity is believed to have physiological importance in keeping laryngeal resistance low in hypoxia and thus facilitating air flow by opposing the constricting effect of negative airway pressures generated by the respiratory muscles of the chest wall. (Bartlett Jr., 1980; Remmers et al, 1978).

There are a numbers of receptors in the laryngeal area. It is known that laryngeal stimulation, particularly in the neonatal period, has a profound influence on ventilation, and may elicit swallowing, arousal, laryngeal adduction, and cardiovascular adjustment (Harding, 1984).

It is thought that laryngeal activity is involved in both sleep apnea and SIDS. This section will deal with the sensory motor aspects of laryngeal function of the upper airway during the newborn period. In particular, the relationship between larynx and hypoglossal motoneurones will be considered.

A key question in this thesis is:
Does hypoxaemia influence upper airway reflexes by altering the balance between excitatory and inhibitory inputs to HMNs?

This has been addressed in experiments by looking at the effects of hypoxaemia on

membrane potential changes in HMNs evoked by mechanical stimulation of the larynx.

1.4.2 Anatomy and function of the larynx

1.4.2.a Anatomy

The larynx, or voice box, is a passageway that connects the pharynx with the trachea. It lies in the midline of the neck anterior to the fourth through sixth cervical vertebrae. The wall of the larynx is composed of nine pieces of cartilage. Three are single and three are paired. The three single pieces are the thyroid cartilage, epiglottic cartilage (epiglottis), and cricoid cartilage. The intrinsic laryngeal muscles are all innervated by vagal motoneurones. The cricothyroid muscle is supplied by motor fibres in the external branch of the superior laryngeal nerve (SLN). All the other muscles are innervated by branches of the recurrent laryngeal nerve (RLN).

1.4.2.b Function of the larynx

In spontaneous breathing, the laryngeal airway is open wide during inspiration, but closes a little during expiration. This phasic change in the upper airway is mostly the result of inspiratory contraction and expiratory relaxation of the posterior cricoarytenoid (PCA) muscles, intrinsic laryngeal muscles that serve to abduct the vocal cords (Murakami & Kirchner, 1974; Bartlett Jr. et al., 1973; Bartlett Jr., 1979). The extrinsic muscles, extending from the thyroid and cricoid cartilages to the hyoid and other extra-laryngeal structures act as a suspensory sling for the larynx (Andrew, 1956; Fink, 1975; Proctor, 1977).

The anatomical relationships of the upper airway muscle or structures change throughout gestation and early postnatal development, particularly in relation to the position of the larynx. (Bosma, 1975). In many species, including man, the neonatal larynx lies higher relative to the cervical vertebrae than in adult, allowing the free portion of the epiglottis to lie behind the soft palate. (Laitman et al., 1977). This

interlocking of the epiglottis and soft palate bestows on the infant the ability to breathe while suckle feeding, and may also be responsible, in part, for the inability of neonates to breathe effectively via the mouth.

1.4.3 Reflexes elicited from the larynx

1.4.3.a The effect on breathing

Depressed breathing and apnea in response to laryngeal stimulation in newborn have been described from many studies (Fleming, 1978; Marlot & Buron, 1979). In newborn lambs, stimulation of the upper airway by liquids produced apnea which could be relieved by sectioning the superior laryngeal nerves (Harned et al., 1978; Tchobroutsky et al., 1969). Many experiments in the newborn have shown that during sleep, a more prolonged, and sometimes fatal, inhibition of breathing occurs (Ariagno et al., 1980; Bosma, 1975; Fleming et al., 1978). These observations have since been extended to piglets (Downing & Lee, 1975), kittens (Harding et al., 1978; Lucier et al., 1979), neonatal monkeys (Harding et al., 1978), and dog pups (Boggs & Bartlett Jr., 1982).

1.4.3.b The cardiovascular responses

Major cardiovascular responses are elicited by laryngeal mucosal stimulation with liquids or by SLN stimulation. In unanesthetized lambs, reflex apnea is accompanied by bradycardia and hypertension, both of which are rapidly reversed when the stimulus is removed and breathing re-established (Johnson, 1974). This has been confirmed in anaesthetized piglets (Lee et al., 1977).

The bradycardia is vagally mediated (Harding et al., 1975) and is due, in part, to the cessation of rhythmical pulmonary afferent traffic from stretch receptors secondary to respiratory arrest. Cardiac slowing, even to the point of arrest, may occur in response to combined stimulation of the SLN and carotid bodies (Angell-James & Daly, 1975).

The inhibition produced by stimulating the upper airway is very powerful. Carotid body stimulation occurs as a result of apnea which develops during laryngeal stimulation. However, the chemoreceptor stimulus to breathing appears to be overridden, especially in the neonate, by afferent inputs from the upper airway. The combined cardiac slowing effects of laryngeal and carotid body stimulation is life threatening in some individuals, and may be of relevance to sudden death in infancy (Daly et al., 1979).

In newborn lambs, laryngeal-induced apnea causes a major redistribution of blood flow; cardiac output falls while flow to the brain and heart increases (Harding et al., 1975). Elsewhere, blood flow is reduced, especially to the small intestines and kidneys. Similar changes in flow occur when hypoxemia is prevented, indicating that the response is not solely due to carotid body stimulation (Harding et al., 1975). However, the changes in heart rate and blood flow are no longer present when the lungs are rhythmically inflated throughout the period of laryngeal stimulation (Harding et al., 1975). These findings are suggestive of a role of pulmonary afferents in cardiac and circulatory reflexes (Angell-James & Daly, 1975).

1.4.4 The properties of laryngeal receptors

With many physiological roles and the need for several types of feedback control, the larynx has a rich sensory innervation. A few afferent fibres run in the RLNs (Glogowska et al., 1974; Susuki & Kirchner, 1969), but most are in the internal branches of the SLN (Mathew et al., 1984; Widdicombe et al., 1988; Wyke & Kirchner, 1976). Many investigators have recorded the activity of single afferent fibres in the SLN (Andrew, 1956; Boggs & Bartlett Jr., 1982; Boushey et al., 1974; Davis, 1986; Davis & Nail, 1987; Mathew et al., 1984; Sampson & Eyzaguirre, 1964; Sant'Ambrogio et al., 1983; Sant'Ambrogio et al., 1985; Suzuki & Kirchner, 1969).

Ventilatory and cardiovascular reflexes are produced by both mechanical and chemical stimulation of the laryngeal mucosa. The characteristics of laryngeal receptors have

been studied with two different methods.

In one set of studies, the effects of laryngeal stimulation using a range of substances have been documented in a number of species. Johnson has established that a wide range of solutions, when passed retrogradely through the larynx of fetal and newborn lambs, caused apnea and swallowing (Johnson, 1974). Stimulants included water, HCL, sucrose and glucose solutions, cow's milk, and allatonic fluid. However, the receptors involved do show some specificity as isotonic saline, amniotic and tracheal fluids, and sheep's milk were without effect. The consistency of the relationship between the ability of a substance to suppress breathing and elicit swallowing has been confirmed in lightly anaesthetized lambs (Kovar, et al. 1979). In other studies, recordings have been made from afferent fibres of the SLN. Characteristics of laryngeal afferent fibres responding to liquids were defined initially in adult cats (Boushey, et al., 1974; Storey & Johnson, 1975) and rabbits (Shingai, 1977). These studies have been performed in kittens, lambs, monkeys (Harding et al., 1978), puppies (Boggs & Bartlett,Jr., 1982) and piglets (Lee et al., 1977).

1.4.4.a Mechanoreceptors of the larynx

The most thoroughly investigated general category of laryngeal receptors are those responding to displacement or deformation, i.e., mechanoreceptors. Sampson and Eyzaguirre (Sampson & Eyzaguirre, 1964) distinguished "touch" receptors, lying in or near the laryngeal mucosa, and "deep" mechanoreceptors, which appeared to be located in the laryngeal muscles or joints. The mechanoreceptors were classified in two groups, depending on their spontaneous activity: group 1 fibres had little or no spontaneous activity and most adapted quickly after mechanical stimulation (Boushey et al., 1974). The units were also simulated by SO_2 and CO_2. The group 2 fibres were spontaneously active and showed slow and incomplete adaptation. The units were unaffected by SO_2 and inhibited by CO_2. Davis and Nail using the similar classification criteria- the "silent" and "tonic" receptors, confirm many of the findings of Boushey et al. and provide a detailed description of the responses of both types of receptors to

static and dynamic mechanical stimulation (Davis et al., 1986; Davis & Nail 1987).

In addition to the studies above, three types of receptors were identified and characterized by Sant'Ambrogio and colleagues in a series of investigations in spontaneously breathing animal preparations (Sant'Ambrogio et al., 1983). 1) Pressure receptors, had tonic activities and slowly adapting increases in activity in response to collapsing or less commonly distending transmural pressures in the larynx (Mathew et al., 1984; Sant'Ambrogio et al., 1983). These receptors are similar to the "group 2" receptors which have been introduced above and the "tonic" units of Davis and Nail (Davis et al., 1986; Davis & Nail, 1987). 2) Flow receptors, initially identified by their response to airflow through the larynx (Sant'Ambrogio et al. 1983). 3) Cold receptors, which are activated by convective and evaporative cooling of the laryngeal mucosa during inspiration (Sant'Ambrogio et al., 1983; Sant'Ambrogio et al., 1985a,b). Furthermore, some of the units were initially silent, but all responded to positive or negative pressure steps and most adapted slowly and incompletely to sustained transmural pressure differences in the isolated upper airway in cats (Hwang et al., 1984b).

1.4.4.b Chemoreceptors of the larynx

The larynx contains receptors with activity that is determined by the chemical composition of fluids placed in the laryngeal lumen. The responses of these receptors to a variety of liquids have been investigated by several researchers (Boggs & Bartlett Jr., 1982; Boushey et al., 1972; Harding et al., 1978; Lucier et al., 1979; Shingai, 1977; Storey & Johnson, 1975). The most striking response of these units is to intra-laryngeal water; this leads to sustained afferent fibre discharge until the water is flushed from the larynx with normal saline or some other non-stimulating fluid. The chemical basis of the water response may vary among species. In dogs and rabbits, the chief stimulus is the reduction in chloride ion concentration (Boggs & bartlett Jr., 1982; Boushey et al., 1974; Shingai, 1977). However, chloride has little influence on the laryngeal water receptors of rats (Shingai, 1980), and those of lambs respond to a

variety of salts and sugars (Harding et al., 1978). The structural nature of the laryngeal chemoreceptors has not been established; some evidence suggests that they may be free, unmyelinated nerve endings (Boggs & Bartlett Jr., 1982; Harding et al., 1978).

1.4.5 The response of superior laryngeal nerve stimulation in neonates

A variety of studies demonstrate important relationships between growth and development of laryngeal reflexes. The immature animal may be more responsive to SLN stimulation than is the adult. Electrical stimulation of the SLN elicits prolonged apnea in piglets but not in more mature piglets greater than 1 month of age (Lee & Downing, 1980). Similarly, the protracted apnea, produced by stimulus to the airway is more powerful in neonates than in adults (Boushey et al., 1972; Lee & Downing, 1980; Marchal et al., 1982; Storey & Johnson, 1975; Trippenbach & Kelly, 1988). Morever, SLN stimulation of piglets results in a protracted apnea that persists even after cessation of the stimulus (Lawson 1981; Lawson, 1982).

1.4.6 The response of larynx to hypoxia and hypercapnia

1.4.6.a The response of larynx to hypoxia

Neural pathways involved in laryngeal function during hypoxia are unclear. Hypoxia elicits expiratory adduction of the cords after vagotomy, and tests with cyanide indicate that this effect is the result of carotid body chemostimulation (Bartlett Jr., 1980; Dixon et al., 1974). This adductor influence of peripheral chemoreceptor stimulation is counteracted in vagally intact animals by pulmonary afferent activity, probably from stretch receptors.

Isocapnic hypoxia in human subjects elicits responses that resemble those in vagotomized animals, the cords are more adducted in expiration than they are at the same level of ventilation in hypercapnia (England et al., 1986). This pattern presumably reflects the expiratory vocal cord adducting influence of peripheral

chemoreceptor stimulation that is revealed by vagotomy in animals (Bartlett Jr., 1980; Dixon et al., 1974). Its appearance in vagally intact humans may reflect the relatively weak reflex responses to pulmonary stretch receptor afferent activity in conscious humans noted by Guz and co-workers (Guz et al., 1970).

When ventilation is stimulated by hypercapnia or hypoxia, upper airway muscle activity increases, particularly during expiration. This response lowers laryngeal airway resistance, thus permitting increased airflow without large increases in driving pressure (Bartlett Jr. et al., 1973; Dixon et al .,1974; Bartlett Jr., 1979).

1.4.6.b The response of larynx to hypercapnia

Laryngeal breathing movements participate in the ventilatory responses to chemical stimulation in both human and animals. The laryngeal response to hypercapnia in most vagally intact animals is similar to that found in hypoxia (Bartlett Jr., 1979; Bartlett Jr., 1980). In hypercapnia, the vocal cords are more widely abducted during inspiration than in the resting state, and the abduction is prolonged through the expiratory period, thus sharply reducing expiratory laryngeal resistance (Bartlett Jr., 1979; Bartlett Jr. et al., 1973; Bianconi & Raschi, 1964; McCaffrey & Kern, 1979; Murakami & Kirchner, 1974). This pattern of response accommodates the increased expiratory flow rates that occur in hypercapnia and is also seen in human subjects during muscular exercise (England & Bartlett Jr., 1982) and in cats during hyperthermia (Dixon et al., 1974).

The responses of the larynx in hypercapnic states, especially during expiration, depend on the relative strengths of several influences. Afferents from central chemoreceptors and from pulmonary stretch receptors and an influence related to ventilatory drive favour expiratory abduction of the cords, with a resultant decrease in the resistance of the larynx to expiratory flow. These influences are opposed by peripheral chemoreceptor afferent activity and by hypocapnia, factors that favour vocal cord adduction during expiration.

1.4.7 Response of hypoglossal activity to SLN stimulation

The laryngeal information carried into the brain stem by SLN nerve afferents is concerned with initiation of both complex and elementary reflex activities and with conscious sensation from the larynx (Sessle, 1973). As expected from the role in upper airway patency, such reflex activity also involves responses evoked in the hypoglossal and genioglossus system. The pathway mediating upper airway reflex activation of the tongue is shown in figure 1.

SLN stimulation could elicit an excitatory effect on GG muscle and hypoglossal nerve (or motoneurones). There is short-latency, low-threshold tongue protrusive reflex in response to SLN nerve stimulation (Lowe et al., 1976; Sessle & Kenny, 1973). A similar excitatory response was also recorded in dog, cat and monkey (Doty & Bosma, 1956) and adult sheep (Amri et al., 1991). Further, electrical stimulation of the SLN elicited a reflex response in the GG muscle with a direct correlation between the firing rate of single GG motor units and the sensory stimulus in the larynx (Miller & Bowman, 1974). As expected from the response in GG muscle, HMNs were strongly depolarized during stimulation of the SLN (Jiang et al., 1991) and excitatory potentials predominate during trains of stimulation of SLN in adult cats (Mifflin, 1989). The averaged latency of EPSPs evoked by SLN nerve stimulation was 30 msec in HMNs (Sumi, 1969a.b).

In contrast to the excitatory influences on the tongue inhibitory reflexes are also evident. SLN nerve stimulation evoked a prolonged inhibitory effect for a prolonged period (400 msec) recorded in hypoglossal nerve (Schmitt et al., 1973; Sessle & Kenny, 1973). These excitatory and inhibitory components are also evident in the complex postsynaptic potentials recorded from HMNs (Sica et al., 1984). SLN stimulation elicit complex patterns of excitatory and inhibitory postsynaptic potentials in respiratory modulated HMNs (Withington-Wray et al., 1988). The effect of single-shock laryngeal stimulation on the hypoglossal discharge was short latency ipsilateral excitation (an EPSP) followed by a bilateral inhibition.

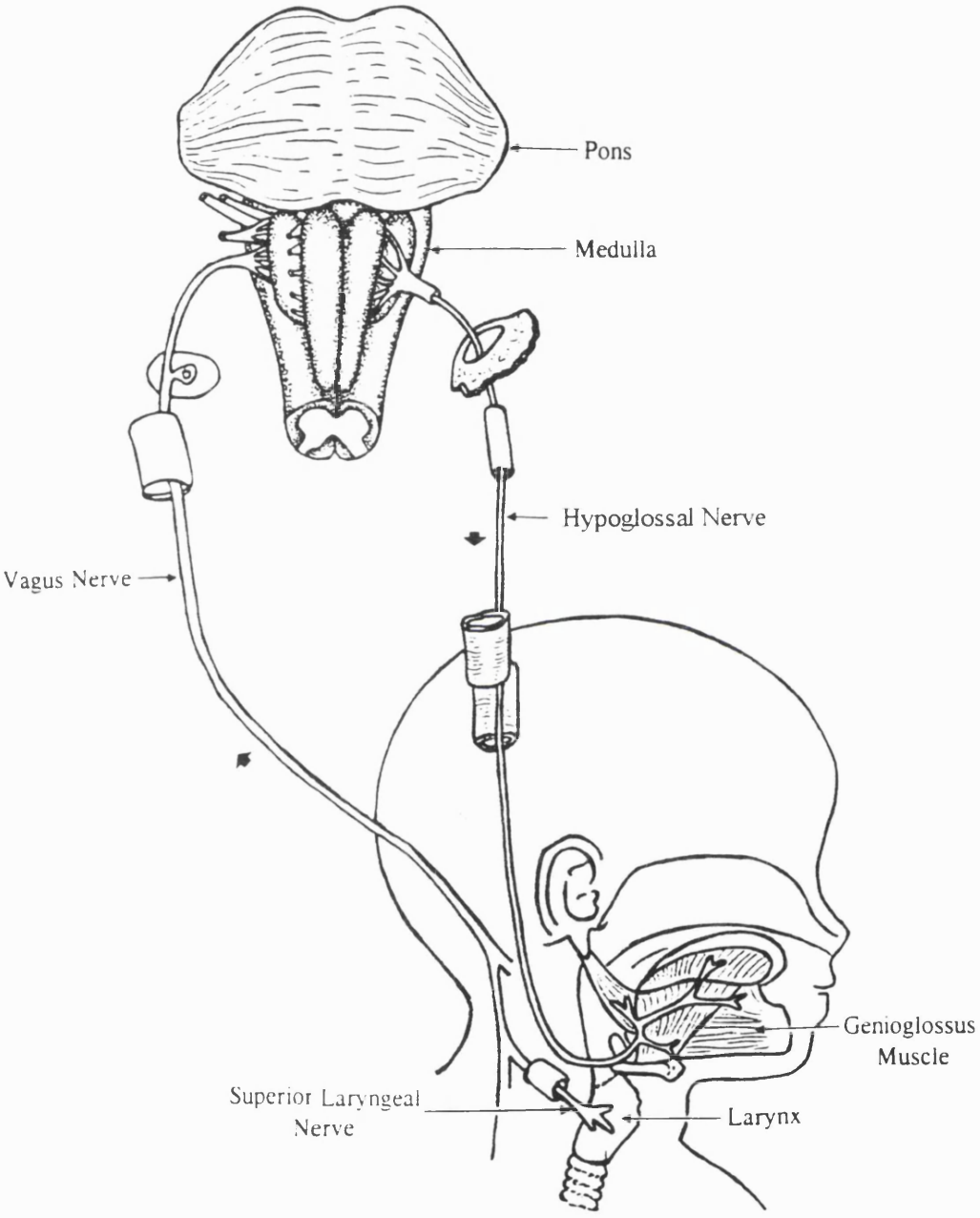


Figure 1. Pathway mediating upper airway reflex activation of the tongue.

Complex evoked potentials were also found in early studies. Stimulation of the central end of SLN induced three types of responses in HMNs: EPSPs, IPSPs and combined response of EPSPs and IPSPs (Sumi, 1969a,b). The first was an EPSP which shortened in latency and increased in amplitude and duration as the intensity of the SLN nerve stimulation was increased. The second type was an IPSP. The latency, duration and amplitude of the response varied from different neurons or even from time to time in the same neuron. The latencies of the IPSPs varied from 5 to 10 msec, durations from 20 to 150 msec. In the third type of response, an IPSP followed by an EPSP. The inhibitory potential preceded the excitatory potential or vice versa in their sequential combination.

SLN stimulation may activate neurons in the reticular formation near the hypoglossal nucleus, eliciting mechanisms causing excitatory activation of HMNs with respiratory modulation via increased synaptic input. Anatomical studies suggest that HMNs receive extensive inputs from the adjacent reticular formation (Amri & Car, 1988; Takada et al., 1984) . Neurons of the reticular formation in the vicinity of the hypoglossal nucleus receive inputs from both glossopharyngeal and superior laryngeal nerves (Sessle, 1973). Stimulation of reticular formation can elicit greater effects on upper airway than on spinal motoneurones, and sometimes elicits prolonged augmentation of respiratory discharge in HMNs (St. John, 1986).

1.4.8 Influence of intra-laryngeal CO_2 on hypoglossal respiratory activity

Intra-laryngeal CO_2 has been shown to elicit a decrease in ventilation (Boushey et al., 1974) or phrenic nerve activity (Bartlett et al., 1992), accompanied by an augmentation of the inspiratory activity of the hypoglossal nerve (Bartlett et al., 1992). This result confirmed that hypoglossal activity increases in hypercapnic states, in keeping with the increased airflow (Hwang et al., 1983a, 1988).

1.4.9 Afferent pathway for hypoglossal response to changes in upper airway pressure

Changes in pharyngeal pressure altered GG and DIA electromyographic activities (Mathew et al., 1982a,b). Hypoglossal activity significantly increased following pressure changes in the upper airway; phrenic discharge declined in most trials in unanesthetized cats (Hwang et al., 1984a). Similar alterations of neural activities were induced by positive pressures change. The effects were greatly decreased by bilateral SLN section. This suggested that hypoglossal-laryngeal reflexes play a role in promoting upper airway re-opening during obstructive apnea.

1.4.10 Influence of SLN stimulation on phrenic activity

In contrast to the excitatory response of hypoglossal activity to SLN stimulation, phrenic activity is inhibited by the stimulation of SLN or laryngeal receptors (Eldridge & Millhorn, 1986; Iscoe, 1988). There is a prolonged inhibition of phrenic discharge following SLN stimulation and this inhibitory effect is more pronounced in infants than in adults (Gerber & Polosa, 1979; Lawson, 1981; Lawson, 1982; Sutton et al., 1978). SLN electrical stimulation has been shown to inhibit phrenic nerve discharges in adult cats (Biscoe & Sampson, 1970). The inhibition of phrenic activity by single SLN stimuli occurs after a short latency (5-10 msec) and persists for 20-40 msec (Biscoe & Sampson, 1970). Jiang and co-worker found that the inhibition was a decrease in phrenic burst frequency without consistent effects on burst amplitude (Jiang et al., 1991). The inhibitory effect on phrenic nerve may be functionally significant in that it stops the build up of negative pressure generated by inspiratory muscles.

1.4.11 The influence of vagal afferent fibres on laryngeal activity

Vagal afferent input must play some part in determining the behaviour of the larynx in hypercapnic states. It would be expected that increases in ventilation will change the pattern and intensity of pulmonary receptor discharge. Lung inflation inhibits laryngeal

abduction during inspiration. Thus the wide inspiratory abduction that occurs in hypercapnia and exercise must reflect an increased central command to the PCA muscles that overcomes the inflation-induced inhibition. However, there are no differences in laryngeal resistance between vagotomized and vagi-intact animals in response to hypercapnia. In both cases, laryngeal resistance is reduced in high CO_2 levels (Dixon et al., 1974).

1.4.12 The effect of sleep states on laryngeal reflexes

Responses to laryngeal stimulation dependent on sleep states. In adult dogs, the threshold for arousal from sleep by laryngeal stimulation was higher in REM sleep than in non-REM sleep and least during wakefulness (Sullivan et al., 1978). The depression of the arousal response in REM sleep is in keeping with the effects of REM sleep on a range of afferent inputs (Jouvet, 1967). In contrast, the respiratory inhibition and bradycardia induced by subarousal stimuli was of greater duration and intensity during REM sleep than in non-REM sleep (Sullivan et al., 1978). A similar study in premature newborn lambs failed to establish a sleep-related difference in the incidence of arousal or in the degree of respiratory depression (Marchal, 1982). However, inhibition of breathing was least marked when arousal occurred from sleep (and during wakefulness), establishing the survival value of the awake state. It is of interest that in this study on unanesthetized premature lambs, prolonged, potentially fatal apnea, did not occur, in contrast to studies in which restraint or anaesthesia were employed (Downing & Lee, 1975: Lucier et al., 1979). Further, the degree of laryngeal stimulation required to produce arousal and coughing was higher in REM sleep than quiet sleep (Sullivan et al., 1978).

1.4.13 Summary

(1) In patients having histories of obstructive sleep apneas, pharyngeal pressures, even in unobstructed periods, may exceed both the negative and positive values (Anch et al., 1981). During an obstructive apnea, large negative intrathoracic and intra-tracheal

pressure will be generated which stimulate the airway "pressure" receptors. There is a reflex augmentation of hypoglossal-genioglossus activity to re-open the upper airway.

(2) There are both excitatory and inhibitory effects in response to superior laryngeal stimulation. Inspiratory activity of the GG muscle is strongly activated by stimulation of afferent fibres in superior laryngeal nerve (Iscoe, 1988; Sant'Ambrogio & Mathew, 1988). From intracellular recording it has also been found that hypoglossal activity is strongly increased during superior laryngeal stimulation in adult cats (Jiang et al., 1991; Withington-Wray et al., 1988).

(3) In contrast to the excitatory effects on hypoglossal activity, phrenic nerve exhibited an inhibitory response to SLN stimulation (Gerber & Solosa, 1979; Jiang et al., 1991).

(4) It is very important that during upper airway occlusion, negative (or positive) pressure stimulates laryngeal receptors which, through a reflex action, inhibits the inspiratory muscles and increases the activity of hypoglossal nerve. Therefore, the laryngeal-hypoglossal reflex pathway is likely to play an important role in maintaining upper airway patency during conditions such as sleep or anaethesia when upper airway activity is reduced (Isoe, 1988; Sant'Ambrogio & Mathew, 1988).

The responses of hypoglossal activity to superior laryngeal stimulation, in neonates who experience repeated or periodic episodes of apnea, and the laryngeal-hypoglossal reflex during hypoxia are not clear. Is there an excitatory or inhibitory response to laryngeal stimulation on hypoglossal activity in newborn? Are apneas due to a decrease of upper airway-hypoglossal reflexes in newborn? Does hypoxia compromise this reflex in neonates?

The present study has examined the effect of mechanical laryngeal stimulation and the laryngeal-hypoglossal reflex during mild levels of hypoxia in newborn kittens.

1.5 THE AIMS OF THIS STUDY

In this thesis I have addressed several issues concerning the control of hypoglossal activity. I have investigated the effect of hypoxaemia on HMNs responses in neonatal kittens.

The aims of this study were to examine:

1) The nature of hypoglossal motoneurones discharge activity and pattern of this activity,
2) The effects of hypoxaemia on hypoglossal output and respiratory activity,
3) Whether such effects are due to direct action in HMNs or are exerted through respiratory drive?
4) Whether the effects on HMNs are due to changes in vagal input?
5) The effects of hypoxaemia on laryngeal-evoked HMNs potentials.

CHAPTER 2
MATERIALS AND METHODS

2.1 Animals

Experiments were carried out using 55 mixed breed kittens aged between 14-25 (20.56±0.46, mean±SEM) days and 245-530g (345.89±9.24) body weight. All the animals were delivered from established cat colonies at Leeds University. All experiments were licensed by the Home Office in accordance with the Animals (Scientific Procedures) Act, 1986. Successful recordings were made from 30 kittens, which were aged from 14 days to 25 days old (21.43±0.55) and weighed from 250 to 440 (343.2±10.51) grams.

2.2 Anaesthesia and drug administration

The kittens were first sedated with an intramuscular injection of ketamine (15-30mg/kg) (Ketalar, Parke-Davis, U.K.) and anaesthesia was induced by inhalation of 1-2% halothane (May & Baker) in 30-45% O_2 by face mask. A catheter was then inserted into the femoral vein and, after the halothane was switched off, an initial dose of α-chloralose (40-50mg/kg, SIGMA. Chemical Co.) was injected slowly into the vein. Further doses were administered as required (10-25mg/kg). The level of anaesthesia was assessed by stability of arterial pressure, persistence of miotic pupils, and absence of the withdrawal reflex or increased arterial pressure in response to a pinch applied to a paw. Before incision, 1 ml of lignocaine (Xylotox, Willows Francis Veterinary) was injected subcutaneously. During the period of recording the animals were paralysed with gallamine triethiodide (7mg/Kg, i.v.). To maintain animals in the best physiological state through the long experiment, a solution of sodium bicarbonate in glucose-saline (2.1-4.2% Sodium Bicarbonate in 5% Glucose and 0.9% Saline) was continuously infused via the femoral vein by a infusion pump (World Precision Instruments Ltd.) at a rate of 0.5 mls/hour. This was found to stabilize blood pressure and blood pH. In cases where a metabolic acidosis occurred an additional bolus of pre-warmed sodium bicarbonate was also administered (0.5-1ml, i.v.).

2.3 General surgical preparation

The animal was placed in the supine position. The trachea was exposed and then intubated below the larynx with a paediatric tracheal tube with an inner diameter of either 2.0 or 2.5mm (Portex) depending on the size of the trachea. After tracheotomy, the animal was artificially ventilated with a respirator (Infant Star Ventilator) using positive pressure. The ventilator was typically set with a frequency of 9-12 breaths per minute, an inspiratory duration of 0.8-0.92 seconds and peak inspiratory pressure (PIP) of 13 mbars. A positive end expiratory pressure (PEEP) was applied to prevent lung collapse. The left femoral artery was also cannulated for monitoring blood pressure and taking blood samples for blood gas analysis. The blood pressure was monitored by a pressure transducer (Harvard Apparatus Pressure Transducer). Blood samples ($<$0.2mls) were taken for arterial blood gas analysis (Instrumentation Laboratory, 1306 PH/Blood Gas Analyzer). Both the femoral arterial and venous cannulae were flushed with heparinised saline (10 units/ml in saline, C.P Pharmaceuticals Ltd, U.K.).

Respiratory gas samples were automatically taken through a narrow diameter tube attached to a 2.5mm paediatric/neonatal airway adaptor (Ohmeda). End-tidal carbon dioxide levels ($P_{ET}CO_2$) were maintained at 3-4% by adjusting lung volume and/or respiratory rate and fractional inspired oxygen (FiO_2) and were continuously monitored (Ohmeda 5250 Respiratory Gas Monitor). The Respiratory Gas Monitor was calibrated with a sample gas (Scott Medical Products).

The rectal temperature was maintained between 36 and 38°C by a homeostatically controlled ventral heating pad (Harvard) and a overhead lamps. A intravenous blood volume expander (Ficall, 0.2-0.5ml, i.v.) was given to maintain blood pressure after thoracotomy.

2.3.1 Insertion of the balloon-tipped catheter in to the larynx

In addition to the tracheotomy, a silicone foley balloon catheter (Sherwood) was

inserted directed rostrally into the larynx. This enabled a constant pressure pulse (20-30psi) (World Precision Instruments, PV 820) to be applied through the balloon catheter to provide mechanical stimulation in the larynx. Before securing the foley balloon catheter it was inflated to ensure that it was positioned correctly in the larynx. During a test the balloon catheter was inflated with air.

2.3.2 Exposure of the hypoglossal nerve

The transverse jugular vein was identified, ligated and cut in to two parts. The tissue beneath the vein was dissected to expose the left hypoglossal nerve. A length of 1.5 cm of hypoglossal nerve was exposed by separating it from the surrounding tissue and a 5.0 silk thread was loosely tied around it for later identification. A bipolar concentric stainless-steel electrode (subminiature electrode, 50-1650, Harvard Electrode) was used to identify the hypoglossal nerve by the evoked protrusion of the tongue in response to stimulation of the nerve. Before suturing the incision, a few drops of paraffin were applied to keep the nerve moist. An incision was then made between the digastric and the masseter muscle on the left side of the neck. This incision was subsequently used to gain access to nerve for electrical stimulation when the animal was held in the stereotaxic frame in the prone position.

2.3.3 Vagotomy

Vagal activity is known to suppress the respiratory activity of hypoglossal nerve (Bartlett Jr., 1980; Bruce, 1986). The vagi were identified by finding the common carotid artery and interal jugular vein which accompanies the vagus at the mid-cervical laryngeal level. Both sides of the vagosympathetic trunk were isolated. The vagus nerves were carefully separated from the cervical sympathetic nerve and sectioned bilaterally.

2.3.4 Exposure of the phrenic nerve

Phrenic nerve activity was taken as an index of the central respiratory rhythmic drive in some experiments. With the kitten in the supine position, an incision was made on the right side using a ventral approach. The midline incision of the neck, which had been made for the tracheotomy, was extended approximately 4 cm caudally along the midline. Forceps were used to hold the tissue so that the right cervical plexus could be exposed. A branch of the phrenic nerve, from the ventral division of the fifth cervical plexus, was identified by electrode stimulation and contraction of the diaphragm. It was then isolated, dissected clear of surrounding tissue and cut peripherally, a loop of thread was loosely tied around the nerve for identification after the animal was placed in the stereotaxic frame. The phrenic nerve is extremely delicate and it was important to take care that the suture did not damage it. The incision was sewn up and animal was put in the prone position and a midline incision was made from the base of the neck and extended about 3 cm caudal in the midline of back. The branch of the phrenic nerve was identified by the thread. After the nerve was cut, the central end of phrenic nerve was mounted onto a pair of bipolar platinum subminiature recording electrodes (50-1650, Harvard Electrodes) for nerve recording. The nerve was covered with paraffin oil to prevent drying.

The animal was placed in a stereotaxic frame with the body in the prone position. The muscles overlying the cranium and upper-cervical vertebral bones were removed, and the brainstem was exposed by occipital craniotomy (figure 2).

2.4 Electrophysiological recording

2.4.1 Preparation of the electrodes

Glass capillary microelectrodes (Clark Electromedical Instruments, GC100F-10) were pulled using a Flaming/Brown micropipette puller (Sutter Instruments) on the day before the experiment. The electrodes were filled with 3M potassium citrate. The

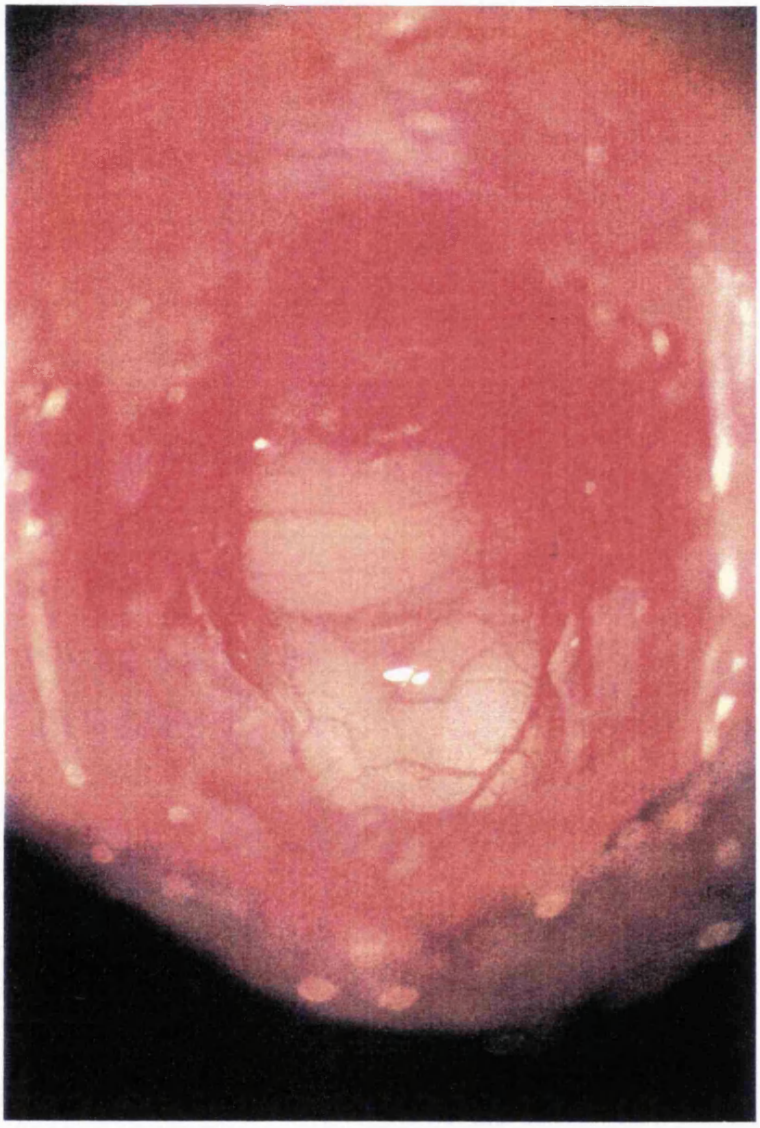


Figure 2. Photograph showing the obex of the medulla oblongata and cerebellum in a neonatal kitten. This part of the brain stem was exposed by an occipital craniotomy.

impedance of each electrode was measured by passing a constant current pulse (10nA) and using a bridging current (Axoclamp, Axon Instruments) to balance the voltage drop across the electrode. The impedance of the electrodes were 70-90 MΩ when filled and this dropped to 30-40MΩ on the second day.

2.4.2 Stability for recording from the brainstem

All experiments were conducted on a nitrogen-suspended antivibration table.

There were three factors influencing the stability of the recordings:
1) Respiratory movement causing movement of the brainstem.
2) Blood pressure-related pulsations of the cerebellum and brainstem.
3) Acid-base imbalance producing changes in pulse pressure with consequent effect on brainstem movement.

Four procedures were used to overcome these problems:

1) To reduce respiratory-related body movement, a bilateral thoracotomy was performed. To prevent damage to the lung, an incision of 1-2 cm was made at the last intercostal space to allow passage of air through the hole into the thoracic cavity. To ensure patency of the thoracotomy the intercostal muscle were pulled apart by two toothed artery forceps. The forceps were attached to the animal procedure frame. A positive end-expiratory pressure (PEEP, 1-2 mbars) was applied to prevent lung collapse.

2) To stabilize the brain tissue, a small horse-shoe shaped metal foot was placed gently on the surface of the medulla using a binocular microscope (Richter et al., 1991) (figure 3). This foot was made from the eye of a stainless steel surgical needle which was shaped after heating with a burner (Arnolds Veterinary Products Ltd, size 17). To prevent tissue damage the foot was only applied during in recording period and was removed immediately after each recording.

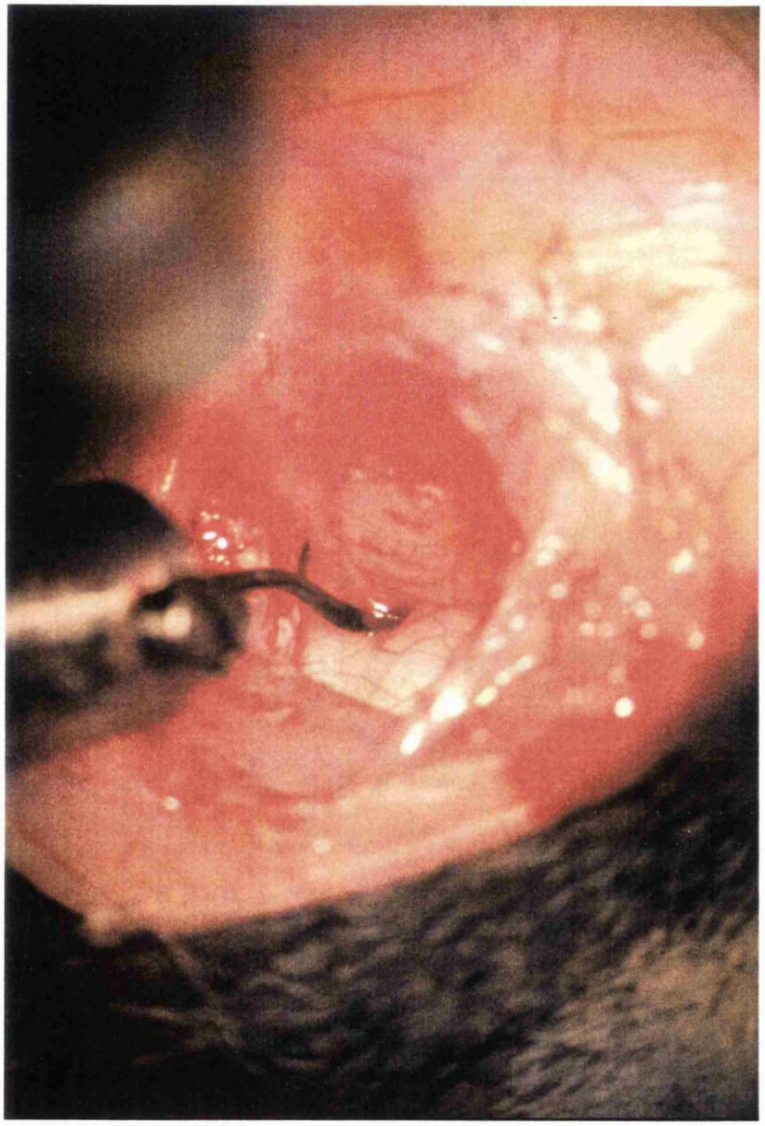


Figure 3. Photograph showing the electrode penetrating the brain stem. Note the horse-shoe shaped foot which was used to help maintain stability of the brain stem during recording. The foot was placed just rostral to the obex.

3) To maintain a good acid-base balance, $P_{ET}CO_2$ and arterial blood base excess were monitored. To prevent respiratory acidosis the ventilatory rate and tidal volume was adjusted as necessary. To prevent metabolic acidosis, a major feature of chloralose anaesthesia, a solution of 2-3% sodium bicarbonate in 5% glucose was continuously infused intravenously.

4) In addition to the procedures above, to reduce body movement when stimulating the nerve, the animal was paralysed with gallamine triethiodide (Flaxedil, May & Baker). The dose of 7mg/kg was administered by intravenous injection. The gallamine was administered as a bolus and its effects were allowed to wear off every 30 mins to check the level of anaesthesia (see above).

2.4.3 Identification of the hypoglossal motoneurones

The left hypoglossal nerve was placed on a bipolar platinum stimulating electrode. After the medulla was exposed, a computer controlled step motor microdrive (Digitimer, with Epson HX-20 computer) was used to move a glass capillary microelectrode (see above) down in 2 or 20 μm steps penetrating the medulla approximately 0.5 mm rostral and caudal to the obex. Hypoglossal motoneurones were identified by antidromic action potentials recorded following electrical stimulation of the ipsilateral hypoglossal nerve (stimuli 2-5 mA pulse every 600 msec with duration 0.1 msec) from a constant-current stimulator (Isostim A320, World Precision Instruments, U.S.). Standard criteria for classifying a response as antidromic were used (Lipski, 1981): 1) constant onset latency; 2) at threshold level of stimulation, the responses were all-or-none, and no underlying potentials were observed when the spike failed; 3) high following frequency; and 4) collision with orthodromic impulses.

2.4.4 Signal processing

Figure 4 shows a schematic diagram of the experimental set up.

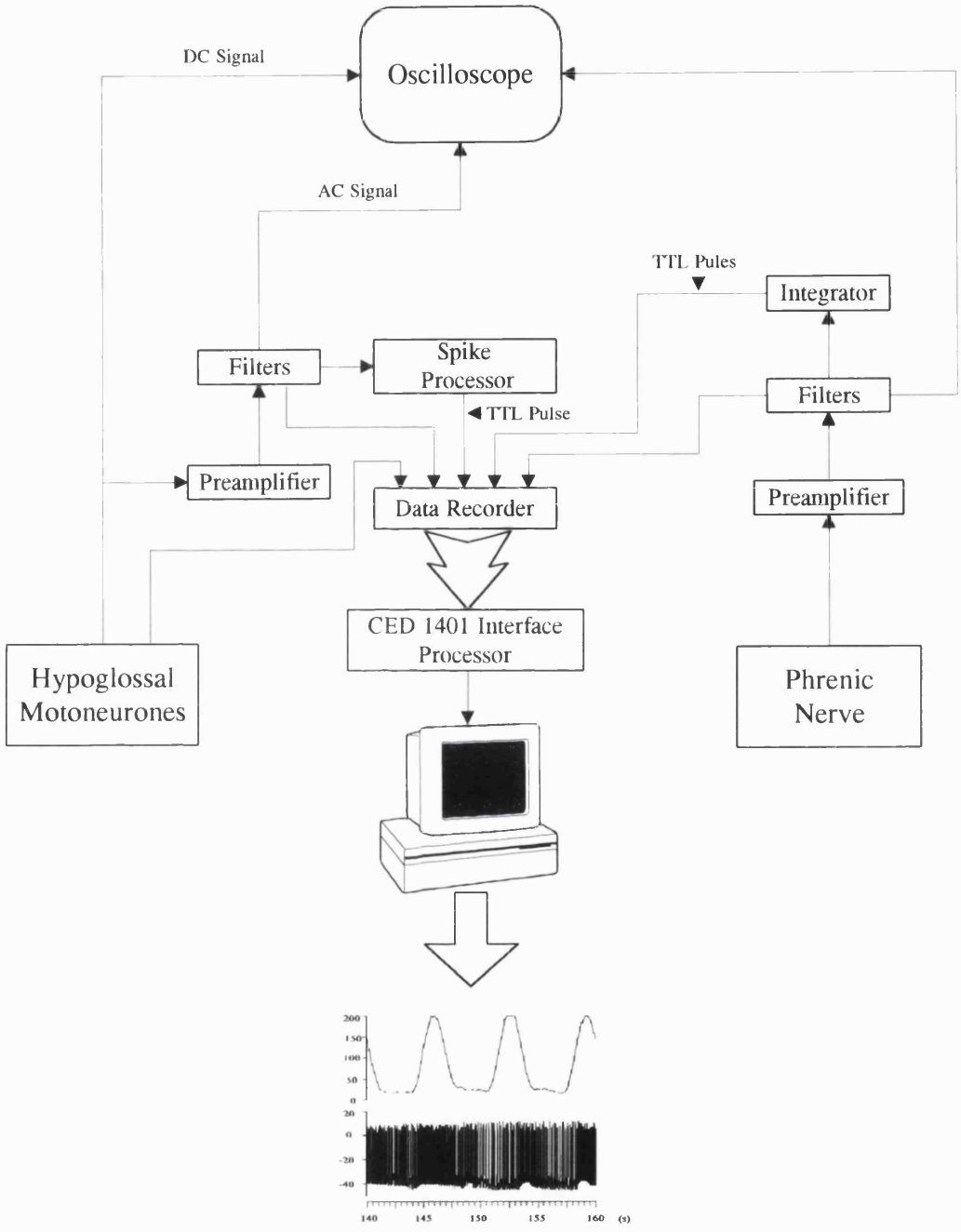


Figure 4. Schematic representation of the recording set up.

The electrode was mounted in a unity gain head stage (Axon Instruments HS-2) and the recordings of the activity of a single unit were made with glass capillary microelectrode (Clark Electromedical Instruments, GC100F-10). The signal was fed into a DC electrometer (Axoclamp, Axon Instruments) with capacitance neutralization and bridge balance facilities. The unprocessed DC signals were displayed on digital oscilloscope for direct on-line analysis. The signals were also fed through a series of AC amplifiers. The signals were amplified (x1000) and then passed through a series of filters with low and high pass characteristics (band width 800Hz-6kHz, Neurolog Ltd., D130). After amplifying and filtering, single unit activity was fed into a spike processor/audio amplifier unit (Spike Processor, Digitimer) to produce standard TTL pulses. The spike processor had a multiplex output. Both the AC trace and the window levels could be viewed on the oscilloscope and the window levels adjusted to discriminate the action potentials of the unit being studied. The AC and DC signals were recorded and stored on digital audio tape (Bio-logic, Digital Tape Recorder TR1800) for later analysis.

2.5 Experimental protocol

Both extracellular and intracellular recordings of hypoglossal motoneurones were made. For extracellular recordings, the discharge frequency during normoxia, normocapnic hypoxia and post-hypoxia (normoxia) were recorded. The membrane potential, changes in membrane impedance and membrane excitability of hypoglossal motoneurones were also recorded using intracellular technology. Normocapnic hypoxia (16-17%O_2 in N_2) was produced by decreasing the oxygen level by substitution with nitrogen.

In addition to recording unit activity, phrenic nerve activity was also recorded (see section 2.3.4). Phrenic nerve activity was amplified (gain 1000-1500k), filtered (band width 600-6000Hz) and fed into a tape recorder.

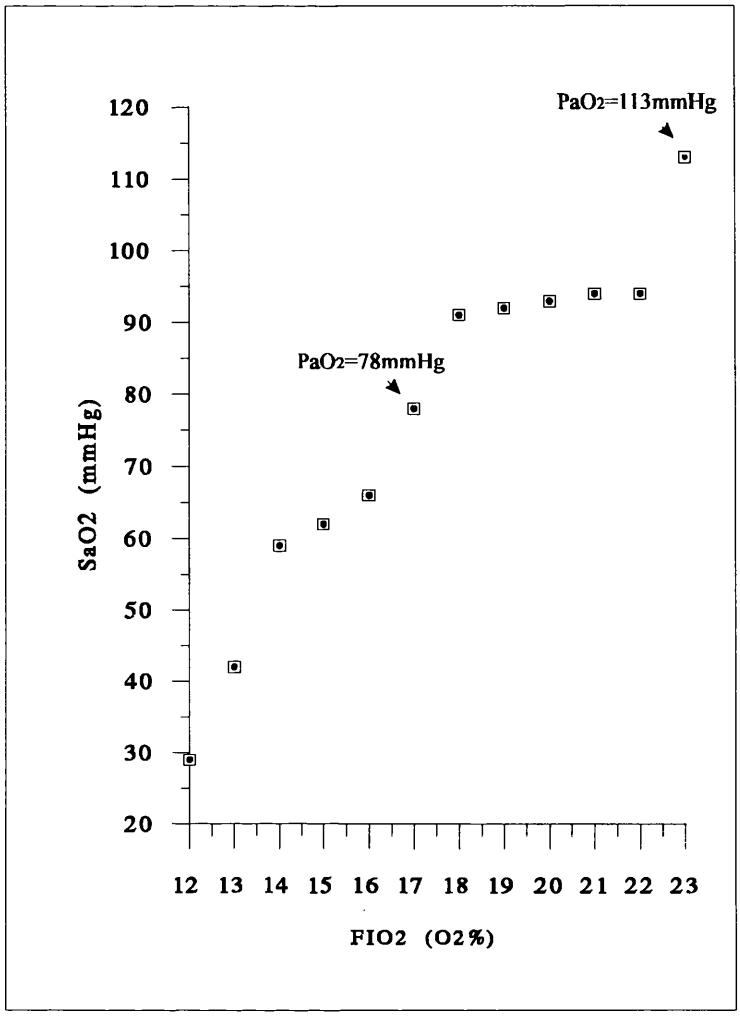


Figure 5. Relationship between FiO_2 and Hb saturation for a kitten aged 18 days and weighing 200g. Haemoglobin saturation (SaO_2) measured with a pulse oximeter (Ohmeda 5250) is plotted against fractional inspired oxygen (FiO_2) measured with a respiratory gas monitor (Ohmeda 5250). Blood samples were taken at an FiO_2 of 0.25 and 0.17 and revealed that the PaO_2 drops from 113 mmHg to 78 mmHg. In the majority of recordings the FiO_2 was reduced to 16 to 17%.

All nerve and motoneurone activities were processed and obtained on line using a C.E.D. 1401 interface (Cambridge Electronic Design) and recorded on digital tape for subsequent analysis using Spike2 programs.

Only stable recordings from neurones were selected for test and analysis. The criteria

for a stable recording was: 1) animal was in good physiological state, 2) the resting membrane potential was stable and more than negative than -40 mV (during intracellular recording). The results were based upon an analysis of 125 neurones.

2.5.1 Extracellular recordings

Extracellular recording were made from 40 hypoglossal neurones.

2.5.1.a The criteria for extracellular recordings

The unit was only tested if:
1) The blood pressure was over 40mmHg.
2) The signal-noise ratio of the antidromic action potential was greater than 1/3.

2.5.1.b Experimental protocol

Hypoglossal motoneurone and phrenic nerve activity was recorded for a period of 3 minutes in normoxia. This was control period (pre-hypoxia). Normocapnic hypoxia was then induced by reduction of the FiO_2 to 15-17% oxygen with nitrogen for 3-6 minutes. Activity of HMNs were then recorded after the FiO_2 returned to normoxia (post-hypoxia).

2.5.1.c Analysis

1) Hypoglossal motoneurones

For the purpose of determining whether hypoglossal discharge frequency decreased and/or increased during different periods, the TTL pulses were processed through a CED 1401 computer interface and analysed using a script program, written by Dr. R. Noble, to run with Spike2 software (Cambridge Electronic design, U.K.). The discharge frequency of the hypoglossal motoneurone were represented as a moving

time average with a time bin of ten seconds. The mean discharge frequencies were also calculated as mean discharge frequency for each successive minute.

2) Phrenic nerve

In order to analyse whether HMNs had a respiratory-related activity pattern, the discharge frequency of phrenic nerve were also fed into an interface (Spike Processor, Digitimer) to generate a TTL pulses. This was analysed as a moving time average with a time bin of 10 seconds.

2.5.1.d Statistical Analysis

To analyse each extracellular recording, the 60 second period to hypoxia was used as a control period. To determine whether hypoxia had an effect on the discharge of the hypoglossal motoneurone, the 60 second period during hypoxia with the largest change in discharge frequency (peak response) was compared to the discharge frequency during the control period. The difference recorded during post-hypoxia was also calculated to compare with the pre-hypoxic level. The change in discharge frequency during normoxia, hypoxia and post-hypoxia were compared using a paired Student's t-test (two-tailed, $P \leq 0.05$). The Bonferroni correction was used to correct for the accumulation of error with multiple t-tests (Slinker & Glantz, 1990).

The Pearson linear correlation test was used to examine the relationship between discharge frequency or discharge frequency changes of HMNs and possible factors, such as age, $PaCO_2$ level, PaO_2 level and blood pressure.

2.5.2. Intracellular recordings

80 neurones were recorded with intracellular electrodes.

2.5.2.a The criteria for an intracellular recording

The recording was only tested if:

1) the blood pressure was over 40mmHg and stable.

2) the membrane potential was more negative than -45mV.

3) the pH > 7.2.

2.5.2.b Experimental protocol

1) The recording of the membrane and evoked potential

After the successful intracellular penetration of a hypoglossal motoneurone, a period of 30 secs or more was given to allow the membrane to seal around the glass electrode. The larynx was stimulated by a balloon catheter (see 2.3.1) using a constant pressure pulse. The membrane potential was then recorded for a control period of 3 minutes in normoxia. After that, a period of hypoxia (FiO_2 between 0.16 and 0.17) was induced for up to 3 minutes followed by a 3 mins period of post-hypoxia. If the intracellular recording was stable enough, a second hypoxic test was given.

In 11 experiments, higher concentrations of oxygen (FiO_2 between 33-41% and 128-170 mmHg in PaO_2) were given before the hypoxic test.

2) Excitability test

After successful penetration of a hypoglossal motoneuron, a depolarizing current was applied through the recording electrode over the range of 0.3-5.5 nA. The stimulus current was increased by 0.1 nA until at least one action potential could be initiated. This minimum current was defined as rheobase. After 1-3 minutes stable recording, normocapnic hypoxia (17%) was induced for 2-3 minutes followed by a return to normoxia in all units.

3) Impedance test

Negative current pulses (0.1 nA, duration 200ms) were applied every 2 seconds through the recording electrode. A period of 3 minutes hypoxia was induced after 1-3 minutes normoxic recording. This was followed by a post-hypoxia recording period of 2-3 mins.

2.5.2.c Data collection and analysis

The activity of hypoglossal motoneurones were recorded on digital tape for further computer analysis using a script program (a program for measuring membrane potential), written by Dr. R. Noble, to run with Spike2 software (Cambridge Electronic design, U.K.). Mean and standard error deviations for all measured variables were calculated for each period.

1) Membrane potential

The maximum change of membrane potentials were measured at the end of normoxia, hypoxia and post-hypoxia by a program that runs in the spike2 program, written by Dr. R. Noble.

2) Respiratory-related EPSPs and IPSPs

The duration and amplitude of the respiratory-related EPSP and/or IPSP activity during hypoxia was measured and compared to the corresponding activity during hypoxia and tested for significance using the paired Student's *t*-test (two-tailed, $P<0.05$). If the recording was long enough to return to post-hypoxia, the amplitude and duration of respiratory-related EPSPs and/or IPSPs were measured and compared with normoxia using the same test.

3) Laryngeal evoked potentials

The amplitude and duration of laryngeal evoked potentials were average by a triggered average program run in the Spike2 system and were also measured. During normoxia and hypoxia, when mean changes were greater than 10% from baseline they were categorised as an increase or a decrease. The median amplitude and duration were compared as a group in normoxia and a group during hypoxia by using Wilcoxon signed nonparametric paired two-tailed test.

4) Excitability and Impedance test

Impedance change

Changes in membrane impedance were indicated by a change in the voltage drops across the membrane in response to the current pulses during normoxia, hypoxia and post-hypoxia. Comparison of the averages from 6-7 pulses were made between normoxia, hypoxia and post-hypoxia. Input resistance was measured at the peak of the voltage response and immediately before the end of the pulses (steady-state). Results presented are mean$\pm$SEM. Paired two-tailed Student's *t*-test was used for comparison of individual observation between normoxia and hypoxia or normoxia and post-hypoxia. Differences in means were considered statistically significant if $P < 0.05$.

Excitability

The changes in excitability during hypoxia compared to normoxia were judged by the ability of a given depolarizing current pulses to generate action potentials. A decreased excitability was indicated by either an inability to generate action potentials or by a shift in onset time of the action potential so generated.

5) The factors which may influence the change of hypoglossal activity during hypoxia

Most of the intracellular results were analysed for possible correlation with possible factors of influence by using the Pearson linear correlation by linear regression. The correlation between two groups of data were considered significant when $P < 0.05$.

CHAPTER 3
RESULTS

Overview of the results

131 hypoglossal motoneurones were recorded; 26 with extracellular electrodes in 9 kittens and 105 with intracellular electrodes in 20 kittens.

The results in this thesis have been divided into four sections:

Section 1 presents the results from extracellular recordings showing the effects of mild levels of hypoxaemia on the discharge frequency of the hypoglossal motoneurones.

Section 2 presents the results from intracellular recordings showing the effects on the membrane potential and rhythmic discharge during mild levels of hypoxaemia.

Section 3 presents the result from intracellular recordings showing the effects of hypoxaemia on laryngeal-evoked potentials.

Section 4 presents the results from intracellular recordings showing the effects of mild hypoxaemia on membrane impedance and hypoglossal motoneurone excitability.

3.1 DISCHARGE FREQUENCY

3.1.1 The general characteristics of hypoglossal motoneurones from extracellular recordings

3.1.1.a Antidromic latency

The latency of the antidromic action potentials for the hypoglossal motoneurones recorded with extracellular electrodes ranged from 1.89 to 3.66 (2.62 ± 0.0097) milliseconds (msecs). One example is shown in figure 6. The latency was measured from the start of the stimulus artifact to the peak of the action potential. The latency from stimulus to the peak of the spike was constant in any given neurone and was not different in normoxia and hypoxia.

3.1.1.b Discharge pattern and frequency

In normoxia, the hypoglossal motoneurones exhibited an irregular pattern of discharge with frequencies ranging from 0 (no spontaneous discharge) to 16.73 ± 2.17 impulses per second (imp/sec).

1) The different levels of discharge frequency

In table 1, the motoneurones are grouped according to their discharge frequency in normoxia. More than half of them (56%) had discharge frequencies of less than 1 imp/sec.

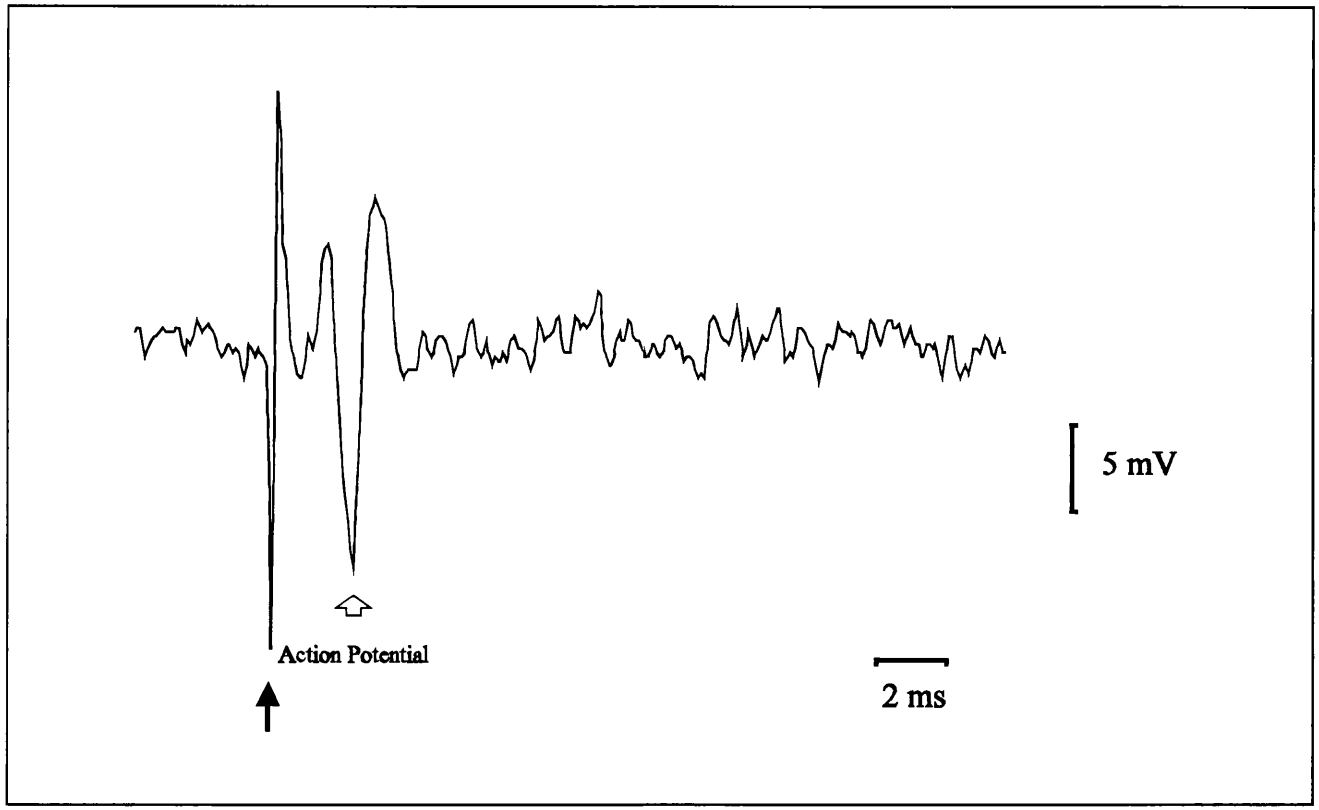


Figure 6. Antidromic activation of a hypoglossal motoneurone evoked by stimulation of the hypoglossal nerve. The arrow points the stimulus artifact and action potential.

Discharge Frequency During Normoxia (imp/sec)	<1	1-2	>2	Total
Number of Motoneurones	15 (55.6%)	6 (22.2%)	6 (22.2%)	27 (100.0%)

Table 1. Summary of the discharge frequency of the hypoglossal motoneurones recorded in 27 hypoglossal motoneurones in 9 kittens (19 to 25 days old, 22.56±0.89) in normoxia.

2) Patterns of discharge in normoxia

During normoxia, 16 of the 27 motoneurones (59.25%) showed clear rhythmic discharge; the remaining 11 of 27 (40.75%) had no obvious rhythmic discharge.

3.1.2 Discharge frequency during hypoxia

The effect of hypoxia (FiO_2 0.16 to 0.18) on discharge frequency was determined from 27 hypoglossal motoneurones, recorded with extracellular electrodes, in 9 kittens.

3.1.2.a The effect of hypoxia on pattern of discharge frequency

The motoneurones were grouped according to their response to hypoxaemia and have been summarized in table 2. Hypoxia evoked four types of response: sustained increase, transient increase, decrease and no effect. For each motoneurone recorded a two-tailed, unpaired Student's t-test was used to determine the significance of the results ($P<0.05$). Of the 27 units tested, 13 units showed an increase in discharge frequency. However, this increase was only sustained in 4 of these throughout the hypoxic period. Nine of these motoneurones showed only a transient increase in discharge frequency. Eleven motoneurones showed a decrease in discharge frequency during hypoxia.

	Sustained Increased	Transient Increase	Decrease	No Effect	Total
Number of Motoneurones	4 (14.8%)	9 (33.3%)	11 (40.7%)	3 (11.7%)	27

Table 2. A summary of the responses of the hypoglossal motoneurones recorded in 9 neonatal kittens (19 to 25 days old, 22.56±0.89) to mild levels of isocapnic hypoxia (FiO_2 0.16 to 0.18, 0.1641±0.0013).

1) Hypoglossal motoneurones responding to hypoxia with a sustained increase in discharge frequency

The discharge frequency of these neurones ranged from 0 to 0.70±0.26 (0.24±0.16) imp/sec in normoxia. The maximum increase in discharge frequency was recorded within 3 to 6 (4.5±0.65) minutes of reducing the FiO_2. The peak frequency of the motoneurones during hypoxia ranged from between 0.23±0.09 and 22.27±3.64 (6.26±5.36) imp/sec.

Two examples of the results are shown in figure 7. In each case, the top trace shows mean discharge frequency represented as a moving time average with a time bin of 10 secs. The bottom trace shows the mean discharge frequency calculated for each minute. The stippled bar represents the period of hypoxia. The arrows indicate when the FiO_2 level had reached 16% in hypoxia and 21% on return to normoxia. In the example shown in figure 7A, the calculated mean discharge frequency over each minute increased significantly ($P<0.05$) from 0.73± 0.19 imp/sec within 4 minutes of hypoxia to 2.28±0.63 imp/sec when the FiO_2 reached 16%. In this case, the onset of the response was relatively early with a significant increase in discharge frequency in the first minute of hypoxia (FiO_2 17). In the case of the other neurone in figure 7B,

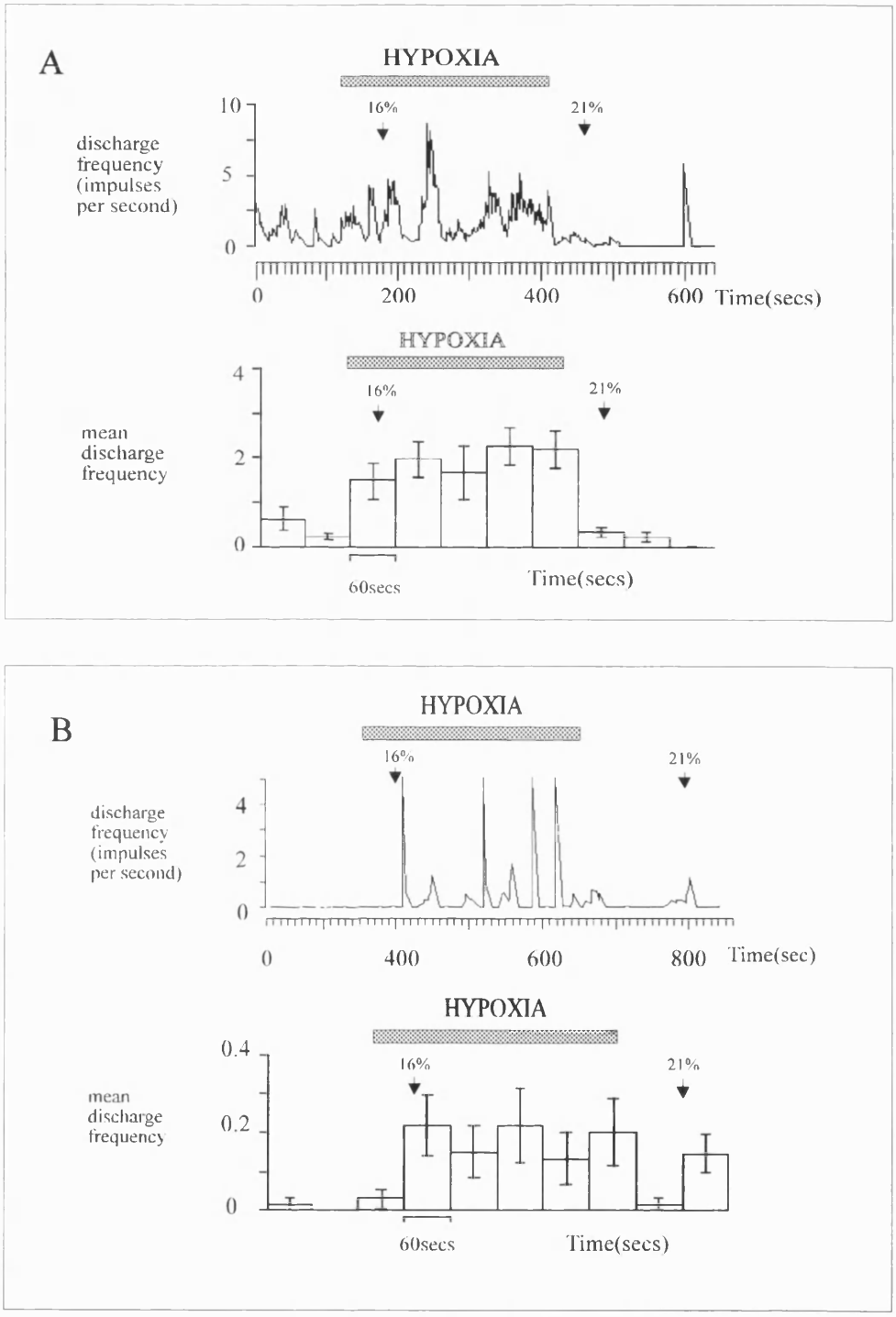


Figure 7. Two examples of hypoglossal motoneurones recorded extracellularly and which responded to hypoxia (stippled bars, FiO_2 0.16) with a sustained increase in discharge frequency. The response is presented as a moving time average (top trace in each case, time bin 10 secs) and discharge frequency (mean ±SEM) over each successive minute (bottom trace in each case). The kittens were 21 and 22 days old and weighed 380 and 340g respectively.

the discharge frequency increased ($P<0.05$) from 0.02 ± 0.02 to 0.22 ± 0.08 imp/sec within 2 minutes of decreasing the FiO_2 and the lowest level during hypoxia in the fourth minute in hypoxia was still significantly greater ($P<0.05$) than the discharge frequency recorded during normoxia. As the FiO_2 level returned to 21%, the discharge frequency was significantly greater than pre-hypoxic levels ($P<0.005$).

2) Hypoglossal motoneurones which responded to hypoxia with a transient increase in discharge frequency

Thirteen hypoglossal motoneurones increased their discharge frequency initially, but this increase was not sustained throughout the hypoxic challenge. In the first one or two minutes of hypoxia, the discharge frequency increased, but the level of discharge had returned to or fallen below the pre-hypoxic level in the later period of hypoxia. Half of the hypoglossal motoneurones recorded were classified as this kind of response. The duration of hypoxia was 3-5 (3.67 ± 0.29) minutes. The discharge frequency of the group with a transient response during hypoxia ranged from 0 to 2.95 ± 0.97 (0.49 ± 0.15) imp/sec in normoxia and the peak increase ranged from 0.23 ± 0.09 to 18.5 ± 5.82 (4.17 ± 1.84) imp/sec. The lowest level of mean discharge frequency following the transient increase in hypoxia ranged from 0 to 2.27 ± 0.55 (0.19 ± 0.07) imp/sec.

Two further examples are shown in figure 8. The hypoglossal motoneurones responded to hypoxia with a transient increase in discharge frequency. In figure 8A, the discharge frequency increased significantly ($P<0.05$) from 0.03 ± 0.02 to 1.75 ± 0.66 imp/sec within two minutes of reducing the FiO_2. However, after one minute the discharge frequency returned to or just below the pre-hypoxic level. In the second of these two examples (figure 8B), the discharge frequency increased significantly ($P<0.001$) from 0.17 ± 0.1 to 3.35 ± 1.2 imp/sec within one minute after the FiO_2 was reduced to 16%. The peak discharge frequency was 20.27 ± 5.82 imp/sec (significant change compared to normoxia, $P<0.001$) and this returned to 0.17 ± 0.06 imp/sec after 4 minutes of hypoxia.

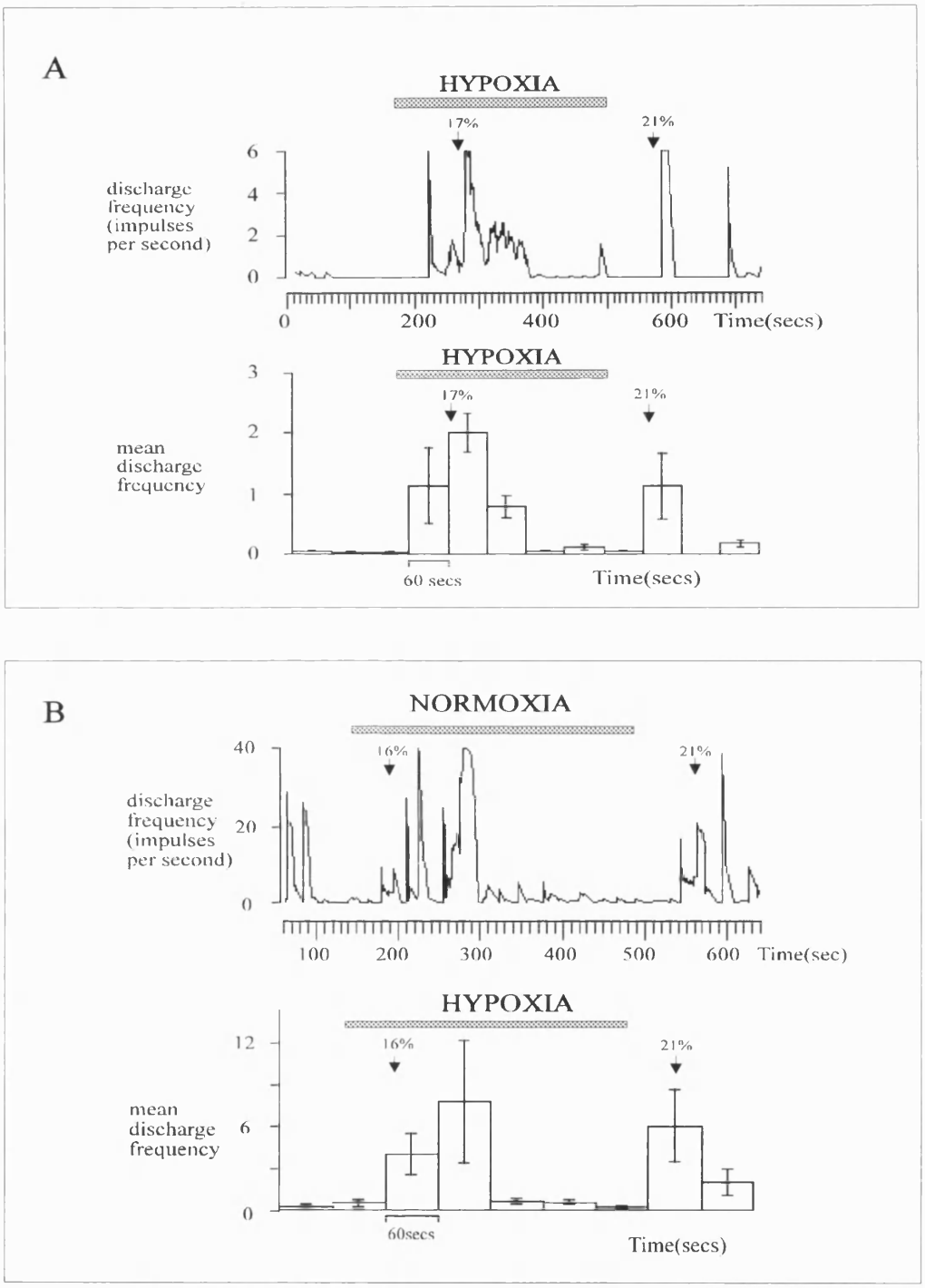


Figure 8. Two examples of hypoglossal motoneurones recorded extracellularly and which responded to hypoxia (stippled bars, FiO_2 0.17 and 0.16 respectively) with only a transient increase in discharge frequency. The response is represented as a moving time average (top trace in each case, time bin 10 secs) and discharge frequency (mean±SEM) over each successive minute (bottom trace in each case). The kittens were aged 19 and 20 days old and weighed 380 and 400g respectively. Note the rebound response when FiO_2 returned to normoxia.

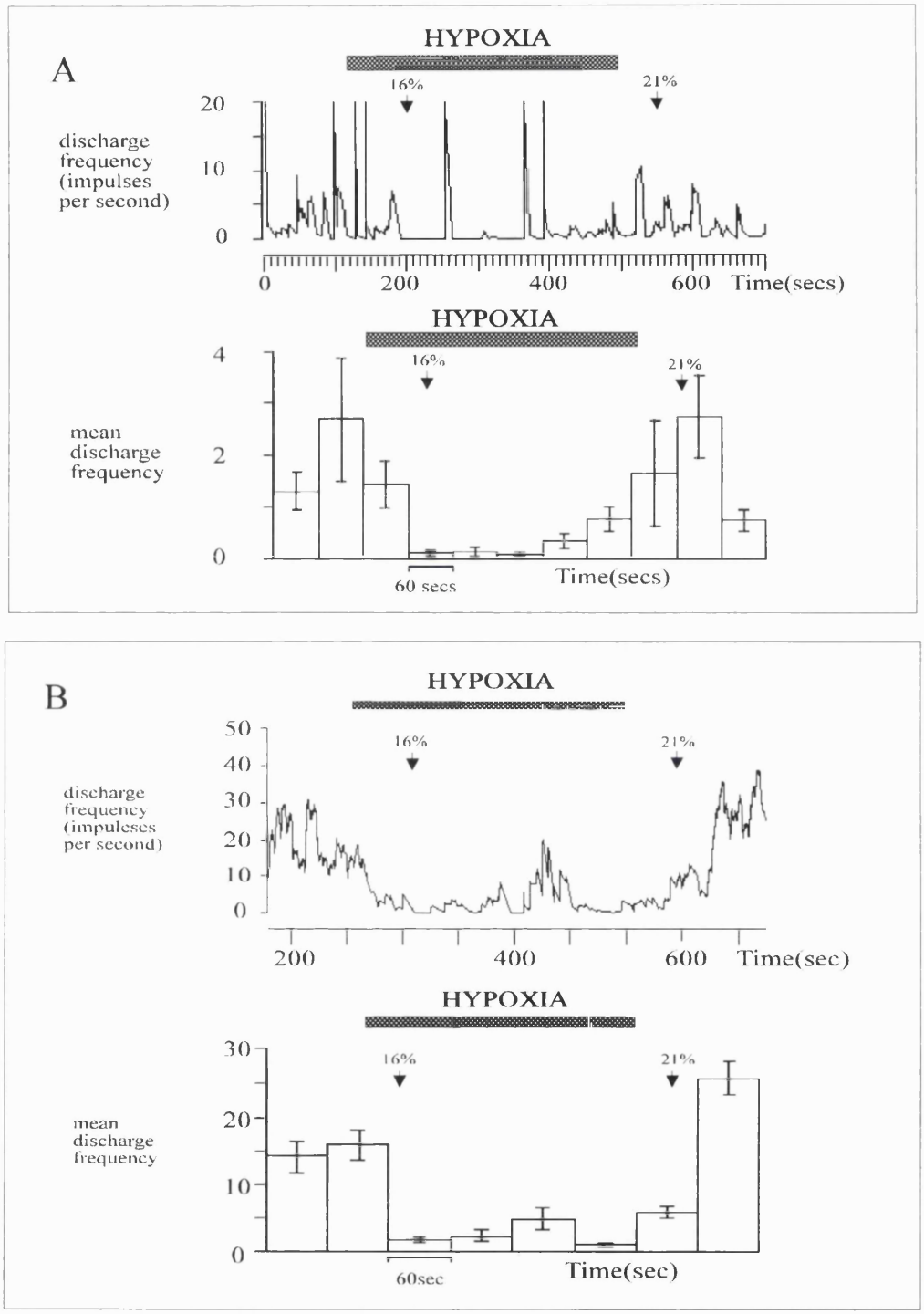


Figure 9. Two examples of hypoglossal motoneurones recorded extracellularly and which responded to hypoxia (stippled bars, FiO_2 0.16) with a decrease in discharge frequency. In both cases the discharge frequency was reduced throughout the hypoxic period. The response is presented as a moving time average (top trace in each cases, time bin 10 secs) and discharge frequency averaged (mean $\pm$SEM) over each successive minute (bottom trace in each case). The kittens were 21 and 20 days old and weighed 380 and 325g respectively.

3) Hypoglossal motoneurones which responded to hypoxia with a decrease in discharge frequency

Nine motoneurones showed a decrease in discharge frequency during hypoxia. The mean discharge frequency of these neurones was 0.28 ± 0.13 to 16.73 ± 2.17 (4.8 ± 1.64) imp/sec in normoxia and was 0 to 2.35 ± 0.53 (0.68 ± 0.23) imp/sec during the hypoxic period. The period of hypoxia was 3 to 5 (3.36 ± 0.2) minutes and the level of hypoxia was 16% to 18%O_2 (16.36 ± 0.15) in FiO_2.

Two examples showing an inhibitory response are presented in figure 9. In the first case (figure 9A) the discharge frequency fell significantly ($P<0.005$) from 1.30 ± 0.37 imp/sec in the first minute of normoxia to 0.10 ± 0.06 imp/sec during the second minute of hypoxia. The fall began 1 minute after the change to the hypoxic mixture, and returned to the pre-hypoxic level (1.63 ± 1.02 imp/sec) after the FiO_2 was returned to 21%. Figure 9B shows a similar response in another hypoglossal motoneuron. The discharge frequency was 11.98 ± 2.37 imp/sec during normoxia and this decreased to 2.87 ± 0.68 imp/sec ($P<0.001$) in the first minute when the FiO_2 reach 16%. When the FiO_2 returned to normoxia, the discharge frequency was 23.88 ± 2.59 imp/sec and this was significantly greater than pre-hypoxic levels ($P<0.001$). In this case then, a clear rebound excitation occurred on return to normoxia.

During the decrease in both the transient and inhibited groups, the motoneurones were still able to generate action potentials either spontaneously or when antidromically activated. Furthermore, there was no significant change in antidromic latency recorded for each motoneurone.

3.1.2.b The patterns of rhythmic discharge during hypoxia

Hypoxia had effects on both frequency and pattern of discharge of the HMN recorded in this study. As described above in section 3.1.1.ii, 16 of 27 (59.26%) HMNs showed clear rhythmic activity (determined by observing the traces). 11 of 27 presented a non-

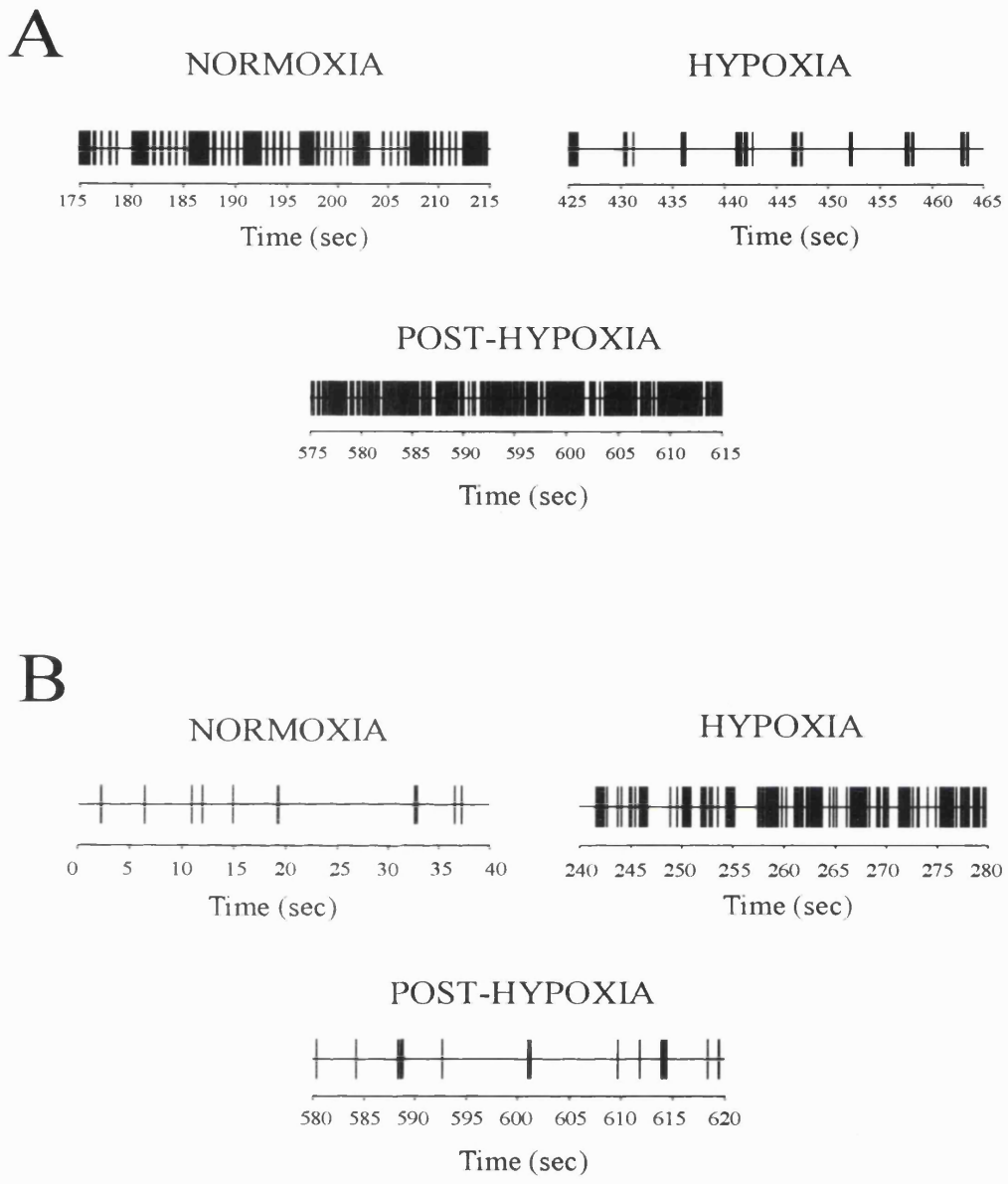


Figure 10. Patterns of discharge of two hypoglossal motoneurones recorded extracellularly during normoxia, hypoxia and post-hypoxia. In each case the impulses are represented as pulses generated by a window discrimination. A. The discharge was rhythmic during normoxia; there was a decrease in discharge frequency during hypoxia (FiO_2, 0.17), but this was still rhythmic. However, the discharge increased and the bursts became indistinguishable in post-hypoxia. B. In this case, there was a low discharge frequency during normoxia. This increased during hypoxia (FiO_2 0.16) when rhythmic activity became apparent.

rhythmic discharge pattern. During hypoxia, 8 of 16 units showed a decrease in the frequency of these rhythmic bursts of action potentials, 4 of 16 had either a transient or sustained increase in such rhythmic discharge whereas 4 of 16 had no change during the hypoxic period. The units which had non-rhythmic pattern of discharge in normoxia had nevertheless exhibited this activity during the hypoxic challenge. Two examples are shown in figure 10. In this case, during hypoxia the mean discharge frequency calculated over each minute significantly decreased from 16.73 ± 2.17 to 5.22 ± 0.82 imp/sec ($P < 0.001$). Nevertheless, the rhythmic activity was still evident. Compared to the post-hypoxic period, both the irregular discharge and rhythmic activity was significantly increased compared to the pre-hypoxic control period (figure 10A, FiO_2 0.16). This is, therefore, another example of a rebound excitation on return to normoxia.

Three additional motoneurones showed no distinct rhythmic activity during normoxia, but the activity appeared during hypoxia. Figure 10B shows the action potentials represented as gated pulses generated by the spike processor during periods of normoxia, hypoxia (FiO_2 0.16) and post-hypoxia. In this case, the mean discharge frequency increased from 0.48 ± 0.33 in control to 22.27 ± 3.64 bursts per second during hypoxia ($P < 0.001$). Notice that during post-hypoxia the discharge frequency returned to pre-hypoxic levels.

3.1.2.c Effect of hypoxia on phrenic activity

In addition to the hypoglossal recordings, phrenic discharge frequency was recorded in 3 preparations. An example is shown in figure 11. In this case, during the last minute of hypoxia the hypoglossal discharge was reduced, but the discharge of phrenic nerve was sustained. In the top part of this figure, hypoglossal motoneurone and phrenic nerve activities are presented as moving time averages with a time bin of 10 seconds in each case. If the time scale is extended, as shown in the bottom of the figure, and the activity of the hypoglossal motoneuron and phrenic nerve are shown with a time bin of 2 seconds, it is clearly seen that the rhythmic discharge of the

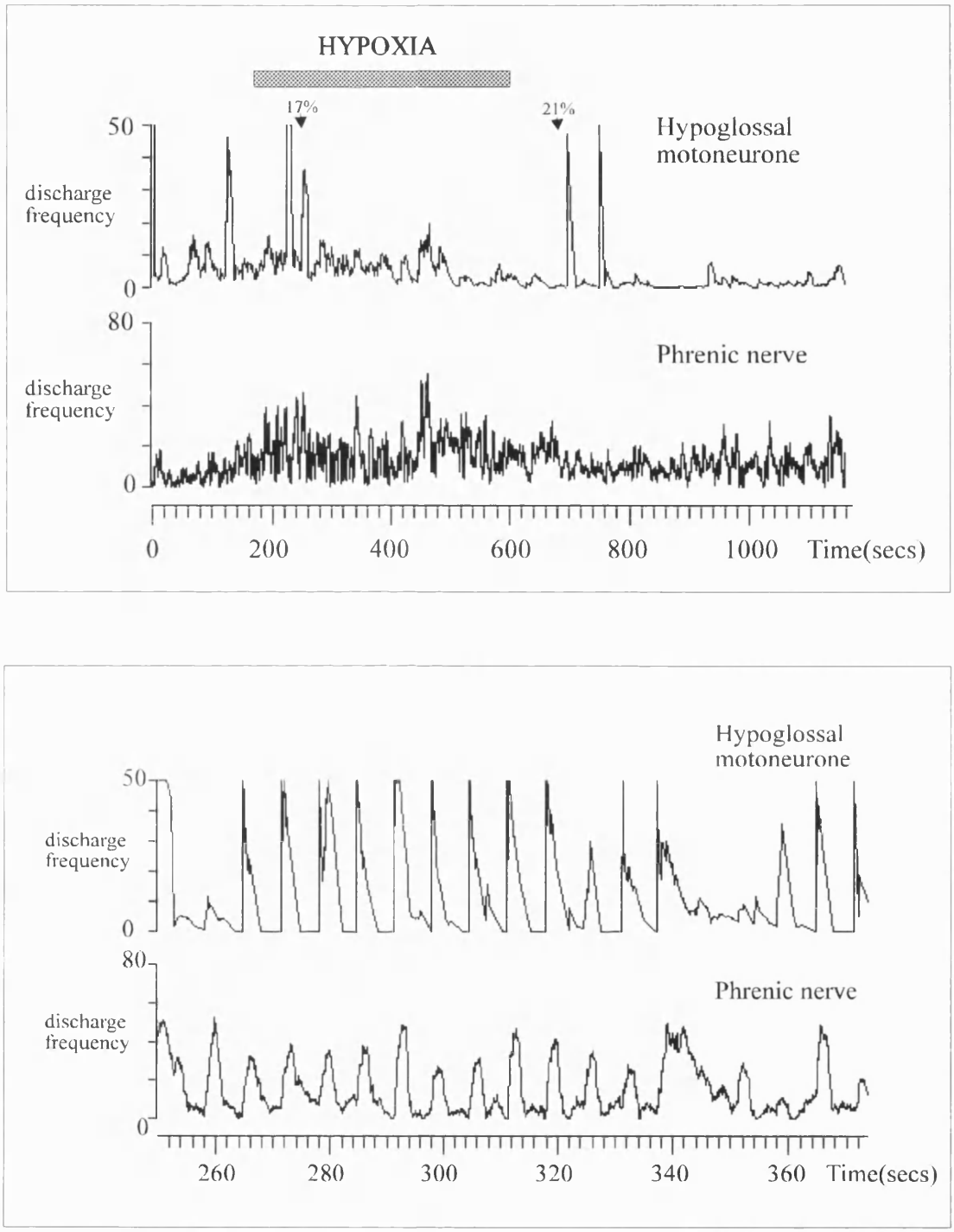


Figure 11. Moving time averages of both rhythmic phrenic (bottom traces) and hypoglossal motoneurone discharge (upper traces) recorded simultaneously. In the top half of the figure, the time bin in set at 10 secs. In the bottom half, the time bin was 2 secs. Hypoglossal rhythmic activity occurred with the inspiratory phase of the respiratory cycle. FiO_2 was 0.17 during hypoxia. This kitten was 25 days old and weighed 440g.

hypoglossal motoneuron was correlated with the inspiratory phase of the respiratory cycle.

3.1.2.d The possible factors influencing the pattern of discharge frequency during hypoxia

1) Animals and age

The type of response to hypoxia was not obviously related to the age of the kittens. The three observed patterns of the response often occurred in the same preparation. For example, 4 transient increases, 1 sustained increase and 2 decreases in discharge frequency were recorded from different motoneurones in one 22 day old kitten. Most kittens were around 20 to 22 days old (21 of 27 motoneurones), however we do not have sufficient numbers of animals in different age groups to statistically examine whether age was a factor in the type of response observed.

2) Hypoxic level

Different levels of hypoxia were used during the extracellular recordings of the 27 motoneurones. The mean of PaO_2 was 94.3 ± 1.32 mmHg during normoxia and 41.9 ± 0.91 mmHg during hypoxia. The range of FiO_2 was between 16% to 18%. 19 out of 27 (70.4%) motoneurones were tested with 16% FiO_2 and 5 out of 27 (18.5%) with 17% and 3 out of 27 (11.1%) with 18% O_2. The grouped level of PaO_2 is shown in table 3.

There is no significant correlation between the response to hypoxia and the change in PaO_2 ($r=0.2737$, $P=0.1671$).

Response During Hypoxia	Sustained Increase	Transient Increase	Decrease	No Effect	Total
30-40 mmHg	0 (0%)	2 (7.4%)	2 (7.4%)	0 (0%)	4 (14.8%)
40-50 mmHg	3 (11.1%)	3 (11.1%)	3 (11.1%)	1 (3.7%)	10 (37.0%)
50-60 mmHg	0 (0%)	3 (11.1%)	3 (11.1%)	0 (0%)	6 (22.2)
60-70 mmHg	1 (3.7%)	1 (3.7%)	4 (14.8%)	1 (3.7%)	7 (25.9%)
Total	4 (14.8%)	9 (33.3%)	12 (44.4%)	2 (7.4%)	27 (100%)

Table 3. Percentage of responses of hypoglossal motoneurones at different levels of PaO_2 (mmHg) in 27 hypoglossal motoneurones.

3) pH

pH was 7.38±0.08 in normoxia and 7.31±0.12 during hypoxia, there was no significant change during hypoxia.

4) $PaCO_2$

The level of $PaCO_2$ for the extracellular recordings ranged between 33.4 and 43.2 mmHg (38.19±0.51 mmHg) during normoxia and 30.3 to 49.4 mmHg (36.43±0.84 mmHg) during hypoxia. Twenty-three of 27 units had a repeated hypoxia test after each recording. Nineteen of 23 tests showed a decrease in $PaCO_2$ during the period of hypoxia (38.73±0.49 in normoxia and 35.17±0.98 during hypoxia). Are the responses of discharge frequency dependent on the changes of $PaCO_2$ during hypoxia? There is

no correlation between the responses of hypoglossal motoneurones in response to hypoxaemia and $PaCO_2$ during hypoxia ($r=0.02008$, $P=0.1590$).

5) Arterial blood pressure

The mean arterial blood pressure (MAP) was ranged 47.5 to 80 mmHg (56.98 ± 2.15) before hypoxaemia. In 26 hypoxic tests (96.3%) it was found that blood pressure decreased during hypoxia. The range of blood pressure was from 34.5 mmHg to 70 mmHg (43.90 ± 2.69 mmHg) during hypoxia. However, only one unit (3.7%) showed an increase in blood pressure during the hypoxic period (from 50 mmHg to 60 mmHg). In this case, because blood pressure decreased in 96.3% of cases, no relationship could be observed between blood pressure changes during hypoxia and the type of responses observed.

6) Range of discharge frequency

Table 4 shows the relationship between discharge frequency in normoxia and the pattern of responses of the HMNs. Most of the units with a sustained or transient excitation had a frequency of $<$ 1 imp/sec. In contrast, most of the units exhibiting a decrease in discharge frequency had normoxic discharge frequencies of 1-2 or 2 imp/sec. In fact those with a frequency greater than 2/sec in normoxia ($n=6$) all showed an inhibition during hypoxia. However, there was not sufficient numbers to examine these relationships statistically.

Discharge Frequency During Normoxia (imp/sec)	Responses to Hypoxia (unit)				
	Sustained Increase	Transient Increase	Decrease	No Effect	Total
<1	4 (14.8%)	7 (25.9%)	2 (7.4%)	2 (7.4%)	15 (55.6%)
1-2	0 (0.0%)	2 (7.4%)	3 (11.1%)	1 (3.7%)	6 (22.2%)
>2	0 (0.0%)	0 (0.0%)	6 (22.2%)	0 (0.0%)	6 (22.2%)

Table 4. Comparison of percentage of HMNs in response to mild levels of hypoxia and discharge frequency during normoxia in vagotomized kittens.

3.1.3 Effect of vagal influences

3.1.3.a The difference between vagotomized and vagi-intact kittens in the pattern of response to hypoxia

In a previous study in this laboratory (Smith et al., 1993a), the effect of hypoxia on discharge frequency was determined for 41 HMNs recorded with extracellular microelectrodes in 18 vagi-intact animals. The motoneurones were grouped according to their response to hypoxia and have been summarized in table 5. There is a bigger proportion of inhibitory effect on discharge frequency recorded in vagotomized kittens.

	Sustained Increase	Transient Increase	Decrease	No effect	Total
Vagi-intact	14 (34.0%)	11 (26.8%)	8 (19.5%)	8 (19.5%)	41
Vagotomized	4 (14.8%)	9 (33.3%)	11 (40.7%)	3 (11.1%)	27

Table 5. Comparison of percentage of HMNs in response to mild levels of isocapnic hypoxaemia in vagotomized and vagi-intact kittens. The level of mild hypoxia was 0.16 to 0.18 FiO_2 in vagotomized kittens and 0.14 to 0.18 FiO_2 in vagi-intact kittens.

3.1.3.b difference in rhythmic discharge

A significantly greater proportion of hypoglossal motoneurones (17 of 27, 62.96% in vagotomized kittens) had a rhythmic discharge, either in normoxia or hypoxia, than was found in vagi-intact kittens (2 of 41, 4.9%).

3.1.4. Summary of section 1

This section has shown that hypoglossal motoneurones in neonatal kittens respond to even a mild reduction in the FiO_2.

Of the 27 hypoglossal motoneurones tested with hypoxia by using extracellular electrodes, three types of response was recorded:

1) 4 (14.8%) showed a sustained increase
2) 9 (33.3%) showed a transient increase
3) 11 (40.7%) showed a decrease
4) and 3 (11.7%) showed no significant change in discharge frequency.

These responses were similar to the results in vagi-intact kittens in the previous study in this laboratory.

A significantly greater proportion of hypoglossal motoneurones (17/27, 62.96%) had a rhythmic discharge, either in normoxia or hypoxia, than was found in non-vagotomized kittens (5/41, 12.20%).

When phrenic discharge was also recorded the rhythmic HMNs were found to be inspiratory related.

There is no significant correlation between the type of responses and age, the levels of PaO_2 and $PaCO_2$. No relationship could be observed between the type of response and pH and blood pressure.

3.2 MEMBRANE POTENTIALS

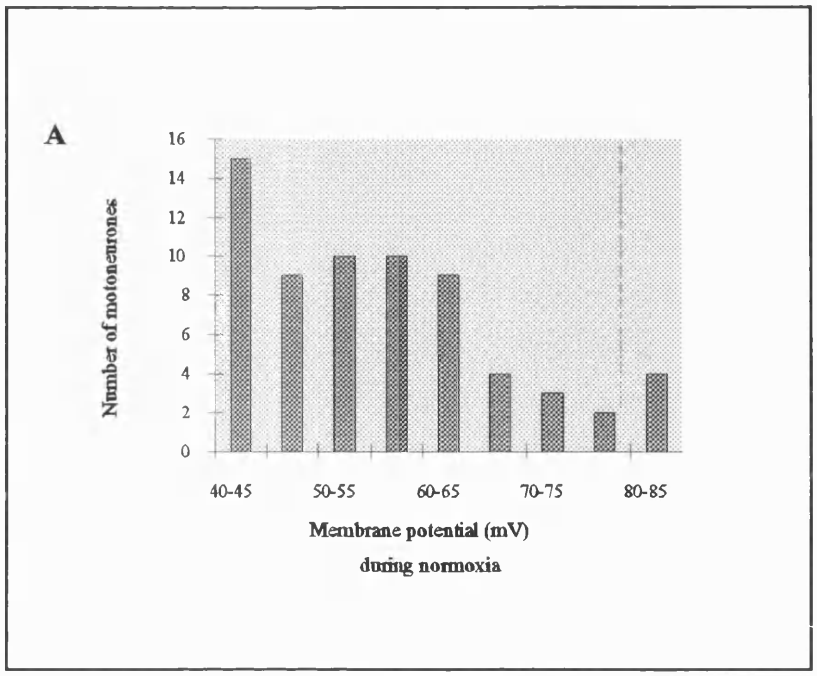
This section presents the results the intracellular recordings investigating the effect of mild levels of hypoxia on the membrane potentials of hypoglossal motoneurones in neonatal kittens. Intracellular recordings were made from 87 hypoglossal motoneurones in 20 kittens. The effects of hypoxia on 42 motoneurones were tested. The effect of mild hypoxia on upper airway evoked potentials were recorded in 26 of these units. The effect of hypoxia on membrane potential was also tested by passage of positive or negative current pulses through the intracellular electrodes during normoxia and hypoxia (shown in section 3.4) recorded in 42 of these motoneurones. 39 hypoglossal motoneurones were recorded only during normoxia. A further 8 motoneurones were recorded initially during hyperoxia followed by a reduction of the FiO_2 to normoxia.

3.2.1 Resting membrane potential during normoxia

The resting membrane potential of the 87 motoneurones recorded during normoxia ranged between -41.43 and -83.57 mV (-53.6±5.04 mV). Figure 12A shows the distribution of the resting membrane potentials (mV) recorded in normoxia in 68 HMNs. Figure 12B shows the distribution of the resting membrane potentials recorded in 42 HMNs during the hypoxic test.

3.2.2 The effects of hypoxia on membrane potential

The resting membrane potentials of the 42 motoneurones which were recorded during



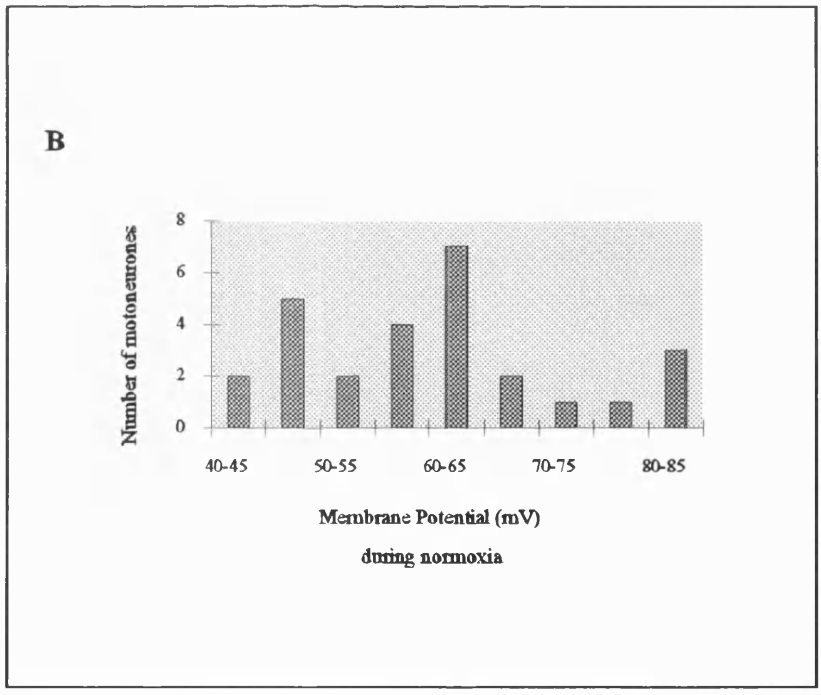


Figure 12. Bar charts showing the distribution of the hypoglossal membrane potentials (mV) recorded during normoxia. For the total sample (68 units, A) and for those subsequently tested with hypoxia (26, B).

hypoxia ranged from between -42.26 and -83.57 mV (-54.94±1.75 mV) during normoxia. 26 motoneurones were considered stable enough to be measured during hypoxia.

3.2.2.a Membrane potential depolarization during hypoxia

Most units showed a membrane potential depolarization during hypoxia.

Seventeen (65.38%) of the 26 HMNs showed a gradual decrease in membrane potential with the onset of hypoxia. The mean membrane potential of these 17 neurones was -60.7±3.14 mV during normoxia and then changed to -47.6±4.34 mV during hypoxia. The average depolarization was 27.65±4.09 mV. Two examples of a HMN which responded to hypoxia in this way are shown in figure 13. The resting membrane potential of this motoneuron during normoxia was approximately -58.21 mV and this depolarized when the FiO_2 was reduced to 16%. The membrane potential was -50.71 mV at end of hypoxia. The hypoxic test was repeated 3 mins after this recording, the FiO_2 was again reduced to 16%, and the PaO_2 fell from 95.6 to 54.3 mmHg. The membrane potential hyperpolarized from approximately -68 mV to -70 mV during the second hypoxic episode.

3.2.2.b Membrane potentials which showed a transient change during hypoxia

Five (19.23%) of these 26 motoneurones showed a transient change in membrane potential during hypoxia.

1) Two of these 5 subsequently repolarized after initial depolarization during hypoxia. The

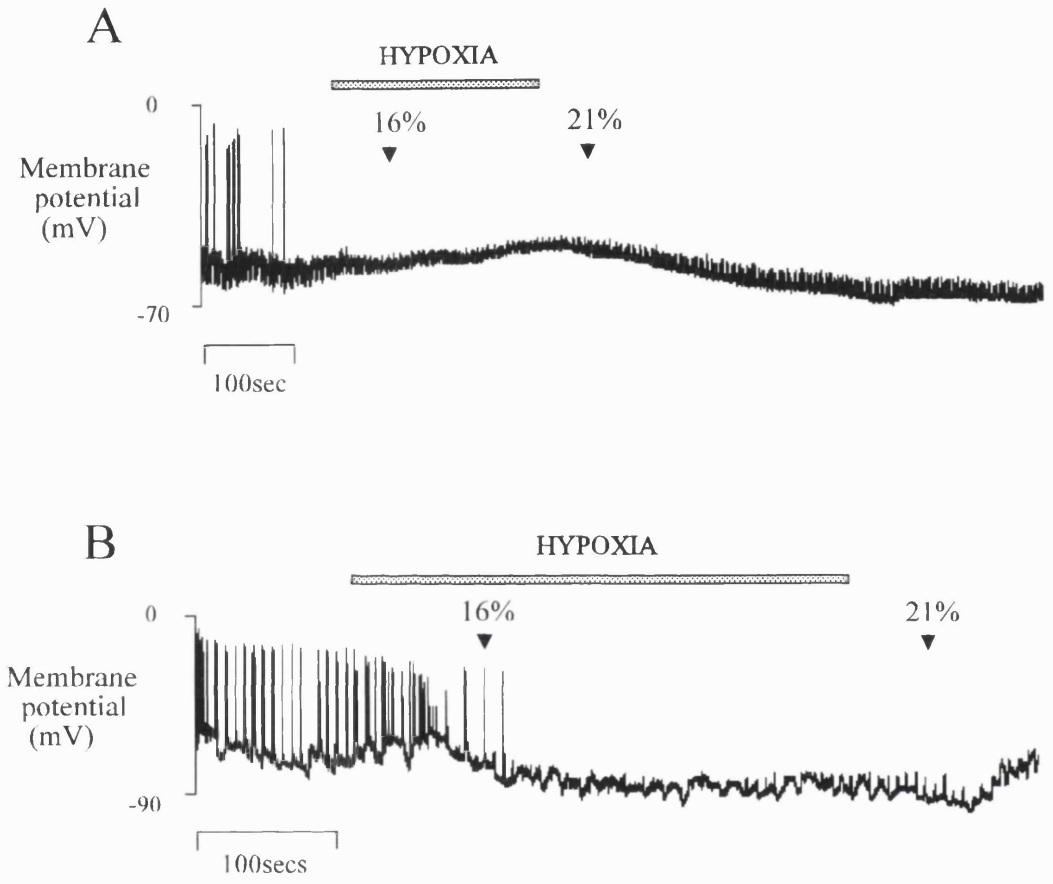


Figure 13. Intracellular recordings from two hypoglossal motoneurones. In the case A, the resting membrane potential was -60 mV during normoxia; this depolarized by approximately 13 mV during hypoxia (FiO_2 0.16). In case B, the membrane hyperpolarized from -58.21 mV in normoxia to -66 mV during hypoxia (FiO_2 0.16). The kittens in which the units were recorded were 25 and 23 days old and weighed 430 and 400g respectively.

membrane potentials of these motoneurones during normoxia were -48.58 and -61.43 mV respectively. In one case the membrane potential changed from -48.58 mV in normoxia to -35.00 mV during the first minute of hypoxia and subsequently hyperpolarized to -51.07 mV in the second minute of hypoxia. In the second unit shown in figure 14A, the membrane potential was -61.70 mV during normoxia. After reduction of the FiO_2 to 0.17, the membrane depolarized to -42.50 mV in early hypoxia and then hyperpolarized to -59.64 mV during late hypoxia. In this case, the membrane potential depolarized when the FiO_2 was returned to the pre-hypoxic level.

2) In three of the 5 hypoglossal motoneurones, there was an initial hyperpolarization followed by membrane depolarization. During the hyperpolarized period, the amplitude of spontaneous action potentials increased in these units. In figure 14B, the membrane potential was -42.8 mV during normoxia and this initially hyperpolarized to -65.00 mV during hypoxia and then depolarized to -28.93 mV. When the FiO_2 was returned to normoxia, the membrane potential gradually repolarized. During normoxia, the PaO_2 was 98 mmHg and it was reduced to 58 mmHg (FiO_2 17%) during hypoxia.

3.2.2.c Membrane hyperpolarization during hypoxia

A further 4 (15.38%) motoneurones were hyperpolarized during hypoxia by 11.08±3.21 mV. The membrane potentials were -64.90±3.89 mV during normoxia and -73.71±2.76 mV during hypoxia. An example of a hypoglossal motoneuron which responded to hypoxia in this manner is shown in figure 13. During hypoxia the membrane of this motoneurone hyperpolarized from approximately -56.16 mV to -66.07 mV. After the FiO_2

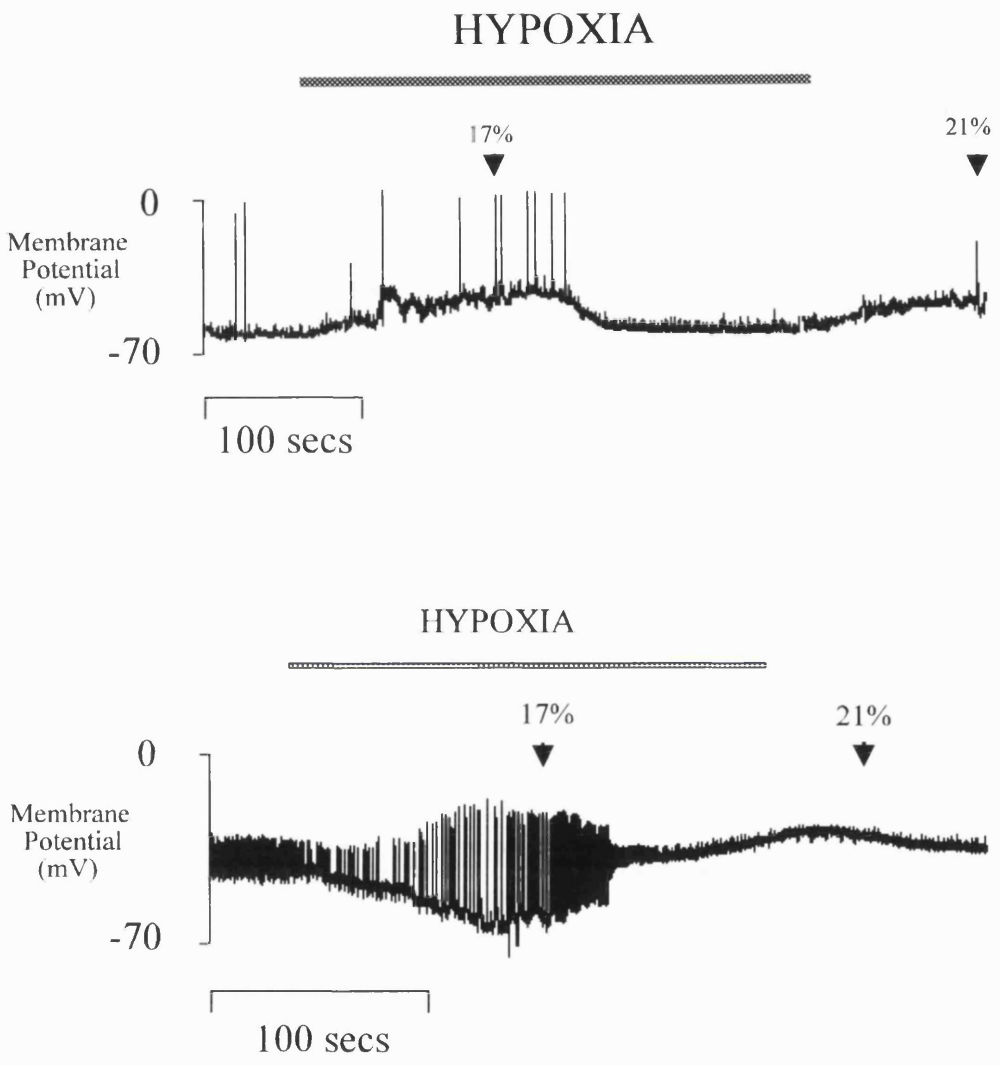


Figure 14. Intracellular recordings from two hypoglossal motoneurones which responded to hypoxia (stippled bars) with transient changes in membrane potential. In A, with the onset of hypoxia (FiO_2 0.17) there was a gradual depolarization of approximately 19 mV followed by a repolarization to approximately -59.64 mV. In case B, the resting membrane potential was -42.8 mV during normoxia. This hyperpolarized to about -65.0 mV initially during hypoxia (FiO_2 0.17) followed by a depolarization to approximately 44 mV. When FiO_2 returned to normoxia, the membrane potential gradually repolarized to pre-hypoxic level. The kittens in which these were recorded were 23 and 19 days old and weighed 280 and 350g respectively.

returned to normoxia, the membrane potential returned to -54.02 mV.

In one motoneuron, the hypoxic challenge was repeated twice and on the first occasion the membrane depolarized. But, on the second occasion there was no change in the membrane potential. On the first occasion, the FiO_2 was reduced to 16% and the membrane was depolarized by approximately 8.5 mV. After approximately 7 minutes when the FiO2 return to 21%, the hypoxic test was repeated and the FiO_2 was again reduced to 16%. The membrane potential changed from -68 to -70.00 mV.

	Sustained Depolarization	Transient Depolarization	Hyperpolarization	No Effect	Total
Vagi-intact	14 (67%)	2 (9%)	4 (19%)	1 (5%)	21 (100%)
Vagotomized	17 (66%)	5 (19%)	4 (15%)	0 (0%)	26 (100%)

Table 6. Comparison of membrane potentials of the HMNs in response to mild levels of hypoxaemia in vagotomized and vagi-intact kittens. The levels of mild hypoxia was 0.16 to 0.18 in FiO_2 in vagotomized kittens (18 to 25 days old) and was 0.14 to 0.19 in FiO_2 in vagi-intact kittens (13 to 26 days old).

In the previous study in this laboratory (Smith et al., 1993b), the results (table 6) from vagi-intact kittens showed that during hypoxia 67% HMNs exhibited a sustained depolarization (66% in the present study), 9% showed a transient depolarization (in comparison to 19% in the present study) and 19% (in comparison 15% in the present study) showed a hyperpolarization. Therefore, the effect of hypoxia on hypoglossal membrane potential is generally similar between vagotomized and vagi-intact kittens.

3.2.3 Effects of hypoxia on rhythmic excitatory postsynaptic potentials (EPSPs)

In normoxia, 22 out of 26 (84.62%) hypoglossal motoneurones displayed rhythmic excitatory postsynaptic potential (EPSP) activity. All 22 were tested with hypoxia (one was tested twice).

3.2.3.a the EPSP amplitude were decreased during hypoxia in most HMNs

The responses to mild hypoxia of the rhythmic EPSP activity are summarized in table 7.

	EPSP amplitude increase	EPSP amplitude decrease	EPSP amplitude no change	Total
EPSP duration increase	0	4	0	4
EPSP duration decrease	3	4	1	8
EPSP duration no change	1	7	3	11
Total	4	15	4	23

Table 7. A summary of the effect of hypoxia (FiO_2 0.16 to 0.18) on rhythmic EPSP activity in 22 hypoglossal motoneurones (23 hypoxic tests) in 9 vagotomized neonatal kittens (age 18 to 25 days old).

In 15 out of 23 units there was a significant decrease in the amplitude ($P<0.05$) and duration ($P<0.05$) of the EPSP activity during hypoxia. The amplitude of EPSPs ranged from 2.17±0.13 mV to 26.24±1.44 mV (10.46±1.84mV) during normoxia. The range during hypoxia was 0 to 16.27±1.32 mV (4.33±1.09 mV).

Of the 15 units in which amplitude decreased during hypoxia, the duration of the EPSPs increased in 4 of them and decreased in 4. However, 7 units showed no significant ($P<0.05$) change in EPSP duration during the hypoxic challenge.

In the 4 units which showed an increase ($P<0.05$) in rhythmic EPSP duration, the change was from 488.3±68.73 to 2526.24±264.58 ms (1407.94±432.04 ms) during normoxia and 1657.86±144.66 ms to 4617.3±244.54 ms (2578.23±685.25 ms) during hypoxia.

In the 4 units which showed a significant decrease ($P<0.05$) in duration of rhythmic EPSP during hypoxia. The duration decreased from 2797.23±339.63 ms during normoxia to 1289.81±474.29 ms during hypoxia. The example in figure 15, shows both EPSP amplitude and duration significantly decreased during hypoxia. The amplitude and duration of EPSP were 26.24±1.44 mV and 3669.8±318.47 ms in normoxia and this fell to 16.27±1.32 mV ($P<0.01$) and 2196.74±230.05 ms ($P<0.01$) during hypoxia. The FiO_2 was 18% and PaO_2 was 66 mmHg during hypoxia. During the post-hypoxic period, both amplitude and duration of EPSPs significantly decreased to 3.18±0.20 mV ($P<0.01$) and 1238.10±74.65ms ($P<0.01$).

In the group (7 of 15) which the duration of rhythmic EPSP failed to show significant

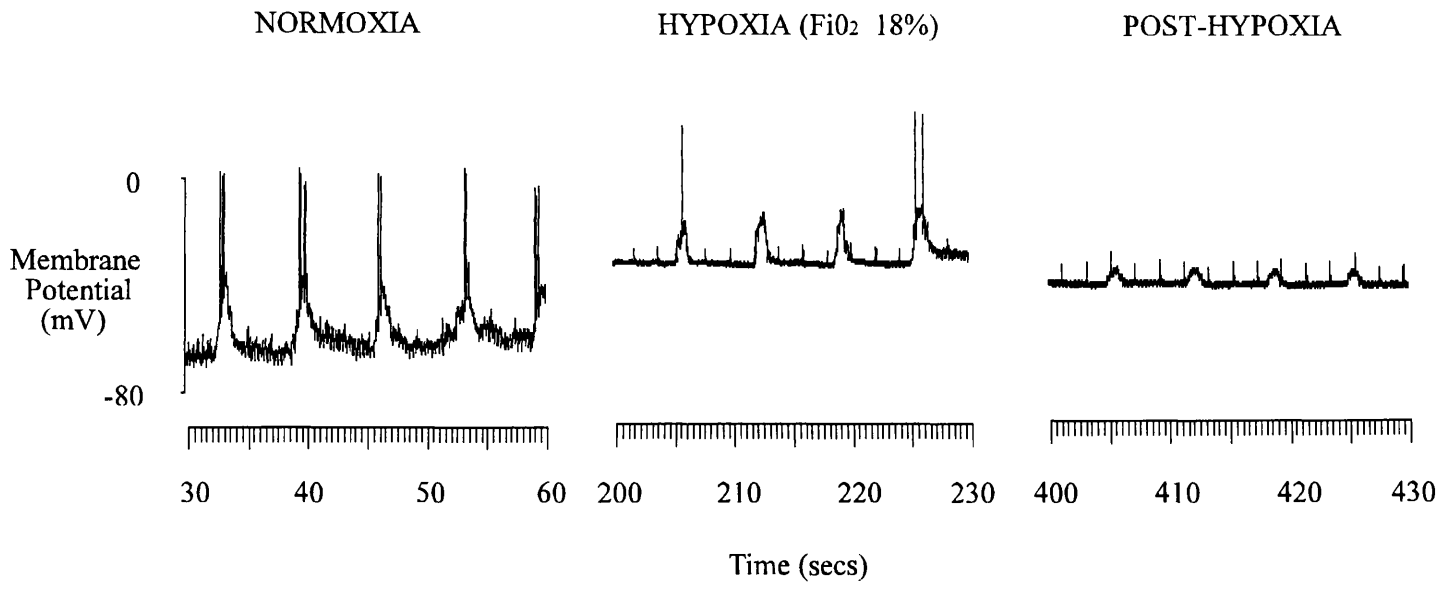


Figure 15. Intracellular recording of one hypoglossal motoneurone showing the effects of hypoxia on the rhythmic EPSPs. In this case, the membrane is depolarized from approximately -62 mV to -27 mV during hypoxia (FiO_2 0.18) and there was a decrease in both amplitude and duration of the rhythmic EPSPs. Note that both amplitude and duration did not return to pre-hypoxic level when FiO_2 has returned to normoxia. The kitten was 25 days old and weighed 430g.

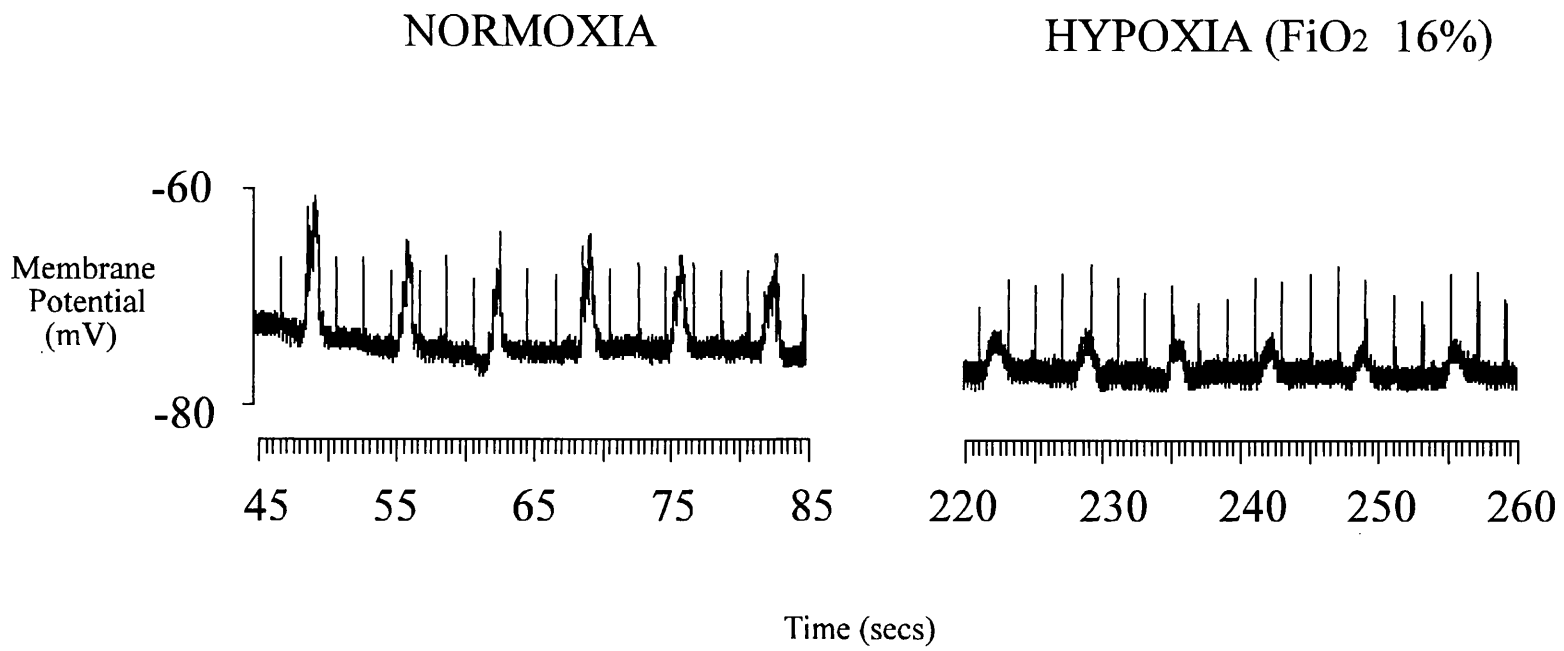


Figure 16. Intracellular recording of one hypoglossal motoneurone showing the effect of hypoxia on the rhythmic EPSPs. This shows that the resting membrane hyperpolarized during hypoxia (FiO_2 0.16), from -75 mV to -79 mV. There is a decrease in the amplitude but not duration of rhythmic EPSPs. The kitten was 25 days old and weighed 430g.

change during hypoxia, the duration was 1147.62±199.43ms to 2297.0±151.71 ms during normoxia and this was 2312.9±79.94 ms during hypoxia. In the example in figure 16, the amplitude of the rhythmic EPSP activity decreased significantly ($P<0.0001$) during the hypoxic challenge from 9.02±0.76 to 3.12±0.24 mV. The duration of the rhythmic EPSP activity was not significantly changed. The FiO_2 was 16% and 64 mmHg (PaO_2) during hypoxia.

3.2.3.b Increase EPSP amplitude during hypoxia

In 4 out of 23 motoneurones, the amplitude of the rhythmic EPSP activity increased significantly ($P<0.05$) during the hypoxic challenge from 2.33±0.24 to 8.14±0.35 mV (5.27±1.19 mV) during normoxia to 7.41± 0.57 and 19.24±0.69mV (11.05±2.80 mV) during hypoxia. The duration of the rhythmic EPSPs decreased significantly ($P<0.05$) from 2257.68±136.26 ms in normoxia to 1538.68±34.20 ms during hypoxia in 3 hypoglossal motoneurones.

In one motoneurone, shown in figure 17, the amplitude of the rhythmic EPSP increased significantly during hypoxia, the change in duration of the EPSPs was also significant. The amplitude of EPSP was 5.31±0.34 mV during normoxia and this increased to 10.09±0.79mV during hypoxia ($P<0.01$). The duration of the EPSP reduced from 2083.3±182.67 ms to 1540.80±91.12 ms during hypoxia ($P<0.05$). Note the inspiratory-expiratory related EPSP-IPSP complex during normoxia. The respiratory-related IPSPs decreased during hypoxia. Only one neurone exhibited no change in the duration of EPSP during hypoxia (figure 18). In figure 18, the membrane potential depolarized and was

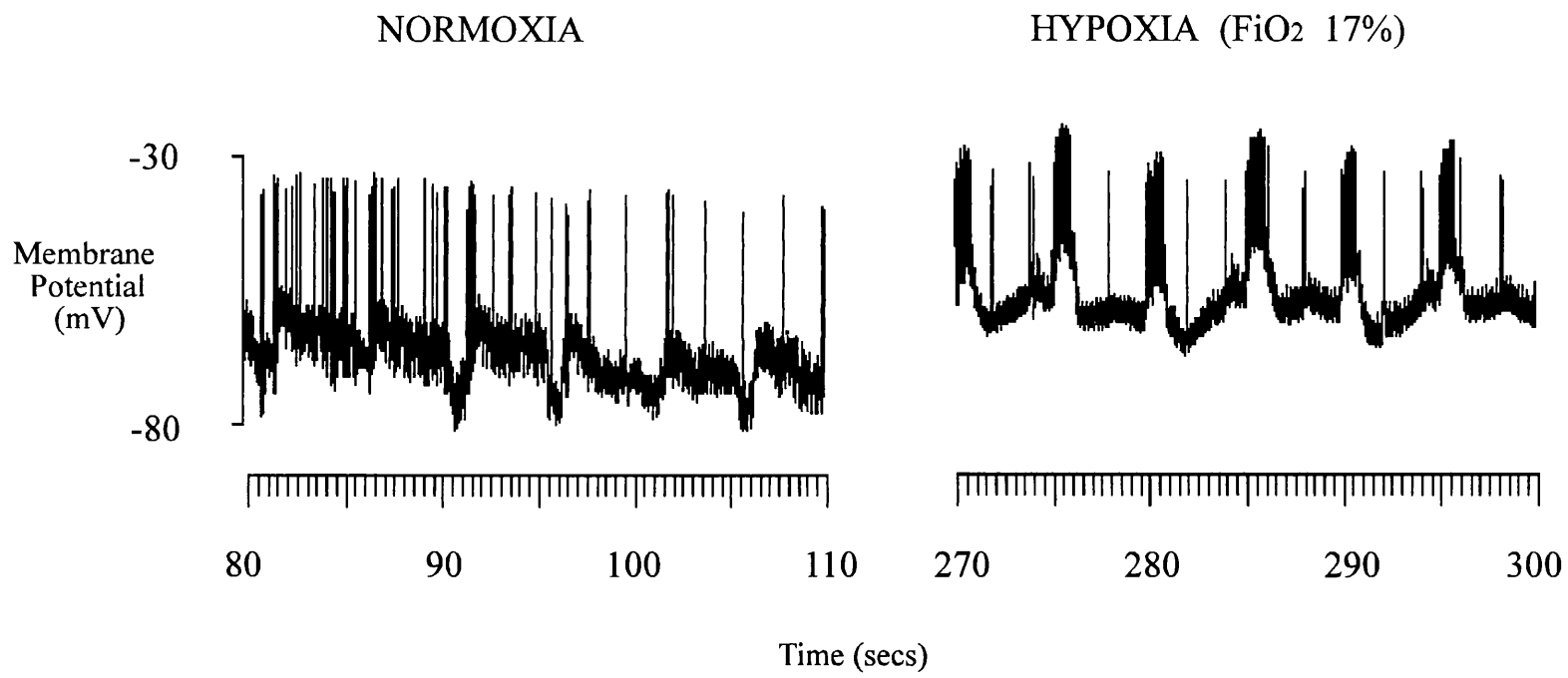


Figure 17. Intracellular recording of one hypoglossal motoneurone showing the effects of hypoxia on the rhythmic EPSPs. This shows that the resting membrane depolarized from approximately -70 mV to -52 mV during hypoxia (FiO_2 0.16) and that there is an increase in the amplitude of the rhythmic EPSP activity. Note that there is an EPSP-IPSP complex in normoxic period. The kitten was 25 days old and weighed 340g.

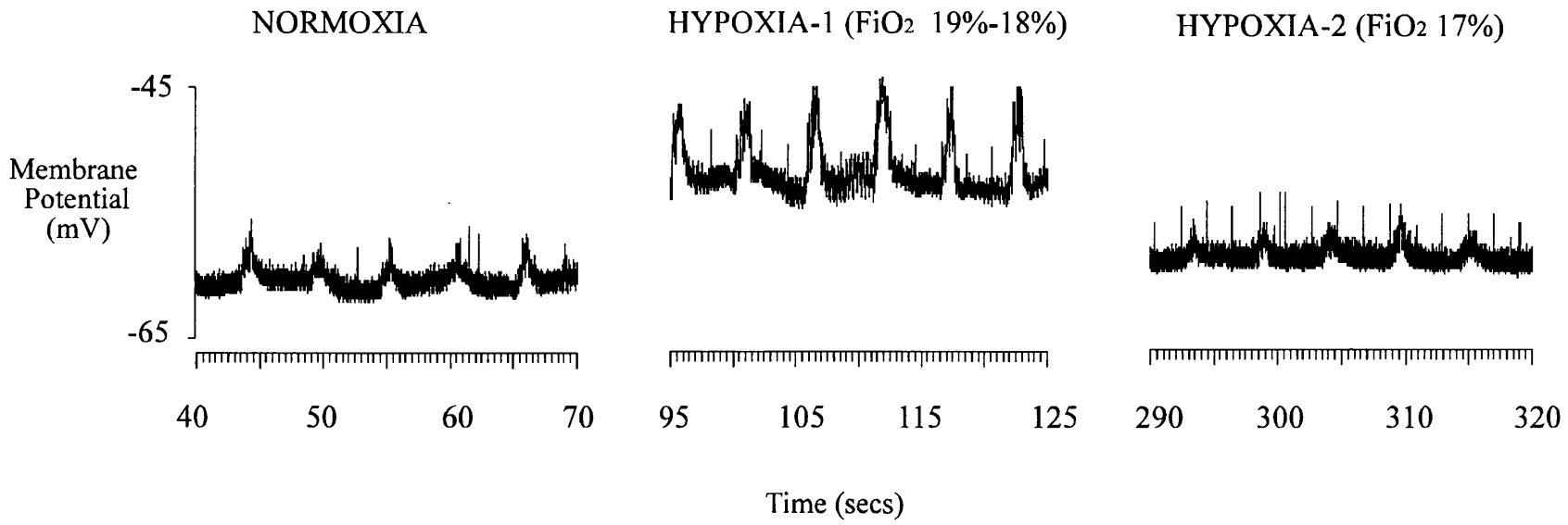


Figure 18. Intracellular recording of a hypoglossal motoneurone showing the effect of hypoxia on the rhythmic EPSP activity. This shows that as the membrane depolarized from approximately -61 mV to -42 mV during hypoxia (FiO_2 0.17) there was an increase in the EPSP amplitude during early hypoxia (FiO_2 0.18-0.19). But this amplitude was reduced during late hypoxia. Note that there was a transient depolarization in membrane potential. The kitten was 23 days old and weighed 280g.

followed by hyperpolarization during hypoxia. The amplitude of the EPSPs increased from 2.33±0.24 mV during normoxia to 7.46±0.57 mV during early hypoxia (FiO_2 19-18%) ($P<0.001$) when the membrane potential was depolarized and there was no change in late hypoxia when the membrane potential repolarized. However, when the FiO_2 was reduced to 17%, the amplitude decreased to pre-hypoxic levels (1.83±0.17mV). The duration, however, was not changed significantly during that time. The membrane potential was -61.43 mV during normoxia and this depolarized to -42.50 mV first and then hyperpolarized to -59.64 mV.

A further 4 out of 23 motoneurones showed no significant change in EPSP amplitude during hypoxia. In 1 of these 4 units, the EPSP duration decreased during hypoxia. Three out of 4 showed no significant change in EPSP duration during hypoxia.

In addition to the motoneurones which showed a rhythmic EPSP activity during normoxia, there were 4 motoneurones which had no rhythmic EPSPs during the normoxic period. In three of these, EPSPs appeared during the hypoxic test (range from 2.22±0.09 mV to 10.08±1.34 mV). The remaining unit had rhythmic EPSP activity after the hypoxic test when the FiO_2 had returned to normoxic levels.

3.2.4 Factors which may have influenced the changes in membrane potential during hypoxia

3.2.4.a Is the change in membrane potential during hypoxia dependent upon the initial resting membrane potential ?

Figure 19A shows the change in membrane potential during hypoxia plotted against the resting membrane potential recorded during normoxia. Taking the group of motoneurones together, there was a correlation between these two factors ($r=0.493$, $P<0.01$). When those motoneurones which depolarized during hypoxia were considered separately (figure 19B) there was also a significant correlation ($r=0.726$, $P<0.01$) between the resting membrane potential recorded during normoxia and the change in membrane potential during the hypoxic challenge.

3.2.4.b Effect of resting membrane potential during normoxia on the presence of rhythmic EPSP activity

Figure 20A shows the change in amplitude of rhythmic EPSPs during hypoxia plotted against the resting membrane potential recorded during normoxia. There was no significant correlation between these two factors ($r=0.1895$, $P>0.05$). However, the effect that changes in the membrane potential during hypoxia may have on the change in amplitude of rhythmic EPSPs has been considered. There was no correlation ($r=0.0922$, $P>0.05$) between the amplitude of EPSP during hypoxia plotted against the change in membrane potential during hypoxia (figure 20B).

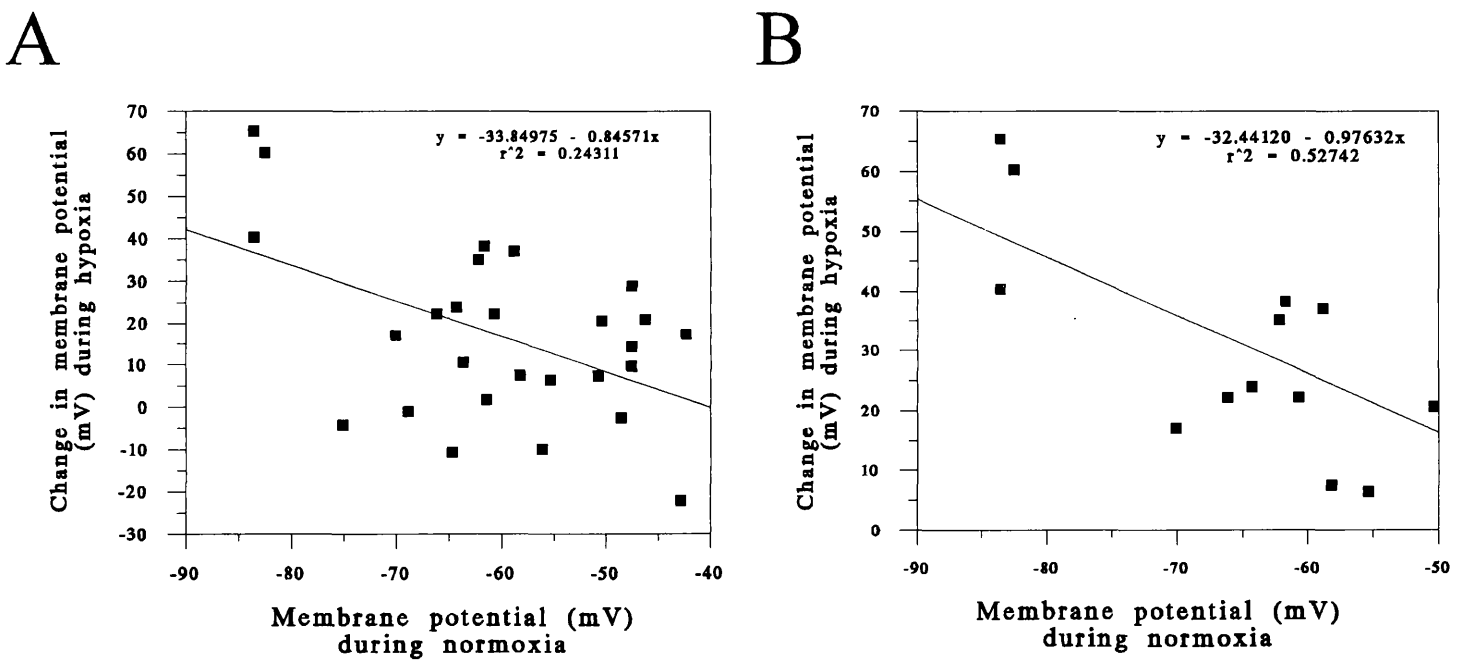


Figure 19. Two graphs showing the change in resting membrane potentials (mV) of the hypoglossal motoneurones during hypoxia as a function of the membrane potentials recorded during normoxia. A. shows all of the motoneurones tested with hypoxia, whereas B shows only those that were depolarized, at least initially during hypoxia. Taking the group of motoneurones together (A), there was a significant correlation ($r=0.493$, $P<0.01$) between the two factors. There was also a significant correlation ($r=0.726$, $P<0.01$) between the change in membrane potential during hypoxia and the membrane potential during normoxia for those that were depolarized (B). The line of best fit has been drawn on the graph.

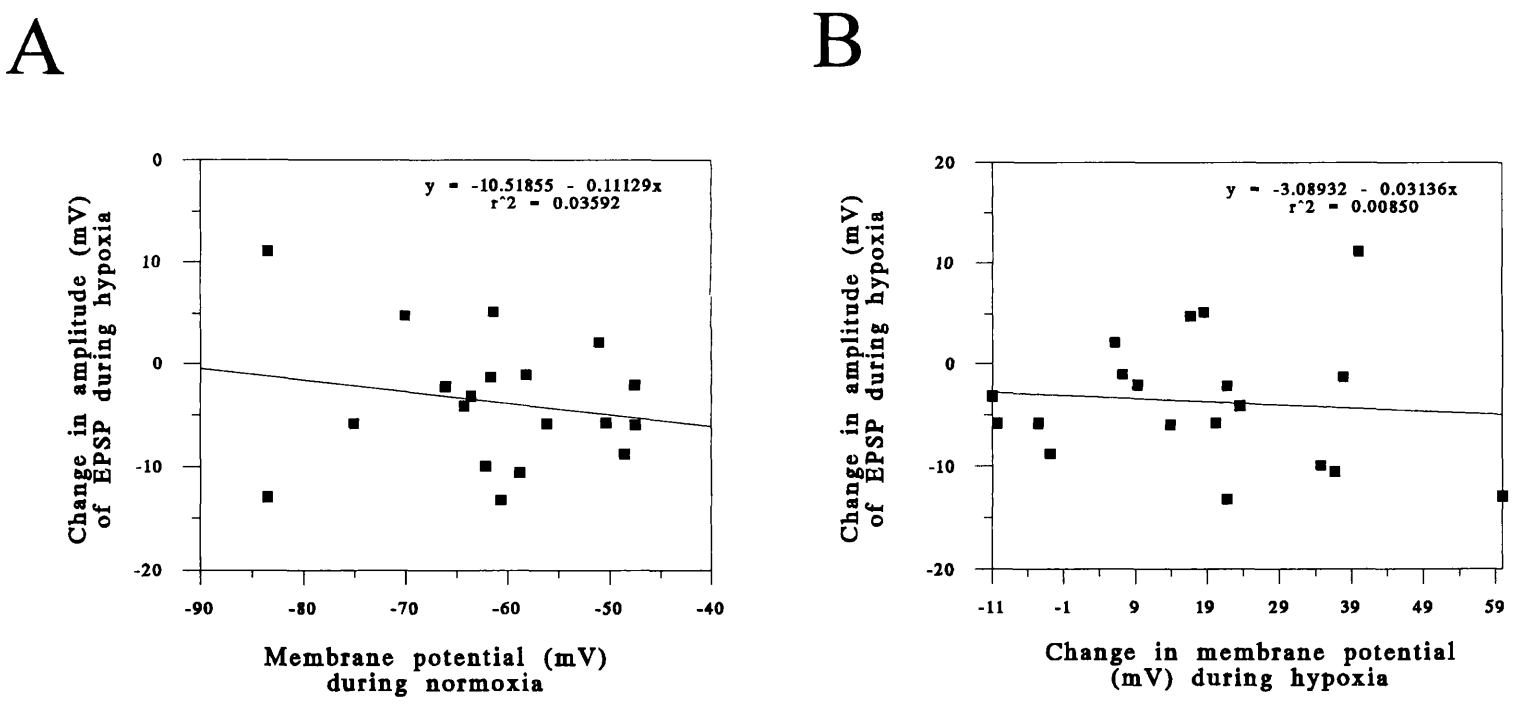


Figure 20. Two graphs showing the change in amplitude of rhythmic EPSP (mV) of the hypoglossal motoneurones during hypoxia as a function of the membrane potentials recorded during normoxia (A) and change during hypoxia (B). There was no significant correlation between the two factors. The line of best fit has drawn on the graph.

3.2.4.c The effect of arterial CO_2 on the response of the hypoglossal motoneurones to hypoxia

Blood samples showed that the $PaCO_2$ during normoxia varied between the animals and ranged from 32.7 and 44.8 mmHg (36.95±0.94 mmHg). During hypoxia, the $PaCO_2$ ranged between 28.2 and 43.6 mmHg (35.86±0.91 mmHg). There was no significant decrease in $PaCO_2$ during hypoxia.

There was no significant correlation ($r=0.058$, $P>0.05$) between the level of $PaCO_2$ during normoxia and the resting membrane potential of the motoneurones.

3.2.5 Changes in arterial blood pressure during hypoxia

The MAP was between 46 and 76 mmHg (60.76±2.41) during normoxia. In the majority (17, 77.3%) of kittens there was a decrease (9.0±1.0 mmHg) in the MAP during hypoxia. 22.7% showed an increase in MAP during hypoxia. The age range is narrow in this study (18 to 25 days, 21.35±2.15). There is no correlation ($r=0.1272$, $P=0.3720$) when the change in MAP during hypoxia was considered as a function of the age of the kittens.

Figure 21 shows an example of a recording in which the MAP decreased during hypoxia (FiO_2 0.18). In this case the membrane depolarized gradually with the onset of hypoxia, but during this time MAP remained unchanged at 62 mmHg. After 2 minutes of hypoxia, MAP gradually decreased to 57 mmHg. When the FiO_2 returned to pre-hypoxic levels, the membrane potential repolarized and the MAP also returned to pre-hypoxic level.

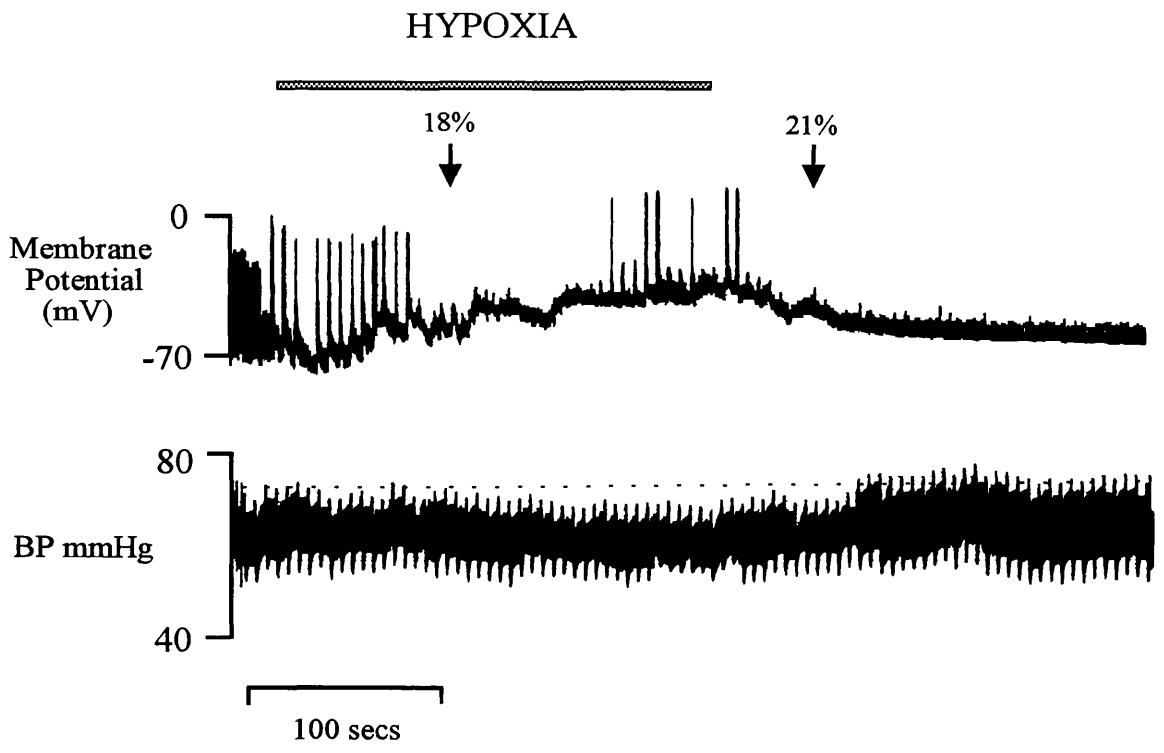


Figure 21. Intracellular recording from a hypoglossal motoneurone which responded to hypoxia with a depolarization. With the onset of hypoxia (FiO_2 0.18) there was a gradual depolarization of approximately 35 mV (from the normoxic level of approximately -62 mV). This figure also shows the blood pressure trace during the same time of the recording. The blood pressure was approximately 62 mmHg during normoxia. Towards the end of hypoxia the mean arterial blood pressure decreased to 57 mmHg. The kitten was aged 25 days and weighed 430g.

3.2.6 Returning to normoxia

3.2.6.a Changes in membrane potential

Twelve hypoglossal motoneurones were maintained for a long enough period of time to return to normoxia following the hypoxic challenge.

1) Seven of these 12 motoneurones were depolarized during hypoxia but they repolarized when the FiO_2 was returned to normoxia. One of these motoneurones is shown in figure 13A. During hypoxia the membrane potential was depolarized by approximately 7.5 mV, from a resting membrane potential of -58.21 mV, but when the FiO_2 was returned to normoxia the membrane potential began to repolarize.

2) Two of these 12 motoneurones were hyperpolarized during hypoxia and their responses were also repolarized when the FiO_2 returned to normoxia. As shown in figure 13B, the membrane potential was -76 mV during normoxia, it hyperpolarized to approximately -85 mV during hypoxia (FiO_2 0.16). This repolarized to pre-hypoxic level when FiO_2 returned to normoxia.

3) The other 3 of the 12 motoneurones had a transient depolarization during hypoxia, two depolarized first followed by a repolarization during the hypoxic period, and one hyperpolarized and then depolarized during hypoxia, as described earlier in sections 3.2.2.a. The membrane potential recorded from one of these motoneurones is shown in figure 14B. In this case, the membrane was depolarized during hypoxia (FiO_2 0.19-0.18)

by approximately 18.93 mV. As the FiO_2 level reached 0.17, the membrane repolarized to 59.64 mV. When FiO_2 was returned to normoxia the membrane gradually depolarized to approximately -46.29 mV.

3.2.6.b Changes in rhythmic EPSP activity during post-hypoxia compared to normoxia

Of the 12 units which were examined (see table 7).

1) Five of the 12 HMN showed a significant decrease in EPSP amplitude during post-hypoxia compared to normoxia (see in table 7). The duration of EPSP increased in 1 of these 5 units. However, 4 of these 5 units showed a significant decrease in EPSP duration. An example is shown in figure 15. The EPSP amplitude reduced significantly ($P< 0.01$) from 26.24±1.44 mV during normoxia to 16.27±1.32 mV during hypoxia. In post-hypoxia, it decreased to 3.18±0.20 mV. The EPSP duration also reduced significantly ($P<0.01$) from 2669.8±318.47 ms in normoxia to 123.10±74.65 ms during post-hypoxia. The rhythmic EPSP activity of one HMNs disappeared completely after 2 minutes of hypoxia and reappeared on return to normoxia.

2) In 4 of these 12 HMNs, the amplitude of rhythmic EPSP increased significantly ($P<0.05$) during post-hypoxia. The EPSP amplitude ranged from 0 to 4.50±0.62 mV during normoxia and ranged from 3.01±0.23 mv to 11.88±1.39 mV during post-hypoxia. The EPSP duration increased in 3 of these 4 units but did not change in the remaining unit.

3) EPSP amplitude was not changed significantly in three of the 12 HMNs during hypoxia. All of these three units showed no change in EPSP duration in post-hypoxia compared to normoxia.

	EPSP amplitude increase	EPSP amplitude decrease	EPSP amplitude no change	Total
EPSP duration increase	3	1	0	4
EPSP duration decrease	0	4	0	4
EPSP duration no change	1	0	3	4
Total	4	5	3	12

Table 7. A summary of the responses of rhythmic EPSP activity during post-hypoxia in 12 hypoglossal motoneurones.

3.2.7 Changing from hyperoxia to normoxia

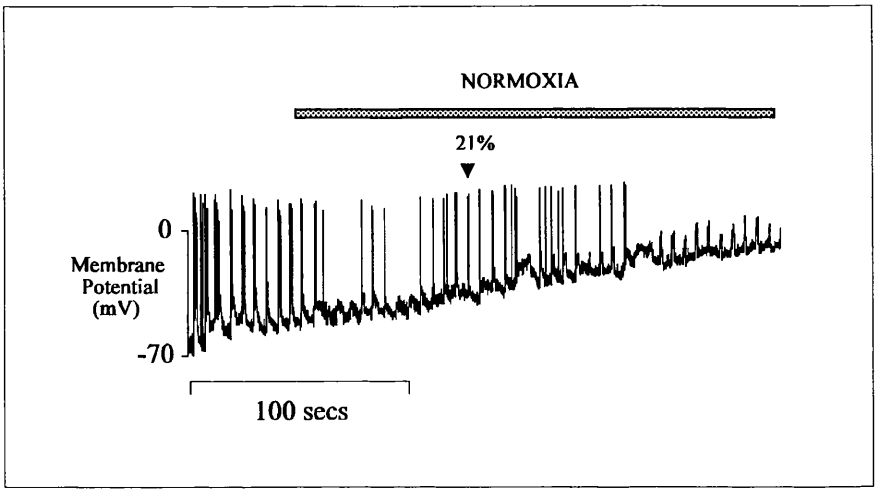


Figure 22. Intracellular recording showing an example of a HMN which responded to the change to normoxia from hyperoxia (FiO_2 0.34) with a depolarization. With the onset of normoxia there was gradual depolarization of approximately 36 mV. The kitten was 20 days old and weighed 300g.

Seven hypoglossal motoneurones were penetrated during hyperoxia (FiO_2 33%-40%, PaO_2 166-202 mmHg). One unit was recorded long enough to change from post-hypoxia to hyperoxia. The resting membrane potentials during hyperoxia were between -50.36 mV and -73.84 mV (62.56±3.58 mV).

1) Four of these 7 hypoglossal motoneurones showed a membrane depolarization when the FiO_2 returned to normoxia. The mean membrane potential was -60.25±3.19 mV during hyperoxia and it depolarized 20.72±7.21 in normoxia. Of this group, one of these units showed a significant increase in the amplitude of rhythmic EPSP during the return to normoxia (duration did not change in one and decreased in one). The other two units,

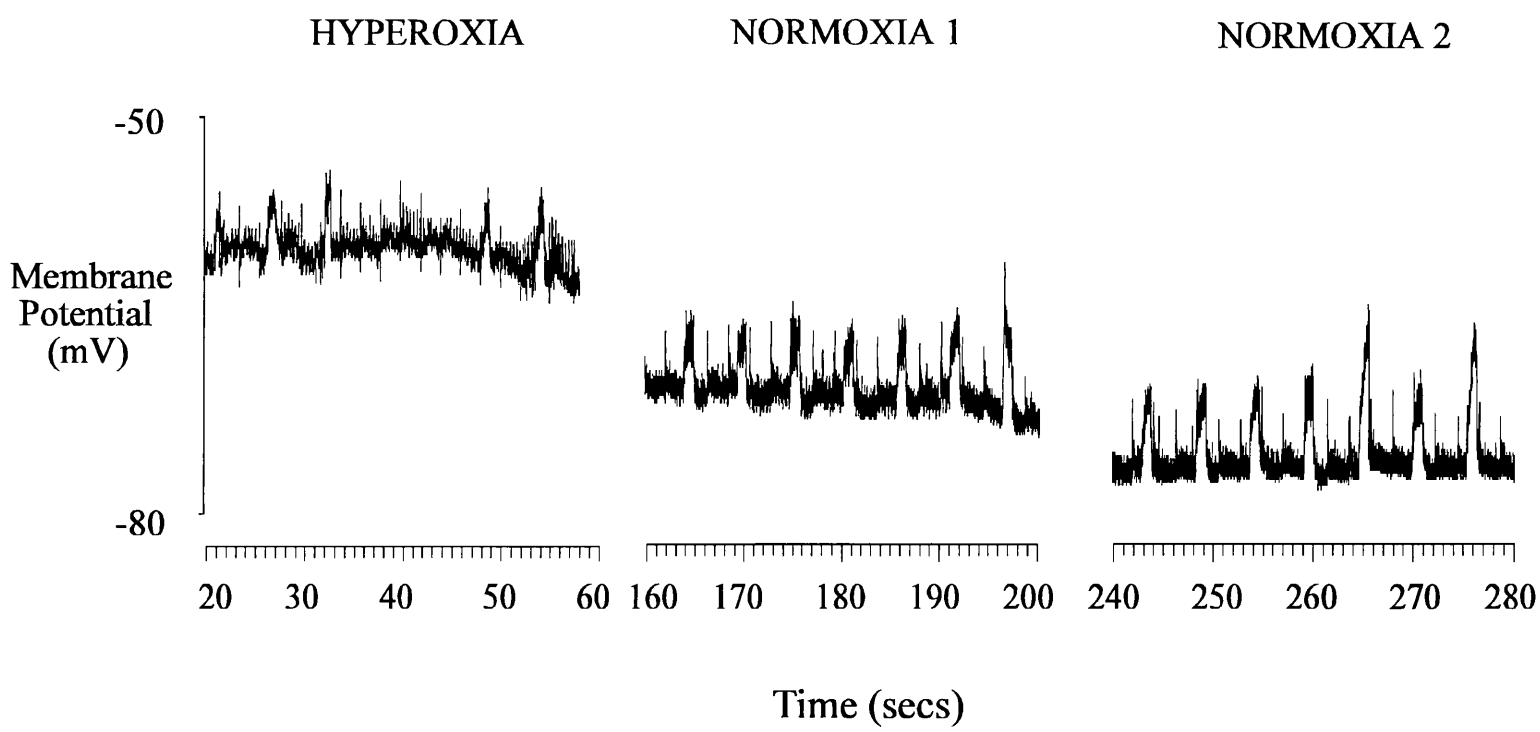


Figure 23. Intracellular recording of one hypoglossal motoneurone initially recorded in hyperoxia. The membrane is hyperpolarized from approximately -62 mV during hyperoxia (FiO_2 0.36) to -77 mV during late normoxia. There was an increase in the amplitude of the rhythmic EPSPs during early and late normoxia. The kitten was 18 days old and weighed 300g.

had rhythmic EPSP activity which appeared until the FiO_2 reached 21%. One showed no significant change in amplitude of rhythmic EPSP during hyperoxia compared to normoxia; however, EPSP duration had decreased. Figure 22 shows an example of a hypoglossal motoneuron which depolarized during normoxia. The membrane potential was -67.29 mV during hyperoxia and this depolarized to -31.55 mV during normoxia.

2) Two out of 7 motoneurones shown a hyperpolarization during normoxia. The membrane potentials were -62.07 and -73.84 mV during hyperoxia and these hyperpolarized to -75.36 and -82.5 mV during normoxia. One unit (figure 23) showed an increase in both amplitude and duration during normoxia and the other had no significant change in normoxia when the FiO_2 returned to 21%.

3) The remaining 2 HMNs exhibited no change in membrane potential when the FiO_2 returned to normoxia. The membrane potentials during hyperoxia were -69.29 and -50.36 mV, compared to control levels of -69.29 and -49.64 mV respectively. The amplitude of rhythmic EPSP activity in these units significantly increased and the duration decreased during normoxia. The other unit showed no change in either amplitude and duration of EPSP activity when the FiO_2 returned to normoxia.

3.2.8 Summary of section 2

This section has shown that the membrane potential of hypoglossal motoneurones in neonatal kittens changes during even mild levels of hypoxaemia.

Of the 26 hypoglossal motoneurones tested with hypoxia:

1) 17 showed a sustained depolarization.

2) 5 showed a transient depolarization.

3) 4 were hyperpolarized.

These responses were similar to the results from vagi-intact kittens in the previous study in this laboratory (Smith, 1993).

23 of 26 motoneurones displayed rhythmic EPSP activity and were tested with hypoxia;

1) 15 showed a decrease in amplitude during hypoxia

2) 4 showed an increase in amplitude of the EPSP activity

3) 4 showed no change in the EPSP activity

The changes in membrane potential during hypoxia were dependent on resting membrane potentials. But, the changes in rhythmic EPSP activity were independent of changes in resting membrane potential and the change during hypoxia.

3.3 THE LARYNGEAL-EVOKED POSTSYNAPTIC POTENTIALS IN HYPOGLOSSAL MOTONEURONES

3.3.1 Introduction

The third part of the results section presents the results of my investigation of the effect of mild levels of hypoxemia on the potentials evoked by laryngeal stimulation in neonatal kittens.

3.3.2 Laryngeal evoked potential

The responses to laryngeal stimulation were recorded in 63 hypoglossal motoneurones in 20 kittens. There were two patterns of response to laryngeal stimulation during normoxia, recorded from both vagotomized and vagi-intact kittens; EPSP or EPSP-IPSP complex (figure24).

3.3.2.a In vagotomized kittens

Forty hypoglossal motoneurones were recorded from 10 vagotomized kittens. Twenty-four (58.54%) units showed an EPSP-IPSP pattern during normoxia and 10 (24.39%) showed an EPSP during normoxia. Examples of the results are shown in figure 25.This shows two patterns of the response recorded. On the left of figure 25, there is a clear EPSP-IPSP complex in response to laryngeal stimulation, an EPSP being followed by an IPSP component. However, in the example shown on the right of figure 25, only an EPSP was recorded. In 7 hypoglossal motoneurones the generation of action potentials during normoxia made it difficult to average accurately the evoked potential.

3.3.2.b In vagi-intact kittens

Twenty-two hypoglossal motoneurones were recorded from 6 vagi-intact kittens. Sixteen

of 22 (72.73%) showed EPSP-IPSP complexes, 4 (18.18%) of them had an EPSP only during normoxia. I had problems generating action potentials during normoxia in 2 of them.

3.3.3 Effects of hypoxia on laryngeal evoked potentials

3.3.3.a In vagotomized kittens

The effect of mild hypoxia was recorded from 27 HMNs in 9 vagotomized kittens (aged from 18 to 25 days old). Five motoneurones had spontaneous action potentials during normoxia and /or hypoxia, and this made it difficult to measure the amplitude and duration of evoked EPSP and IPSP accurately. Thirteen of 22 neurones were recorded EPSP-IPSP complex and EPSPs only were recorded in 9 of these.

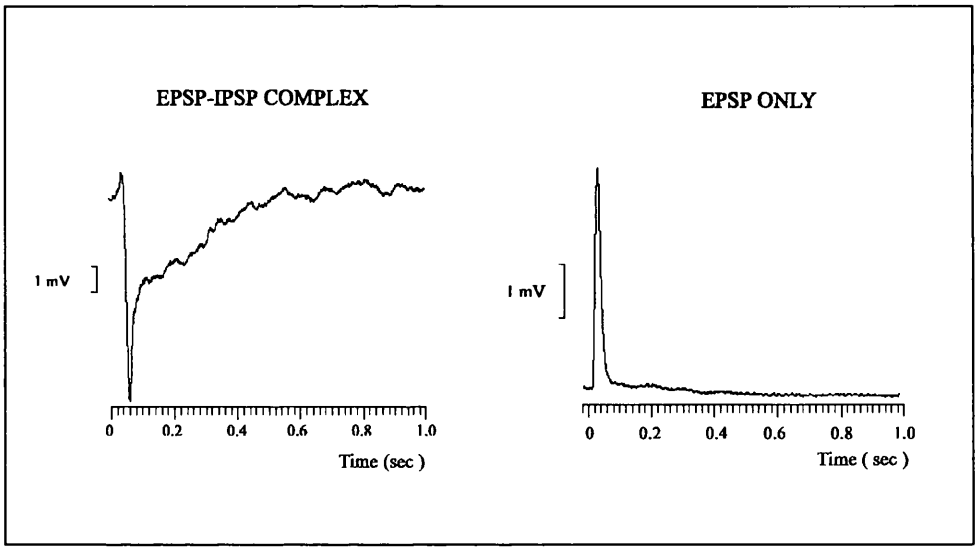


Figure 24. Two patterns of laryngeal evoked potentials in hypoglossal motoneurones during normoxia in two vagotomized kittens. This shows the triggered averages (sweeps=20) of the postsynaptic potentials in two kittens. The membrane potentials were -50.4mV and -48.6mV. The kittens were 25 and 18 days old and weighed 400 and 300g respectively.

Blood pressure and blood gases have been previously described in section 3.2.4.iv and 3.2.5.

1) Changes in evoked potential amplitude:

In most of the HMN, the EPSPs and IPSPs were reduced in amplitude.

	Increase	Decrease	No Effect	Total
EPSP Duration (ms)	5	4	7	16
IPSP Amplitude (mV)	2	6	0	8
IPSP Duration (ms)	2	6	0	8

Table 8. The relationship between an increase in the amplitude of the laryngeal evoked EPSP with the change of EPSP duration and the change in amplitude and duration of the laryngeal evoked IPSP during hypoxia in 16 hypoglossal motoneurones in vagotomized kittens (age from 18 to 25 days old).

1) In sixteen out of 22 (72.7%) HMNs there was a decrease in the amplitude of the laryngeal evoked EPSP activity during hypoxia (see table 8).

Of these 16, 8 showed an EPSP-IPSP complex and 8 showed only an EPSP. The amplitude of the EPSPs ranged from 0.21 to 8.91 mV (3.45±0.56 mV) during normoxia and from 0.16 to 4.19 mV (2.24±0.37 mV) during hypoxia. There was a significant decrease in amplitude during hypoxia ($P<0.0005$). The percentage change during hypoxia was significantly decreased (38.85±7.63%, $P<0.0005$).

Figure 25 shows the triggered averages of the laryngeal evoked potentials recorded. During normoxia, the averaged EPSP was 0.92 mV in amplitude, and this was reduced in hypoxia to 0.3 mV. The IPSP amplitude was decreased from 0.9 mV in normoxia to 0.79 mV in hypoxia. The IPSP duration was increased from 400 ms to 500 ms. The duration of the EPSP was not significantly changed during hypoxia.

A further example is shown in figure 26. An EPSP-IPSP complex was recorded in normoxia. The triggered averages (sweeps=20) of the laryngeal evoked EPSP was 1.37 mV during normoxia, and this was reduced to 0.48 mV during hypoxia. The EPSP duration increased from 18 ms to 35 ms. The IPSP amplitude decreased from 4.2 mV to

3.35 mV during hypoxia. However, the IPSP duration was 54 ms in normoxia and the membrane potential did not return to pre-hypoxic values. During post-hypoxia, the amplitude of EPSP was greater than that recorded during normoxia or hypoxia. The IPSP duration was not changed and amplitude increased. The membrane potential depolarized -62.2mV to -27.1mV during hypoxia and repolarized to -48.5mV during post-hypoxia. An example in which only IPSP was recorded during normoxia is shown in figure 27. The amplitude of laryngeal evoked EPSP decreased from 6.2 mV during normoxia to 1.9 mV during hypoxia. However, the duration of IPSP was not significantly changed. The membrane depolarized from -64.7mV to -75.3mV during hypoxia.

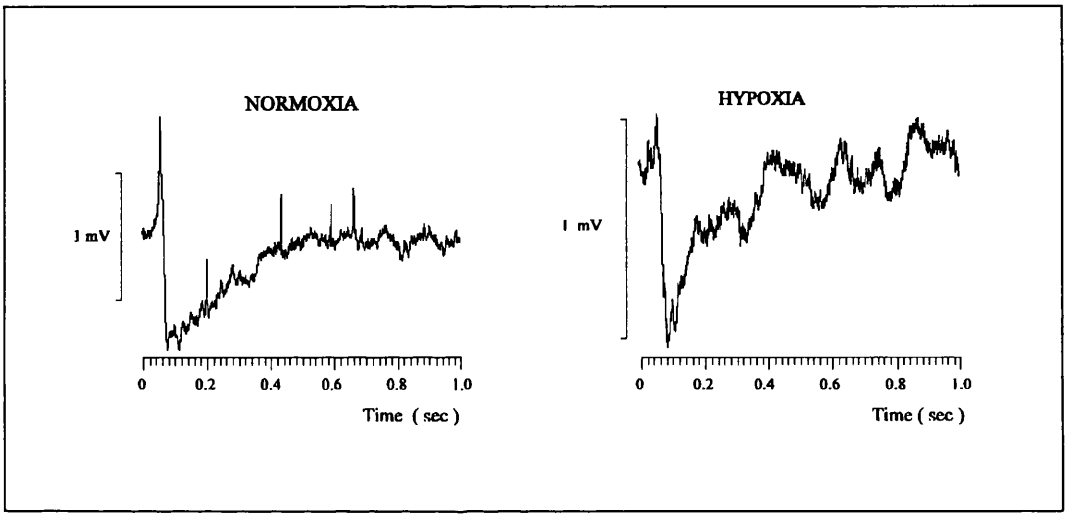


Figure 25. Intracellular recording of a hypoglossal motoneurone showing the effects of hypoxia on laryngeal-evoked potentials in a vagotomized kitten. The triggered averages (sweeps=30) of the amplitude of EPSP and IPSP were 0.92 mV and 0.9 mV respectively during normoxia to 0.3 mV and 0.79 mV during hypoxia (FiO_2 0.17). The duration of laryngeal evoked IPSP increased from 400 ms to 500 ms during hypoxia. However, the duration of EPSP has not significantly changed during hypoxia. In this case, the membrane potential depolarized from -46.3mV during normoxia to -25.4mV during hypoxia.

2) Two of the 22 (9.1%) units showed an increase in the amplitude of the laryngeal-evoked EPSP activity during the hypoxic challenge. During normoxia, the range of the amplitude of EPSPs was from 5.73 to 0.82 mV (3.45±0.56 mV). This increased to 6.60 mV (an increase of 15.2%) to 0.95 mV (an increase of 15.9%) during hypoxia. In one of these two neurones, only EPSPs were recorded. The duration of the EPSP of this neurone

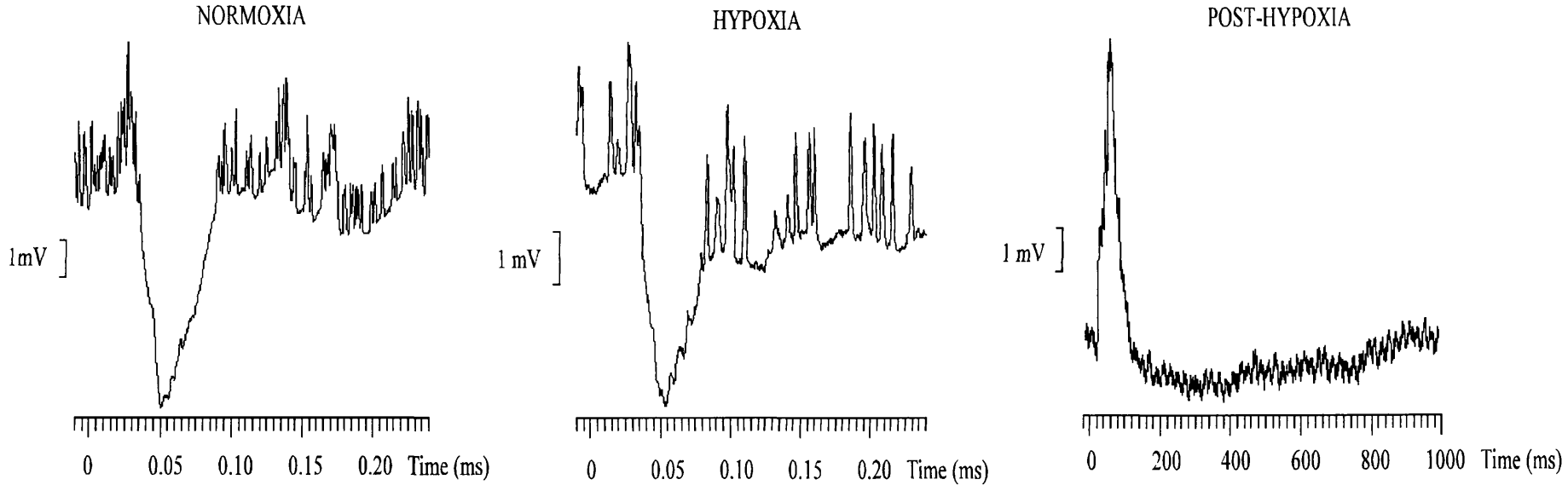


Figure 26. Intracellular recording of one hypoglossal motoneurone showing the effects of hypoxia on laryngeal-evoked potentials in a vagotomized kitten. The amplitude of the evoked EPSP reduced from 1.37 mV during normoxia to 0.48 mV during hypoxia (FiO_2 0.16). The duration of the EPSP increased from 18 ms to 35 ms during hypoxia. There was also a reduction in amplitude of laryngeal evoked IPSP from 4.2 mV to 3.5 mV during hypoxia. The duration of IPSP was 54 ms during normoxia. Note that there was a rebound response (increased EPSP) when FiO_2 returned to normoxia. The kitten was 25 days old and weighed 430g. (sweeps=20).

was also increased. The other neurone showed an EPSP-IPSP complex and the duration of the EPSP did not change when the amplitude increased in hypoxia. In this case, both the duration and amplitude of IPSP were reduced during hypoxia.

3) Four out of 22 units had no significant change in the amplitude of evoked EPSP during hypoxia. These four neurones all had a EPSP-IPSP pattern evoked by laryngeal stimulation during normoxia. An example is shown in figure 27. There was no significant change in the amplitude of the EPSP compared to normoxia (1.55 to 1.50 mV). The duration of EPSP increased from 26.80 mV to 39.60 mV (an increase of 47.2%). Both duration and amplitude of IPSP fell from 4.93 mV in amplitude, 907.6 ms in duration during normoxia to 1.0 mV and 506.8ms during mild hypoxia (FiO_2 0.17). Note that the amplitude of EPSP significantly increased in the post-hypoxic period when the FiO_2 returned to normoxia. In this group, the duration of the EPSP increased in one and decreased in 2 of the HMNs. The amplitude of the triggered average of laryngeal evoked IPSP decreased in 4 units, whereas the duration was increased in one but was reduced in 3 of them. These results are shown in Table 9.

	Increase	Decrease	No Effect	Total
EPSP Duration (ms)	1	2	1	4
IPSP Amplitude (mV)	0	4	0	4
IPSP Duration (ms)	1	3	0	4

Table 9. This table shows the effect of hypoxia on the duration of EPSP and IPSP and the amplitude of IPSP in 4 HMNs. There was no effect of hypoxaemia on the amplitude of IPSP.

4) The amplitude of rhythmic IPSP activity during hypoxia decreased in 11 of 13 motoneurones. The amplitude of IPSP ranged from 0.12 mV to 4.93 mV (1.50 ±0.43 mV) during normoxia. This decreased significantly ($P<0.05$) from 0 to 1.32 (0.57±0.17 mV) during hypoxia. The amplitude of the IPSP increased in 2 HMNs. The amplitude increased from 0.5 mV and 0.39 mV during normoxia to 1.85 mV (an increase of 270.07%) and 0.73 (an increase of 87.2%) during the hypoxic challenge.

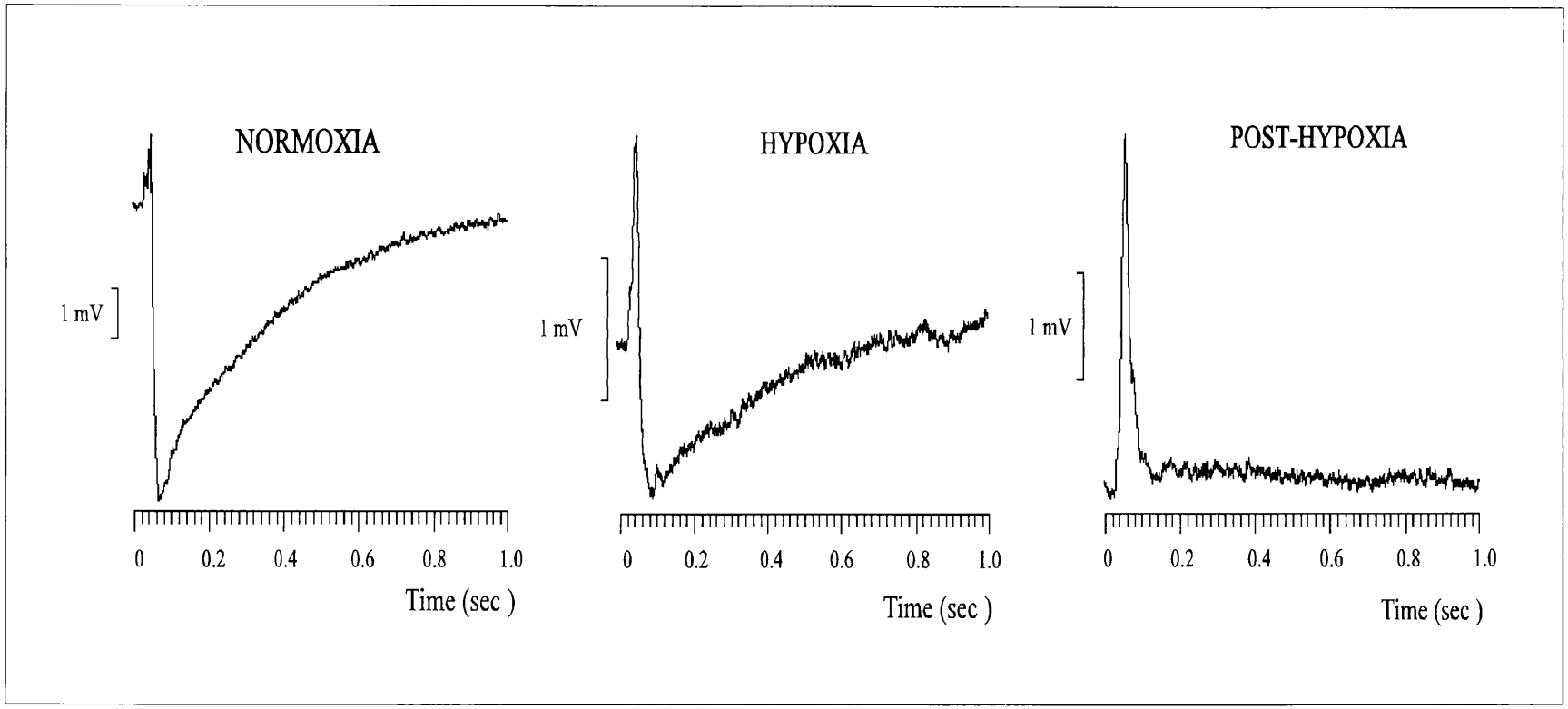


Figure 27. Intracellular recording of one hypoglossal motoneurone showing the effects of hypoxia on laryngeal-evoked potentials in a vagotomized kitten. The laryngeal-evoked potentials are shown as triggered averages (sweeps=23). There was no obvious change in the amplitude of EPSP during hypoxia (FiO_2 0.17). The duration reduced during hypoxia. Both amplitude and duration of IPSP reduced from 4.93 mV and 907.6 ms to 1.0 mV and 506.8 mV during hypoxia. Note the rebound response (increased EPSP) when FiO_2 returned to normoxia. The kitten was 25 days old and weighed 430g.

2) Changes in evoked potential duration:

In most of HMNs, the laryngeal evoked EPSP and IPSP duration decreased.

1) Of the 16 units which had a decrease in the amplitude of EPSP described above, the duration of these evoked EPSPs increased in 5 of them. The duration ranged from 21.6 to 174.8 ms (78.72±26.7 ms) during normoxia and from 36.0 to 382.8 ms (176.2±61.2 ms) during hypoxia. 4 HMNs showed a decrease in the duration of EPSP activity during hypoxia. The duration ranged from 23.6 to 72.8 ms (42.4±11.8 ms) during normoxia and from 0 to 46.4 ms (20.7±9.6 ms) during hypoxia. A further 7 units showed no change in the duration of the EPSP during hypoxia. An example is shown in figure 28. In this case, only an EPSP was recorded in response to the laryngeal stimulation during normoxia. The amplitude of the triggered EPSP was 8.91 mV in normoxia, and this fell to 1.20 mV in hypoxia. The duration of the EPSP did not change in hypoxia.

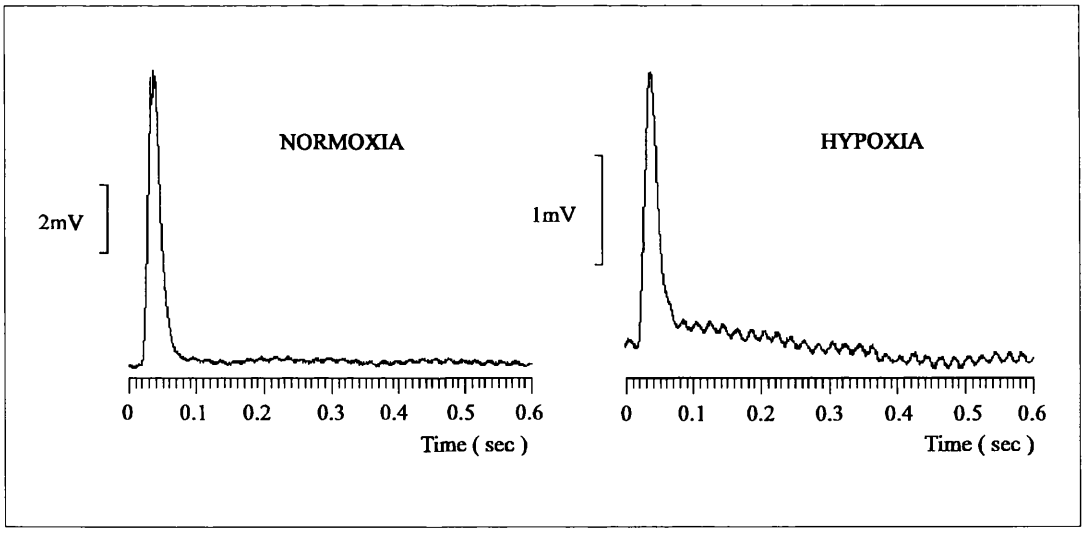


Figure 28. Intracellular recording of one hypoglossal motoneurone showing the effects of hypoxia on laryngeal evoked potentials in a vagotomized kitten. In this case only EPSP activity was recorded. The triggered averages (sweeps=20) of the amplitude of laryngeal evoked EPSP decreased from 6.8 mV to 1.9 mV during hypoxia (FiO_2 0.17). There was no change in the duration of the EPSP. The kitten was 21 days old and weighed 260g. The membrane potential hyperpolarized from -66.2mV to -44.0mV during hypoxia.

2) Ten of 13 neurones showed a decrease in the duration of rhythmic IPSP. The range of duration of IPSP was from 103.2 ms to 907.6 ms (417.96±80.21 ms) during normoxia to 0 and 506.8 ms (219.0±54.72 ms) during hypoxia.

The results obtained are summarized in table 10.

	Number of Hypoglossal Motoneurones	Normoxia (Median)	Hypoxia (Median)
EPSP Amplitude(mV)	22	3.24	2.05 ($P<0.01$)
EPSP Duration (ms)	22	72.40	80.40
IPSP Amplitude(mV)	13	0.91	0.73 ($P<0.05$)
IPSP Duration (ms)	13	264.0	240.0 ($P<0.05$)

Table 10. Summary of the effects of averaged EPSP and IPSP response to laryngeal air pressure stimulation in mild isocapnic hypoxia (FiO_2 0.16 to 0.18) recorded in 22 hypoglossal motoneurones in 9 vagotomized kittens (18 to 25 days old).

Evoked potentials were recorded in normoxia and hypoxia in 22 motoneurones. In vagotomized kittens, laryngeal stimulation evoked an EPSP-IPSP complex in 13 units during normoxia while 9 displayed only EPSP activity. In normoxia, the median EPSP amplitude was 3.24 mV and this was significantly ($P<0.01$) reduced to 2.05 mV during hypoxia. In contrast, the EPSP duration, which was 72.4 ms in normoxia, was not significantly changed in hypoxia. The IPSP amplitude was 0.91 mV in normoxia and this was significantly ($P<0.05$) reduced to 0.73 mV. The IPSP duration was also significant ($P<0.05$) reduced from 264.0 ms to 240.0 ms during hypoxia.

3.3.3.b In vagi-intact kittens

The responses of HMNs to laryngeal stimulation during mild hypoxia was recorded in 9 units in 4 vagi-intact kittens. EPSP-IPSP complexes were recorded in 6 units. During normoxia one hypoglossal motoneurone showed EPSP alone. Two units could not be determined because of spontaneous action potentials. All neurones were tested with hypoxia. Most of the HMNs showed a decrease in amplitude and duration of laryngeal evoked potential during hypoxia (table 11).

	Increase	Decrease	No Effect	Total
EPSP amplitude	1	6	0	7
EPSP Duration	0	2	5	7
IPSP Amplitude	1	4	1	6
IPSP Duration	1	2	3	6

Table 11. A summary of the changes in EPSP and IPSP of the hypoglossal motoneurones, recorded with intracellular electrodes, to mild levels of isocapnic hypoxia (FiO_2 0.16 to 0.18) recorded in 7 HMNs in 4 vagi-intact neonatal kittens (21 days old).

1) Six of 7 HMNs showed a decrease in the amplitude of evoked EPSP activity. The EPSP amplitude ranged between 0.43 mV and 4.47 mV during normoxia and from 0.34 mV to 2.33 mV during hypoxia. Five of these 6 had EPSP-IPSP complexes and only one had EPSP activity during normoxia. In these six units, the mean amplitude was 2.57±0.52 mV during normoxia and significantly ($P<0.005$) decreased to 1.36±0.30 mV during hypoxia. There was also a significant ($P<0.005$) decrease in the percentage change between normoxia and hypoxia. The EPSP duration ranged from 1.01 ms to 4.47 ms during normoxia and 0.34 ms to 2.33 ms during hypoxia. In this group, EPSP duration decreased in 2 units, but there no effect in 4 units. two out of 6 motoneurones exhibited a decrease in duration of EPSP during hypoxia, but there was no change in the remaining 4. Four out of 6 motoneurones showed a decrease in the amplitude of IPSP activity during

hypoxia. An example of an EPSP-IPSP complex, recorded in vagi-intact kittens, during normoxia is shown in figure 29.

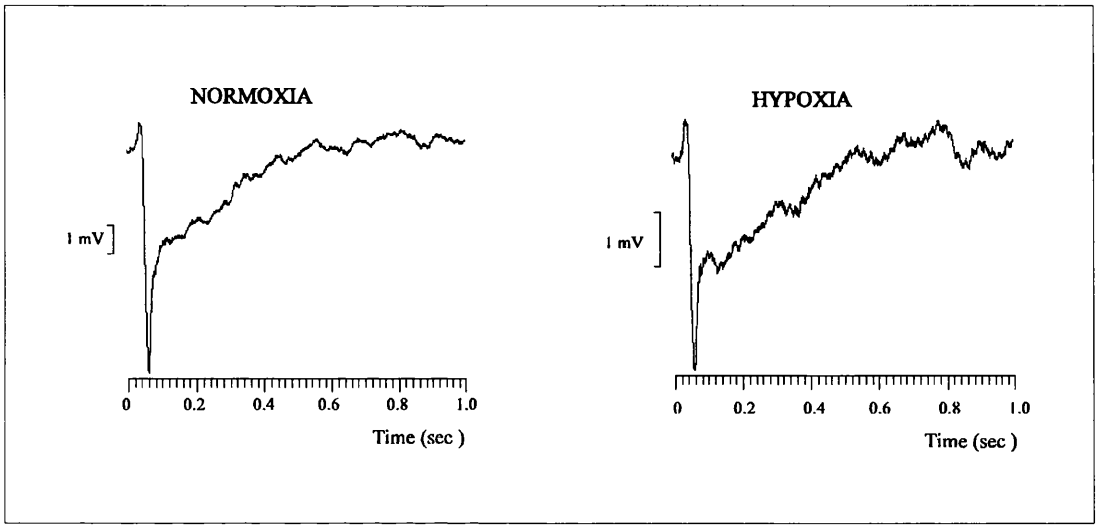


Figure 29. Intracellular recording of one HMN showing the effects of hypoxia on the laryngeal evoked potential in a vagi-intact kitten. The triggered averages (sweeps=30) of laryngeal evoked EPSP was 1.12 mV in amplitude during normoxia and this decreased to 0.75 mV during hypoxia (FiO_2 0.16). Both amplitude and duration of IPSP decreased during hypoxia. There was no change in the duration of EPSP during the hypoxic challenge. The kitten was 21 days old and weighed 270g. The membrane depolarized from-50.4mV to -29.8mVduring hypoxia.

In this case, the amplitude of EPSP was 1.12 mV during hypoxia, and this decreased to 0.75 mV during hypoxia. The EPSP duration was 45 ms in normoxia , but was not obviously changed when the FiO_2 was reduced to 16%. Both amplitude and duration of the IPSP decreased from 7.54 mV and 460 ms in normoxia to 6.24 mV and 400 ms respectively during hypoxia. The duration ranged from 0.28 mV to 6.36 mV (2.57±0.52) during normoxia and decreased to 0 to 4.74 mV (1.36±0.30 mV) during hypoxia.

2) Only one of the 7 HMNs showed an increase in the amplitude of EPSP in hypoxia (figure 30). In this case, the EPSP amplitude increased from 0.43 mV in normoxia to 1.84 mV during hypoxia (an increase of 327.9%). The duration of the EPSP and both

amplitude and duration of IPSP were not significantly changed during hypoxia.

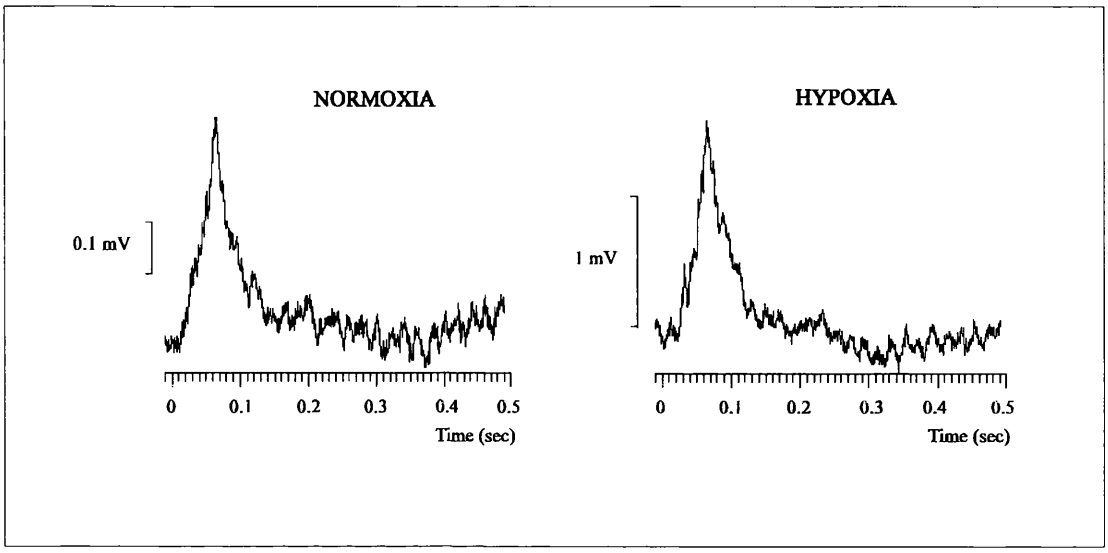


Figure 30. Intracellular recording of one HMN showing the effects of hypoxia on laryngeal evoked potential in vagi-intact kitten. The triggered averages (sweeps=34) of the amplitude of EPSP increased from 0.43 mV to 1.84 mV during hypoxia (FiO_2 0.16). The duration of the EPSP and IPSP and the amplitude of the IPSP did not changed. The membrane potential depolarized from -66.2mV during normoxia to -44.0mV during hypoxia. The kitten was 21 days old and weighed 380g.

3) Four out of 6 units showed a decrease in IPSP amplitude during hypoxia. The amplitude of these 4 units ranged from 0.31 mV to 6.36 mV in normoxia and from 0 to 4.74 mV in hypoxia. Of the remaining 2 units, there was a increase in the amplitude of IPSP during hypoxia. During hypoxia, the IPSP duration decreased in three units, increased in one and one remained the same.

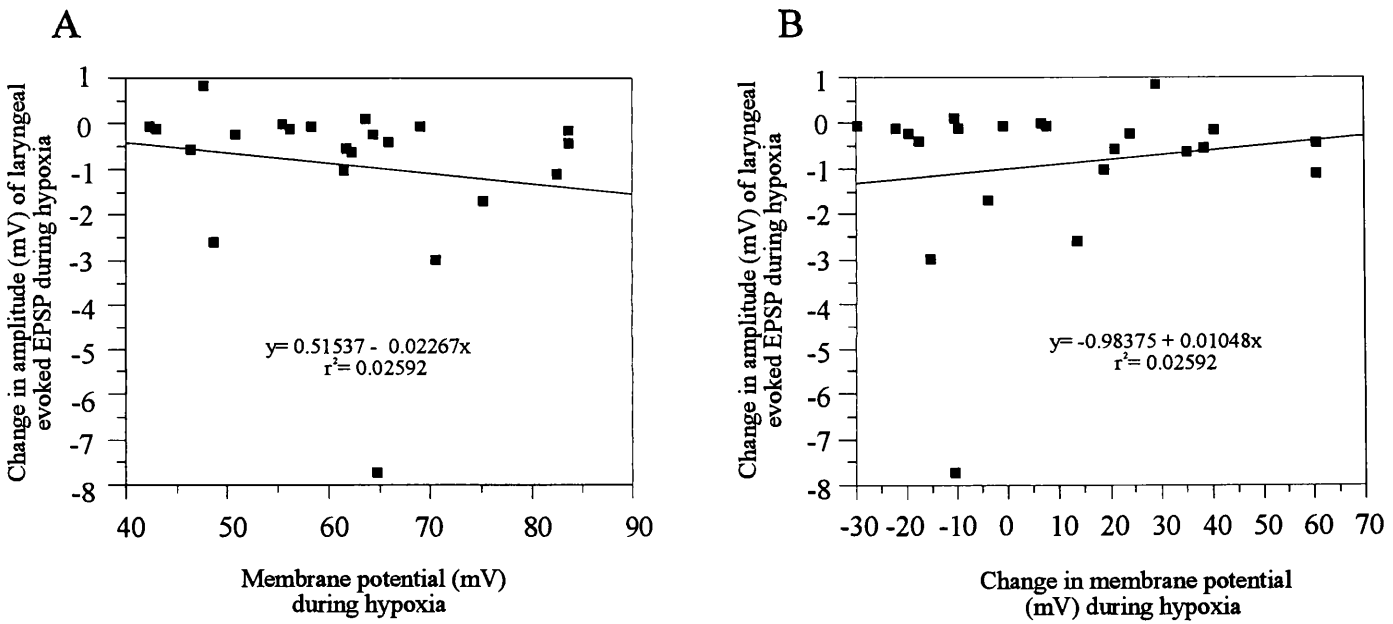


Figure 31. Two graphs showing the change in amplitude of laryngeal evoked EPSP during hypoxia as a function of the membrane potential (A) and change in membrane potential (B) during hypoxia. There was no significant correlation ($r=0.00067$, $P>0.05$) between the change in amplitude of laryngeal evoked EPSP and the membrane potential during hypoxia. Also there was no correlation ($r=0.00061$, $P>0.05$) between the amplitude of laryngeal-evoked EPSP and the change in membrane potential during hypoxia. The line of best fit has been draw on each graph.

3.3.4 Factors which may have influenced the changes in laryngeal evoked potential during hypoxia

3.3.4.a Is the change in laryngeal evoked potential during hypoxia dependent upon the initial resting membrane potential or the change of membrane potential during hypoxia?

There was no relationship between the amplitude of evoked EPSPs and resting membrane potentials. There was also no correlation between the change in the amplitude of the triggered average of the laryngeal evoked EPSPs during hypoxia compared to the resting membrane potential during normoxia ($r=0.1257$, $n=22$, $P>0.05$).

There was no significant correlation between the change of amplitude of laryngeal evoked EPSP during hypoxia and the membrane potential during hypoxia (figure 31A). Furthermore, there was also no correlation between the change of amplitude of laryngeal evoked EPSP during hypoxia and the membrane potential change in hypoxia.

There was no relationship between the change of IPSP amplitude during hypoxia and resting membrane potential during normoxia ($r=0.3096$, $n=13$, $P>0.05$). There also was no correlation between change of IPSP amplitude in hypoxia and membrane potential change during hypoxia ($r=0.0454$, $n=13$, $P>0.05$).

There was no correlation between the change of duration of IPSP during hypoxia and resting membrane potential during normoxia ($r=0.0318$, $n=13$, $P>0.05$). There was also no correlation between the change of duration and membrane potential during hypoxia ($r=0.0747$, $n=13$, $P>0.05$).

3.3.4.b The effect of age on the response of the laryngeal evoked potential to hypoxia

There were no significant ($r=0.2216$, $P>0.05$) correlations between the change and percentage of amplitude of EPSP during hypoxia and the age of the kittens on the day of

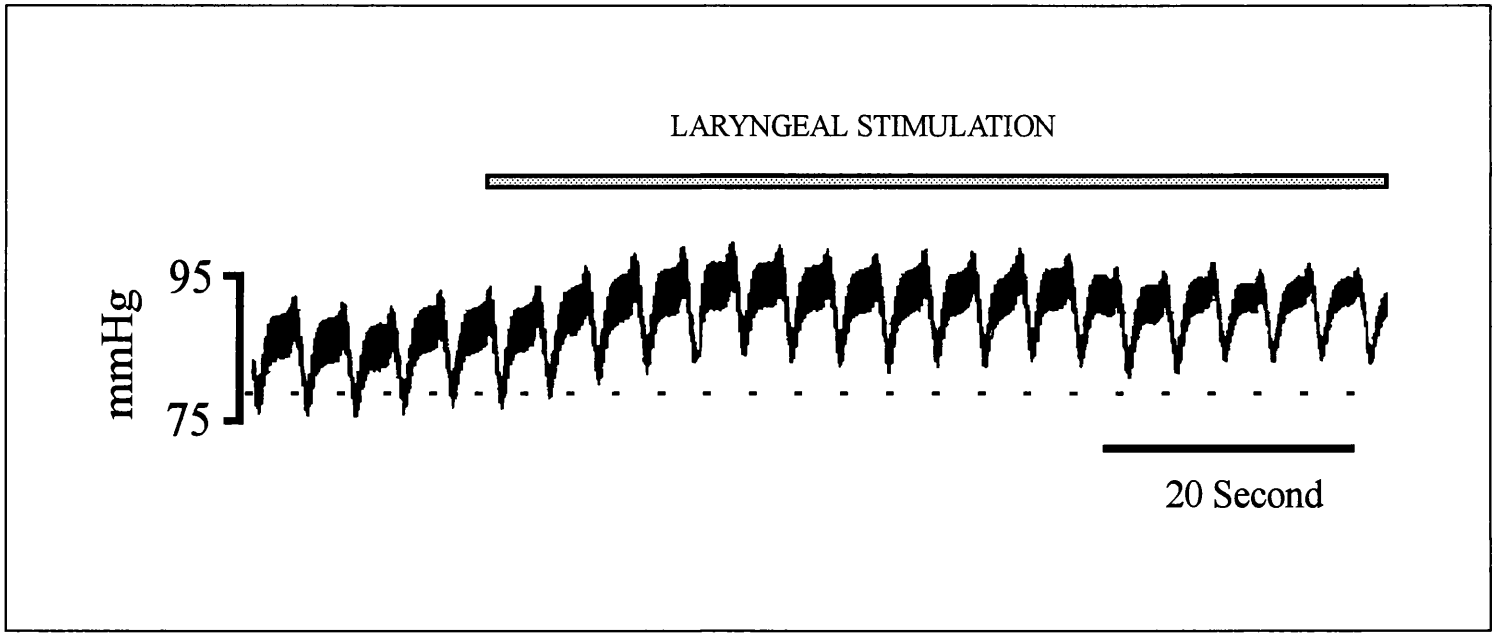


Figure 32. An example of the change of blood pressure before and after laryngeal stimulation. The blood pressure was approximately 80 mmHg before laryngeal stimulation and it increased to approximately 85 mmHg on the peak of pressure pulses after laryngeal stimulation has given. Note the arrhythmia was associated with the positive pressure ventilation. The kitten was 19 days old and 280g.

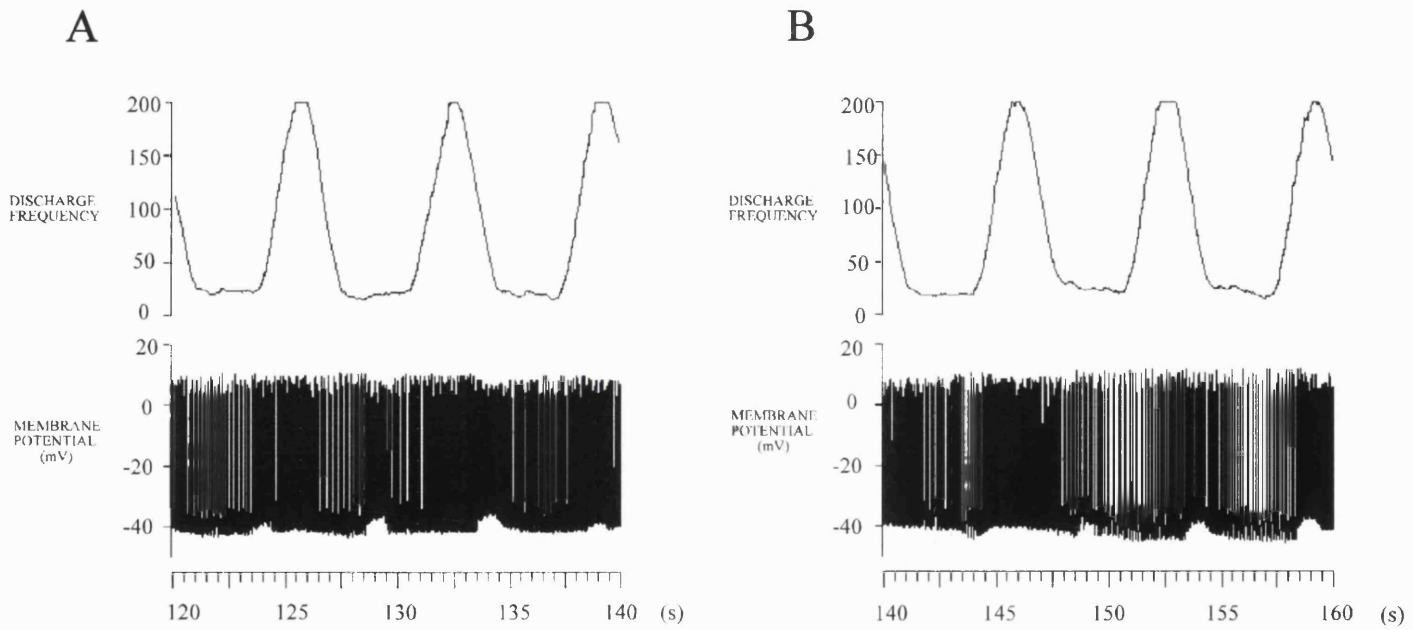


Figure 33. Intracellular recording from a hypoglossal motoneurone (bottom trace) which had rhythmic EPSP activity associated with integrated phrenic nerve recording (top trace). The kitten was 25 days old and weighed 440g.

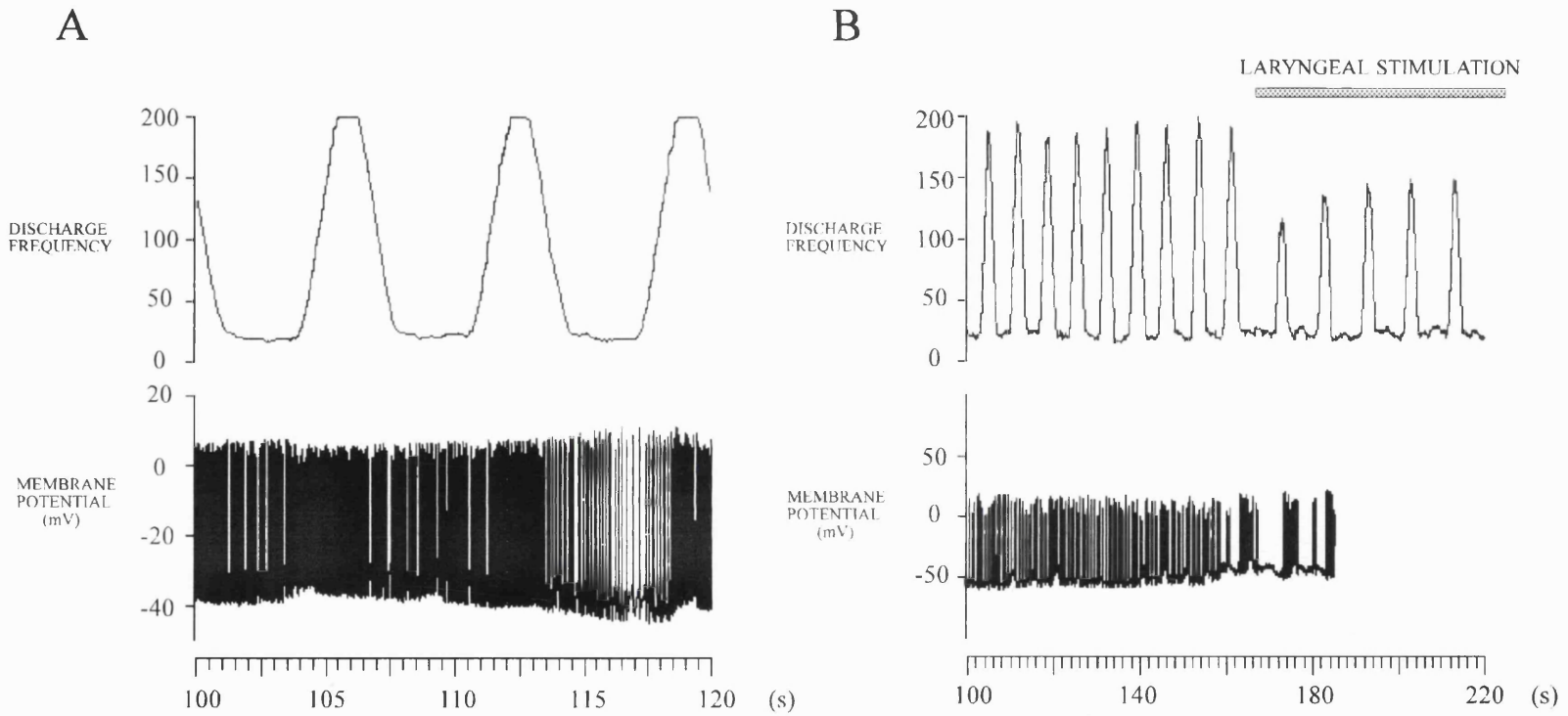


Figure 34. An example of intracellular recording from a hypoglossal motoneurone (bottom trace) with integrated phrenic discharge (top trace). In A, the rhythmic activity of this hypoglossal motoneurone occurred mostly in the inspiratory phase of the respiratory cycle. In B, laryngeal stimulation (indicated by stippled bar) caused both hypoglossal rhythmic and integrated phrenic activity to slow. The kitten was 25 days and weighed 320g.

recording. There was also no correlation between the change of duration or amplitude of IPSP in hypoxia and the age at recording ($r=0.1421$, $n=13$, $P>0.05$).

3.3.4.c The effect of arterial CO_2 on the response of the laryngeal evoked potential to hypoxia

There was no significant correlation between the change of amplitude of laryngeal evoked EPSP during hypoxia and $PaCO_2$ during hypoxia ($r=0.1302$, $P>0.05$). There was also no significance between the percentage change of EPSP amplitude during hypoxia and $PaCO_2$ levels in hypoxia ($r=0.0167$, $P>0.05$).

3.3.5 Changes in blood pressure during laryngeal stimulation and hypoxia

The mean arterial blood pressure has been described in section 3.2.5. MAP increased immediately with the laryngeal stimulation in 76.9% of 40 cases, and this increase was sustained. It ranged from 40 and 86 mmHg (56.62±3.11 mmHg) before stimulation and from 44 and 90 mmHg (61.77±3.69 mmHg) after stimulation ($P<0.05$). In 23.1% of 40 cases MAP fell after the start of laryngeal stimulation. An example is shown in figure 32. MAP increased to 85mmHg from 80mmHg during laryngeal stimulation.

3.3.6. The effect on discharge frequency of phrenic nerve

Three hypoglossal motoneurones were recorded together with phrenic recordings during normoxia. All these units had rhythmic EPSPs associated with the integrated phrenic discharge. An example is shown in figure 33. This figure shows the integrated phrenic activity (top trace) and hypoglossal membrane potential (bottom trace) during normoxia.

One unit was recorded during laryngeal stimulation (figure 34). In this case, the hypoglossal rhythmic EPSPs occurred during the inspiratory phase of the respiratory cycles (figure 34A). With the onset of laryngeal stimulation, both the integrated phrenic and HMN rhythmic activity slowed (figure 34B).

3.3.7. Summary of section 3

This section has demonstrated the activity of laryngeal evoked EPSPs and IPSPs of HMNs in vagotomized neonatal kittens in normoxia and hypoxia. In normoxia, laryngeal stimulation evoked two pattern of responses; EPSP-IPSP complex and EPSP only.

22 HMNs were examined before and during hypoxia. 13 units exhibited an EPSP-IPSP complex and 9 units showed an EPSP pattern during normoxia.

1) 16 showed a decrease in amplitude evoked by laryngeal stimulation during hypoxia.

2) 2 units showed an increase in amplitude during hypoxia.

3) 5 showed no change during hypoxia.

Mild levels of hypoxia (FiO_2 0.16-0.18) reduced the amplitude of laryngeal evoked EPSP and both amplitude and duration of IPSP in neonatal kittens were also decreased.

The changes in activity of laryngeal evoked potential were independent of changes in resting membrane potential and the change of membrane potential during hypoxia.

There was no correlation between the change of laryngeal evoked EPSP amplitude during hypoxia and the age of the kittens on the day of recording. There was also no correlation between the change of laryngeal evoked EPSP amplitude during hypoxia and $PaCO_2$ during hypoxia.

In most cases (77%), MAP increased during laryngeal stimulation.

3.4 MEMBRANE CHARACTERISTICS

3.4.1 Introduction

The fourth part of the results section presents the investigation of the effect of mild levels of hypoxemia on the membrane input resistance and excitability.

3.4.2 Change in resting membrane potential during hypoxia

Fifteen tests were performed on twelve HMNs in 4 kittens. Three of these motoneurones were tested twice in hypoxia. The resting membrane potentials recorded during normoxia ranged from -41.43 mV to -73.34 mV and -22.23 mV to -55.05 mV during hypoxia. In 14 of 15 tests there was a depolarization during hypoxia. The mean membrane potential decreased significantly ($P<0.001$) from -59.72±2.19 mV in normoxia to -40.34±2.40 mV during hypoxia. The range of the membrane potential decrease during hypoxia was from 9.94 mV to 35.96 mV (mean 21.02±1.76 mV). A typical example is shown in figure 35. In this case, as the threshold was reached the discharge increased. However, discharge subsequently ceased even when the membrane continued to depolarize. This HMN was tested twice. The membrane potential depolarized from -57.68 mV in normoxia to -32.23 mV during hypoxia in the first hypoxic test. During the second test, the membrane potential gradually depolarized from -73.48 mV to -50.18 mV. This second test was conducted 4 mins after the first. In the second test, no action potentials were generated during the depolarization. One unit showed a transient depolarization in the hypoxic challenge. This unit showed a hyperpolarization from -56.63 mV in normoxia to -62.46 mV in early hypoxia but then depolarized to -46.56 mV in late hypoxia.

3.4.3 Effects of hypoxia on rhythmic excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs)

There were two patterns of rhythmic activity recorded during normoxia, rhythmic EPSP and an EPSP-IPSP complex. Two of 12 units showed a rhythmic EPSP-IPSP complex

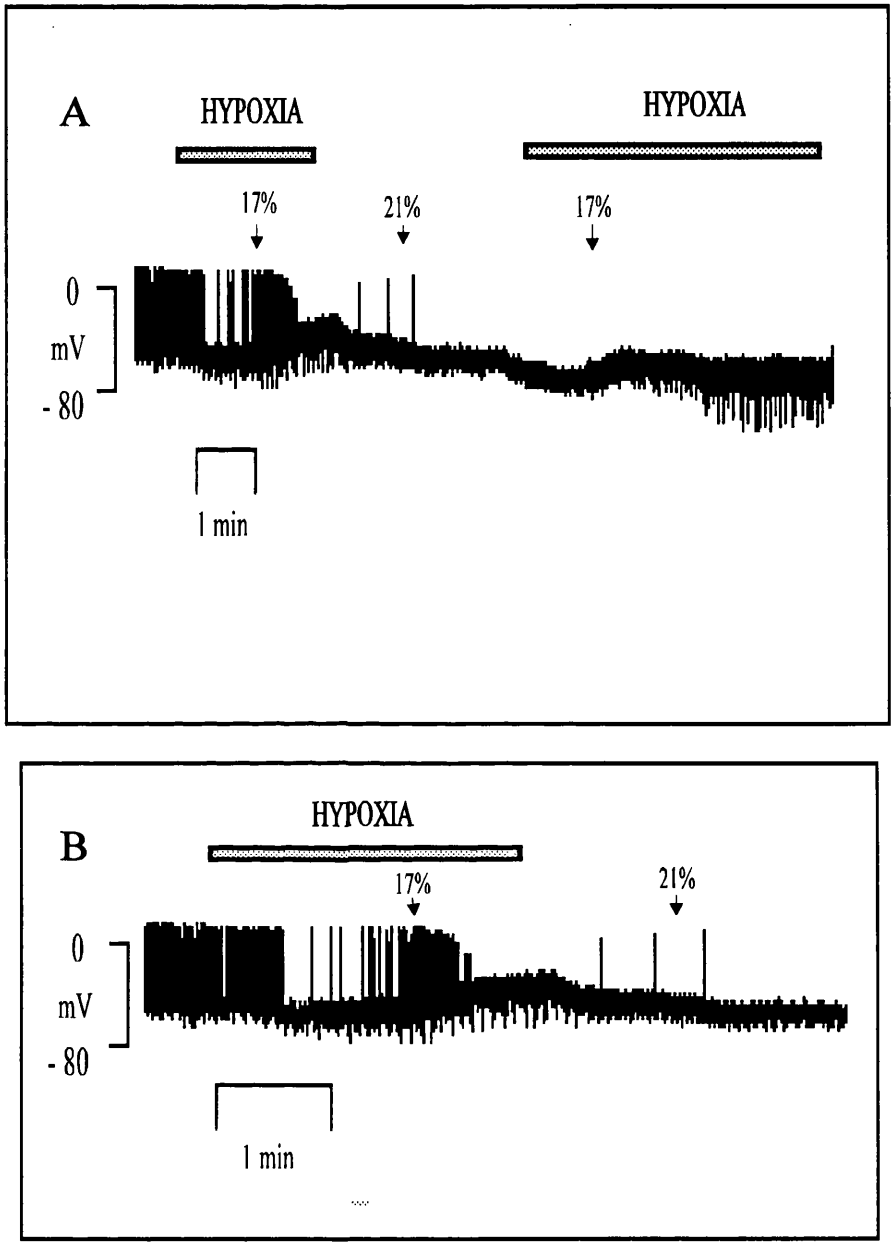


Figure 35. Effects of systemic hypoxia on hypoglossal membrane input resistance. Short hyperpolarizing current pulses (2 msecs, -1nA) were passed through the intracellular electrode. Two hypoxic tests (stippled bars) were performed on this motoneurone. In A, the membrane depolarized from approximately -67 mV to -42 mV during first hypoxia (FiO_2 0.17) and from -81 mV to -60 mV during second hypoxia (FiO_2 was 0.17). In B, when the time scale has been extended, it is clear that the input resistance increased during the hypoxic periods with an increased voltage drop across the membrane associated with the current pulses. The kitten was 23 days old and weighed 280g.

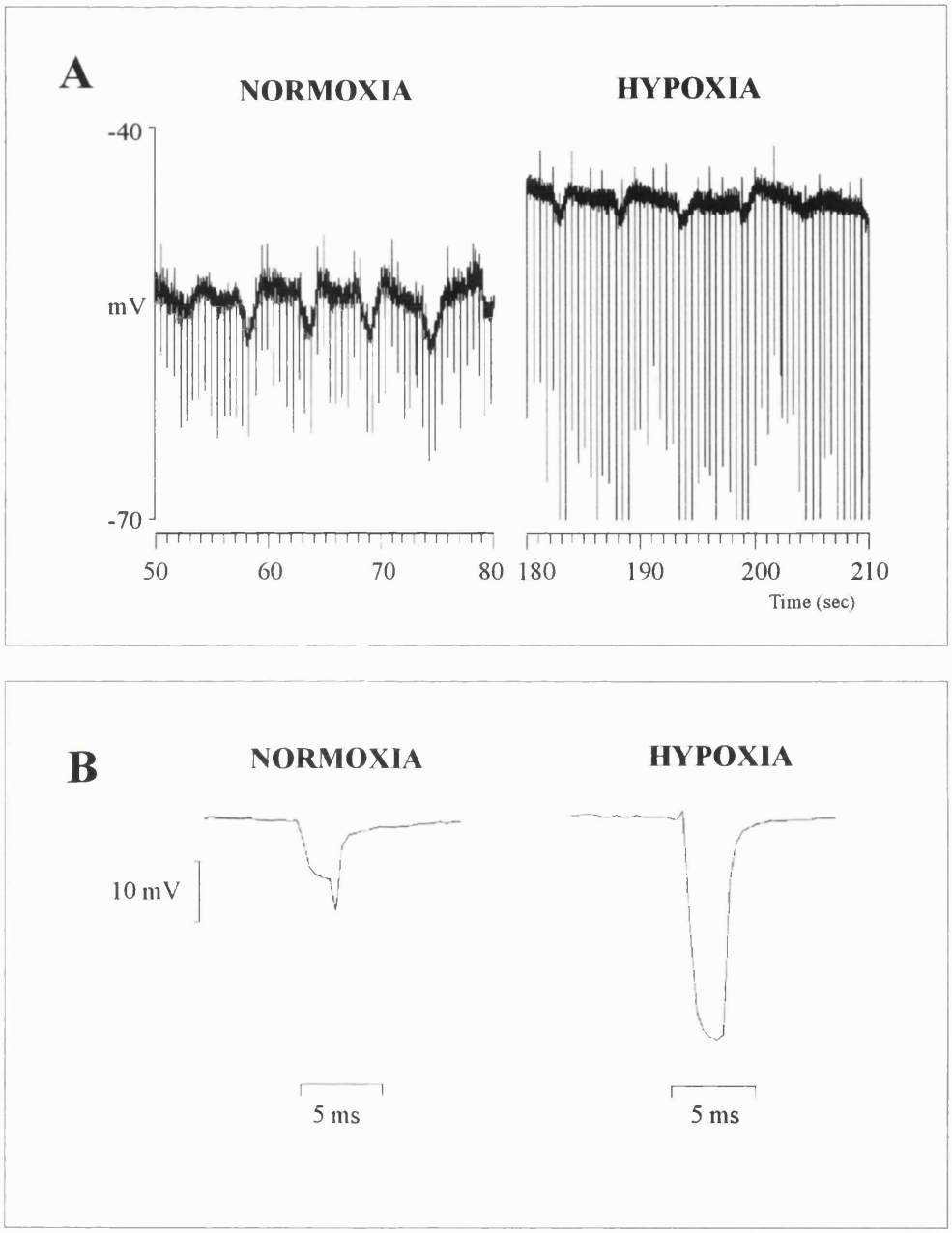


Figure 36. Intracellular recording of one hypoglossal motoneurone showing the effects of hypoxia (FiO_2 0.17) on rhythmic EPSP activity (A) and on hypoglossal membrane input resistance. A. shows the rhythmic EPSP-IPSP complex. The membrane potential was depolarized (by approximately 13 mV) and the amplitude of rhythmic EPSP reduced significantly from 2.28 mV to 1.25 mV during hypoxia. B. Increase in input resistance shown by voltage drop across the membrane associated with the current pulses (2msec, -1nA). The kitten was 23 days old and weighed 280g.

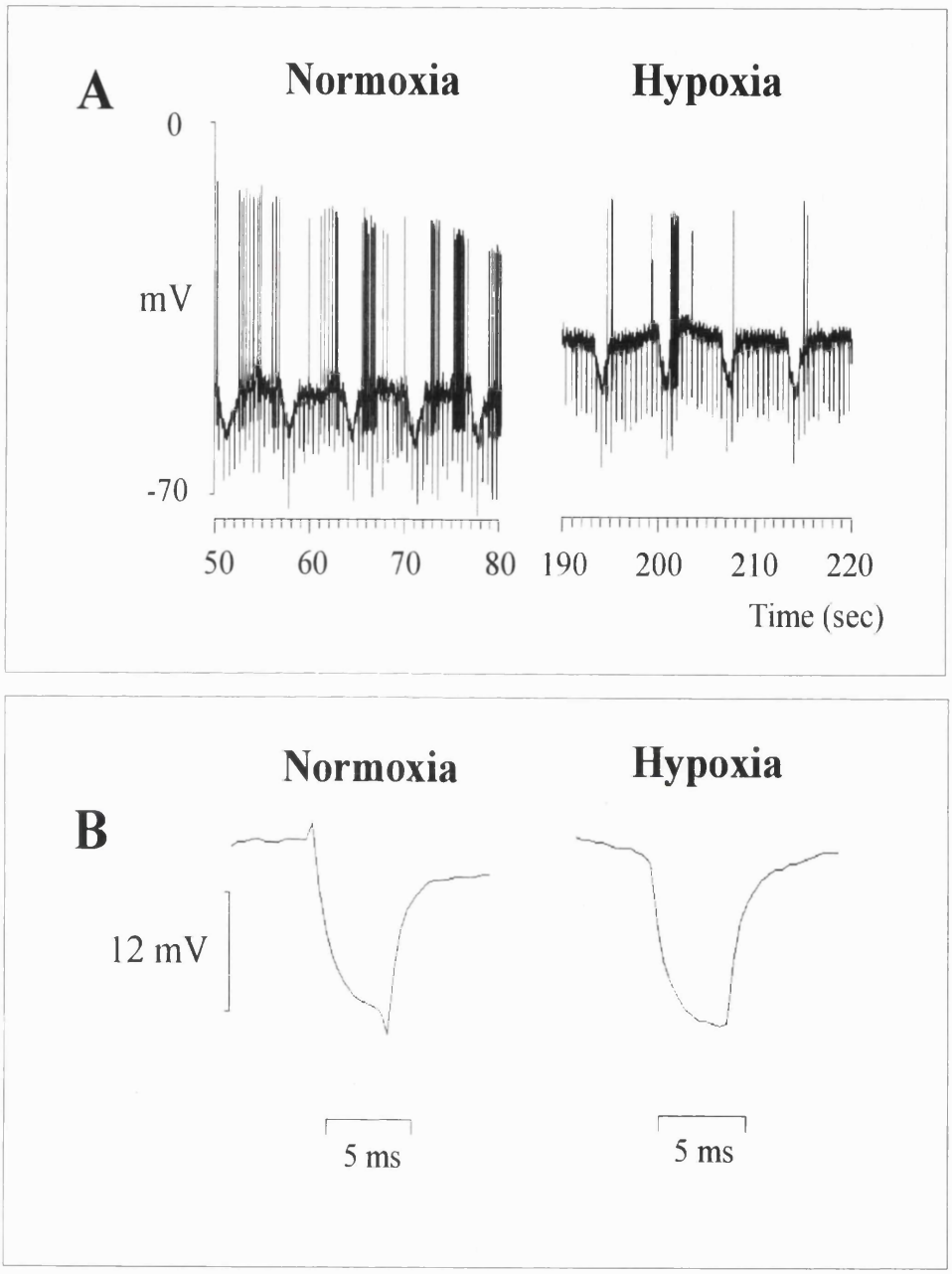


Figure 37. Intracellular recording of one hypoglossal motoneurone showing the effect of hypoxia (FiO_2 0.17) on rhythmic IPSP activity (A) and on hypoglossal membrane input resistance. A shows there was only rhythmic IPSP recorded. The membrane potential depolarized from approximately -58 mV to -45 mV during hypoxia. There was no significant change in amplitude of rhythmic IPSPs during hypoxia. B. There was no change in input resistance as determined by voltage drop across the membrane associated with the current pulses (2 msec, -1nA). The kitten was 18 days old and weighed 430g.

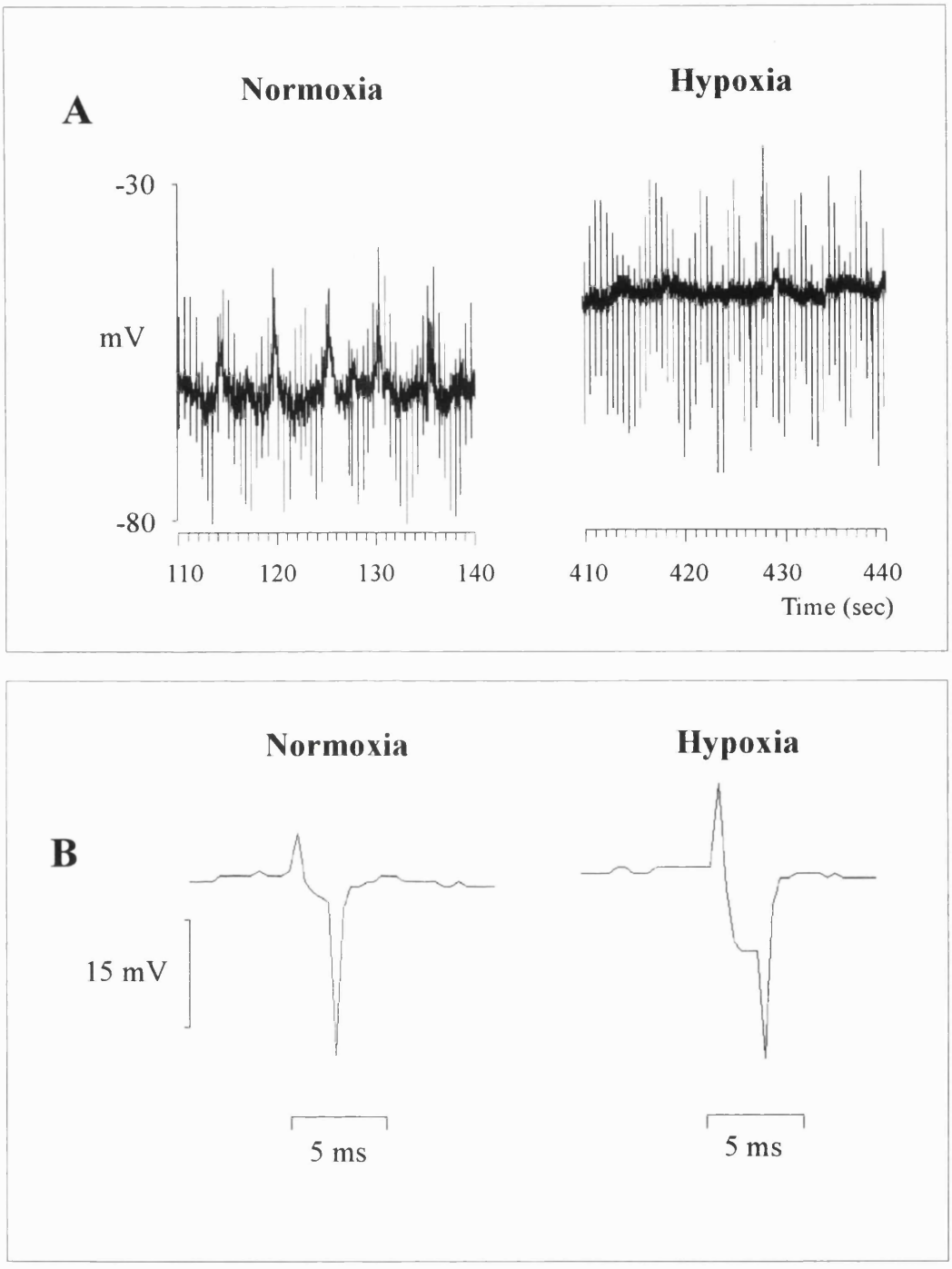


Figure 38. Intracellular recording of one hypoglossal motoneurone showing the effects of hypoxia (FiO_2 0.17) on rhythmic EPSP activity (A) and on hypoglossal membrane input resistance. A. shows the membrane was depolarized from approx. -64 mV to -46 mV during hypoxia and rhythmic EPSPs decreased from 5.05 mV to 1.56 mV during hypoxia. B. Increase in input resistance shown by voltage drop across the membrane associated with the current pulses (2 msec, -1 nA). The kitten was 23 days old and weighed 280g.

(an example is shown in figure 36A), 8 units exhibited only EPSP (an example is showed in figure 38A), 2 exhibited only IPSP (see example in figure 37A). Two units shown no rhythmic EPSPs or IPSPs during normoxia.

3.4.3.a Effects of hypoxia on rhythmic EPSP amplitude and duration

1) The amplitude of the rhythmic EPSP activity during hypoxia

In most of the HMN, the EPSPs were reduced in amplitude.

Seven of 11 units exhibited a significant decrease in the amplitude of rhythmic EPSPs. An example is shown in figure 38A. The EPSP amplitude was 5.05±0.21 mV during normoxia. It significantly ($P<0.05$) decreased to 1.56±0.11 mV during hypoxia. A further example is shown in figure 36A. Both amplitude and duration of EPSP decreased significantly ($P<0.05$) from 2.28±0.14 mV and 2098.8±78.12 ms in normoxia to 1.25±0.09 mV and 1528.12±85.92 ms during hypoxia. In this case, membrane potential decreased from -64.29 mV in normoxia to -46.79 mV during hypoxia.

The amplitude of rhythmic EPSPs increased in 2 of 11 units and there was no change in 3 of 11 during hypoxia.

2) Changes in the duration of rhythmic EPSPs in hypoxia

There was a significant decrease in the duration of the rhythmic EPSPs during hypoxia in 5/11 units. There was no change on the remaining 6 units.

3.4.3.b Effects of hypoxia on rhythmic IPSP amplitude and duration

In addition to the rhythmic EPSPs recorded, rhythmic IPSPs was also recorded. The amplitude of the IPSPs ranged from 1.94±0.09 mV to 4.44±0.49 mV during normoxia and from 0 to 3.88±0.46 mV during hypoxia. The amplitude of the rhythmic IPSP decreased

in 3 of 5 HMNs. 2 units exhibited no significant change during hypoxia. An example is shown in figure 37A. The amplitude and duration of IPSP did not change during hypoxia.

The duration of rhythmic IPSPs decreased significantly in 4 of 5 units and did not change in the remaining unit. An example is showed in figure 36A. The IPSP amplitude was not significantly changed during hypoxia. However, the duration of IPSPs was reduced significantly from 1439.56±35.75 ms in normoxia to 1209.42±51.55 ms during hypoxia. Another example is shown in figure 37A. Neither the amplitude nor duration of IPSP were changed during hypoxia.

3.4.4 Changes in input resistance

Changes in membrane input resistance during hypoxia and hyperoxia were tested in 9 units in 2 kittens by measuring the change in voltage drop across the membrane when a constant current pulse was passed through the recording electrode. Seven units were recorded the changes from normoxia to hypoxia and 2 units were recorded from hyperoxia to normoxia.

3.4.4.a Changes in the membrane input resistance during hypoxia

Most HMNs showed an increase in input resistance during hypoxia.

Hypoxia induced a gradual increase in input resistance in 5 of 7 HMNs. This was evident in the significant change in membrane voltage drop in response to the constant current pulses. The average drop was 10.42±1.84 mV during normoxia and then increased significantly ($P<0.05$) to 24.17±5.64 mV during hypoxia. An example is shown in figure 38. The mean voltage drop was 7.35±0.18 mV during normoxia and this significantly ($P<0.05$) increased to 10.49±0.45 mV during hypoxia (FiO_2 0.17). A further example is shown in figure 36B. The mean voltage drop was 13.17±0.93 mV during normoxia, but by 2 minutes of hypoxia (FiO_2 0.17) it had increased to 35.60±1.94 mV.

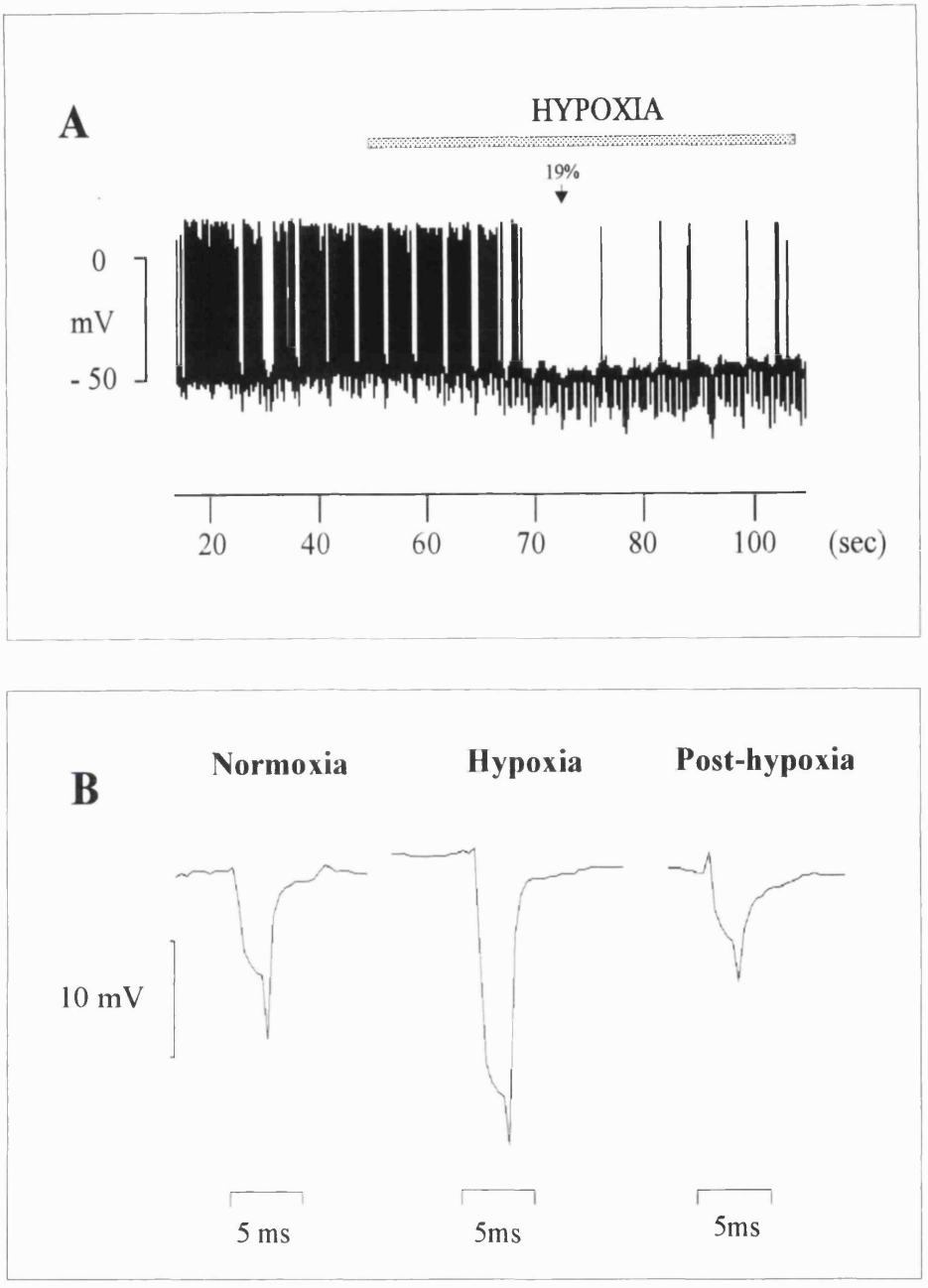


Figure 39. Intracellular recording of one hypoglossal motoneurone showing the effects of hypoxia on membrane input resistance change in hypoxia (FiO_2 0.17) and post-hypoxia. A shows the membrane depolarized during hypoxia. B. Increase in input resistance shown by voltage drop across the membrane associated with the current pulses (2 msec, -1 nA). The kitten was 23 days old and weighed 280g.

The impedance did not change significantly during hypoxia in 2 of 7 units. An example of this type of response is shown in figure 37B. In this case, the voltage drop did not change significantly from 12.83 mV during normoxia to 13.32 mV during hypoxia.

3.4.4.b Change in the membrane input resistance during post-hypoxia

I was able to record from 5 units long enough to examine input resistance changes during normoxia, hypoxia and post-hypoxia. The impedance of all 5 units increased during the hypoxic period. Three of 5 units did not change the input resistance during post-hypoxia compared to normoxia. In one unit the voltage drop decreased ($P<0.005$) during post-hypoxia compare to the normoxia (see figure 39). The voltage drop was significantly ($P<0.005$) increased during hypoxia. The remaining unit showed that there was significant ($P<0.005$) increase in impedance during post-hypoxia.

3.4.4.c Changes in the membrane input resistance during hyperoxia

The changes in input resistance of 2 HMNs were examined during hyperoxia. During hyperoxia, the FiO_2 was 32% and 44%. Figure 40 shows the membrane potential hyperpolarized from -57.68 mV in hyperoxia to -61.17 mV during normoxia. The voltage drop was 17.98±0.56 mV during hyperoxia and it significantly ($P<0.05$) increased to 57.54±2.83 mV during normoxia. The other unit which the voltage drop was 3.45±0.12 mV during hyperoxia and that significantly ($P<0.005$) increased to 5.88±0.19 mV during normoxia.

3.4.5 Change in membrane excitability

In response to depolarizing current pulses, the HMNs displayed one or more action potentials when threshold was reached.

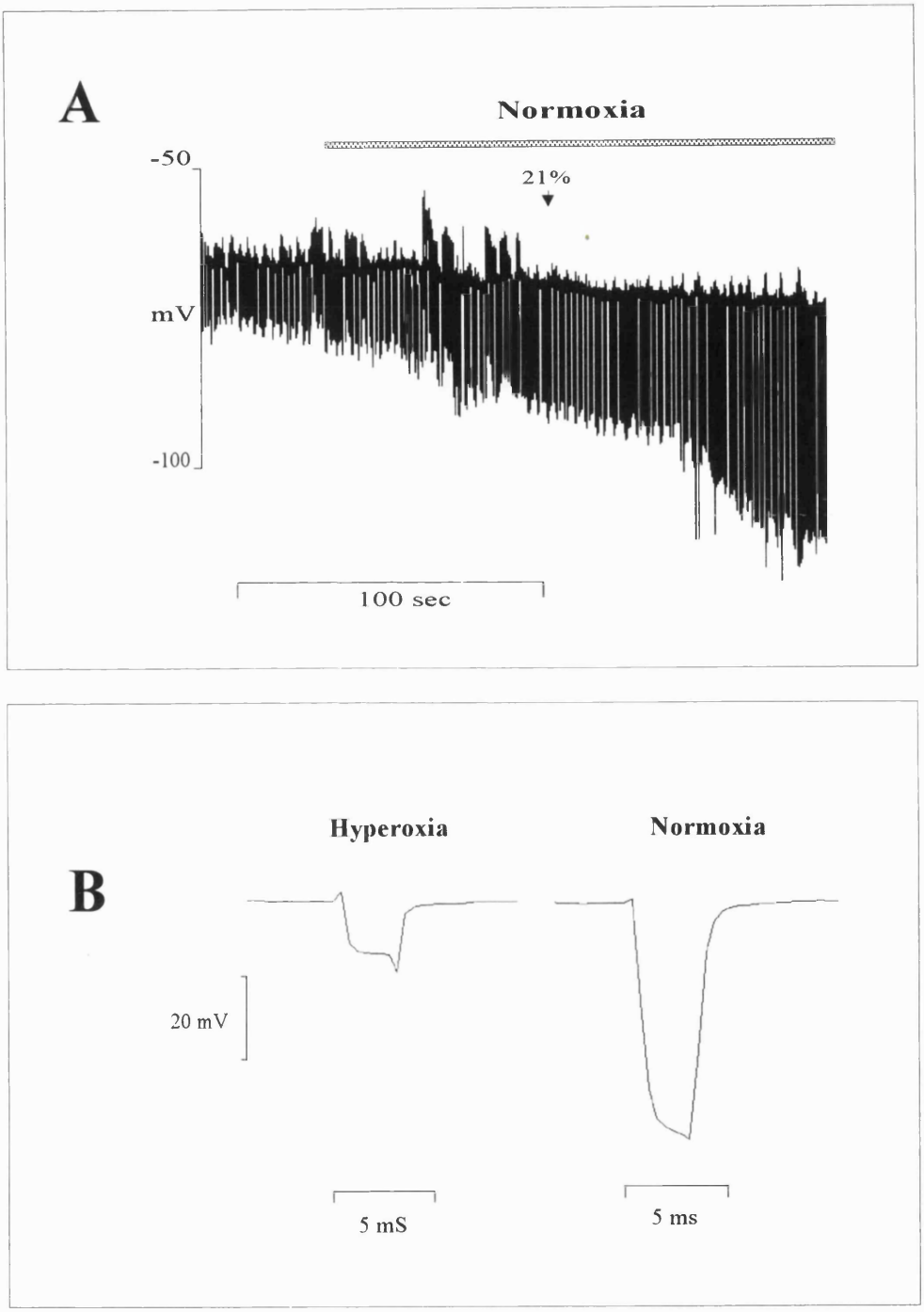


Figure 40. Intracellular recording of one hypoglossal motoneurone showing the effect on membrane potential on change from hyperoxia (FiO_2 0.35) to normoxia (A) and membrane input resistance (B). A, the membrane was hyperpolarized from approximately -60 mV during hyperoxia to -57 mV during normoxia. B, increase in input resistance shown by voltage drop across the membrane associated with current pulses (2msec, -nA). The kitten was 23 days old and weighed 280g.

3.4.5.a Effect of hypoxia on membrane excitability

Most HMNs showed a decrease in membrane excitability during hypoxia as measured by the ability of given depolarizing current pulses to generate action potentials. A decreased excitability was indicated by either an inability to generate action potentials or by a shift in onset time of the action potential so generated.

Eight HMNs were tested during hypoxia with depolarizing current pulses. The depolarizing current pulses varied from unit to unit (0.3 to 7.4 nA) depending on the threshold at which action potential were generated.

The majority of the HMNs (7 of 8, 88%) exhibited a decrease in membrane excitability during late hypoxia. An example is shown in figure 41. In this case, the amplitude of the generated action potential was decreased 3 minutes after the FiO_2 was changed to 17%. During post-hypoxia, the generated action potential had not returned to pre-hypoxic level even 2 after the FiO_2 had returned to normoxia. The reduced impulse amplitude is most readily explained by a failure of the IS (initial segment) spike to invade the soma. This was similar to the result of a previous study when it was found that in hypoxia antidromic impulses often failed to invade the soma (Smith, 1993).

Another example is shown in figure 42. This shows action potentials generated by 0.3 nA depolarization current pulses. During late hypoxia, these pulses failed to generate action potentials but had done so readily in normoxia and early hypoxia (i minute). In this case, the excitability returned in the early post-hypoxia period as is evident by the action potentials were evoked by the current pulses.

Figure 43 shows a case where the onset of the action potentials had shifted during early hypoxia. In this case, the latency of the action potentials were delayed during hypoxia and no action potentials could be evoked in late hypoxia.

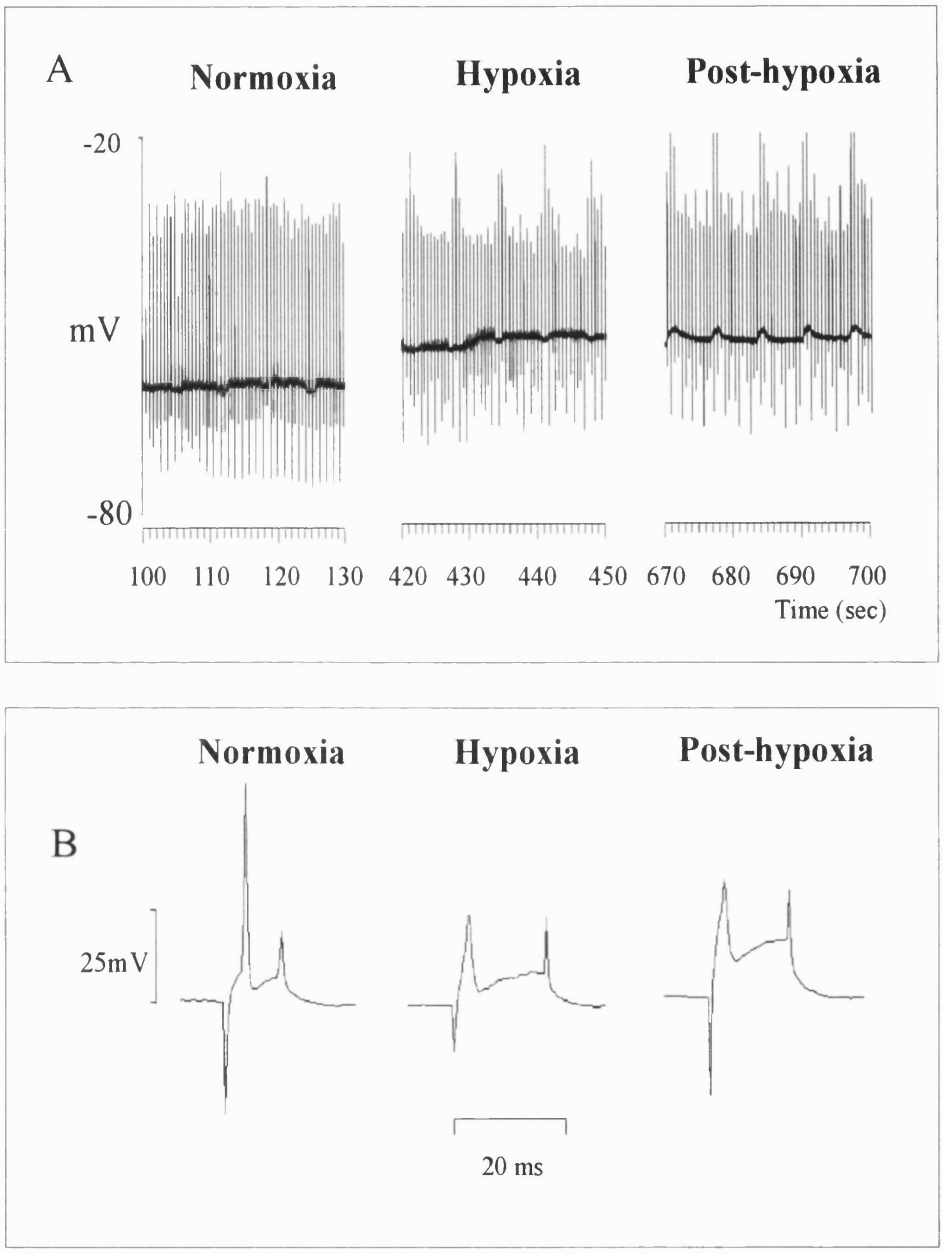


Figure 41. Intracellular recording of a hypoglossal motoneurone showing the effects of hypoxia on rhythmic EPSP and IPSP activity and membrane excitability. In A, only rhythmic IPSPs were recorded during normoxia. The membrane depolarized from approximately -57 mV to -42 mV during hypoxia and the rhythmic IPSPs disappeared, EPSPs appeared in post-hypoxia. In B, action potentials were generated by depolarizing current pulses (2 msec, +2.5 nA) passed though the recording electrode. Note the amplitude of generated action potential was reduced during hypoxia. The kitten was aged 24 days old and weighed 290g.

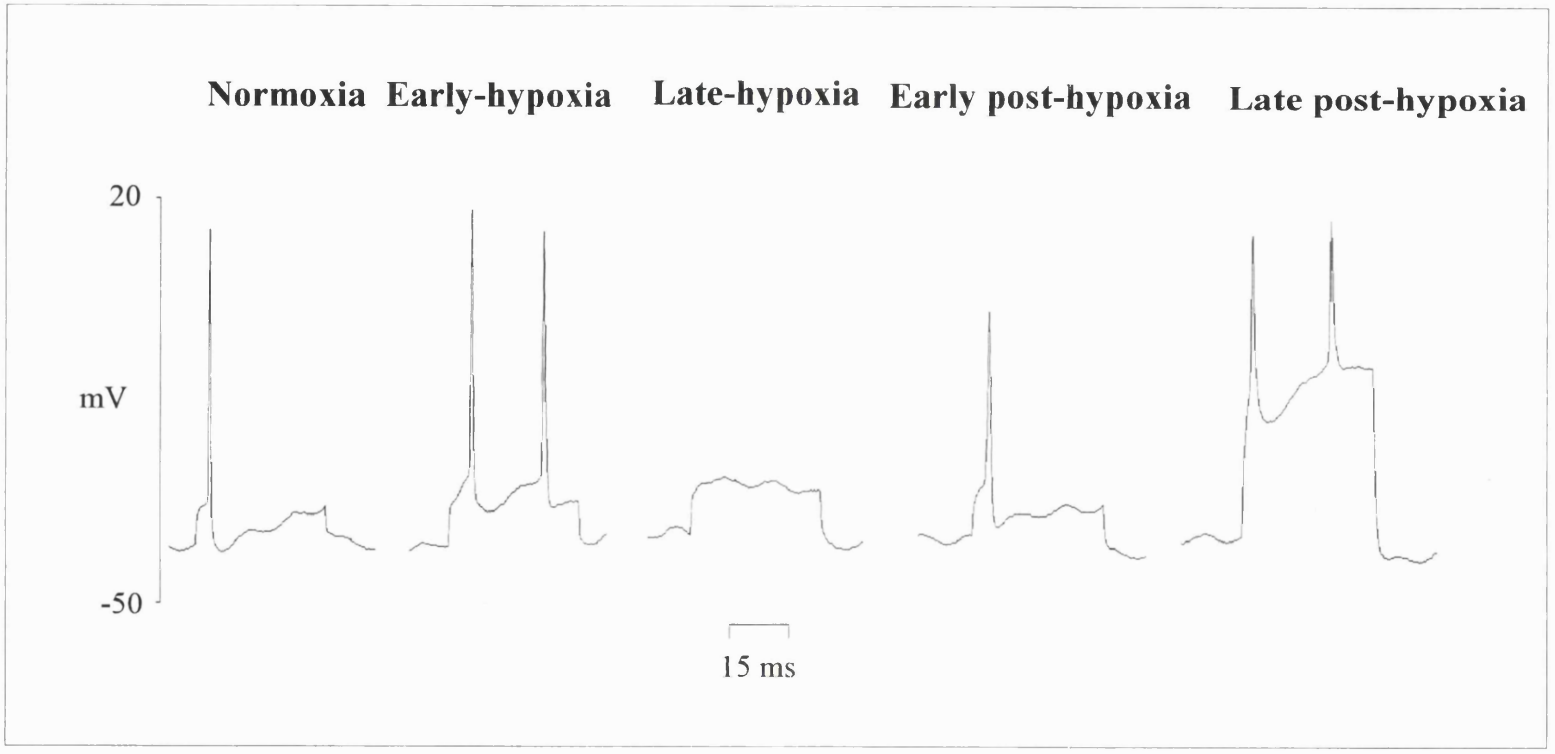


Figure 42. An example of one hypoglossal recording showing the effects of hypoxia on membrane excitability during early hypoxia (FiO_2 0.19-0.18), late hypoxia (FiO_2 0.16), early post-hypoxia and late post-hypoxia. Action potentials were generated by constant positive current pulses (2 msec, 0.3 nA). Note membrane excitability increased during early hypoxia but decreased in late hypoxia as indicated by failure to generate action potentials with the current pulses. When FiO_2 returned to normoxia, membrane excitability was restored. The impedance of the recording electrode increased during late post-hypoxia. The kitten was 24 days old and weighed 410g.

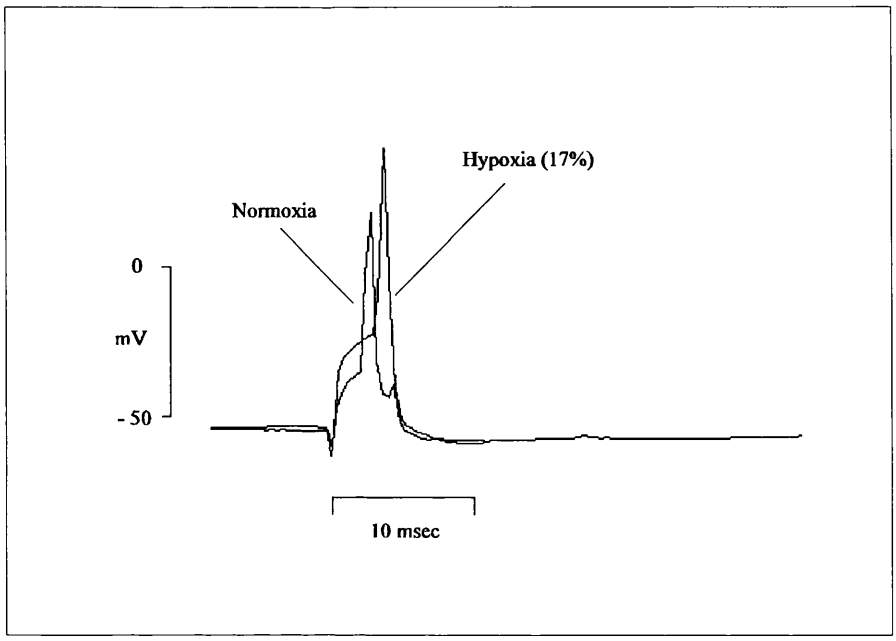


Figure 43. An example of a hypoglossal motoneurone showing the effects of hypoxia on membrane excitability during hypoxia (FiO_2 0.17). Action potentials were generated by a constant positive current pulses (2 msec, 0.9 nA). The onset of the generated action potential shifted during hypoxia. The kitten was 24 days old and weighed 290g.

3.4.5.b Membrane excitability in post-hypoxia

Seven of 8 units were held for a long enough time to examine membrane excitability during immediate post-hypoxic period.

Five of 8 units showed a decrease in membrane excitability during post-hypoxia compared to normoxia. Two of these 5 units exhibited a decrease further when compared to hypoxia. Three of these 5 showed membrane excitability return in the period of post-hypoxia but it was still less than in the pre-hypoxic period (for example see figure 42).

Two units exhibited an increase in membrane excitability during post-hypoxia when compared to normoxia.

3.4.6 Effect of change in membrane potential during hypoxia on the change with membrane excitability during hypoxia

In 7/8 units the membrane potential depolarized during hypoxia and in 1 of 8 units there was a transient depolarization during hypoxia. This unit exhibited a decrease in membrane excitability during hypoxia.

3.4.7 Effect of $PaCO_2$ and PaO_2 on the responses of the input impedance and membrane excitability

The $PaCO_2$ was 93.5±1.46 mmHg during normoxia and fell to 44.0±2.94 mmHg during hypoxia. There was no significant change in mean $PaCO_2$ (37.03±0.95 mmHg during normoxia, 36.17±1.98 mmHg during hypoxia). pH was 7.36±0.37 in normoxia and did not significantly changed during hypoxia (7.28±0.01).

3.4.8 Summary of section 4

This section has shown that changes in the input resistance by measuring the voltage drop across the membrane and changes in membrane excitability by measuring the ability to generate action potentials during mild hypoxia in neonatal kittens.

The input resistance increased in 5 of 7 hypoglossal motoneurones.

The excitability decreased in 8 of 9 hypoglossal motoneurones.

CHAPTER 4

DISCUSSION

4.1 A summary of the results of this thesis

The results of this study show that the output of HMNs is affected by even mild levels of hypoxaemia. Most of these motoneurones (74%) responded with either a decrease or only transient increase in discharge frequency, 11% had no significant change. Only a small proportion of these motoneurones (15%) showed a sustained increase in discharge frequency during hypoxia.

Intracellular recordings were made to determine to what extent these changes in HMN output could be explained by either a change in membrane excitability or a hyperpolarization of the motoneurones.

To address further the hypothesis that the decrease or transient nature of any increase in discharge frequency is due to inhibitory mechanisms producing a hyperpolarization, I have recorded the membrane potential of HMNs during mild levels of arterial hypoxia similar to those used in the extracellular studies. In intracellular recordings, three distinct responses were recorded. Most (65%) of the motoneurones showed a sustained depolarization in membrane potential in hypoxia. 20% of motoneurones had a transient depolarization similar in time course to the transient increase in discharge frequency in extracellular recordings. Furthermore, 15% motoneurones were hyperpolarized. This is similar to the previous study in this laboratory (Smith et al., 1993b).

This study therefore shows that in neonatal kittens the activity of HMNs is altered during mild levels of hypoxaemia by changes in membrane potential. But, the proportion of HMNs with inhibition in extracellular recordings was not the same as that with hyperpolarization during the hypoxic challenge in intracellular recordings. It is concluded that the changes in HMN output in most cases cannot be the result of hyperpolarization.

In most of the HMNs, it was also found that in hypoxia membrane excitability was

reduced and membrane impedance increased. This supports the hypothesis that such changes underlie the changes in HMN output recorded with extracellular electrodes during hypoxia.

The results of this study show that hypoxaemia produces changes in respiratory-related rhythmic EPSPs. In most units the amplitude of rhythmic EPSPs was decreased. This suggests that hypoxaemia has an inhibitory effect on hypoglossal output through a mechanism which is dependent on respiratory control.

Previous studies of hypoglossal motoneurones or nerve in adult cats have shown both excitatory and inhibitory effects evoked by electrical stimulation of superior laryngeal nerve. The laryngeal stimulation evoked two patterns of responses: EPSP-IPSP complex and EPSP only.

Mechanical stimulation of the larynx in this study also evoked EPSPs, EPSP-IPSP complexes or IPSPs alone. These laryngeal-evoked potentials were reduced in amplitude and duration in most cases during hypoxia.

In this thesis it will be argued that the response of HMN to hypoxia may be the result of both direct effects on the motoneurones themselves and on excitatory and inhibitory projection to these neurones. The results clearly show that hypoxaemia has both effects on respiratory-related and non-respiratory-related HMN activity with changes in resting membrane potential, impedance and excitability as well as reductions of rhythmic EPSP amplitude.

4.2 Overall view of the discussion

This thesis has addressed the following central questions:

1) What are the effects of mild levels of hypoxaemia on hypoglossal motoneurone output and discharge pattern?

2) Does hypoxaemia affect both respiratory and non-respiratory related activity in these motoneurones?

3) What are the effects of systemic hypoxia on hypoglossal membrane potentials and excitability?

4) How do these effects of hypoxia compare to the effect previously observed in hypoglossal motoneurones recorded in *in vitro* brain slices?

5) What are the effects of hypoxaemia on laryngeal-evoked potentials recorded in hypoglossal motoneurones?

In this chapter, I will discuss the response characteristics of the hypoglossal motoneurones during mild levels of hypoxia. This will be followed by a consideration of the excitatory and inhibitory influences which may affect HMNs activity. I will consider the changes in respiratory related rhythmic EPSP activity recorded in hypoxia and will discuss some possible mechanisms underlying these changes. Furthermore, a general discussion of the possible central mechanisms contributing to the observed changes will be included. Finally, the clinical implications and future directions for research in this area will be presented.

4.3 The levels of hypoxia

The levels of hypoxaemia used in this study were relatively mild. The partial pressure of the arterial blood (PaO_2) fell from 92.4 ± 2.1 during normoxia to the level of PaO_2 48.85 ± 3.9 mmHg during hypoxia. The haemoglobin oxygen saturation (SaO_2) was reduced no more than 15%. In a previous study such hypoxia did not change the oxygen saturation of the haemoglobin circulating in the brain tissue in neonatal puppies (Nioka et al., 1990). However, this was not measured in the present study.

Many previous studies have used more severe levels of hypoxia or anoxia. Such levels were not used the present study because they profoundly affect the stability of the preparation. Nor would these kittens survive such a severe challenge if applied repeatedly.

But another reason why mild levels of hypoxaemia were used was because such levels are not uncommon in newborn babies or infants during apnoeic episodes (Haidmayer et al., 1982 a,b; McCulloch et al., 1982). Further, such levels are not uncommon in newborn babies in respiratory distress or with persistent fetal circulation (Ariagno et al., 1980; Brady et al., 1978; Harding, 1994). It was not known what effect such levels had on respiratory-related reflexes.

Severe tissue acidosis has been viewed as a damaging component of cerebral hypoxia. Acidosis is a hallmark of cerebral hypoxia and has received considerable attention since its description (Crowell & Kaufmann, 1961; Liunggren et al., 1974). Except for certain cells in the brain stem and spinal cord, acidosis appears to depress the excitability of a majority of central neurones (Meyer et al., 1961; Speckman and Caspers, 1974; Jarolinek et al., 1990). Tissue acidosis was not measured in this study and this cannot be ruled out even with the mild levels of hypoxic test. However, cerebral acidosis was found in studies using very severe levels of hypoxia.

4.4 The effects of hypoxia on the discharge frequency of the HMNs in neonatal kittens

The results of this present study show that systemic hypoxia produces complex changes in HMN output; 15% of the HMNs recorded with extracellular electrodes exhibited a sustained increase in discharge frequency, but for most (74%) the change during hypoxia was either only a transient increase or a decrease in impulse frequency.

Thus the effect of hypoxia on HMN discharge frequency is similar to its effect on ventilation. In neonates, the recorded increase in ventilation during hypoxia is not

sustained (Blanco et al., 1984). This 'biphasic' ventilatory response to hypoxia is similar in time course to the transient response of HMNs in the present study, with an increase during the first minute of hypoxia, with a subsequent return to or to below the normoxic level.

These results of this thesis are consistent with and no doubt underlie the effects of hypoxia on genioglossus recruitment in neonatal kittens (Watchko et al. 1989). Such recruitment is age related. In young kittens (<1 month old) recruitment is either non-existent or is only transient. In older kittens (2 month old), recruitment is sustained. The response of HMNs in the present study were similar in time course to the recruitment of genioglossus in hypoxia in the younger kittens.

4.5 Effects of hypoxia on the membrane potential of HMNs in neonatal kittens

This study shows that in neonatal kittens the activity of HMNs is altered during mild levels of hypoxaemia by changes in membrane potential. But, the proportion of HMNs with inhibition in extracellular recordings was not the same as that with hyperpolarization during hypoxia in intracellular recordings. Possibly, the sample in extracellular recordings was more representative of the population of HMNs; the intracellular recordings may have been biased by ease of penetration to HMNs with depolarizing responses.

But equally there are reasons to suppose that the transient response or inhibition of HMNs in hypoxia are not due to hyperpolarization of the membranes.

The depolarization seen in most HMNs in this study is similar to the response of these motoneurones during hypoxia in brain slices *in vitro* (Haddad & Donnelly, 1990). Thus, hypoxia may be having similar effects in both *in vitro* and *in vivo* preparations.

In contrast, hyperpolarization of HMNs during hypoxia was not seen *in vitro*, and in the present study it was observed in only 15% of HMNs. Hyperpolarization in response

to hypoxia has been recorded in other neurons in CNS. For example, in adults, anoxia induces a hyperpolarization in hippocampal CA3 neurones (Ben Ari, 1989; Mourre et al., 1989). Anoxia produces a consistent hyperpolarization in CA1 cells in newborn rats (Cherubini et al., 1989).

In present study, the transient excitation during mild levels of hypoxia showed two patterns, repolarization after depolarization and repolarization after hyperpolarization. The first pattern is similar in time course to the transient response of the whole nerve in a hypoxic episode (Watchko et al., 1989) and also the "biphasic" ventilatory response in infants (Edelman et al., 1966; Kagawa et al., 1982; Rigatto et al., 1975; Rigatto, 1979; Sankaran et al., 1979).

However, as emphasized above, these changes were not recorded in sufficient numbers to fully explain the transient increase in discharge frequencing recorded in extracellular studies.

4.6 The direct effect of hypoxia on the HMNs

If hyperpolarization of HMNs cannot fully account for the changes in HMN output in hypoxia then other mechanisms have to be found. One possibility addressed in this thesis is that there are direct effects influence the excitability of HMNs.

4.6.1 Evidence of direct change on the HMNs

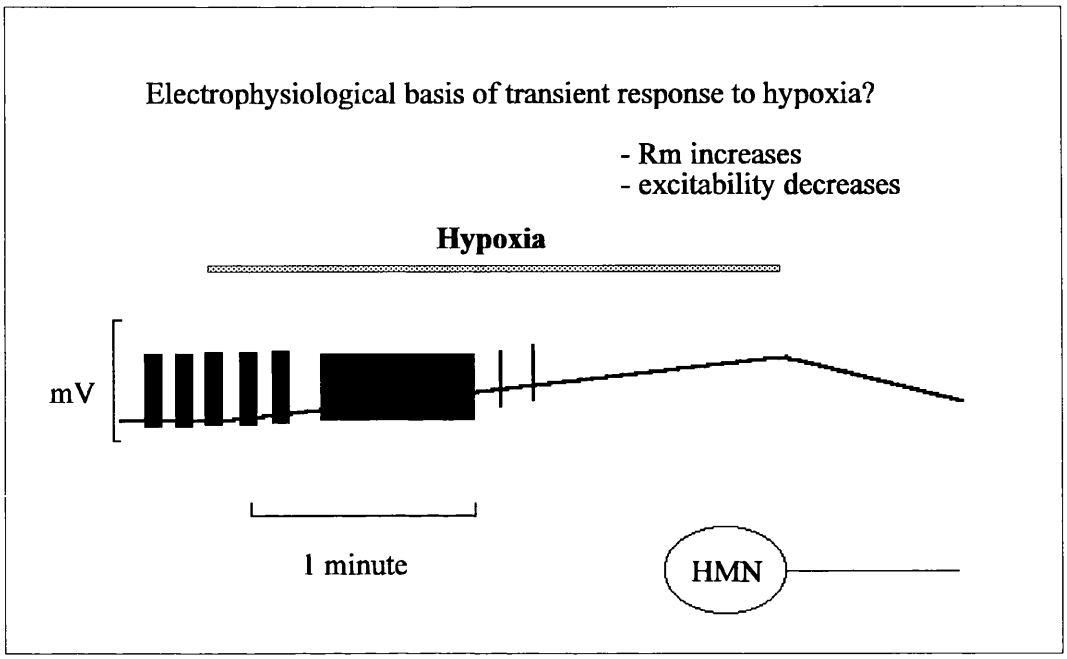


Figure 44. A theoretical model of a direct effect of mild levels of hypoxia on hypoglossal motoneurones.

At least some of the changes in hypoglossal output shown in the present study may be due to direct effects of hypoxia on HMNs. It has been shown previously in *in vitro* studies that the membrane impedance and membrane excitability are changed during hypoxia (Haddad & Donnelly, 1990; Jiang et al., 1994). Equally, in the present study, membrane impedance of HMNs increased during hypoxia and also we have evidence of changes in excitability. The results of this thesis are the first to show that the changes observed in the *in vitro* studies are also occurring in an *in vivo* neonatal preparation. Furthermore, they are occurring even with mild levels of hypoxaemia. The transient increases in discharge frequency could therefore be explained by such changes. In this model (figure 44), as the membrane depolarizes there is an increased discharge frequency over the first minute of hypoxia. Subsequently, excitability is gradually reduced. This change in excitability makes it harder for the neuron to discharge. Thus despite a sustained depolarization discharge frequency falls or ceases.

This would account for the transient increase in discharge frequency in extracellular recordings during hypoxic challenge.

Previous studies have found that the membrane excitability was reduced and membrane impedance increased in HMNs during hypoxia *in vitro*. This is also true in the present study. This shows that even during mild levels of hypoxaemia as in the present study, similar effect are observed in HMNs *in vivo*.

4.6.2 Comparison of effects on membrane impedance and excitability of HMNs during hypoxia between in vivo and in vitro studies

In a previous study of HMNs recorded in rat brain slices *in vitro* input resistance was found to be higher in HMNs recorded in neonatal slices than in adult slices (Haddad & Donnelly, 1990). Input resistance increased in HMNs recorded in slices of adult brain stem during hypoxia but remained constant in those recorded in the slices taken from neonates. The reason of a lower input resistance in adult could be the result of an increase in soma size and dendritic arborization (Haddad et al., 1990).

Data from Donnelly and Haddad show that the excitability increases in early hypoxia followed by a decrease in some HMNs in adult brain slice (Donnelly et al., 1992). This is consistent with the present result (an example is shown in figure 42).

4.6.3 The effect of ATP change during hypoxia

If the inhibitory effects of hypoxia are caused by changes in excitability then how might these occur? One possibility is a modulation of K^+ channel. During hypoxia, K^+ concentration outside the cells increases when recording in slice preparations. K^+ concentration shows a sustained decrease inside of the cell during anoxia. Furthermore, K^+ channel modulation has been shown to be an integral and important cellular response to decrease in O_2 (Jiang & Haddad, 1991; 1994).

ATP-sensitive K^+ channels appear to be responsible for the sustained output of K^+ current in HMNs (Jiang et al., 1991). K^+-ATP channel activator can increase the sustained K^+ currents by 20%. Further, in cell-free excised membrane patches, a K^+ channel was inhibited by a decrease in ATP during anoxia in neonatal rat in vitro preparation (Jiang & Haddad, 1994; Jiang et al., 1992; Jiang et al., 1994).

In the present study, the low O_2 in the CNS may reduce K^+ channel activity by the decreasing of ATP level. This might explain why most HMNs depolarized with an accompanying increase membrane impedance during hypoxia.

4.7 Possible mechanisms which may be involved in the response of the HMNs to mild levels of hypoxia

The results in the present investigation show that systemic hypoxia produces changes in the impedance and excitability of HMN in anaesthetized neonatal kittens. These are similar to the effects of hypoxia recorded in HMNs in brain slices *in vitro* (Haddad & Donnelly, 1990). The similarity in the response in in vitro and in the in vivo preparation are striking. In both preparation the membrane is depolarized, impedance increased and excitability falls during hypoxia. However, there are equally striking differences in the responses recorded in the two preparations. In particular, the hyperpolarization, recorded in a significant proportion of HMNs in the neonatal kittens have not been recorded *in vitro*. Furthermore, respiratory-related EPSPs were reduced in amplitude. Direct effects may not therefore account all the changes occurring during systemic hypoxia.

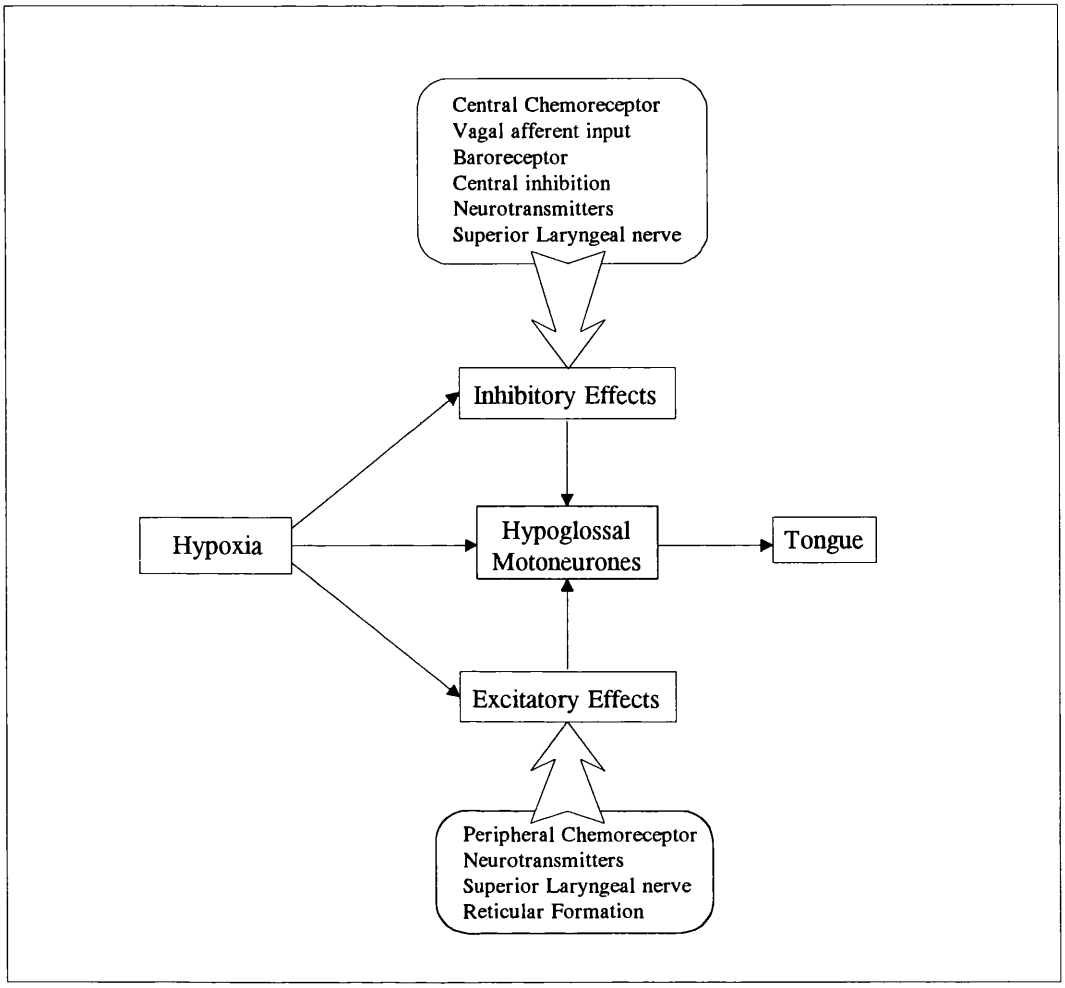


Figure 45. A theoretical model of the way in which mild levels of hypoxaemia may influence hypoglossal motoneurone output. It is proposed that hypoxia influences both excitatory and inhibitory inputs to hypoglossal motoneurones. During hypoxia, the activity of hypoglossal motoneurones will determined by the changes in the balance of excitatory and inhibitory mechanisms during hypoxia.

The changes recorded in the present study during mild levels systemic hypoxia might be produced by changes in the balance between excitatory and inhibitory input to HMNs. A striking feature was that even with the same level of hypoxia, in the same kitten, as the similar levels of reduced FiO_2 some HMNs were excited, some transiently excited and some inhibited during hypoxia. Different motoneurones may have at any given time different levels of excitability drive or inhibitory influences (see

figure 45). The differing components of this balance might explain the different effects of hypoxia in present study. For example it has been shown at superior laryngeal excitatory inputs are profoundly depressed during systemic hypoxia in the present study. From our model, a number of possible mechanisms would therefore have to be considered in determining the effect of hypoxia on HMN output. These include:

1) variations in the degree of hypoxia,

2) changes in chemoreceptor activity,

3) changes in lung compliance,

4) changes in cerebral blood flow,

5) brain stem inhibitory mechanisms,

6) the age of the kittens.

Each of them is discussed below.

4.7.1 Variations in the degree of hypoxia

The balance between excitation and inhibition in the presence of a hypoxic stimulus depends on the severity of the hypoxia, on the age of the subject and the degree of maturation of the peripheral and central chemoreceptors and on factors that would influence the synthesis or metabolism of mediator substances. Thus, the response of the HMNs may vary depending upon the degree of hypoxia. With mild levels of hypoxia HMNs may be inhibited and at more severe levels they may be excited, or vice versa. However, in the present study in a given kitten, at any level of FiO_2, motoneurones could be recorded with the different response characteristics. Nevertheless, this does not show how an individual motoneurone would respond at different levels of FiO_2.

This can only be established by holding a HMN for a long enough period of time to be able to lower the level of FiO_2 systematically and record the response at each level.

Even if the same level of FiO_2 were used in the hypoxic test, the blood samples showed that PaO_2 levels varied. As we expect, the levels of PaO_2 were lower than the levels of mild hypoxia. This would have been dependent upon the rate of alveolar ventilation, the rate of oxygen diffusion across the lungs and the distribution of pulmonary blood flow. These factors may have been different between animals and may have altered over the course of an experiment. However, it must be remembered that the different response characteristics could be recorded in the same kitten and even in the same motoneurone and the sequence in which they were recorded was not consistent. For example, even in one intracellular preparation during the first hypoxic test the membrane depolarized and the amplitude of respiratory related rhythmic EPSP increased; however, during the second hypoxic test the membrane hyperpolarized and the amplitude of rhythmic EPSPs decreased.

Another consideration is the levels of PO_2 experienced by the individual motoneurones. This will very depending upon the blood flow in the microcirculation and the position of the motoneurones in relation to the capillaries. Thus, if hypoxia has a direct effect, the different response characteristics may reflect the PO_2 experienced by each motoneurone as a result of differences in their position within the tissue.

4.7.2 Changes in chemoreceptor discharge

A most likely explanation for at least some of the excitation of the HMNs in vagotomized kittens, recorded in the present study during the mild levels of hypoxaemia, is that there was an increase in the discharge of the peripheral chemoreceptors; particularly as stimulation of the peripheral chemoreceptors in adult cats produces a depolarization of HMNs (Mifflin, 1990) and was manifested as an increase in either the amplitude or duration of the inspiratory membrane potential depolarization. Furthermore, several studies have shown, that the discharge of carotid

sinus nerve fibres increases during hypoxia in anaesthetized neonatal kittens (Blanco et al., 1984, Marchal et al., 1992; Morray et al., 1991, 1992). With levels of hypoxaemia similar to the levels used in the present study, the discharge of the carotid chemoreceptors increased from 17.3±6.1 to 36.9±12.2 impulses per second in 4 week old kittens anaesthetized with α-chloralose (Morray et al., 1992). Furthermore, peripheral chemoreceptors play an active role in maintaining normal ventilation and avoidance of prolonged apnea in for example piglets (Donnelly & Haddad, 1986). It is therefore extremely likely that the mild levels of hypoxaemia used in the present study would have increased the discharge of the peripheral chemoreceptors.

If the peripheral chemoreceptor discharge was not active enough to maintain the activity of HMNs in the kittens in the present study during the mild levels of hypoxaemia, it may explain why most of the motoneurones were unable to sustain an increase in activity and others were inhibited. Again, the decreased excitability recorded in the present study supports thus idea. On the other hand, central chemoreceptors do respond to changes of $PaCO_2$ in newborn animals and human infants (Guthrie et al., 1980; Rigatto et al., 1980; 1981). In adult cat, the decrease of $PaCO_2$ reduces the hypoglossal activity (Hwang et al., 1988). The result in this thesis showed that $PaCO_2$ decreased during hypoxia in most of the recordings. This decrease in $PaCO_2$ may have altered the balance of activation of central nervous peripheral chemoreceptor inputs with an increased contribution from the peripheral chemoreceptors.

However, if the changes in HMNs activity are be fully explained by changes in chemoreceptor activity, this hypothesis would have to account for the fact that the different responses, for example excitation, transient excitation and inhibition, could be recorded in the same kitten. One possible explanation is that not all of the peripheral chemoreceptor fibres respond in the same manner to hypoxia, or do so over the same time course. Indeed, in a recent study of single fibres, some fibres sustained the increase in discharge frequency whereas in others, after the initial increase, the discharge frequency declined (Marchal et al., 1992). If there is a differential

distribution of the types of fibres involved in the hypoglossal system it might explain the different response characteristics recorded. Because peripheral chemoreceptor discharge is still elevated above control it is more difficult to see how it could produce the effect on those HMNs which were simply inhibited. Thus, it is doubtful that the patterns of response during hypoxia are only due to changes in the chemoreceptor input. Again it must be emphasized that most HMNs were depolarized in hypoxia in the present study.

4.7.3 Change in lung compliance

Changes in vagal input may be caused by changes in many parameters including blood pressure and changes in lung compliance. One question that has been addressed in present study is wether the inhibitory effects are due to changes in vagal afferent input.

The present result showed that the HMNs had a much stronger respiratory related rhythmic activity in normoxia compared to the results from a previous study in this laboratory in vagi-intact kittens (Smith, 1993; Smith et al., 1993c). In a number of previous studies, the influence of vagal feedback mechanisms on respiratory-modulated activities of cranial nerves has been assessed (Bartlett Jr. & St. John, 1988; Hwang et al., 1989; Hwang et al., 1988; Sica et al., 1984; St. John & Zhou, 1992; Van Lunteren et al, 1984). The phasic discharge of pulmonary stretch receptors ubiquitously inhibited these activities. The pulmonary stretch activity is an important determinant of the presence and magnitude of both inspiratory and expiratory cranial neural activities (St. John & Zhou, 1992; St. John, 1986). When pulmonary inflations are withheld, both inspiratory and expiratory discharges are greatly enhanced. For the inspiratory activities, the depression during cycles with lung inflations might simply reflect inhibition by pulmonary stretch receptors (Kuna, 1986). A greater activity of pulmonary stretch receptors might also underlie the depression of expiratory cranial neural activities. This has been confirmed on HMN which have greatly increased activity after vagotomy in cats (Bradley et al., 1976). This might explain why the respiratory activity was less and rhythmic IPSPs were not observed in non-vagotomized

kittens in a previous study in this laboratory (Smith, 1993; Smith et al., 1993c).

A possible interpretation of the inhibition of the HMNs during hypoxia in previous studies was that it was due to an increase in vagal afferent input. The effect of pulmonary afferent activity on hypoglossal discharges was inhibitory, as shown by the increase of activity when inflation was withheld in adult cats (Sica et al., 1984). Lung inflation has been reported to induce both excitatory and inhibitory effect in upper airway muscles in different circumstances (Bartlett et al., 1973). Furthermore, the effects of pulmonary afferent activity on hypoglossal discharge is inhibitory and vagotomy produces a greater increase in hypoglossal activity (Fukuda & Honda, 1982; Weiner et al., 1982). Contradictory results have been reported on the effects of hypoxia on dynamic lung compliance and pulmonary resistance in the newborn (La Frambiose et al., 1983; Cote et al., 1988; Blanco et al., 1984). Nevertheless, given the effects of pulmonary afferent input on hypoglossal discharge it was always possible that inhibition of HMN during hypoxia was due to such changes.

In a previous report in this laboratory (Smith, 1993; Smith et al., 1993a), 34% HMNs showed a sustained increase, 27% of HMNs a transient increase and 20% had a decrease in discharge frequency during hypoxia in extracellular recordings. At that time we could not rule out the possibility that the inhibition was a result of a change in vagal afferent input due say to decreased lung compliance. However, the present study shows a much large proportion (40.7%) of HMNs had a decrease in activity during hypoxia in vagotomized preparation. Thus the inhibitory effect is not due to changes in vagal feedback as a result of decreased lung compliance (Li & Noble, 1994).

4.7.4 Changes in cerebral blood flow

Cerebral blood flow increases in hypoxia in newborn (Giussani et al., 1992) and this rise will produce a fall in brain tissue PCO_2 which could lead to reduced activity of central chemoreceptors. It has been suggested that central depression of ventilation during moderate levels of hypoxia (PaO_2 45-55 mmHg) is due to an increase in brain

blood flow and, as a consequence, a ventral medullary alkalosis (Lee & Milhorn, 1975). In support of this idea, medullary alkalosis was found to occur in chemodenervated adult cats during mild hypoxaemia ($PaO_2 > 60mmHg$) (Neubauer et al., 1981) and in neonatal piglets (0.5 to 28 days old) when the FiO_2 was reduced to between 0.10 and 0.15 (Brown & Lawson, 1988).

In the present study, although there was very little change in the $P_{ET}CO_2$, as measured by the respiratory gas monitor, the effects of hypoxia on the cerebral blood flow and the level of $PaCO_2$ at the medulla were not measured. From the results of the above studies, it is possible that the levels of hypoxia used would have induced medullary alkalosis secondary to increased cerebral blood flow. In this case, the stimulation to the central chemoreceptor will be reduced. Also the CO_2 transport by haemoglobin may have increased under the hypoxic condition (Haldane effect) again leading to medullary alkalosis. Thus it may be that the mild levels of hypoxia used in the present study induced a medullary alkalosis and this may be the underlying reason for the repolarization or hyperpolarization, or even the decrease in rhythmic EPSP activity, recorded in present study. Indeed, if such alkalosis reduced synaptic transmission then this could itself account for the increase in HMN membrane impedance recorded in the present study.

4.7.5 Brain stem inhibitory mechanisms

There is now strong cumulative evidence that active brain stem inhibitory mechanisms are evoked during hypoxaemia. In particular they appear to be involved in the biphasic ventilatory response in neonates (Hanson & Williams, 1989; Noble & William, 1989; Noble et al., 1990). Equally, the inhibitory effects recorded in present study might be attributed to such active brain stem inhibitory mechanisms.

Recent evidence has shown that the red nuclei are involved in the biphasic ventilatory response in neonate. Transection in the brain stem of neonatal rats and rabbits removes the secondary fall in ventilation in hypoxia (Martin-Body & Johnston, 1988; Williams

& Hanson, 1989). Thus, after transection of the rostral brain stem, increased ventilation is sustained throughout the hypoxic episode. Later, Heywood and coworker (Heywood et al, 1992). shown that an impaired CO_2 sensitivity occurred with a unilateral brain stem lesion.

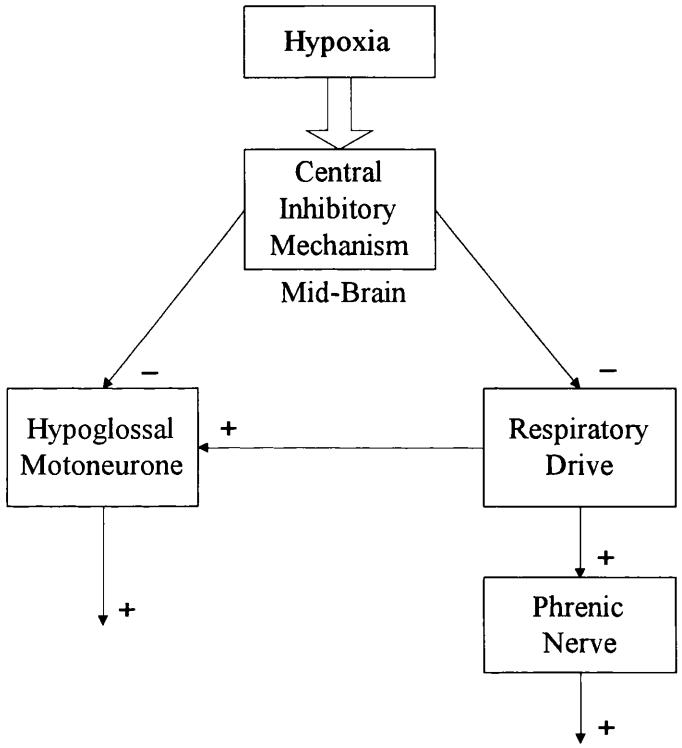


Figure 46. Possible pathways by which mild levels of hypoxaemia may influence hypoglossal motoneurone output. It is proposed that hypoxia has an effect on hypoglossal output by a mechanism which is independent of , or in addition to, the effects exerted through respiratory rhythm.

Cooling of the intermediate area of the ventral medullary surface is an experimental technique that has been shown to inhibit central neural pathways mediating CO_2 sensitivity in adult animal models (Schläfka & Loeschcke, 1967; Cherniack et al., 1979; Millhorn and Eldridge, 1986). Martin suggested that ventrolateral medulla surface cooling and the resultant decrease in central chemosensitivity enhances hypoxic respiratory depression during early postnatal life (Martin et al., 1994). Furthermore,

increased ventilation during hypoxia is maintained in newborn lambs with focal cooling in the rostral pons (Moore et al., 1991). Most recently it has been shown that increased ventilation in hypoxia is sustained after bilateral lesions in the red nuclei (Ackland et al., 1995).

There are circumstantial reasons for proposing that such inhibitory mechanisms are involved. Not least, the transient excitation of genioglossus and hypoglossal activity during hypoxia in neonates is similar in time-course to the "biphasic" ventilatory response, where an initial increase during the first two minutes is followed by a decline back to pre-hypoxic levels (Rigatto, 1984). Furthermore, we might expect that HMN respiratory output would also be affected by such a mechanism. In the present study HMNs were depolarized by hypoxia but the respiratory-related EPSPs were decreased. It is therefore possible that the changes in respiratory-related activity were mediated by brain stem inhibitory projections; but it is difficult to see how they account for the changes in membrane potential and excitability recorded in the present study.

In the present study, hypoxia had effects on HMNs in addition to, or independent of any effects occurring through respiratory drive. Most motoneurones studied were depolarized during the hypoxic challenge, a result similar to that obtained in in vitro studies (Haddad & Donnelly, 1990; Viana et al., 1993). Such a result is clearly not dependent on the respiratory network. Equally, some HMNs were hyperpolarized during hypoxia and again this appeared to be independent of any effect on respiratory output. Thus, even accepting the presence of active brain-stem inhibitory mechanisms, such a mechanism is unlikely to fully explain all the changes observed in HMN output during hypoxia. It is the contention of this thesis that the effects of hypoxia on HMN output are caused by many factors. Some of these factors have been discussed in the previous section.

4.7.6 The influence of age on the response of the HMNs

The age range used in the present study is quite narrow. In the present study on

neonates, a large proportion of the HMNs were depolarization under mild hypoxic stimulation. This at least agrees with the studies previously *in vitro* (Haddad et al., 1990) in neonatal (< 28 days) and adults rats, when HMNs showed a sustained depolarization after 5 of minutes hypoxia (15-20 mmHg) (Haddad & Donnelly, 1990).

In a previous study, the response of the GG muscle to hypoxia has been shown to be different depending upon the age of the kittens (Watchko et al., 1989); Thus, although the two month old kittens studied could sustain an increase in GG muscle activity throughout a period of hypoxia (FiO_2 0.10), the increase was sustained in only half of the one month old kittens (Watchko et al., 1989).

An age-related change in HMN input resistant was found in neonatal and adult rats brain slices *in vitro* (Haddad et al., 1990; Viana et al., 1993). The significant findings were changes in subthreshold responses including a decrease in input resistance and an increase in membrane potential with age. The low value of rheobase current and higher input resistance found at early postnatal stages suggest a higher excitability to synaptic input. Input resistance of most adult but not neonatal HMNs increased during hypoxia (Haddad & Donnelly, 1990). However, in the present study, input resistance increased in most HMNs in neonates in vivo. This may be due to species differences (rat vis cat). But it might also be due to the synaptic inputs which would be intact in the *in vivo* preparation. If hypoxia reduced synaptic activation then this might explain the increased hypoglossal membrane impedance recorded in the present study.

The resting membrane potentials were lower recorded in neonate than adult brain slices *in* vitro. It was -73±2 mV in neonates and -80±2 mV in adults in Haddad's study (Haddad & Donnelly, 1990). But, there was no difference in Viana's study, -69.6±0.7 mV in neonates and 69.6 mV in adults (Viana et al., 1993). The resting membrane potential were somewhat lower (-53.6±5.04 mV) in my preparation than those recorded in adult cats in vivo (62±2 mV) (Miffin, 1990). However the resting membrane potentials of the present study recorded was -41.4 mV to -83.57 mV during normoxia in vagotomized kittens. This was not different to the range of membrane

potential recorded in vagi-intact kittens in the previous study in this laboratory (Smith, 1993) nor was it different to the range of membrane potentials recorded in studies on adult cats (Green & Neigishi, 1963, Sumi, 1969a).

4.8 Laryngeal evoked postsynaptic potentials during hypoxia on hypoglossal motoneurones in neonatal kittens

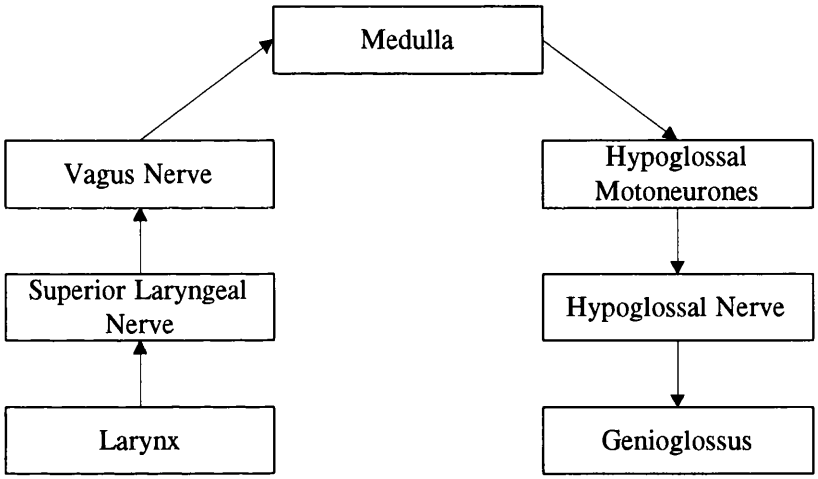


Figure 47. Components of laryngeal-hypoglossal reflex.

The present observation shows that hypoxia compromises the balance of laryngeal evoked EPSP and IPSP in neonatal kittens (Li & Noble, 1995).

Upper airway reflexes play a vital role in maintaining upper airway patency during breathing. Evidence also shows that hypoxia compromise this reflex. In a clinical study, apnea is promoted by the decreases in inspired oxygen in human infants (Johnson & Andrew, 1994). It has been already suggested that one way in which hypoxia might be having an effect on hypoglossal output is by altering the balance of excitatory and inhibitory projections. Laryngeal stimulation evokes both excitatory and inhibitory effects on hypoglossal output. In this study therefore it has been investigated whether mild levels of systemic hypoxia alters the laryngeal-evoked membrane

potential changes. Particularly, to see if hypoxia affects the excitatory potentials more than inhibitory potentials or vice versa.

It is generally accepted that laryngeal stimulation plays a role in hypoglossal activity. In 1 to 2 week old kittens, laryngeal afferent input has an inhibitory effect on ventilation. But this effect is usually very small in normal conscious subjects. However, during special circumstances, including anaesthesia and sustained upper airway flows and pressure, the ventilatory inhibition can be disproportionately magnified (Mortola & Rezzonico, 1989). The greater ventilatory inhibition in response to laryngeal chemoreceptor stimulation in the first postnatal week may be related to a low carotid body sensitivity to changes in arterial O_2 pressure (Grogaard et al. 1986). Both excitation and inhibition of hypoglossal activity was seen consistently in cats in response to cold air flowing through the larynx (Ukabam et al., 1992). In contrast to the study above, SLN stimulation causes prolonged augmentation of hypoglossal activity via increased synaptic inputs to HMNs in adult cats (Jiang et al., 1991).

In the present study, there were two distinct patterns of response to laryngeal stimulation: EPSP-IPSP complex and EPSP alone. This is similar to the result recorded in adult cats (Withington-Wray et al. 1988). In other studies, laryngeal stimulation had no effect on some HMNs (Jiang et al., 1991; Withington-Wray et al., 1988). In the present study, a laryngeal-evoked response was observed in all HMNs studied. The difference may reflect the different thresholds for activation of SLN.

If is thought that laryngeal evoked EPSPs and IPSPs in HMNs essentially increase the dynamic range over which synaptic input can induce an increase in neuronal firing frequency. The effects of SLN stimulation are not changed in hypercapnia; in contrast, the effects are decreased with low O_2 levels (Nishino et al., 1986). The regulatory contribution of laryngeal stimulation is small during normoxia and insignificant during hypoxic hyperventilation (Mortola & Piazza, 1987). The hypoglossal nerve activity increases during laryngeal chemical stimulation, and hypoxia reduces this response in adult cats (Bartlett Jr. & Knuth, 1994). In neonates, acute hypoxia augments the

laryngeal reflex apnea response in studies performed on anaesthetized piglets (Lanier et al., 1983).

The present results are similar to those described above from hypoglossal nerve in adult. It is clear from the data presented in this thesis that the amplitude of laryngeal evoke postsynaptic potentials in HMNs decreases during mild levels of hypoxia.

The effects of SLN stimulation are not dependent on the background respiratory activity in hypoglossal nerve (Nishino et al., 1986). In agreement with this, changes of amplitude of laryngeal evoked potentials in hypoxia in the present study were not clearly related to resting membrane potential or the changes in membrane potential during hypoxia.

4.9 Effects of hypoxia on rhythmic hypoglossal activity

It has been investigated whether or not hypoxia has a differential effect on respiratory-related activity. In the present study on vagotomized neonatal kittens, a majority of HMNs recorded with intracellular microelectrodes had rhythmic EPSPs during normoxia. When phrenic nerve was recorded simultaneously, these EPSPs were respiratory-related.

4.9.1 Effect of hypoxaemia on rhythmic EPSPs

The results of this thesis show that there is a reduction of rhythmic EPSP activity in amplitude and duration in most HMNs in hypoxaemia. The amplitude of rhythmic EPSP ranged from 2.21 ± 0.22 mV to 26.24 ± 1.44 mV. This is different to the result from non-vagotomized kittens. In the vagi-intact kittens, most HMNs increased the amplitude during hypoxia. However, a comparison is very difficult because respiratory-related potentials are rarely observed in the vagi-intact kittens. Vagal input in the preparation clearly suppresses such respiratory activity.

4.9.2 Effect of hypoxaemia on rhythmic IPSPs

One important finding is that there were rhythmic IPSPs in a few of the HMNs in the present study. 5 of 15 HMNs showed such rhythmic IPSPs during normoxia. The amplitude decreased in 3 and 4 decreased the duration during hypoxic challenge.

4.9.3 Respiratory-related activity

The most likely interpretation of the rhythmic activity recorded from extracellular and intracellular recordings in the present study is that they were respiratory-related. This was found to be the case in the present study when phrenic nerve discharge was also recorded. Many units in the present study showed a respiratory-related EPSP in normoxia in young kittens. In the present study, it was not possible to record the phrenic activity in all cases. Because in some preparation particularly in the smallest kittens, it was not possible to isolate a sufficient length of phrenic nerve without damage it.

In the isolated newborn rat brain stem spinal cord preparation, periodic discharges can be recorded from cranial and cervical ventral roots and particularly from the phrenic nerve (Suzue, 1984). Intracellular studies have shown that in adult cats the phasic discharge of action potentials and rhythmic depolarization of the membrane in the HMNs were correlated with central respiratory rhythm (Withington-Wray et al., 1988; Mifflin, 1990; Jiang et al., 1991). In a neonatal study *in vitro*, respiratory related hypoglossal activity was record in 0 to 3 days old rats (Morin et al. 1990b). Also both hypoglossal motoneurones and phrenic nerve have inspiratory patterns recorded in the neonatal kittens. The networks driving these neurons must receive inputs from the central generators (Sica et al., 1984).

This observation shows that hypoxia affects hypoglossal output by both effects on resting membrane potential and on respiratory activity. This might explain why during mixed apnea in spontaneously breathing infants, there is evidence for decreased central

respiratory drive, as expressed by an association with central respiratory pauses and an attenuated diaphragm EMG in the obstructed inspiratory efforts (Gauda et al., 1991, 1989). This is supported by the fact that the transient inhibitory response of respiratory activity to hypoxia is paralleled by disturbance of synaptic interaction between medullary respiratory neurones (Richter et al., 1987).

This respiratory modulation of hypoglossal activity related to the extrinsic muscles of the tongue and particular to the genioglossus muscle (Lowe, 1978; Remmers et al., 1978). The respiratory activity of the HMNs during inspiration, results in an inspiratory phasic contraction of the GG muscle and protrusion the tongue. The respiratory-related EPSP activity is reduced in hypoxia and this suggests that input of central respiratory generator is decreased during hypoxia. In this case, in neonatal kittens during hypoxia, the respiratory protection of the airway would be reduced.

4.9.4 Is the tonic hypoglossal activity related to the respiratory activity?

In the present study it is clear that the changes in membrane potentials would produce change in any on going tonic activity on HMNs. From extracellular recording, it is very difficult for us to record that change because them was not a high level of activity. In a previous study, hypoglossal inspiratory related bursts were recorded against a background of tonic activity (Sauerland & Mitchell, 1970). However, in a further study, some hypoglossal nerve fibres had tonic discharge but most of them were silent, being recruited in hypercapnia or hypoxia (Hwang et al., 1983b; Mitra & Cherniack, 1983).

In the present study, unexpectedly, discharge frequency recorded in extracellular recordings in vagotomized animals was much less than in the previous study with vagi-intact animal. This is surprising because vagal input is known to inhibit HMNs. It was therefore expected the HMN activity would increase with vagotomy. However, respiratory-related activity did increase as expected and it is possible that associated inhibitory respiratory-related inputs suppressed background tonic discharge. Supporting

this idea is the fact that respiratory-related IPSPs were recorded in some of the HMNs in the present study.

4.10 Possible mechanisms involved in the changes of the respiratory-related rhythmic EPSP activity during hypoxia

This is also discussed in further detail in section 4.7.5. The changes in the respiratory-related rhythmic EPSP activity during hypoxia may be due to a number of mechanisms.

4.10.1 Stimulation of carotid chemoreceptors

For the reasons we have discussed in 4.6.5 an increase in amplitude and /or duration of respiratory-related rhythmic EPSPs activity during hypoxia could be due to an increase in chemoreceptor discharge. Equally, the decrease in these EPSPs might due to a decrease in chemoreceptor activity during hypoxia by a reduction of $PaCO_2$.

4.10.2 Laryngeal stimulation

It is possible that the relative high levels of respiratory-related activity recorded in the present study was itself produced by laryngeal stimulation. Central respiratory drive potentials in HMNs increase following SLN stimulus in cats (Jiang et al., 1992). In agreement with this result, the respiratory-related hypoglossal activity was much more common in the present study than the result in non-laryngeal stimulated kittens used in this laboratory previously. However, not all the units recorded in this study were done with the larynx being stimulated. Even in this case, there was a high level of respiratory-related activity. This suggests that laryngeal stimulation was not responsible for this activity in the preparation presented in this thesis.

In contrast to the excitatory effect on HMNs, SLN activation inhibits the phrenic neurogram and this inhibition outlasts the period of stimulation in adults animals (Lawson, 1981; 1982; Jiang et al., 1991; Sutton et al., 1978). This was also observed

in the present study when phrenic nerve was also recorded. The respiratory-related activity of HMNs was slowed in response to laryngeal stimulation. In adult, these changes in the hypoglossal discharge pattern were often independent of events in phrenic nerve (Jiang et al., 1991; Withington-wray et al. 1988). The results of the present study demonstrated that the inhibitory effect on phrenic nerve is present in neonatal kittens.

4.11 A physiologically stable in vivo preparation

In recent years a number of researchers have used *in vitro* brain slice or brain stem preparations to record HMNs. However, the aim of the present study was to record these motoneurones in an *in vivo* preparation with the peripheral chemoreceptors and cardiovascular responses still intact. Our laboratory is the first to recorded such neurones systematically in neonatal animals *in vivo*. The first aim of this thesis was therefore to establish a physiologically stable preparation which allowed these motoneurones to be recorded.

4.11.1 Surgery and body temperature

The kittens had noticeably less chance of survival the longer they were maintained on the halothane anaesthetic and it was crucial that surgery during this stage was completed as quickly as possible. The longer the surgery continued the lower the temperature became, particularly in the younger kittens. In these animals the temperature could only be maintained by wrapping them completely in the homeothermic blanket between recordings.

4.11.2 Blood gas analysis

Blood gas analysis was extremely important. By assessing the gas tension, metabolic and respiratory acidosis could be detected and either the ventilator adjusted or sodium bicarbonate administered. Maintaining the blood volume is also very important.

Because of the small blood volume, it is easier to cause anaemia and metabolic acidosis. Thus, the number of blood samples taken was strictly limited. In this case, blood samples could not be taken after every recording if the experiment had more than ten recordings. In these circumstance to maintain volume, 2 mls dextrose was given i.v. The respiratory gas monitor used (Ohmeda 5250) was specifically designed for human babies in intensive care and not for small animal research. Nevertheless, in spite of the small size of the kittens, the respiratory gas monitor, gave readings which were comparable with arterial blood gas analysis and therefore provided a good indication of gaseous exchange.

4.11.3 Prevention of metabolic acidosis

Mild acidosis (pH=6.9-7.3) can cause a reversible depression of the neuronal activity in adult rat brain slices (Tombaugh, 1994). The study in this laboratory previously found that, most of the kittens were susceptible to non-respiratory acidosis under chloralose anaesthesia. A previous study also reported that kittens of less than 3 weeks old were more susceptible to acidosis than the kittens older than 4 weeks (Schwieler, 1968). It was suggested that this was due to anaesthesia. In the present study, anaesthesia could not be avoided. Decerebration was not attempted as further surgery with possible blood loss was considered to be detrimental to the physiological state and survival of the kittens at such a young age. In the present study, sodium bicarbonate with glucose was continually given as soon as possible after venous cannulation through a perfusion pump. Therefore, the pH level was much higher than in the previous studies in our laboratory.

4.11.4 The stability of the brain stem

The pulsation of the cerebellum and brainstem was the biggest problem encountered in keeping stable recordings. As described in the method section four procedures were employed to overcome this: 1) the use of a pressure foot, 2) paralysing the animal, 3) performing a bilateral thoracotomy and 4) continuous perfusion with sodium

bicarbonate to prevent acidosis.

To prevent the lungs collapsing after the bilateral thoracotomy, a positive end-expiratory pressure was applied. The lungs in the younger kittens are less compliant than those of older animals and, contrary to what may be expected from their size, the tracheal pressure and positive end-expiratory pressure were set to the same pressures as those used for older animals.

4.12 Factors which may have influenced the activity of the HMNs and their response to hypoxia

It is impossible in these *in vivo* preparations to maintain the conditions constant between animals. Furthermore, the condition in the same preparation may alter over time. In this section, several factors have been considered which may have altered the activity of the HMNs during normoxia and this may influence the response of HMNs to hypoxia.

4.12.1 Anaesthesia

A number of studies have shown that anaesthesia depresses the activity of the hypoglossal nerve (section 1.3.6.a) and this may explain the low levels of discharge which were recorded from these motoneurones during normoxia.

Does anaesthesia influence the response of the HMNs to hypoxia?

I cannot at present define the role played by anaesthesia in my experiments. As anaesthesia is known to depress the activity of the GG muscle and hypoglossal nerve, the obvious question is: does it alter the response of the HMNs to hypoxia? Hwang and colleagues (Hwang et al., 1983b) have shown that 0.5% halothane in decerebrated adult cats decreases the response of the hypoglossal nerve to hypoxia. In this case, it is likely that the increase in discharge frequency recorded in some of the HMNs in the

present study, would have been greater if the kittens had not been anaesthetized. However, the present study used halothane for only 30 minutes and was switched off at least 3 hours before the recording. The depression caused from halothane anaesthesia does not seem to be so severe since in our experiment, hypoglossal respiratory-related activity was obvious in most recordings in the present study. Nevertheless, the long term effect of halothane on these motoneurones is not known. Furthermore, the influence of α-chloralose and the paralysing agent, gallamine triethiodide, on the response of the individual HMNs to hypoxia is uncertain, and studies are required therefore to investigate this further.

In addition to the above, ketamine suppresses the increased respiratory drive during hypoxia (Hirshman et al., 1975) and inhibits carotic body respiratory stimulation by blocking central synaptic transmission, which is necessary for integration of the chemoreceptor reflex (Timms, 1982). In other kittens (4 weeks old) the increase in ventilation and phrenic nerve discharge during hypoxia (PaO_2 35-45mmHg) were sustained in kittens anaesthetized with α-chloralose after initial induction with halothane (Morray, Noble & Hanson, 1991; Morray et al., 1992). However, when 1% halothane was added to the inhaled gas mixture the increase in ventilation and phrenic nerve discharge was not sustained. In this case then, the response resembled the "biphasic" response recorded in young neonates. The mechanism by which anaesthesia alters the ventilatory response to hypoxia remains unclear. One possibility is that the halothane decreases the sensitivity of peripheral chemoreceptors (Knill & Gelb, 1982).

Notwithstanding these consideration, we do not think that the level of anaesthesia was responsible for the different characteristic response in this study. The excitatory, transient excitatory and inhibitory effects could be recorded from the same preparation and in no particular order in time. For example, in one preparation, there were one excitatory , two transient excitatory, three inhibitory effects and one showed no effect on discharge frequency recorded from HMNs.

4.12.2 Influence of the end-tidal CO_2 on the discharge frequency of the HMNs during hypoxia

It is tempting to speculate that the decrease in respiratory-related EPSPs is the result of chemoreceptor activation. In this study inspired CO_2 was not controlled. Although during each test the $P_{ET}CO_2$ was constant during normoxia, it did vary between different tests. A small but statistically significant ($P<0.0001$) reduction of $PaCO_2$ was found during hypoxia in the recordings. This is similar to the result showed in Blanco's experiment on kittens (Blanco et al., 1984). In the extracellular recordings, due to the small number of HMNs recorded, it is difficult to compare the relationship between excitatory, transient excitatory and inhibitory group. However, in the intracellular recordings, at least there was no significant correlation between the level of $PaCO_2$ during normoxia and the membrane potential during hypoxia. Nor was a correlation between the change in laryngeal-evoked potentials and $PaCO_2$.

Decreased $PaCO_2$ in response to hypoxia was common in the present study. This may be because hypoxia causes constriction in pulmonary arteries and a decrease the lung compliance and is followed by a hypoventilation (Morrell et al., 1994; Weir & Archer, 1995).

As described in the introduction, the discharge of the hypoglossal nerve is usually absent at lower levels of chemical drive, but once the level of CO_2 increases, and the CO_2 threshold reached, the discharge frequency of the hypoglossal nerve rapidly increases. Single hypoglossal nerve fibres differ from each other in their sensitivity to CO_2 and the threshold required to induce respiratory-related activity (Hwang et al., 1983a; Mitra & Cherniack, 1983). In the present study, the change in $PaCO_2$ was relatively small and may not have been high enough to reach the threshold of the activation of the majority of HMNs. This would explain why the level of discharge frequency of these motoneurones during normoxia was relatively lower (<2 impulses per second).

4.12.3 Influence of the end-tidal CO_2 on the response and discharge frequency of the HMNs during hypoxia

It is possible that the response of the individual motoneurones to hypoxia may be influenced by the level of CO_2 changes. This can only be established by holding the motoneurones for a long enough period of time to be able to systematically alter the level of $P_{ET}CO_2$ during the hypoxia. The result in this thesis show that most animals reduced end-tidal CO_2 during the short period of mild hypoxia. Haddad and colleagues suggest that the $PaCO_2$ decreases as a result of a decrease in metabolic rate during hypoxia (Haddad et al., 1982, 1984).

Further studies are required to investigate the effects of altering $PaCO_2$ level on the discharge frequency and the membrane potential of the individual HMNs, and to determine the effect that this has on their response to hypoxia.

4.12.4 Category of motoneurone recorded

The different HMN responses to hypoxia may reflect different HMN pools. The hypoglossal nerve innervates a number of upper airway muscles, including the hypoglossus and styloglossus and the different responses to hypoxia may therefore reflect the different muscles which the individual motoneurones innervate. Although all of the motoneurones were recorded at the level of the obex and 0.5mm rostral to it, and all were between 1.1 and 1.4 mm from the surface of the medulla, we can not rule out this possibility. Further studies, involving a combination of labelling and recording techniques, are required to determine which muscles the specific motoneurones innervate.

Although the medial branch of the hypoglossal nerve carries fibres to a number of muscles, stimulating this part of the nerve instead of the whole nerve increases the probability that the motoneurones innervate the genioglossus muscle. Therefore, in future experiments which investigate the effect of hypoxia on the control of the GG

muscle, it would be preferable to antidromically stimulate this branch of the nerve.

4.12.5 Blood pressure recordings

4.12.5.a Blood pressure change in hypoxia

It may be considered that the changes in HMNs output recorded in the present study on the result of changes in blood pressure. Both the mean arterial blood pressure and the changes during hypoxia were comparable to those previously recorded in anaesthetized neonatal kittens (Morray et al, 1992). The mean blood pressure was 59 ± 9 mmHg recorded in 4 weeks old kittens. During hypoxia (PaO_2 40-50 mmHg), the blood pressure increased to 82 ±2 mmHg. This preparation was similar to that used in the present study in that the kittens were initially anaesthetized with halothane followed by α-chloralose. Therefore, the levels of hypoxia used were also the similar.

Further, respiratory-related hypoglossal activity was inhibited during baroreceptor stimulation while tonic hypoglossal activity was unchanged (Salamone et al., 1983; Wasicko et al., 1993). However, these responses were not influenced by bilateral vagotomy in adult cats (Salamone et al., 1983). Also, HMNs had a hyperpolarization and a decreased discharge frequency in response to arterial blood pressure increase in adult cats (Mifflin, 1990). In human subject, GG activity decreased even with blood pressure raised 15-25 mmHg above the baseline (Garpestad et al., 1990).

In the present study, most recordings showed a decreased blood pressure in response to hypoxia. In this case, it would be expected that the respiratory-related hypoglossal activity would increase during the hypoxic period because the fall in blood pressure would reduce any inhibitory influence it may exert. But, in most cases, HMN activity was reduced or only transiently increased.

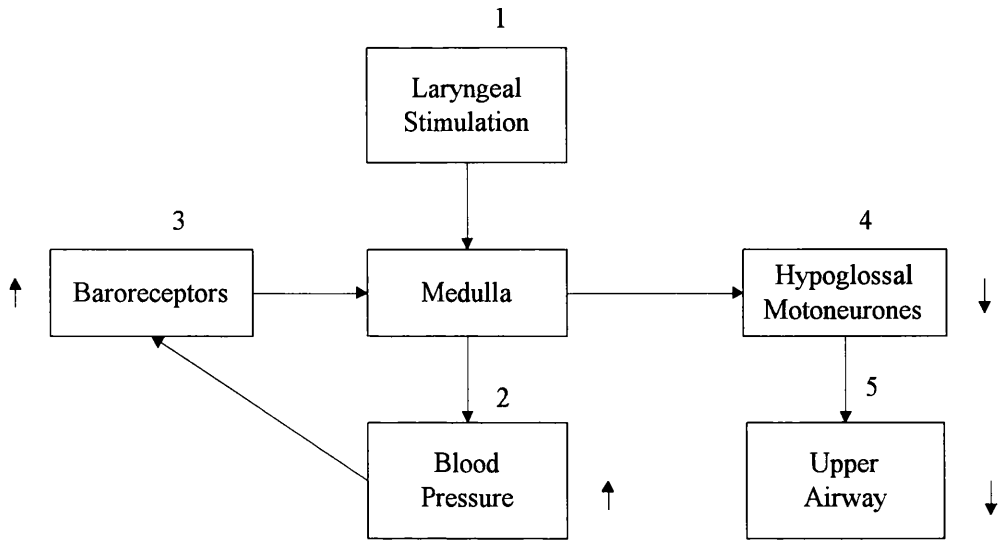


Figure 48. Sequence of the possible pathways by which laryngeal stimulation increases blood pressure and inhibits upper airway stability.

However, because there is no clear correlation in time between the response of HMNs and a fall in arterial blood pressure during hypoxia, there is no evidence for a direct causative link between the two phenomena. Also, the responses recorded was not different in those few cases when blood pressure increased. It is unlikely therefore that the changes could be attributed entirely to altered blood pressure. However, an influence of blood can not be entirely ruled out because in intracellular recordings most HMNs were depolarized. This is exactly what in expected it the fall in present removed an inhibition on the HMNs.

Respiratory-related activity was much evident in the vagotomized than those in the vagal-intact kittens in a previous study (Smith, 1993). This may in part be due to a removal of baroreceptor input. Although the carotid baroreceptor input remains intact. Also, more HMNs were inhibited during hypoxia.

4.12.5.b Effect of blood pressure on laryngeal-evoked response

In the present study, there was an increase in blood pressure during laryngeal stimulation. This also occurred in infant monkeys with SLN stimulation (Sutton et al., 1978). In this case the animals, anaesthetized with ketamine-pentobarbital, lost their cardiovascular responses to SLN stimulation after vagotomy (Sutton et al., 1978).

In the present study in mid-cervical vagotomized kittens, there was an increase in arterial blood pressure in response to laryngeal stimulation. This may be the reason why the tonic activity of HMNs was lower compared to the result in the previous study in this laboratory (Smith, 1993). This could be tested by SLN section but this has not been done.

4.12.6 Effect of vagotomy on HMNs

As we expected, the respiratory-related hypoglossal activity increased with vagotomy compared to the previous study in this laboratory (Smith, 1993). This is consistent with previous study that hypoglossal activity is absent with intact vagi but appears after bilateral vagotomy (Bruce, 1986).

However, the influence of vagal afferents on the laryngeal responses to hypoxia is uncertain. Vagotomy increased expiratory laryngeal resistance slightly and diminished or reversed the laryngeal responses to hypercapnia and hypoxia, suggesting an appreciable influence of vagal afferents on these responses in intact animals (Dixon et al., 1974). In unilateral vagotomized cats, section of the remaining vagus caudal to the origin of recurrent laryngeal nerve resulted in an increase in laryngeal muscle to hypercapnia (Sherry & Megirian, 1975). However, the hypoglossal excitatory response to intra-laryngeal CO_2 are most clearly demonstrable after bilateral vagotomy (Bartlett Jr. et al., 1992). Also vagotomy led to an increase in expiratory laryngeal resistance during hypoxia due to a decrease in expiratory posterior cricoarytenoid muscle (Bartlett Jr., 1980).

In contrast to hypoxia, bilateral vagotomy lowered the respiratory frequency, but did not consistently alter either inspiratory or expiratory laryngeal resistance in a hyperoxic control state (Bartlett Jr., 1980). SLN section caused ventilation to deepen and slow compared to SLN intact newborn kittens (1 to 2 weeks) after vagotomy (Mortola & Rezzonico, 1989). The vagotomy did not consistently alter either inspiratory or expiratory laryngeal resistance in the hyperoxia control state (Dixon et al., 1974).

4.12.7 Influence of artificial movement of the experiment

The animals used in the present study were artificially ventilated. It is always possible that rhythmic changes in potential recorded in HMNs were artefacts cause by movement of the chest or body during ventilation. There are several reasons to support the assumption that this is not the case. Firstly, if this were so it is difficult to see why hypoxia reduced the amplitude and duration of these potentials whilst the membrane itself was depolarized in the majority of cases. Not only that, but when phrenic nerve was recorded together with HMNs these rhythmic potentials were found to be correlated with the respiratory activity of this nerve.

4.13 The clinical relevance and implications of the result of this thesis

These observations of reduced activity of HMNs in response to mild hypoxia take on added importance in light of recent observations of upper airway abnormalities described in SIDS victims. Upper airway muscles, especially the GG muscle, innervated by the hypoglossal nerve, play an equally important role to that of the diaphragm for providing adequate ventilation. For instance, failure to properly activate GG muscle is a prevalent cause of obstructive apnea. The results of this study show that inhibitory mechanisms are activated during mild levels of hypoxia. If the similar inhibition of HMNs occurs in human babies during apnoeic episodes, the patency of the airway may be compromised and the survival of the infant threatened.

Tissue markers of chronic hypoxia were found in many victims of SIDE studied

postmortem (Valdes-Dapena, 1983, 1981; Naeye, 1977, 1976). Hypoxia is a common feature in infants who stop breathing for short periods of time (Henderson-Smart, 1981). Abnormalities of neurologic control of breathing have been reported in babies with apnea (Guilleminault et al., 1975; Shannon et al., 1977; Hunt et al., 1981). Abnormal ventrolateral responses to hypoxia and hypercapnia have also been shown in some babies with apnea (Shannon et al., 1977; Hunt et al., 1981). As noted in the introduction, 90% of babies with apnea failed to arouse from quiet sleep in response to hypoxia comparing to 30% of control infants (McCullonch et al., 1982).

4.13.1 The hypoglossal respiratory-related rhythmic EPSP reduced during hypoxia

It is accepted that, under natural conditions, inspiratory activity of the hypoglossal nerve and GG muscle serve to prevent a relapse of the tongue during inspiration (Remmers et al., 1978; Sauerland & Misuno, 1970). This progressively earlier onset of these activity as ventilatory drive increases and thus, as inspiratory pressure in the oropharynx progressively decreases, would appear necessary in preventing obstruction, especially early in inspiration (Remmers et al., 1978; Sauerland & Mizuno, 1970; Hwang et al., 1983b). Therefore, a reduction of rhythmic hypoglossal activity would be unable to maintain upper airway patency during inspiration by causing relapse and increase airway resistance (Mifflin, 1990).

4.13.2 The laryngeal evoke-EPSP on HMNs reduced during hypoxia

In normal babies, during upper airway occlusion, negative or positive pressure stimulate laryngeal receptor which through laryngeal-hypoglossal reflex could augment hypoglossal nerve discharge in an effort to restore airway patency. If this reflex is compromised during hypoxia then may cause a prolonged apnea and lead to SIDE.

4.14 Proposals for future work

The above discussion documents the direct effect and some factors which influence the effect of hypoxia on HMNs in neonates. In this case, two points remain to be discussed. Generally speaking, the brain is metabolically one of the most active organs in the body and the neuronal energy stores are limited. The integrity of brain function depends on a continuous energy supply. It has been found that HMNs lose their cytosolic K^+, which substantially contributes to the increase in K^+ out of the cell. The loss of neuronal intracellular K^+ is mediated to a large degree by the activation of ATP-sensitive K^+ channels in hypoglossal motoneurones in adult cat brain slices during anoxic conditions *in vitro*. Research on ionic balance during anoxia has suggested that K^+ is much better maintained in neonatal rats.

Quite a few studies have found that rhythmic respiratory drive potentials involve synaptic excitation mediated by glutamate (Denavit-Saubié et al., 1978; Feldman et al., 1992) and chloride-dependent synaptic inhibition (Richter et al., 1979; Ballantyne & Richter, 1986) involving GABA or glycine as transmitters (Champagnat et al., 1982; Haji et al., 1990). Also, a $GABA_B$ receptor-mediated inhibition acting via potassium currents (Lalley, 1986; Pierrefiche et al., 1993). Further, neuromodulation seems to occur through both cholinergic (Morin-Surun et al., 1984; Böhmer et al., 1989) and monoaminergic mechanisms (Champagnat et al., 1979).

It has been found that membrane potential changes, not only affect, but also critically depend on transmitter-controlled and voltage-regulated ion conductances (Champagnat & Richter, 1994; Mifflin et al., 1985; Richter et al., 1987). In this process, non-inactivating potassium conductances seem to be of essential importance (Richter et al., 1993). The potassium conductance exerts inhibitory functions at different periods of the respiratory cycle in brainstem expiratory neurones (Champagnat & Richter, 1994). In an *in vitro* study, total block of Na^+-K^+ ATPase increased the spontaneous K^+ loss but did not abolish the additional anoxia-induced increase in K^+ (Jiang & Haddad, 1991). Whether the K^+ leak from neurones happens during mild hypoxia in neonates

is still not known. Is it possible there is a Na^+-K^+. ATPase effect in neonates *in vivo* as in adults *in vivo*? Although a considerable amount of central and peripheral influence of this ATP-sensitive K^+ should not be ruled out.

As a start to determine potassium activity in response to hypoxia, intracellular recording could be made. Recordings could be made during normoxia and mild levels of hypoxia in kittens in *vivo*. The potassium current blockers calcium and tetraethylammonium could be then injected intracellularly by ionophoretic current into hypoglossal motoneurones.

4.15 Conclusion

In this thesis, the effects of mild levels of hypoxaemia on hypoglossal motoneurones have been studied in an *in vivo* preparation of neonatal kittens. The major findings of this study are:

1) In extracellular recordings, three types of response were recorded during hypoxia: sustained increase in discharge frequency, a transient increase in discharge frequency and inhibition. Most neurones showed either transient excitation or inhibition. These are similar to the results in the previous study of vagi-intact kittens in this laboratory.

2) Hypoglossal motoneurones were depolarized during hypoxia.

3) There is a reduction of membrane excitability during hypoxia. There is also an increase of membrane impedance.

2) and 3) are similar to the effects of hypoxia on hypoglossal motoneurones *in vitro*.

4) Respiratory-related hypoglossal activity decreased during hypoxia.

5) The EPSP-IPSP components of laryngeal-evoked potentials were reduced during hypoxia.

A major conclusion of this thesis is that many of the changes in hypoglossal motoneurone output during hypoxia can be explained by changes in membrane properties of hypoglossal motoneurones. These changes lead to an increase in input impedance accompanied by depolarization of the membrane and a reduced excitability. Such changes would certainly explain the transient nature of increased discharge frequency recorded in the hypoglossal system. However, it is clear that hypoxia is having other effects on hypoglossal motoneurone output. In particular through an action on respiratory-related activity which is also reduced. The hypoxia has effects on both respiratory-related and non-respiratory-related activity in hypoglossal motoneurones. In particular it has been shown in this thesis that hypoxaemia has a profound influence on laryngeal-evoked potentials.

CHAPTER 5
REFERENCES

ACHER, H., LUBBERS, D.W., PURVES, M.H., TAN, E.D. (1980). Measurements of the partial pressure of oxygen in the carotid body of fetal sheep and newborn lambs. *J. Dev. Physiol.* **2:** 323-238.

ACKLAND, G.L., WAITES, B.A., NOBLE, R. AND HANSON, M.A. (1995). Bilateral lesions in the red nucleus abolish the biphasic respiratory response to hypoxia in decerebrate newborn rabbits. *J. Physiol.* **483:** 89P.

AMOROSO, S., SCHMID-ANTOMARCHI, H., FOSSET, M. AND LAZDUNSKI, M. (1990). Glucose, antidiabetic sulphonylureas and neurotransmitter release. Role of ATP-sensitive K^+ channels. *Science Wash. DC* **247:** 852-854.

AMRI, M., LAMKADEM, M. AND CAR, A. (1991). Effects of lingual nerve and chewing cortex stimulation upon activity of the swallowing neurons located in the region of the hypoglossal motor nucleus. *Brain Res.* **548(1-2):** 149-155.

AMRI,M. AND CAR,A. (1988). Projection from the medullary swallowing centre to the hypoglossal motor nucleus: a neuroanatomical and electrophysiological study in sheep. *Brain Res.* **441**: 119-126.

ANCH, A.M., REMMERS, J.E., SAUERIAND, E.K., DE GROOT, W.J. (1981). Oropharyngeal patency during waking and sleep in the pickwickian syndrome: electromyographic activity of the tensor veli palatini. *Electromyogr. Clin. Neurophysiol.* **21:** 317-330.

ANDREW, B.L. (1956). A functional analysis of the myelanated fibres of the superior laryngeal nerve of the rat. *J. Physiol. (London)***133**: 420-432.

ANGELL-JAMES, J.E., DALY, M. DE. B. (1975). Some aspects of upper respiratory tract reflexes. *Acta Oto-Laryngol.* **79:** 242-252.

ARIAGNO, R., NAGEL, L. AND GUILLEMINAULT, C. (1980). Waking and ventilatory responses during sleep in infants near-miss for sudden infant death syndrome. *Sleep* **3:** 351.

ARITA, H., KOGO, N. AND ICHIKAWA, K. (1988). Locations of medullary neurons with non-phasic discharges excited by stimulation of central and/or peripheral chemoreceptors and by activation of nociceptors in cat. *Brain Res.* **442:** 1-10.

ASHFORD, M.L.J., STURGESS, N.C., TROUT, N.J., GARDNER, N.J., AND HALES, C.N. (1988). Adenosine-5-triphosphate-sensitive ion channels in neonatal rat cultured central neurons. *Pfluegers Arch.* **412:** 297-304.

ASTRUP, J., SKOVSTED, P., GJERRIS, F. AND RAHBEK-SORENSEN, H. (1981). Increase in extracellular potassium in the brain during circulatory arrest: effects of hypothermia, lidocaine, and thiopental. *Anaesthesiology,* **55:** 256-262.

BAIRAM, A., HANNHART, B., CHONE, C. AND MARCHAL, F. (1993). Effects of dopamine on the carotid chemosensory response to hypoxia in newborn kittens. *Respir. Physiol.* **94:** 297-307.

BAKER. R.L. AND McGINTY, D.J. (1979). Sleep apnea in hypoxia and normal kittens. *Dev. Psychobiol.* **12(6):** 577-594.

BALLANTYNE, D. AND RICHTER, D.W. (1986). The non-uniform character of expiratory synaptic activity in expiratory bulbospinal neurones of the cat. *J. Physiol.* **370:** 433-456.

BARTLETT, D. Jr. AND KNUTH, S.L. (1994). Influence of hypoxia on ventilatory responses to intra-laryngeal CO_2 in cats. *Respir. Physiol.* **96(1):** 61-69.

BARTLETT, B., KNUTH,S.L. AND LEITER,J.C. (1992). Alteration of ventilatory

activity by intra-laryngeal CO_2 in cat. *J. Physiol.* **457:** 177-185.

BARTLETT, D. Jr. (1990). Control and action of the genioglossus muscle. (Review). *Prog. Clin. Biol. Res.* **345:** 99-107.

BARTLETT, D. Jr. AND ST. JOHN, W. M. (1988). Influence of lung volume on phrenic nerve activities. *Respir. Physiol.* **73:** 97-110.

BARTLETT. D. Jr., KNUTH, S.L. AND WARD, D.K. (1987). Influence of extreme hypercapnia on respiratory motor nerve activity in cats. *Respir. Physiol.* **70:** 173-181.

BARTLETT.D. Jr. (1980). Effects of vagal afferent on laryngeal responses to hypercapnia and hypoxia. *Respir. Physiol.* **42:** 189-198.

BARTLETT, D. Jr. (1979). Effect of hypercapnia and hypoxia on laryngeal resistance to airflow. *Respir. Physiol.* **37(3):** 293-302.

BARTLETT, D. JR., REMMERS, J.E. AND GAUTIER, H. (1973). Laryngeal regulation of respiratory airflow. *Respir. Physiol.* **18(2):** 194-204.

BASSAL, M. AND BIANCHI, A.L. (1982). Inspiratory onset or termination induced by electrical stimulation of the brain. *Respir. Physiol.* **50(1):** 23-40.

BEN ARI, Y. (1989). Effect of glibenclamide, a selective blocker of an ATP-K^+ channel, on the anoxic response of hypocampal neurons. *Pfluegers Arch.* **414:** S11-114.

BERMAN, A.L. (1968). The brain stein of the cat. A cytoarchitectronic atlas with stereotaxic coordinates. 79 pp. Univ. of Wisconsin Press, Madison.

BERTHON-JONES, M. AND SULLIVAN, C.E. (1982). Ventilatory and arousal

responses to hypoxia in sleeping humans. *Am. Rev. Respir. Dis.* **126:** 632-639.

BIANCONI, R. AND RACHI, F. (1964). Respiratory control of motoneurones of the recurrent laryngeal nerve and hypocapnic apnoea. *Arch. Ital. Biol.* **102:** 56-73.

BISCOE, T.J. AND SAMPSON, S.R. (1970). Responses of cells in the cat to stimulation of the sinus, glossopharyngeal, aortic and superior laryngeal nerves. *J. Physiol.* **209pp:** 359-373.

BLANCO, E.B. (1994). Peripheral control of breathing. In: Fetus and Neonate Physiology and Clinical Applications. Vol. 2. Breathing. pp. 85-108. Edited by M. A. Hanson, J. A. D. Spencer, C. H. Rodeck and D. Walters. pp, 85-108. Printed in Great Britain at University Press, Cambridge.

BLANCO ,C.E., HANSON, M.A., JOHNSON, P. AND RIGATLO,H. (1984). Breathing pattern of kittens during hypoxia. *J. Appl. Physiol.* **56(1):** 12-17.

BLANCO, C.E., DAWES, G.S., WALKER, D.W. (1983). Effect of hypoxia in conscious newborn lambs before and after denervation of the carotid bifurcation. *J. Physiol.* **339:** 467-474.

BLANCO, C.E., DAWES, G.S., HANSON, M.A., McCOOKE, H.B. (1982). The arterial chemoreceptors in fetal sheep and newborn lambs. *J. Physiol.* **330:** 38P.

BLANK, W.F. AND KIRSCHINER, H.S. (1977). The kinetics of extracellular potassium changes during hypoxia and anoxia in the cat cerebral cortex. *Brain Res.* **123:** 113-124.

BODDY, K., AWES,G. S., FISHER, R., PINTER, S. AND ROBINSON, J.S. (1974). Foetal respiratory movements, electrocortical and cardiovascular responses to hypoxemia and hypercapnia in sheep. *J. Physiol.* **243**: 599-618.

BODDY, K. AND DAWES, G.S. (1975). Fetal breathing. *Br. Med. Bull.* **31:** 3-7.

BODDY, K. AND ROBINSON, J.S. (1981). External method for detection of fetal breathing in utero. *Lancet,* **2:** 1231-1233.

BOGGS, D.F. AND BARTLETT, D. Jr. (1982). Chemical specificity of a laryngeal apneic reflex in puppies. *J. Appl. Physiol.* **53(2):** 455-462.

BÖHMER, G., SCHMID, K. AND BAUMANN, M. (1989). Evidence for a respiration-modulated cholinergic action on the activity of medullary respiration-related neurons in the rabbit. An iontophoretic study. *Pflügers Archiv* **415:** 72-80.

BOLTON, D.P.G., AND HERMAN, S. (1974). Ventilation and sleep state in the newborn. *J. Physiol.* **240:** 67-77.

BONORA, M. AND BOULÉ, M. (1994). Breathing during sleep in fetus and newborn. In: Fetus and Neonate Physiology and Clinical Applications. Vol. 2. Breathing. pp. 153-191. Edited by M.A. Hanson, J.A.D. Spencer, C.H. Rodeck and D. Walters. Printed in Great Britain at University Press, Cambridge.

BONORA, M., ST. JOHN, W.M. AND BLEDSOE, T.A. (1985). Deferential elevation by protriptyline and depression by diazepam of upper airway respiratory motor activity. *Am. Rev. Respir. Dis.* **131:** 41-45.

BONORA, M., MARLOTM, D., GAUTIER, H., AND DURON, B. (1984). Effects of hypoxia on ventilation during postnatal development in conscious kittens. *J. Appl. Physiol.* **56:** 1464-1471.

BOSMA, J.F. (1975). Introduction to the Symposium. In Development of Upper Respiratory Anatomy and Function. Edited by J.F. Bosma and J. Showacre, pp. 5-49. Washington: US Dept. Health, Educ. Welfare.

BOUSHEY, H.A., RICHARDSON, P.S., WIDDICOMBE, J.G. AND WISE, J.C.M. (1974). The response of laryngeal afferent fibres to mechanical and chemical stimuli. *J. Physiol.* **240:** 153-175.

BOUSHEY, H.A., RICHARDSON, P.S. AND WIDDICOMBE, J.G. (1972). Reflex effects of laryngeal irritation on the pattern of breathing and total lung resistance. *J. Physiol.* **224:** 501-513.

BOWES, G., WILKINSON, M.H. DOWLING, M., RITHCHIE, B.C, BRODECKY, V. AND MALONEY, J.E. (1981). Hypercapnic stimulation of respiratory activity in unanesthetized fetal sheep in utero. *J. Appl. Physiol.* **50:** 693-700.

BRADLEY, G.W. (1976). The effect of CO_2, body temperature and anaesthesia on the response to vagal stimulation. In: Respiratory Centres and Afferent Systems. Edited by B. Duron. Paris. Inerm. pp, 139-154.

BRADY, J.P., ARIAGNO, R.L., WATTS, J.L., GOLDMAN, S.L. AND DUMPIT, F.M. (1978). Apnea, hypoxemia, and aborted sudden infant death syndrome. *Pediatrics,* **62:** 686.

BRADY, J.P. AND DUNN, P.M. (1970). Chemoreceptor reflexes in the newborn infant: effect of CO_2 on the ventilatory response to hypoxia. *Pediatrics.* **45:** 206-215.

BRADY, J.P., CERUTI, E. (1966). Chemoreceptor reflexes in the newborn infant: Effect of varying degree of hypoxia on heart rate and ventilation in a warm environment. *J. Physiol.* **184:** 631-645.

BRICE, A.G., FORSTER, H.V., PAN, L.G., BROWN, D.R., FORSTER, A.L. AND LOWRY, T.F. (1991). Effect of cardiac denervation on cardiorespiratory responses to exercise in goats. *J. Appl. Physiol.* **70:** 1113-1120.

BROUILLETTE, R.T. AND THACH, B.T. (1980). Control of genioglossus muscle inspiratory activity. *J. Appl. Physiol.* **49(5)**: 801-808.

BROUILETTE, R.T. AND THACH, B.T. (1979). A neuromuscular mechanism maintaining extrathoracic airway patency. *J. Appl. Physiol.* **46:** 772-779.

BROWN, D.L. AND LAWSON, E.E. (1988). Brain stem extracellular fluid pH and respiratory drive during hypoxia in newborn pigs. *J. Appl. Physiol.* **64:** 1055-1059.

BRUCE, E.N. (1986). Hypoglossal and phrenic nerve responses to chemical stimuli in kittens. In: Neurobiology of the control of breathing. Edited by von Euler, C. and Lagererant, Z. New York. Raven Pross.

BRUCE, E.N., MITRA, J. AND CHERNIACK, N.S. (1982). Central and peripheral chemoreceptor inputs to phrenic and hypoglossal motoneurones. *J. Appl. Physiol.* **53(6):** 1504-1511.

BRUNNER, M.J., SUSSMAN, M.S., GREENE, A.S., CULMEN, C.H. AND SHOUKAS, A.A. (1982). Carotid sinus baroreceptor reflex control of respiration. *Circ. Res.* **51:** 624-636.

BUDZINSKA, K., von EULER, C., KAO, F.F., PANTATEO, T. AND YAMAMOTO, Y. (1985). Effect of graded focal cold block in rostral areas of the medulla. *Acta Physiol. Scan.* 124: 329-340.

BUREAU, M.A. AND BEGIN, R. (1982). Biphasic ventilatory response to hypoxia in newborn lams: evidence against the theory of central depression. *Am. Rev. Respir. Dis.* **125:** 186.

CALDER, N.A., KUMAR, P. AND HANSON, M.A. (1995). Maturation of carotid chemoreceptor responses to CO_2 in the anaesthetized newborn lamb. *J. Physiol.* **489P:**

162.

CARLO, W.A. AND DIFIORE, J.M. (1990). Respiratory muscle responses to changes in chemoreceptor drive in infants. *J. Appl. Physiol.* **68(3):** 1041-1047.

CARLO, W.A., MARTIN, R.J. AND DIFIOR, J.M. (1988). Differences in CO_2 threshold of respiratory muscle in preterm infants. *J. Appl. Physiol.* **65(6):** 2434-2439.

CARLO, W.A., MILLER, M.J. AND MARTIN, R.J. (1985). Differential response of respiratory muscles to airway occlusion in infants. *J. Physiol.* **59(3):** 847-852.

CARROLL, J.L., BAMFORD, O.S. AND FITZGERALD, R.S. (1993). Postnatal maturation of carotid chemoreceptor responses to O_2 and CO_2 in the cat. *J. Appl. Physiol.***75(6):** 2383-2391.

CHAMPAGNAT, J. AND RICHTER, D.W. (1994). The roles of K^+ conductance in expiratory pattern generation in anaesthetized cats. *J. Physiol.* **479(1):** 127-138.

CHAMPAGNAT, J., DENAVIT-SAUBIE, M., MOYANOVA, S. AND RONDUIN, G. (1982). Involvement of amino acids in periodic inhibitions of bulbar respiratory neurones. *Brain Res.* **327:** 351-365.

CHAMPAGNAT, J., DENAVIT-SAURIÉ, M., HENRY, J.L. and LEVIEL. V. (1979). Catecholaminergic depressant effects on bulbar respiratory mechanisms. *Brain Res.* **160:** 57-68.

CHERNIACK, N.S., HAXHIU, M.A., MITRA, J., STOHL, K. AND VAN LUNTEREN, E. (1984). Responses of upper airway, intercostal and diaphragm muscle activity to stimulation of oesophageal afferents in dogs. *J. Physiol.* **349:** 15-25.

CHERNIACK, N.S., VON EULER, C., HOMMA, I. AND KAO, F.F. (1979).

Graded changes in central chemoreceptor input by local temperature changes on the ventral surface of medulla. *J. Physiol. Lond.* **287:** 191-211.

CHERUBINI, E., BEN-ARI, Y. AND KRNJEVIC, K. (1989). Anoxia produces smaller changes in synaptic transmission, membrane potential, and input resistance in immature rat hippocampus. *J. Neurophysiol.* **62:** 882-895.

COATES, E. L., LI, A. AND NATTIE, E.E. (1993). Widespread sites of brain stem ventilatory chemoreceptors. *J. Appl. Physiol.* **75:** 5-14.

COHEN, M.I. (1979). Neurogenesis of respiratory rhythm in the mammal. *Physiol. Rev.* **59:** 1105-1173.

COHEN, G. AND HENDERSON-SMART, D.J. (1989). Upper airway muscle activity during nasal occlusion in newborn babies. *J. Appl. Physiol.* **66(3):** 1328-1335.

COLES, S.K., KUMAR, P AND NOBLE, R. (1989). Pontine sites inhibiting breathing in anaesthetized neonatal lams. *J. Physiol.* **409:** 66P.

CONNELLY, C.A., ELLENBERGER, H.H. AND FELDMAN, J.L. (1990). Respiratory activity in retrotrapezoid nucleus in cat. *Am. J. Physiol.* **258:** L33-L44.

COOPER, M.H., FRITZ, H.P. AND HAUCK, A.T. (1981). Input of the rat hypoglossal nucleus from the reticular formation. *Anat. Rec.* **199:** 58A.

COSGROVE, J.F., NEUNBURGER, N., BRYAN, M.H., BRYAN, A.C., AND LEVINSON, H. (1975). A new method of evaluating the chemosensitivity of the respiratory center in children. *Pediatr.* **56:** 972-980.

COTE, A., YUNIS, K., BLANCHARD, P.W., MORTOLA, J.P. AND BUREAU, M.A. (1988). Dynamics of breathing in the hypoxic awake lamb. *J. Appl. Physiol.*

64(1): 354-359.

COTTON, E.K. AND GRUSNTEIN, M.M. (1980). Effects of hypoxia on respiratory control in neonates at high altitude. *J. Appl. Physiol.* **48:** 487-495.

COZINE, R.A. AND NGAI, S.H. (1967). Medullary surface chemoreceptors and regulation of respiration in the cat. *J. Appl. Physiol.* **22:** 117-121.

CROWELL, J.W. AND KAUFMANN, B.N. (1961). Changes in tissue pH after cardiac arrest. *Am. J. Physiol.* **200:** 743-745.

DALY, M. DE. B., ANGELL-JAMES, J.E. AND ELSNER, R. (1979) Role of carotid-body chemoreceptors and their reflex interactions in bradycardia and cardiac arrest. *Lancet,* **1:** 764-767.

DAULT, S.H. AND SMITH, R.D. (1969). A quantitative study of the nucleus of the mesencephalic tract of the trigeminal nerve of the cat. *Anat. Rec.* **165:** 79-88.

DAVIS, P.J. AND NAIL, B.S. (1987). Quantitative analysis of laryngeal mechanosensitivity in the cat and rabbit. *J. Physiol.* **388:** 467-485.

DAVIS, P.J., MACEFIELD, G. AND NAIL, B.S. (1986). Respiratory muscle activity during asphyxic apnoea and opisthotonus in the rabbits. *Respir. Physiol.* **65(3):** 285-294.

DAWES, G.S., FOX, H.E., LEDUC, B.M., LIGGINS, G.C. AND RICHARDS, R.T. (1972). Respiratory movements and rapid eye movement sleep in the foetal lamb. *J. Physiol.* **220:** 119-143.

DECKER, M.J., HAAGA, J., ARNOLD, J.L., ATZBERGER, D. AND STROHL. K.P. (1993). Functional electrical stimulation and respiration during sleep. *J. Appl.*

Physiol. **75(3):** 1053-1061.

DENAVIT-SAUBIÉ, M., CHAMPAGNAT, J. AND ZIEGLGÄNSBERGER, W. (1978). Effects of opiates and methionine-enkephalin on pontine and bulbar respiratory neurones of the cat. *Brain Res.* **155:** 55-67.

DIXON, M., SZEREDA-PRZESTASZEWDKA, M., WIDDICOMBE, J.G. AND WISE, J.C.M. (1974). Studies on laryngeal calibre during stimulation of peripheral and central chemoreceptors, pneumothorax and increased respiratory loads. *J. Physiol. Lond.* **239:** 347-363.

DOBLE, E.A., LEITER, J.C., KNUTH, S.L., DAUBENSPECK, J.A. AND BARTLETT, D. Jr. (1985). A noninvasive intraoral electromyographic electrode for genioglossus muscle. *J. Appl. Physiol.* **58:** 1378-1382.

DONNELLY, D.F., JIANG, C. AND HADDAD, G.G. (1992). Comparative responses of brain stem and hippocampal neurons to O_2 deprivation: in vitro intracellular studies. *Am. J. Physiol.* **262:** L549-L554.

DONNELLY, D.F. AND HADDAD, G.G. (1986). Respiratory change induced by prolonged laryngeal stimulation in awake piglets. *J. Appl. Physiol.* **61(3):** 1018-1024.

DOWNMAN, C.B. (1939). Afferent fibres of the hypoglossal nerve. *J. Anat.* **73:** 387-395.

DOWNING, S. E. AND LEE, J. C. (1975). Laryngeal chemosensitivity: a possible mechanism for sudden infant death. *Pediatrics,* **55(5):** 640-649.

DOTY, R.W. AND BOSMA, J.F. (1956). An electromyographic analysis of reflex deglutition. *J. Neurophysiol.* **19:** 44-60.

EDELMAN, N.H., CHERNIACK, N.S., LAHIRI, S. AND FISHMAN, A.P. (1966). Response of goats to acute, chronic, and life-long hypoxia. *Fed. Proc.* **28:** 1223-1227.

ELDRIDGE, F.L. AND MILLHORN, D.E. (1986). Oscillation, gating, and memory in the respiratory control system. In A.P. Fishman (Ed), Handbook of Physiology, Section 3, vol, 2 American Physiol. Soc., Bethesda, MD, pp. 93-114.

ENGLAND, S.J. AND BARTLETT, D. Jr. (1982). Changes in respiratory movements of the human vocal cords during hypercapnia. *J. Appl. Physiol.* **52:** 780-785.

ESCANDE, D., THURINGER, D., LEGUERN, S., COURTEIX, J., LAVILLE, M. AND CABERO, I. (1989). Potassium channel openers act through an activation of ATP-sensitive K^+ channels in guinea-pig cardiac myocytes. *Pluegers Arch.* **414:** 669-675.

FAGENHOLZ, S.A., O'CONNELL, K., AND SHANNON, D.C. (1976). Chemoreceptor function and sleep state in apnea. *Pediatrics.* **58:** 31-36.

FEDORKO, L., KELLY, E.N. AND ENGLAND, S.J. (1988). Importance of vagal afferents in determining ventilation in newborn rats. *J. Appl. Physiol.* **65:** 1033-1039.

FEDORKO, L. AND MERRILL, E.G. (1984). Axonal projections from rostral expiratory neurones of the Bötzinger complex to medulla and spinal cord in the cat. *J. Physiol. Lond.* **350:** 487-496.

FELDMAN, J.L., WINDHORST, U., ANDERS, K. AND RICHTER, D.W. (1992). Synaptic interaction between medullary respiratory neurones during apneusis induced by NMDA-receptor blockade in cat. *J. Physiol.* **154:** 179-184.

FINK, B.R., MARTIN, R. AND LAVIGNE, A.B. (1975). Spring mechanics of the human larynx. In: Development of Upper Respiratory Anatomy and Function. pp, 63-

76. Edited by J.F. Bosma et al.

FLEMING,P.J. (1978). Functional immaturity of pulmonary irritant receptors and apnea in newborn preterm infants. *Pediatrics,* **61:** 515-518.

FLORES-GUEVARA, R. (1982). Sleep apneas in normal neonates and infants during the first three months of life. *Neuronpediatrics,* **13:** 21-28.

FORSTER, H.V., OHTAKE, P.J., PAN, L.G., LOWRY T.F., KORDUCKI, M.J., AARON, E.A., AND FORSTER, A.L. (1995). Effects on breathing of ventrolateral medullary cooling in awake goats. *J. Appl. Physiol.* **78(1):** 258-265.

FUKUDA, Y. AND HONDA, Y. (1982). Differences in respiratory neural activities between vagal (superior laryngeal), hypoglossal and phrenic nerves in the anaesthetized rat. *Jap. J. Physiol.* **32:** 387-398.

FUKUDA, Y., SEE, W.R. AND HONDA, Y. (1980). H^+-sensitivity and pattern of discharge of neurons in the chemosensitive areas of the ventral medulla oblongata of rats in vitro. *Pfluegers Arch.* **388:** 53-61.

GABRIEL, M., ALBANI, M. AND SHULTE, F. J. (1976). Apneic spells and sleep state in preterm infants. *Pediatrics,* **57:** 142-147.

GARPESTAD, E., BASNER, R., RINGLER, J., LILLY, J., SCHWARTZSTEIN, R., WEINBERGER, S. AND WEIS, J.W. (1990). Elevation of blood pressure decreases genioglossus activity in awake normals. (Abstract). *Am. Rev. Respir. Dis.* **141:** A669.

GAUDA, E.B., CARROLL, T.P., SCHWARTZ, A.R., SMITH, P.L. AND FITZGERALD, R.S. (1994). Mechano- and chemoreceptor modulation of respiratory muscles in response to upper airway negative pressure. *J. Appl. Physiol.* **76(6):** 2656-

2662.

GAUDA, E.B., CARROLL, J.L., McCOLLEY, S. AND SMITH, P.L. (1991). Effect of oxygenation on breath-by-breath response of the genioglossus muscle during occlusion. *J. Appl. Physiol.* **71(4):** 1231-1236.

GAUDA, E.B., MILLER, M.J., CARLO, W.A., DIFORE, J.M., JOHNSEN, D.C. AND MARTIN, R.J. (1987). Genioglossal response to airway occlusion in apneic versus nomorcapneic infants. *Pediatr. Res.* **22(6):** 683-687.

GERBER, U. AND POLOSA, C. (1979). Some effects of superior laryngeal nerve stimulation on sympathetic neuron firing. *Can. J. Physiol.* **57:** 1073-1081.

GIUSSANI, D.A., MOORE, P.J., BENNET, L., SPENCER, J.A.D. AND HANSON, M.A. (1992). Phentolamine increases the incidence of fetal breathing movements both in normoxia and hypoxia in tern fetal sheep. *J. Physiol.* **452:** 320P.

GLOGOWSKA, M, STRANSKY, A. AND WIDDICOMBE, J.G. (1974). Reflex control of discharge in motor fibres to the larynx. *J. Physiol. Lond.* **239:** 365-379.

GOODCHILD, A.K., DAMPNEY, R.A. AND BANDLER, R. (1982). A method for evoking physiological responses by stimulation of cell bodies, but not axons of passage within localized regions of the central nervous system. *J. Neurosci. Methods,* **6(4):** 351-363.

GREEN, J.D. AND NEGISHI, K. (1963). Membrane potentials in hypoglossal motoneurones. *J. Neurophysiol.* **26:** 835-856.

GRODAARD, J., KREUGER, E., LINDSTROM, D. AND SUDDELL, H. (1986). Effect of carotid body maturation and berbutaline on the laryngeal chemoreflex in newborn lambs. *Pediatr. Res.* **20(8):** 724-729.

GRUNSTEIN, M.M., HAZINSKI, T.A. AND SCHLUETER, M.A. (1981). Respiratory control during hypoxia in newborn rabbits: implied action of endophins. *J. Appl. Physiol.* **51:** 122-130.

GRUNSTEIN, M.M., DERENNE, J.P. AND MILIC-EMILI, J. (1975). Control of depth and frequency of breathing during baroreceptor stimulation in cats. *J. Appl. Physiol.* **39:** 395-404.

GRUNSTEIN, M.M., YOUNES, M. AND MILIC-EMILI, J. (1973). Control of tidal volume and respiratory frequency in anaesthetized cats. *J. Appl. Physiol.* **35:** 463-476.

GUILLEMINAULT, C., PERAITA, R., SOUQUET, R. AND DEMENT, W.C. (1975). Apneas during sleep in infants: possible relationship with sudden infant death syndrome. *Science,* **190:** 677-679.

GUTHRIE, R.D., STANDAERT, T.A., HODSON, W.A. AND WOODRUM, D.E. (1980). Sleep and maturation of eucapnic ventilation and CO_2 sensitivity in the premature primate. *J. Appl. Physiol.* **48:** 347-354.

GUZ, A., NOBLE, M.I.M., EISELE, J.H. AND TRENCHARD, D. (1970). The role of vagal inflation reflexes in man and other animals. In: Breathing: Hering-Breuer Centenary Symposium. Edited by R. Porter. London: Churchill, pp. 17-40.

HADDAD, G.G. AND DONNELLY, D.F. (1990). O_2 deprivation induces a major depolarization in brain stem neurons in the adult but not in the neonatal rat. *J. Physiol.* **429:** 411-428.

HADDAD, G.G., DONNELLY, D.F. AND GETTING, P.A. (1990). Biophysical properties of hypoglossal neurons in vitro: intracellular studies in adult and neonatal rats. *J. Appl. Physiol.* **69(4)**: 1509-1517.

HADDAD, G.G. AND MELLINS, R.B. (1984). Hypoxia and respiratory control in early life. *Annu. Rev. Physiol.* **46**: 629-643.

HADDAD, G.G., GANDHI, M.R. AND MELLINS, R.B. (1982). Maturation of ventilatory response to hypoxia in puppies during sleep. *J. Appl. Physiol.* **52**: 309-314.

HAIDMAYER, R., KURZ, R., KENNER, T., WURM, H. AND PFEIFFER, K.P. (1982a). Physiological and clinical aspects of respiration control in infants with relation to the sudden infant death syndrome. *Klinische Wochenschrift*, **60(1):** 9-18.

HAIDMAYER, R., PFERFFER, K.P., KENNER, T. AND KURZ, R. (1982b). Statistical evaluation of respiratory control infants to assess possible risk for the sudden death syndrome (SIDS). *Eur. J. Pediatr.* **138(2):** 145-150.

HAJI, A., REMMERS, J.E., CONNELLY, C. AND TAKEDA, R. (1990). Effect of glycine and GABA on bulbar respiratory neurons of cat. *J. Neurophysiol.* **63(5):** 955-965.

HANSEN, A.J. (1985). Effect of anoxia on ion distribution in the brain. *Physiol. Rev.* **65:** 101-148.

HANSON, M. AND KUMAR, P. (1994). Chemoreceptor function in the fetus and neonate. (Review). *Adv. Exp. Med. & Biol.* **360:** 99-108.

HANSON, M., CALDER, N., WATANABE, T. AND KUMAR, P. (1993). The drive to breath and sudden infant death syndrome. (Review). *Acta Paediatr.* Supplement. 82 Suppl **389:** 47-49.

HANSON, M.A. AND WILLIAMS, B.A. (1989). The effect of decerebration and brain-stem transection on the ventilatory response to acute hypoxia in normoxic and chronically hypoxic newborn rats. *J. Physiol.* **414**: 25p.

HARDING, R. (1994). Fetal breathing: relation to postnatal breathing and lung development. In: Fetus and Neonate Physiology and Clinical Applications. Vol. 2. pp. 109-136. Edited by M.A. Hanson, J.A.D. Spencer, C.H. Rodeck and D. Walters. Printed in Great Britain at University Press, Cambridge.

HARDING, R., BUTTRESS, J.A., CADDY, D.J. AND WOOD, G.A. (1987). Respiratory and upper airway responses to nasal obstruction in awake lambs and ewes. *Respir. Physiol.* **68:** 177-188.

HARDING, R. (1984). Function of the larynx in the fetus and newborn. *Ann. Rev. Physiol.* **46:** 645-659.

HARDING, R., JOHNSON, P., AND McCLELLAD, M.E. (1978). Liquid sensitive laryngeal receptors in the developing sheep, cat and monkey. *J. Physiol.* **277:** 409-422.

HARDING, R., JOHNSON, P., McCLELLAND, B.M. AND WILDINSON, A.R. (1975). Cardiovascular changes in newborn lambs during apnea induced by stimulation of laryngeal receptors with water. *J. Physiol.* **256:** 35-36P.

HARNED, H.S., MYRACLE, J. AND REIRO, J. (1978). Respiratory suppression and swallowing from introduction of fluids into the laryngeal region of the lamb. *Pediatr. Res.* **12:** 1003-1009.

HARPER, R.M., LEAKE, B., HOFFMAN, H., WALTER, D.O. AND HOPPENBROUWERS, T. (1981). Periodicity of sleep states is altered in infants at risk for the sudden infant death syndrome. *Science,* **213:** 1030-1032.

HASEBE, N., ONODERA, S., YAMASHITA, Y., HANEDA, T. AND TOBISE, K. (1992). Site of hypoxic pulmonary vasoconstriction in pulsatile perfused canine lung lobes. *Jap. Circ. J.* **56(8):** 837-846.

HAXHIU, M.A., LUNTEREN, E.V., MIREA, J. AND CHERNIACK, S. (1984). Responses to chemical stimulation of upper airway muscles and diaphragm in awake cats. *J. Appl. Physiol.* **56(2):** 397-403.

HAYASHIMOTO, I. (1960). Comparative anatomical studies on the hypoglossal nucleus of the carnivore. *Bull. Kobe Med. Coll.* **20:** 394-408.

HENDERSON-SMART, D.J. (1981). The effect of gestational age on the incidence and duration of recurrent apnoea in newborn babies. *Aust. Pediatr. J.* **17:** 273-276.

HENDERSON-SMART, D.J., AND READ, D.J.C. (1979). Reduced lung volume during behavioral active sleep in the newborn. *J. Appl. Physiol.* **46:** 1081-1085.

HENDERSON-SMART, D.J. AND SOHNSON, P. (1979). Ventilatory response to hypoxaemia during sleep in the newborn. *J. Dev. Physiol.* **1:** 195-108.

HEYWOOD, P., MOOSAVI, S. AND GUZ, A. (1992). CO_2 sensitivity and breathing at rest and during exercise in human subjects with unilateral brainstem lesions. *J. Physiol.* **459:** 353P.

HIRSHMAN, C.A., McCULLOUGH, R.E., COHEN, P.J. AND WEIL, J.V. (1975). Hypoxic ventilatory drive in dogs during thiopental, ketamine, or pentobarbital anaesthesia. *Anaesthesiology,* **43(6):** 628-634.

HODGMAN, J. E. & HOPPENBROUWERS, T. (1983). Cardio-respiratory behaviour in infants at increased epidemiological risk for SIDS. In Sudden Infant Death Syndrome. Edited by Tilden J., Roeder L. M., Steinschneider A. pp: 669-679. New York: Academic.

HOLLOWELL, D.E., BHANDARY, P.R., FUNSTEN, A.W. AND SURATT, P.M. (1991). Respiratory-related recruitment of the masseter: response to hypercapnia and

loading. *J. Appl. Physiol.* **70(6):** 2508-2513.

HOLLOWELL, D.E., JENSEN, P., FUNSTEN, A.W. AND SURATT, P.M. (1987). Activation of masseter muscles during sleep in obstructive sleep apnea. *Am. Rev. Respir. Dis.* **139:** A448.

HOLSTEGE, G. AND KUYPERS, H.G. (1977). Propriobulbar fibre connections to the trigeminal, facial and hypoglossal motor nuclei. An anterograde degeneration study in the cat. *Brain,* **100(2):** 239-264.

HOLSTEGE, G., KUYPERS, H.G.M. AND DEKKER, J.J. (1977). The organization of the bulbar fibre connections to the trigeminal, facial and hypoglossal motor nuclei, II: an autoradiographic tracing study in cat. *Brain,* **100(2):** 227-268.

HORNER, R.L., INNES, J.A., MORRELL, M.J., SHEA, S.A. AND GAZ, A. (1994). The effect of sleep on reflex genioglossus muscle activation by stimuli of negative airway pressure in humans. *J. Appl. Physiol.* **476(1):** 141-151.

HUDGEL, D.W. (1992). The role of upper airway anatomy and physiology in obstructive sleep apnea. (Review). *Clin. Chest Med.* **13(3):** 383-398.

HUDGEL, D.W., CHAPMAN, K.R., FAULKS, C. AND HENDRICKDS, C. (1987). Changes in inspiratory muscle electrical activity and upper airway resistance during periodic breathing induced by hypoxia during sleep. *Am. Rev. Respir. Dis.* **135:** 899-906.

HUNT, C.E., McCULLOCH, K. AND BROUILLETTE, R.T. (1981). Diminished hypoxic ventilatory responses in near-miss sudden infant death syndrome. *J. Appl. Physiol.* **50:** 1313-1317.

HWANG, J.C., SHOU, D. AND ST, JOHN, W.M. (1989). Characterization of

expiratory intercostal activity trangularis stemi in cats. *J. Appl. Physiol.* **67(4):** 1518-1528.

HWANG, J.C., CHIEN, C.T. AND ST. JOHN, W.M. (1988). Characterization of respiratory-related activity of the facial nerve. *Respir. Physiol.* **73(2):** 175-187.

HWANG, J.C., ST.JOHN, M. AND BARTLETT, D. Jr. (1984a). Afferent pathway for hypoglossal and phrenic responses to changes in upper airway pressure. *Respir. Physiol.* **55**: 341-354.

HWANG, J.C., ST.JOHN, W.M. AND BARTLETT, D. Jr. (1984b). Receptors responding to changes in upper airway pressure. *Respir. Physiol.* **55**: 355-366.

HWANG, J.C., BARTLETT, D. Jr. AND ST.JOHN, W.M. (1983a). Characterization of respiratory-modulated activities of hypoglossal motoneurones. *J. Appl. Physiol.* **55(3)**: 793-798.

HWANG, J.C., ST.JOHN, W.M. AND BARTLETT,D. (1983b). Respiratory-related hypoglossal nerve activity: influence of aesthetics. *J. Appl. Physiol.* **55(3):** 785-792.

INSCORE, S.C., STENMARK, K.R., ORTON, C. AND IRVIN, C.G. (1991). Neonatal calves develop airflow limitation due to chronic hypobaric hypoxia. *J. Appl. Physiol.* **70(1):** 384-390.

ISCOE, S.D. (1988). Central control of the upper airway. In: Respiratory Function of the Upper Airway. Edited by O.P. Mathew and G. Sant'Ambrogio. New York: Dekker, 1988. p. 125-192.

ISSA, F.G. AND REMMERS, J.E. (1992). Identification of a subsurface area in the ventral medulla sensitive to local changes in PCO_2. *J. Appl. Physiol.* **72:** 439-446.

ISSA, F.G., EDWARDS, P., SZETO, E., LAUFF, D. AND SULLIVAN, C. (1988). Genioglossus and breathing responses to airway occlusion: effect of sleep and route of occlusion. *J. Appl. Physiol.* **64(2):** 543-549.

ISSA, F.G., McNAMARA, S.G. AND SULLIVAN, C.E. (1987). Arousal responses to airway occlusion in sleeping dogs: comparison of nasal and tracheal occlusions. *J. Appl. Physiol.* **62(5):** 1832-1836.

JAROLINEK, W., MISGELD, U. AND LUX, H.D. (1990). Neurons sensitive to pH in slices of the rat ventral medulla oblongata. *Pflugers Arch Eur J. Physiol.* **416:** 247-253.

JEFFRIES, B., BROUILLETTE, R.T. AND HUNT, C.E. (1984). Electromyographic study of some accessory muscles of respiration in children with obstructive sleep apnea. *Am. Rev. Respir. Dis.* **129:** 696-702.

JEFFREY, H.E. AND READ, D.J. (1980). Ventilatory responses of newborn calves to progressive hypoxia in quite and active sleep. *J. Appl. Physiol.* **48:** 892-895.

JIANG, C., CUMMINS, T.R. AND HADDAD, G.G. (1994). Membrane ionic currents and properties of freshly dissociated rat brainstem neurons. *Exp. Brain Res.* **100(3):** 407-420.

JIANG, C. AND HADDAD, G.G. (1994). Oxygen deprivation inhibits a K^+ channel independently of cytosolic factors in rat central neurons. *J. Physiol.* **481:** 15-26.

JIANG, C., AGULAIAN, S. AND HADDAD, G.G. (1992). CL^- and Na^+ homeostasis during anoxia in rat hypoglossal neurons: intracellular and extracellular in vitro studies. *J. Physiol.* **448:** 697-708.

JIANG, C., MITCHELL, G.S. AND LIPSKI, J. (1991). Prolonged augmentation of

respiratory discharge in hypoglossal motoneurones following superior laryngeal nerve stimulation. *Brain Res.* **538**: 215-225.

JIANG, C. AND HADDAD, G.G. (1991). Effect of anoxia on intracellular and extracellular potassium activity in hypoglossal neurons in vitro. *J. Neurophysiol.* **66(1):** 103-111.

JOHNSON, P. AND ANDREWS, D.C. (1994). Non-invasive cardiorespiratory monitoring at home and in hospital. In: Fetus and neonate physiology and clinical applications. Edited by Mark A. Hanson, John A.D. Spencer, Charles H. Rodeck and Dafydd Walters. Vol.2, pp, 343-363. Printed in Great Britain at University Press, Cambridge.

JOHNSON, P. (1974). Laryngeal induced apnea. In SIDS: Proceedings of the Francis E. Camps Symposium. Edited by R.R. Robinson, pp. 231-242. Toronto: Can. Found. Study of Infant Death.

JOHNSTON, B.M., GUNN, T.R. AND GLUCKMAN, P.D. (1986). In fetal lambs, genioglossus alae nasi activity in fetal sheep. *J. Dev. Physiol.* **8:** 323-331.

JOUVET, M. (1967). Neurophysiology of sleep states. *Physiol. Rev.* **47:** 117-177.

KAGAWA, S., STAFFORD, M.J., WAGGENER, T.B. AND ERINGHAUS, J.W. (1982). No effect of naloxone on hypoxia-induced ventilatory depression in adults. *J. Appl. Physiol.* **52:** 1030-1041.

KAHN, A., BLUM, D. AND HENNART, P. (1984). A critical comparison of the history of sudden-death infants and infants hospitalised for near-miss for SIDS. *Eur. J. Pediatr.* **143:** 103-107.

KAPPERS, C.U.A., HUBER, G.C. AND CROSBY, E.C. (1960). The effectory

system of mammals. In: The comparative anatomy of the nervous system of vertebrates including man. pp. 578-586. Hafner, New York.

KAWASAKI, K., TRAYNELIS, S.F. AND DINGLEDINE, R. (1990). Different responses of CA1 and CA3 regions to hypoxia in rat hippocampal slice. *J. Neronphysiol.* **63:** 385-394.

KIMURA, H., NIIJIMA, M., EDO, H., TATSUMI, K., HONDA, Y. AND KURIJAMA, T. (1993). The effect of hypoxic depression on genioglossus muscle activity in healthy subjects and obstructive sleep apnea patients. *Sleep,* **16(8):** S135-S136.

KNILL, R.L. AND GELB, A.W. (1982). Peripheral chemoreceptors during anaesthesia: Are the watchdogs sleeping? *J. Anesthesiol.* **57(3):** 151-152.

KOVAR, I., SELSTAM, U., CATTERTON, W.Z., STAHLMAN, M.T. AND SUNDELL, H.W. (1979). Laryngeal chemoreflex in newborn lambs: Respiratory and swallowing response to salt, acids, and sugars. *Pediatr. Res.* **13:** 1144-1149.

KRAUS, A.N., SOLONOM, G.E. AND AULD, P.A.M. (1977). Sleep state, apnea, and bradycardia in preterm infants. *Dev. Med. Child. Neurol.* **19:** 160-168.

KROL, R.C., KNUTH, S.L. AND BARTLETT, D. Jr. (1984). Selective reduction of genioglossus muscle activity by alcohol in normal human subject. *Am. Rev. Respir. Dis.* **129(2):** 247-250.

KUMAR, P., THOMAS, T. AND ELNAZIR, B.K. (1992). The reflex ventilatory responses of conscious, newborn rats to alternations of inspiratory oxygen concentration. *J. Dev. Physiol.* **17(3):** 109-118.

KUNA, S.T. AND SANT'AMBROGIO, G. (1991). Pathophysiology of upper airway

closure during sleep. *JAMA.* **266(10):** 1384-1389.

KUNA, S.T. AND SMICKLEY, J. (1988). Response of genioglossus muscle activity to nasal airway occlusion in normal sleeping adults. *J. Appl. Physiol.* **65:** 1332-1339.

KUNA, S.T. (1986). Inhibition of inspiratory upper airway motoneurone activity by phasic volume feedback. *J. Appl. Physiol.* **60(4):** 1373-1379.

LaFRAMBOISE, W.A., GUTHRIE, R.D., STANDAERT, T.A. AND WOODRUM, D.E. (1983). Pulmonary mechanics during the ventilatory response to hypoxemia in the newborn monkey. *J. Appl. Physiol.* **55(3)**: 1008-1014.

LAHIRI, S., MARET, K. AND SHERPA, M.G. (1983). Dependence of high altitude sleep apnea on ventilatory sensitivity to hypoxia. *Respir. Physiol.* **52:** 281-301.

LAITMAN, J.T., CRELIN, E.S., CONLOGUE, G.J. (1977). The function of the epiglottis in monkey and man. *Yale J. Biol. Med.* **50:** 43-48.

LALLEY, P.M. (1986). Response of phrenic motoneurones of the cat to stimulation of medullary raphe nuclei. *J. Physiol.* **380:** 349-385.

LANDAUER, R.C., PEPPER, D.R. AND KUMAR, P. (1995). Effect of chronic hypoxaemia from birth upon chemosensitivity in the adult rat carotid body in vitro. *J. Physiol.* **485(2):** 543-550.

LAWSON, E.E. (1982). Recovery from central apnea: effect of stimulus duration and end-tudak CO_2 partial pressure. *J. Appl. Physiol.* **53(1):** 105-109.

LAWSON, E.E. (1981). Prolonged central respiratory inhibition following reflex-induced apnea (in piglets). *J. Appl. Physiol.* **50(4)**: 874-879.

LEATHY, F.A.N., CATES, D., MaeCLLUM, M. AND TIGATTO, H. (1980). Effect of CO_2 and 100% O_2 on cerebral blood flow in preterm infants. *J. Appl. Physiol.* **48:** 468-472.

LEE, J.C. AND DOWNING, S.E. (1980). Laryngeal reflex inhibition of breathing in piglets: influences of anemia and catecholamine depletion. *Am. J. Physiol.* **239:** R25-R30.

LEE, J.C., STOLL, B.J. AND DOWNING, S.E. (1977). Properties of the laryngeal chemoreflex in neonatal piglets. *Am. J. Physiol.* **233(1):** R30-R36.

LEE, L.Y. AND MILHORN, H.T. Jr. (1975). Central ventilatory responses to O_2 and CO_2 at three levels of carotid chemoreceptor stimulation. *Respir. Physiol.* **25:** 319-333.

LEITER,J.C. AND DAUBENSPECK, J.A. (1990). Selective reflex activation of the genioglossus in human. *J. Appl. Physiol.* **68(6):** 2581-2587.

LEITER, J.C., KNUTH, S.L. AND BARTLETT, D. Jr. (1985). The effect of sleep deprivation on activity of the genioglossus muscle. *Am. Rev. Respir. Dis.* **132(6):** 1242-1245.

LEWIS, P.R., FLUMERFELT, B.A. AND SHUTE, C.C.D. (1971). The use of cholinesterase techniques to study topographical localization in the hypoglossal nucleus of the rat. *J. Anat.* **110:** 203-490.

LI, F. AND NOBLE, R. (1994). Effects of mild levels hypoxaemia on hypoglossal motoneurones in anaesthetized neonatal kittens. *J. Physiol.* **475:** 120P.

LI, F. AND NOBLE, R. (1995). Effects of mild systemic hypoxia laryngeal-evoked potentials in hypoglossal motoneurones in anaesthetized neonatal kittens. *J. Physiol.* **483:** 88-89P.

LIPSKI, J. (1981). Antidromic activation of neurones as an analytic tool in the study of the central nervous system. *J. Neuronsci. Methods,* **4:** 1-32.

LIPSKI, J. AND MERRILL, E.G. (1980). Electrophysiological demonstration of the projection from expiratory neurones in rostral medulla to contralateral dorsal respiratory group. *Brain Res.* **197:** 521-524.

LITMANOVITZ, I., DRESHAJ, I., MILLER, M.J., HAXHIU, M.A. AND MARTIN, R.J. (1994). Central chemosensitivity affects respiratory muscle responses to laryngeal stimulation in the piglet. *J. Appl. Physiol.* **76(1):** 403-408.

LIUNGGREN, B., NORGERG, K. AND SIESJO, B.K. (1974). Influence of tissue acidosis upon restitution of brain energy metabolism following total ischemia. *Brain Res.* **77:** 173-183.

LODGE, D., DUGGAN, A.W., BISCOE, T.J. AND CADDY, K.W. (1973). Concerning recurrent collateral and afferent fibbers in the hypoglossal nerve of the rat. *Exp. Neurol.* **41:** 63-75.

LOWE, A.A. (1981). The neural regulation of tongue movements. *Prog. Neurobiol.* **15:** 295-344.

LOWE, A.A. (1978). Excitatory and inhibitory inputs to hypoglossal motoneurones and adjacent reticular formation neurons in cats. *Exp. Neurol.* **62:** 30-47.

LOWE, A.A., GURZA, S. AND SESSLE, B.J. (1976). Excitatory and inhibitory influences on tongue muscle activity in cat and monkey. *Brain Res.* **21:** 417-422.

LOWE, A.A. AND SESSLE, B.J. (1973). Tongue-activity during respiratory, jaw opening and swallowing in cat. *Can. J. Physiol. Pharmacol.* **51:** 1009-1011.

LUCIER, G.E., STOREY, A.T. AND SESSLE, B.J. (1979). Effects of upper respiratory tract stimuli on neonatal respiration: reflex and single neuron analyses in the kitten. *Biol. Neonate,* **35:** 82-89.

MANAKER, S., TISCHLER, L.J. AND MORRISON, A.R. (1992). Raphespinal and reticubspinal axon collateral to the hypoglossal nucleus in the rat. *J. Comp. Neurol.* **322:** 68-78.

MARCHAL, F., BAIRAM,A., HAOUZI, P., HASCOET, J.M., CRANCE, J.P., VERT, P. AND LAHIRI, S. (1992). Dual responses of carotid chemosensory afferents to dopamine in the newborn kitten. *Respir. Physiol.* **90(2):** 173-183.

MARCHAL, F., BAIRAM, A. AND VERT, P. (1987). Neonatal apnea and apneic syndromes. *Clin. Perinatol.* **14(3):** 509-530.

MARCHAL, F., CORKE, B.C. AND SUNDELL, H. (1982). Reflex apnea from laryngeal chemo-stimulation in the sleeping premature newborn lamb. *Pediatr. Res.* **16:** 621-627.

MARLOT, D., AND DURON, B. (1979). Postnatal development of vagal control of breathing in the kitten. *J. Physiol. Paris.* **75:** 891-900.

MARTIN, R.J., DRSHAJ, I.A., MILLER, M.J. AND HAXHIU, M.A. (1994). Hypoglossal and phrenic responses to central respiratory inhibition in piglets. *Respir. Physiol.* **97(1):** 93-103.

MARTIN, R.J., LUNTEREN, E.V., HAXHIU, M.A. AND CARLO, W.A. (1990). Upper airway muscle and diaphragm responses to hypoxia in the piglet. *J. Appl. Physiol.* **68(2).** 672-677.

MARTIN-BODY, R.L. AND JOHNSTON, B.M. (1988). Central origin of the

hypoxic depression of breathing in the newborn. *Respir. Physiol.* **71:** 25-32.

MATHEW, O.P., ANDERSON, J.W., ORANI, G.P., AND SANT'AMBROGIO, F.B. AND SANT'AMBROGIO, G. (1990). Cooling mediates the ventilatory depression associated with airflow through the larynx. *Respir. Physiol.* **82(3):** 359-367.

MATHEW, O.P., SANT'AMBROGIO, G., FISHER, J.T. AND SANT'AMBROGIO, F.B. (1984). Laryngeal pressure receptors. *Respir. Physiol.* **57**: 113-122.

MATHEW, O.P. AND REMMERS, J.E. (1984). Respiratory function of the upper airway. In: Sleep and Breathing. Edited by N.A. Saunders and C.E. Sullivan. New York: Dekker, p.163-200.

MATHEW,O.P., ABU-OSBA,Y.K. AND THACH, B.T. (1982a). Influence of upper airway pressure changes on respiratory frequency. *Respir. Physiol.* **49**: 223-233.

MATHEW,O.P., ABU-OSBA,Y.K. AND THACH, B.T. (1982b). Influence of upper airway pressure changes on genioglossus muscle respiratory activity. *J. Appl. Physiol.* **52(2):** 438-444.

MATHEW, O.P., SANT'AMBROGIO, G., FISHER, J.T. AND SANT'AMBROGIO, F.B. (1984). Laryngeal pressure receptors. *Respir. Physiol.* **57**: 113-122.

McCAFFREY, T.V. AND KERN, E.B. (1979). Response of nasal airway resistance to hypercapnia and hypoxia in the dog. *Acta. Oto-Laryngol.* **87(5-6):** 545-553.

McCULLOCH, K., BROUILLETTE, R.T., GUZZETTA, A.J. AND HUNT, C.E. (1982). Arousal responses in near-miss sudden infants death and in normal infants. *J. Pediatr.* **101:** 911-917.

MEGIRIAN, D., HINRICHSEN, C.F., SHERREY, J.H. (1985). Respiratory roles

of genioglossus, stemothyroid, and stemothyoid muscle during sleep. *Exp. Neurol.* **90(1):** 118-128.

MEYER, J.S., GOTOH, F. AND TAZAKI, Y. (1961). Inhibitory action of carbon dioxide and acetazolamide in seizure activity. *Electroencephalogr. Clin. Neurophysiol.* **13:** 762-775.

MIFFLIN, S.W. (1990). Arterial chemoreceptor input to respiratory hypoglossal motoneuron. *J. Appl. Physiol.* **69(2):** 700-709.

MIFFLIN, S.W. (1989). Frequency dependence of superior laryngeal nerve inputs to inspiratory hypoglossal motoneurones. *Faseb. J.* **3:** 149.

MIFFLIN, S.W., BALLANTYNE, D., BACKMAN.S.B. AND RICHTER, D.W. (1985). Evidence for a calcium activated potassium conductance in medullary respiratory neurones. In Neurogenesis of Central Respiratory Phythm. Edited by Biahchi, A. and Denavit-Saubié, M. pp. 179-182. MTP Press, Lancaster.

MIKI, H., HIDA, W., CHONAN, T., KIKUCHI, Y. AND TAKISHIMA, T. (1989). Effects of submental electrical stimulation during sleep on upper airway patency in patients with obstructive sleep apnea. *Am. Rev. Respir. Dis.* **140:** 1285-1289.

MILLER, A.J. AND BOWMAN, J.P. (1974). Divergent synaptic influences affecting discharge patterning of genioglossus motor units. *Brain Res.* **78:** 179-191.

MILLHORN, D.E. AND ELDRIDGE, F.L. (1986). Role of ventrolateral medulla in regulation of respiratory and cardiovascular systems. *J. Appl. Physiol.* **61:** 1249-1263.

MITRA, J., PRABHAKAR, N.R., HAXHIU, M. AND CHEMIACK, N.S. (1986). Comparison of the effects of hypercapnia on phrenic and hypoglossal activity in anaesthetized decerebrate and decorticate animals. *Brain Res. Bull.* **17(2):** 181-187.

MITRA ,J. AND CHERNIACK, N.S. (1983). The effects of hypercapnia and hypoxia on single hypoglossal nerve fibre activity. *Respir. Physiol.* **54:** 55-66.

MIZUNO, N., AKIMOTO, C., MOCHIZUKI, K. AND MATSUSHIMA, R. (1973). Experimental studies of afferent fibbers in the hypoglossal nerve in cat: a scanning electron microscope observation on the lingual mucosa following transection of the nerve and degenerated study with silver impregnation methods. *Arch. Histol. Jap.* **35:** 99-113.

MONTEAU, R., MORIN, D., HENNEQUIN, S. AND HILAIRE, G. (1990a). Deferential effects of serotonin on respiratory activity of hypoglossal and cervical motoneurones: an in vitro study on the newborn rat. *Neurosci. Lett.* **111**: 127-132.

MOORE, P.J. AND HANSON, M.A. (1994). Control of breathing: central influences. In: Fetus and Neonate Physiology and Clinical Applications. Vol. 2. pp. 109-136. Edited by M.A. Hanson, J.A.D. Spencer, C.H. Rodeck and D. Walters. Printed in Great Britain at University Press, Cambridge.

MOORE, P.J., PARKES, M.J., NOBLE, R. AND HANSON, M.A. (1991). Reversible blockade of the secondary fall of ventilation during hypoxia in anaesthetized newborn sheep by focal cooling in the brain stem. *J. Physiol.* **438:** 91P.

MORIMOTO, T. AND KAWAMURA, Y. (1972). Inhibitory postsynaptic potentials of hypoglossal motoneurones of the cat. *Exp. Neurol.* **37(1)**: 188-198.

MORIN, D., HENNEQUIN, S., MONTEAU, R. AND HILAIRE, G. (1990a). Serotonergic influences on central respiratory activity: an in vitro study in the newborn rat. *Brain Res.* **535:** 281-287.

MORIN, D., HENNEQUIN, S., MONTEAU, R. AND HILAIRE, G.(1990b). Depressant effect of raphe stimulation on inspiratory activity of the hypoglossal nerve:

in vitro study in the newborn rat. *Neurosci. Lett.* **116**: 299-303.

MORIN-SURUN, M.P., JORDAN, D., CHAMPAGNAT, J., SPYER, K.M. (1984). Excitatory effects of iontophoretically applied substance P on neurons in the nucleus tractus solitaries of the cat: lack of interaction with opiates and opioids. *Brain Res.* **307:** 388-392.

MORRAY, J., BENNET, R. AND HANSON, M.A. (1992). Effect of halothane on the carotid chemoreceptor response to hypoxia in anaesthetized kittens. *J. Physiol.* **446:** 605-629.

MORRAY, J., NOBLE, R. AND HANSON, M.A. (1991). The effects of halothane on the ventilatory response to hypoxia in chloralose-anaesthetized kittens. *J. Physiol.* **438:** 244P.

MORRELL, N.W., NIJRAN, K.S., BIGGS, T. AND SEED, W.A. (1994). Changes in regional pulmonary blood flow during lobar bronchial occlusion in man. *Clin. Science*, **86(5):** 639-644.

MORTOLA, J.P. AND PIAZZA, T. (1987). Breathing patten in rats with chronic section of the superior laryngeal nerves. *Respir. Physiol.* **70:** 51-62.

MORTOLA, J.P. AND REZZONICO, R. (1988). Metabolic and ventilatory rates in newborn kittens during acute hypoxia. *Respir. Physiol.* **73:** 55-68.

MORTOLA, J.P. AND REZZONICO, R. (1989). Ventilation in kittens with chronic section of the superior laryngeal nerves. *Respir. Physiol.* **76:** 369-382.

MOURRE, C., BEN ARI, Y., BEMARDI, H., FOSSET, M. AND LAZDUNSKI, M. (1989). Antidiabetic sulfonylureas: localization of binding sites in the brain and effects on the hyperpolarization induced by anoxia in hippocampal slices. *Brain Res.*

486(1): 159-164.

MOURRE, C., BEN ARI, Y, BERNARDI, H., FOSSET, M. AND LAZDUNSKI, M. (1983). Antidiabetic sulfonylurea: location of binding sites in the brain and effects on the hyperpolarization induced by anoxia in hippocampal slices. *Brain Res.* **486:** 159-164.

MURAKAMI, Y. AND KIRCHNER, J.I. (1974). Respiratory activity of the external laryngeal muscles: An electromyographic study in the cat. In: Ventilatory and Phonatory Control Systems, ed. by Wyke, B. Oxford University Press, London, pp. 430-448.

NAEYE, R.L. (1977). The sudden infant death syndrome: a review of recent advances. (Review). *Arch. Pathol. Lab. Med.* **10(4):** 165-167.

NAEYE, R.L. (1976). Brain-stem and adrenal abnormalities in the sudden-death syndrome. *Am. J. Clin. Pathol.* **66(3):** 526-530.

NATTIE, E.E., BLANCHFORD, C. AND LI, A. (1992). Retrofacial lesions: effects on CO_2-sensitive phrenic and sympathetic nerve activity. *J. Appl. Physiol.* **73:** 1317-1325.

NATTIE, E.E., LI, A. AND ST.JOHN, W.M. (1991). Lesions in retrotrapezoid nucleus decrease ventilatory output in anaesthetized or decerebrate cats. *J. Appl. Physiol.* **71:** 1364-1375.

NATTIE, E.E. AND LI, A. (1990). Fluorescent location of RVLM kainate microinjections that alter the control of breathing. *J. Appl. Physiol.* **94:** 35-50.

NATTIE, E.E., MILLS, J.W., OU, L.C. AND ST. JOHN, W.M. (1988). Kainic acid on the rostral ventrolateral medulla inhibits phrenic output and CO_2 sensitivity. *J. Appl.*

Physiol. **65:** 1525-1534.

NEUBAUER, J.A., SANTIAGO, T.V. AND EDELMAN, N.M. (1981). Hypoxic arousal in intact and carotid chemodenervated sleeping cats. *J. Appl. Physiol.* **51:** 1294-1299.

NELIN, L.D., MOSHIN, J. THOMAS, C.J., SASIDHARAN, P. AND DAWSON, C.A. (1994a). The effect of inhaled nitric oxide on the pulmonary circulation of the neonatal pig. *Pediatr. Res.* **35(1):** 20-24.

NELIN, L.D., RICHABY, D.A., LINEHAN, J.H. AND DAWSON, C.A. (1994b). The vascular site of action of hypoxia in the neonatal pig lung. *Paediatr. Res.* **35(1):** 25-29.

NIOKA, S., CHANCE, B., SMITH, D.S., MAYEVSKY, A., RELLY, M.P., ALTER, C. AND ASAKURA, T. (1990). Cerebral energy metabolism and oxygen state during hypoxia in neonate and adult dogs. *Pediatr. Res.* **28(1):** 54-62.

NISHINO, T., SUGIMORI, K., HIRAGA, K. AND HONDA, Y. (1986). Effects of tracheal irritation and hypercapnia on tracheal smooth muscle in humans. *J. Appl. Physiol.* **69(2):** 419-423.

NOBLE, R., WILLIAMS, B.A., HANSON, M.A. AND SMITH, J.A. (1990). Transient, intense chemoreceptor stimulation does not affect discharge of rostral pontine neurones found to be excited during hypoxia in anaesthetized newborn lams. *J. Physiol.* **429:** 81P.

NOBLE, R. AND WILLIAMS, B.A. (1989). Excitation of neurones in the rostral pons during hypoxia in anaesthetized neonatal lambs. *J. Physiol.* 146P.

OCHIAI, R., GUTHRIE, R.D. AND MOTOYAMA, E.K. (1992). Differential

sensitivity to halothane anaesthesia of the genioglossus, intercostal and diaphragm in kittens. *Anaesthesia & Analgesia,* **74(3):** 338-344.

OCHIAI, R., GUTHRIE, R.D. AND MOTOYAMA, E.K. (1989). Effects of varying concentrations of halothane on the activity of the genioglossus, intercostal, and diaphragm in cats: an electromyographic study. *Anaesthesiology,* **70:** 812-816.

OKABE, S., HIDA, W, KIKUCHI, Y., KUROSAWA, H., MIDORIKAWA, J., CHONAN, T., TAKISHIMA, T. AND SHIRATO, K. (1993). Upper airway muscle activity during sustained hypoxia in awake humans. *J. Appl. Physiol.* **75(4):** 1552-1558.

OKADA, Y., MÜCKENHOFF, K. AND SCHEID, P. (1993). Hypercapnia and medullary neurons in the isolated brain stem-spinal cord of the rat. *Respir. Physiol.* **93:** 327-336.

OLINSKY, A.M., BRYAN, H. AND BRYAN, A.C. (1974). Influence of lung inflation on respiratory control in neonates. *J. Appl. Physiol.* **36:** 426-429.

ÖNAL, E., BURROWS, D.L., HART, R.H. AND LOPATA, M. (1986). Induction of periodic breathing during sleep causes upper airway obstruction in humans. *J. Appl. Physiol.* **61(4):** 1438-1443.

ÖNAL, E., LOPATA, M., GINZBURG, A.S. AND O'CONNOR, T.D. (1981a). Diaphragmatic EMG and transdiaphragmatic pressure measurements with a single catheter. *Am. Rev. Respir. Dis.* **124(5):** 563-565.

ÖNAL, E., LOPATA, M. AND O'CONNOR, T.D. (1981b). Diaphragmatic and genioglossal electromyogram responses to CO_2 rebreathing in humans. *J. Appl. Physiol.* **50:** 1052-1055.

ONIMARU, H., ARATA, A. AND HOMMA, L. (1988). Primary respiratory rhythm generator in the medulla of brainstem-spinal cord preparation from newborn rat. *Brain Res.* **445:** 314-324.

PARISI, R.A., SANTIAGO, T.V. AND EDELMAN, N.H. (1988). Genioglossal and diaphragmatic EMG responses to hypoxia during sleep. *Am. Rev. Respir. Dis.* **138:** 610-616.

PATRICK, G.B., STROHL, K.P., RUBIN, S.B. AND ALTOSE, M.D. (1982). Upper airway and diaphragm muscle responses to chemical stimulation and loading. *J. Appl. Physiol.* **53:** 1133-1137.

PEPPER, D.R., LANDAUER, R.C. AND KUMAR, P. (1995). Postnatal development of CO_2-O_2 interaction in the rat carotid body in vitro. *J. Physiol.* **285(2):** 531-541.

PHILLIPSON, E.A., BOWES, G. (1983). Control of breathing during sleep. In Handbook of Physiology, Respiration, Vol. 1. Control of Breathing. Edited by N.S. Cherniack, J.D. Widdicombe. Wash. DC: Am. Physiol. Soc.

PHILLIPSON, E.A. AND SULLIVAN, C.E. (1978). Arousal: the forgotten response to respiratory stimuli. *Am. Rev. Respir. Dis.* **118:** 807-820.

PIERREFICHE, O., FOUTZ, A.S. AND DENAVIT-SAUBIÉ, M. (1993). Effect of $GABA_B$ receptor agonist and antagonists on the bulbar respiratory network in the cat. *Brain Res.* **605:** 77-84.

POETS, C.F. AND SOUTHALL, D.P. (1994). Apnoea, sudden infant death and respiratory control. In: Fetus and neonate physiology and clinical applications. Edited by Mark A. Hanson et al. Cambridge University Press.

PROCTOR, D.F. (1977). The upper airways. II. The larynx and trachea. *Am. Rev. Respir. Dis.* **115:** 315-342.

REED, W.R., ROBERTS, J.L. AND THACH, B.T. (1985). Factors influencing regional patency and configuration of the human infant upper airway. *J. Appl. Physiol.* **58(2):** 635-644.

REMMERS, J.E., DECROOT, W.J., SAVERLAND, E.K. AND ANCH, A.M. (1978). Pathogenesis of upper airway occlusion during sleep. *J. Appl. Physiol.* **44(6):** 931-938.

RICHTER, D.W., CHAMPAGNAT, J., JACQUIN, T. AND BENACKA, R. (1993). Calcium currents and calcium-dependent potassium currents in mammalian medullary respiratory neurones. *J. Physiol.* **470:** 23-33.

RICHTER, D.W., BISCHOFF, A., ANDERS, K., BELLINGHAM, M. AND WINDHORST, U. (1991). Response of the medullary respiratory network of the cat to hypoxia. *J. Physiol.* **443:** 231-256.

RICHTER, D.W., CHAMPAGNAT, J. AND MIFFLIN, S.W. (1987). Membrane properties of medullary respiratory neurones of the cat. In Respiratory muscles and their neuromotor control. Edited by Sieck, G.C., Gandevia, S.C. and Cameron, W.E. pp. 9-15. Alan R. Liss Ine, New York.

RICHTER, D.W., CAMERER, H., MEESMANN, M. AND RÖHRIG, N. (1979). Studies on the synaptic interconnection between bulbar respiratory neurones of cats. *Pflügers Arch.* **380:** 245-257.

RIGATTO, H. (1984). Control of ventilation in the newborn. *Ann. Rev. Physiol.* **46:** 661-674.

RIGATTO, H. (1982). Apnea. *Pediatr. Clin. N. Am.* **29:** 1105-1116.

RIGATTO, H, DESAI, U., LEATHY, F., KALAPESI, Z. AND CATES, D. (1981). The effect of 2% CO_2, 100% O_2, theophylline, and 15% O_2 on "inspiratory drive" and "effective" timing in preterm infants. *Early Hum. Develop.* **5:** 63-70.

RIGATTO, H., DALAPESI, Z., LEATHY, F.N., DURAND, M., MaeCALLUM, M., AND CATES, D. (1980). Chemical control of respiratory frequency and tidal volume during sleep in preterm infants. *Respir. Physiol.* **41:** 117-25.

RIGATTO, H. (1979). A critical analysis of the development of peripheral and central respiratory chemosensitivity during the neonatal period. In Central Nervous Control Mechanisms in Breathing. Edited by C. von Euler, H. Lagercrantz, pp. 137-148. Oxford: Pergamon.

RIGATTO, H. (1977). Ventilatory response to hypoxia. *Semin. Perinatol.* **1:** 375-362.

RIGATTO, H., BRADY, J.P. AND VERDUZCO, R. T. (1975). Chemoreceptor reflexes in preterm infants: I. The effect of gestational and postnatal age on the ventilatory response to inhalation of 100% and 15% oxygen. *Pediatrics,* **55:** 604-613.

ROBERTS, J.L., REED, W.R., MATHEW,O.P. AND THACH, B.T. (1986). Control of respiratory activity of the genioglossus muscle in micrognathic infants. *J. Appl. Physiol.* **61(4):** 1523-1533.

SALAMONE, J.A., STROHL, K.P., WEINER, J.M. AND CHERNIACK, N.S. (1983). Cranial and phrenic nerve responses to changes in systemic blood pressure (dog). *J. Appl. Physiol.* **55(1)**: 61-68.

SAMPSON, S. AND EYZAGUIRRE, C. (1964). Some functional characteristics of mechano-receptors in the larynx of the cat. *J. Neuronphysiol.* **27:** 464-480.

SANKARAN, K., WIEBE, H., SESHIA, M.M.K., BOYCHUK, R.B., CATES, D. AND TIGATTO, H. (1979). Immediate and late ventilatory response to high and low O_2 in preterm infants and adult subjects. *Pediatr. Res.* **13:** 875-878.

SANT'AMBROGIO, G. AND MATHEW, O.P. (1988). Control of upper airway muscles. *NIPS,* **3**: 167-172.

SANT'AMBROGIO, G., MATHEW, O.P., SANT'AMBROGIO, F.B. AND FISHER, J.T. (1985a). Laryngeal cold receptors. *Respir. Physiol.* **59**: 35-44.

SANT'AMBROGIO. G., METHEW. O.P. AND SANT'AMBROGIO, F.B. (1985b). Role of intrinsic muscles and tracheal motion in modulating laryngeal receptors. *Res. Physiol.* **61(3):** 289-300.

SANT'AMBROGIO, G., MATHEW, O.P., FISHER, J.T. AND SANT'AMBROGIO, F.B. (1983). Laryngeal receptors responding to transmural pressure, airflow and local muscle activity. *Respir. Physiol.* **54**: 317-330.

SAUERLAND, E.K. AND HARPER, R.M.(1976). The human tongue during sleep: electromyographic activity of the genioglossus mascle. *Exp. Neurol.* **51:** 160-170.

SAUERLAND,E.K. AND MITCHELL, S.P. (1970). Eletromygraphic activity of the human genioglossus muscle in response to respiration and to positional changes of the head. Reprinted from the bulletin of the Los Angeles Neurological Societies, **Vol.35. No.2,** April. 1970. Printed in U.S.A.

SAUERLAND, E.K. AND MIZUNO, N. (1970). A protective mechanism for the tongue: suppression of genioglossus activity induced by stimulation of trigeminal proprioceptive afferents. *Experientia,* **26:** 1226-1227.

SCHLÄFKE, M. AND LOESCHCKE, H.H. (1967). Localisation eines an der

Regulation von Atmung und Kreislauf beleiligten Gebeites an der ventralen Oberfläche der Medulla oblongata durch Kälteblockade. *Pflügers Arch.* **287:** 201-220.

SCHLÄFKE, M., FILLE, J.F. AND LOESCHCKE, H.H. (1979). Elimination of central chemosensitivity by coagulation of a bilateral area on the ventral medullary surface in awake cats. *Pfluegers Arch.* **378:** 231-241.

SCHMITT, A., YU, K.J. AND SESSLE, B.J. (1973). Excitatory and inhibitory influences from laryngeal and orificial areas on tongue position in cat. *Arch, Oral Biol.* **18:** 1121-1130.

SCHULTE, F.J., BUSSE, C., EICHHORN, W. (1977). Rapid eye movements sleep, motoneurone inhibition, and apneic spells in preterm infants. *Pediatr. Res.* **11**: 709-713.

SCHULZE, K., KAIRAM, R., STEFANSKI, M., SCIACCA, R., BATEMAN, D. (1981). Spontaneous variability in minute ventilation oxygen consumption and heart rate of low birth weight infants. *Pediatr. Res.* **15:** 1111-1116.

SCHWEILER, G. H. (1968). Respiratory regulation during postnatal development in cats and rabbits and some of its morphological substrate. *Acta. Physiol. Scan.* **304:** 1-123.

SESSLE, B.J. (1973). Presynaptic excitability changes induced in single laryngeal primary afferent fibres. *Brain Res.* **53:** 333-342.

SESSLE, B.J. AND KENNY, D.J. (1973). Control of tongue and facial motility: neural mechanisms that may contribute to movements such as swallowing and sucking. In: fourth Symposium and Perception: Development in the Fetus and Infant, pp. 222-231. ed, J.F. Bosma. D.H.E.W. Bethesda, Md.

SHANNON, D.C., KELLY, D.H. AND O'CONNELL, K. (1977). Abnormal regulation of ventilation in infants at risk for sudden-infant-death-syndrome. *N. Engl. J. Med.* **297:** 747-750.

SHARPARD, J.W., Jr. (1989). Cardiorespiratory changes in obstructive sleep apnea. In: Principles and Practice of Sleep Medicine. Edit by M.H. Kryger, T. Roth and W.C. Dement. Philadelphia, PA: Saunders. p. 537-551.

SHERREY, J.H. AND MEGIRIAN, D. (1977). State dependence of upper airway motoneurones: functions of the cricothyroid and nasolabial muscles of the unanesthetized rat. *Electroencephalography Clin. Neuronphial.* **43(2):** 218-228.

SHERREY, J.H. AND MEGIRIAN, D. (1975). Analysis of the respiratory role of pharyngeal constrictor motoneurones of cat. *Exp. Neurol.* **49(3):** 839-851.

SHINGAI, E. (1980). Water fibbers in the superior laryngeal nere of the rat. *Jap. J. Physiol.* **30:** 305-307.

SHINGAI, E. (1977). Ionic mechanism of water receptors in the laryngeal nucosa of the rabbit. *Jap. J. Physiol.* **27:** 27-42.

SICA, A.L., STEELE, A.M., GANDHI, M.R. AND PRASAD, N. (1988). Factors affecting central inspiratory modulation of hypoglossal motoneuron activity in newborn pigs. *J. Dev. Physiol.* **10:** 285-295.

SICA, A.L., COHEN, M.I., DONNELLY, D.F. AND ZHANG, H. (1984). Hypoglossal motoneuron responses to pulmonary and superior laryngeal afferent inputs. *Respir. Physiol.* **56:** 339-357.

SICK, T.J., ROSENTHAL, M., LAMANNA, J.C. AND LUTZ, P.L. (1982). Brain potassium ion homeostasis, anoxia, and metabolic inhibition in turtles and rats. *Am. J.*

Physiol. **243:** R2281-2288.

SLINKER, B.K. AND GLANTZ, S.A. (1990). Missing data in two-way analysis of variance. *Am. J. Physiol.* **258(2Pt2):** R291-297.

SMIDER, R.S. AND NIEMER, W.T. (1961). A stereotaxic atlas of the cat brain. Univ. of Chicago Press, III.

SMITH, J.A, LI, F. AND NOBLE, R. (1993a). The effect of mild hypoxia on discharge frequency of hypoglossal motoneurones in anaesthetized vagotomized neonatal kittens. *J. Physiol.* **459:** 143P.

SMITH, J.A., LI, F. AND NOBLE, R. (1993b). Effect of isocapnic hypoxia on membrane potentials of hypoglossal motoneurones recorded in anaesthetized neonatal kittens. *J. Physiol.* **459:** 335.

SMITH, J.A., LI, F. AND NOBLE, R. (1993c). Evidence of inhibitory mechanisms mediating hypoglossal motoneuron response to mild hypoxia in anaesthetized neonatal kittens. *J. Physiol.* **473:** 62P.

SMITH, J.A. (1993). The effects of mild hypoxia on hypoglossal motoneurones in neonates. Ph.D thesis.

SPECKMAN, E.J. AND CASPERS, H. (1974). The effect of O_2- and CO_2- tensions in the nervous tissue on neuronal activity and DC potentials. In: Handbook of electroencephalography and clinical neurophysiology. Vol. 2, pp, 71-89. New York: Elsevier.

SPRUCE, A.C., STANDEN, N.B. AND STANFIELD, P.R. (1987). Studies on unitary properties of adenosine-5 triphosphate-regulated potassium channels of frog skeletal muscle. *J. Physiol. Lond.* **382:** 213-237.

ST.JOHN, W.M. AND ZHOU, D. (1992). Reductions of neural activities to upper airway muscles after elevations in static lung volume. *J. Appl. Physiol.* **73(2):** 701-707.

ST.JOHN, W.M. (1986). Influence of reticular mechanisms upon hypoglossal, trigeminal and phrenic activities. *Respir. Physiol.* **66:** 27-40.

ST. JOHN, W.M. AND WANG, S.C. (1976). Integration of chemoreceptor stimuli by caudal pontine and rostral medullary sites. *J. Appl. Physiol.* **41:** 612-622.

STOREY, A.T., AND JOHNSON, P. (1975). Laryngeal water receptors initiating apnea in the lamb. *Exp. Neurol.* **47:** 42-55.

STOTHERS, J.K. AND WARNER, R.M. (1978). Oxygen consumption and neonatal sleep states. *J. Physiol.* **278:** 435-440.

SULLIVAN, C.E. (1980). Breathing in sleep. In Physiology in Sleep. Edited by J. Orem, C.D.Barnes, pp. 213-272. New York: Academic.

SULLIVAN, C.E., MURPHY, E., KOZAR, L.F. AND PHILLIPSON, E.A. (1978). Waking andventilatory responses to laryngeal stimulation in sleeping dogs. *J. Appl. Physiol.* **45(5):** 681-689.

SUMI, T. (1969b). Synaptic potentials of hypoglossal motoneurones and their relation to reflex deglutition. Jap. *J. Physiol.* **19pp**: 68-79.

SUTTON, D., TAYLOR, E.M. AND LINDEMAN, R.C. (1978). Prolonged apnea in infant monkeys resulting from stimulation of superior laryngeal nerve. *Pediatrics,* **61**: 519-527.

SUZUE, T. (1984). Respiratory rhythm generation in the in vitro brainstem-spinal cord preparation of the neonatal rat. *J. Physiol.* (Lond.) **354:** 173-183.

SUZUKI, M. AND KIRCHNER, J.A. (1969). The posterior cricoarytenoid as an inspiratory muscle. *Ann. Otol. Rhinol Laryngol.* **78:** 849-864.

TABER, E. (1961). The cytoarchitecture of hypoglossal motoneurones by stimulation of jaw-opening muscle afferents. *J. Physiol. Soc. Jap.* **35:** 496-497.

TAKADA, M., ITOH, K., YASUI, Y., MITANI, A., NOMURA, S. AND MIZUNO, N. (1984). Distribution of premotor neurons for the hypoglossal nucleus in the cat. *Neurosci. Lett.* **52:** 141-146.

TANGEL, D.J., MEZZANOTTE, W.S. AND WHITE, D.P. (1991). Influence of sleep on tensor palatini EMG and upper airway resistance in normal men. *J. Appl. Physiol.* **70(6):** 2574-2581.

TARKHAN, A.A. (1936). Uber das vorhandensein afferenter fasern in nervus hypoglossus. *Arch. Psychiatr. (Berlin).* **105:** 475-483.

TCHOBROUTSKY, C., MERIET, C. AND REY, P. (1969). The diving reflex in rabbit, sheep and newborn lambs and its afferent pathways. *Respir. Physiol.* **8:** 108-117.

THACH, B.T. (1983). The role of pharyngeal airway obstruction in prolonging infantile apneic spells. In Sudden Infant Death Syndrome. Edited by Tilden, J. Roeder, L. M. and Steinschneider, A. pp: 279-292. New York: Academic.

TIMMS, R.J. (1982). Central blocking action of ketamine anaesthesia on the visceral alerting and chemoreceptor reflex responses in the cat. *Plugers Arch. Eur. J. Physiol.* **394(1):** 12-15.

UKABAM, C.U., KNUTH, S.L. AND BARTLETT, D. Jr. (1992). Phrenic and

hypoglossal neural responses to cold airflow in the upper airway. *Respir. Physiol.* **87(2):** 157-164.

VALDES-DAPENA, M. AND STEINSCHNEIDER, A. (1983). Sudden infant death syndrome (SIDS), apnea, and near miss for SIDS. (Review). *Emerg. Med. Clin. N. Am.* **1(1):** 27-44.

VALDES-DAPENA, M. (1981). Latrogenic disease in the perinatal period as seen by the pathologist. *Monographs Pathol.* **22:** 382-418.

VAN DER HAL, A.L., RODRIGUEZ, A.M., ARGENT, C.W., PLATZKER, A.C. AND KEENS, T.G. (1985). Hypoxia and hypercapneic arousal responses and prediction of subsequent apnea in apnea of infancy. *Pediatrics,* **75(5):** 848-854.

VAN LUNTEREN, E. AND DICK, T.E. (1992). Intrinsic properties of pharyngeal and diaphragmatic respiratory motoneurones and muscles. *J. Appl. Physiol.* **73(3):** 787-800.

VAN LUNTEREN, E., SALOMONE, R.J., MANUBAY, P., SUPINSKI, G.S. AND DICK, T.E. (1990). Contractile and endurance properties of geniohyiod and diaphragm muscles. *J. Appl. Physiol.* **69:** 1992-1997.

VAN LUNTEREN, E. MARTIN, R.J., HAXHIU, M.A. AND CARLO, W.A. (1989). Diaphragm, genioglossus, and traingularis sterni responses to piokilocapnic hypoxia. *J. Appl. Physiol.* **67(6):** 2303-2310.

VAN LUNTEREN, E. AND STROHL, R.J. (1986). The muscles of the upper airways. *Clin. Chest. Med.* **7:** 171-188.

VAN LUNTEREN, E., VAN DE GRAAF, W.B., PARKER,D.M., MITRA, J., HAXHIU, M.A., STROHL, K.P. AND CHERNIACK, N.S. (1984). Nasal and

laryngeal reflex responses to negative upper airway pressure. *J. Appl. Physiol.* **56**: 746-752.

VIANA, F., BAYLISS, D.A. AND BERGER, A.J. (1993). Calcium conductances and their role in the firing behaviour of neonatal rat hypoglossal motoneurones. *J. Neurophysiol.* **69(6):** 2137-2149.

VIANA, F., GIBBS, L. AND BERGER, A.J. (1990). Double- and triple- labelling of functionally characterized central neurons projecting to peripheral targets studied in vitro. *Neuronscience,* **38(3):** 829-841.

WAITES, B.A., ACKLAND, G.L., NOBLE, R. AND M.A. HANSON (1995). Microinjection of L-glutamate in the red nucleus produces inhibition of respiratory output in decerebrate neonatal rabbits. *J. Physiol.* **489P:** 56p.

WASICKO, M.J.J., GIERING, R.W., KNUTH, S.L. AND LEITER, J.C. (1993). Hypoglossal and phrenic nerve responses to carotid baroreceptor stimulation. *J. Appl. Physiol.* **75(3):** 1395-1403.

WATCHKO, J.F., KLESH, K.W., O'DAY, T.L., WEISS, M.G. AND GUTHRIE, R. (1989). Genioglossus recruitment during acute hypoxia and hypercapnia in kittens. *Pediatr. Dulmonol.* **7**: 235-243.

WEINER, D., MITRA, J., SALAMONE, J. AND CHERNIACK, S. (1982). Effect of chemical stimuli on nerves supplying upper airway muscles. *J. Appl. Physiol.* **52(2):** 530-536.

WEINER, D., MITRA, J., SALAMONE, J., NOCHOMOVITZ, M. AND CHERNIACK, N.S. (1980). Effect of chemical drive on the electrical activity and force of contraction of the tongue and chest wall muscles. *Am. Rev. Respir. Dis.* **121(418):** 418-419.

WEIR, E.K. AND ARCHER, S.L. (1995). The mechanism of acute hypoxic pulmonary vasoconstriction: the tale of two channels. (Review). *FASEB Journal,* **9(2):** 183-189.

WEISS, J.N. AND LAMP, S.P. (1987). Glycolysis preferentially inhibits ATP-sensitive K^+ channels in isolated guinea pig cardiac myocytes. *Science Wash. DC* **238:** 67-69.

WHITWAN, J.G., KIDD, C. AND FUSSEY, I.F. (1969). Activity in efferent sympathetic nerves evoked by stimulation of the hypoglossal nerve. *Brain Res.* **14:** 756-758.

WIDDICOMBE, J.G., SANT'AMBROGIO, G. AND MATHEW. O.P. (1988). Nerve receptors of the upper airway. In: Respiratory Function of the Upper Airway. Edited by O.P. Mathew and G. Sant'Ambrogio. New York: Dekker. p. 193-231.

WIEGAND, L., ZWILLICH, C.W., WIEGAND, D. AND WHITE, D.P. (1991). Changes in upper airway muscle activation and ventilation during phasic REM sleep in normal man. *J. Appl. Physiol.* **71(2):** 488-497.

WILLIAMS, B.A. AND HANSON, M.A. (1989). The effect of decerebration and brain-stem transection on the ventilatory response to acute hypoxia in normoxic and chronically hypoxic newborn rats. *J. Physiol.* **414:** 25P.

WITHINGTON-WRAY, D.J., MIFFLIN, S.W. AND SPYER, K.M. (1988). Intracellular analysis of respiratory-modulated hypoglossal motoneurones in the cat. *Neuroscience,* **25(3):** 1041-1051.

WOODALL, D.L., HOKANSON, J.A. AND MATHEW, O.P. (1989). Time of application of negative pressure pulses and upper airway muscle activity. *J. Appl. Physiol.* **67(1):** 366-370.

WOODRUM, D.E., STANDAERT, T.A., MAYOCK, D.E. AND GUTHRIE, R.D. (1981). Hypoxic ventilatory response in the newborn monkey. *Paediatr. Res.* **15:** 367-370.

WYKE, B.D. AND KIRCHNER, J.A. (1976). Neurology of the larynx. In: Scientific Foundations of Otolaryngology. ed, R. Hindheliffe and Harrison, D. Chicago: Heinemann, chapt, 40, pp. 546-574.

ZHANG, S. AND MATHEW, O.P. (1992). Decrease in lung volume-related feedback enhances laryngeal reflexes to negative pressure. *J. Appl. Physiol.* **73(3):** 832-836.