THE EFFECTS OF MILD HYPOXIA ON HYPOGLOSSAL MOTONEURONES IN NEONATES

A thesis submitted for the degree of

Doctor of Philosophy

of the

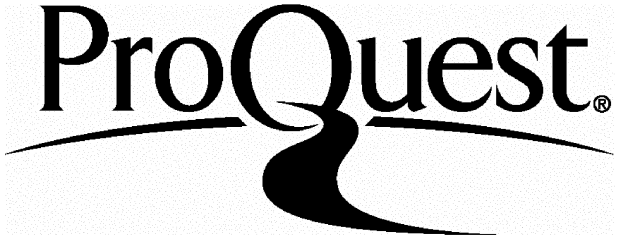
University of London

by

Julie Ann Smith

Departments of Physiology and Obstetrics & Gynaecology
Faculties of Science and Clinical Sciences
University College London

Submitted September 1993



This thesis is dedicated with love to Nic,
for his patience and understanding,
and to
my parents, who
have given me
support and
encouragement
throughout my education.

ABSTRACT

The patency of the upper airway is dependent on the activity of the genioglossus muscle, the main protrusor muscle of the tongue. The force generated by this muscle opposes the negative intraluminal pressure produced by the contraction of the diaphragm during inspiration. Recent studies suggest that there is an immaturity in genioglossus muscle control in neonates and obstructive apnoea may occur when the activity of this muscle is reduced or absent without a corresponding decrease in the activity of the diaphragm. However, little is known of the processes mediating and influencing the activity of the hypoglossal nerve, the motor nerve of the genioglossus muscle, at this stage in development.

In newborn babies, central apnoea (when there is no inspiratory effort) is usually followed by obstructive apnoea (when although there is inspiratory effort there is no inspiratory flow). It is therefore possible that hypoxia which develops during central apnoea, inhibits the activity of the genioglossus muscle and as a consequence the airway becomes obstructed.

The aim of this study was therefore to determine whether hypoglossal motoneurones are inhibited during hypoxia in neonates.

This study has investigated the effect of mild levels of hypoxaemia (PaO_2 47.2 ± 3.8mmHg) on the activity of hypoglossal motoneurones in anaesthetized neonatal kittens (≤ 27 days old).

The results showed that the majority of hypoglossal motoneurones increased in

discharge frequency during hypoxia but for a substantial proportion the increase was only transient. Furthermore, some motoneurones showed a decrease in discharge frequency. Intracellular recordings showed that during similar levels of hypoxia, although a large proportion of the motoneurones were depolarized, at least some of these repolarized despite the continuing hypoxia. In addition, some hypoglossal motoneurones were hyperpolarized.

This is the clearest evidence that inhibitory mechanisms, in addition to excitatory mechanisms, mediate the effects of hypoxia on hypoglossal output in neonates. Furthermore, the results suggest that hypoxia has an effect on the hypoglossal motoneurones independently of, or in addition to, its effect through respiratory rhythm. In some preliminary studies, the transmembrane input resistance increased during the hyperpolarization in response to hypoxia. One possibility is that the inhibition is mediated by the removal of an excitatory input.

If the inhibition found in this study occurs in human babies it may be a compounding factor in apnoeas of the newborn.

TABLE OF CONTENTS

SECTION 2

CHAPTER 2 MATERIALS AND METHODS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

This thesis has been the most difficult project I have set out to achieve. At different times, and in different ways, I have needed and received help from numerous friends and colleagues; without this support the task would have been impossible.

I am first grateful to both Professor Woledge and Professor Rodeck, the heads of Physiology and of Obstetrics and Gynaecology, respectively, for allowing me to do the Ph.D within their departments. My Ph.D was funded by the Medical Research Council, and the project by The Wellcome Trust.

In particular I would like to thank:-

Dr.Ray Noble
For introducing me to such an interesting and challenging area of physiology, and for the constant encouragement and support he has given me throughout the course of the PhD.

Dr Laura Bennet & Professor Mark Hanson
For reading this thesis and giving me such valid criticism. I would particularly like to thank Laura for her help and advice with the surgery and her friendship throughout the last four years.

Fei
For her help in the lab and her company during the long experiments.

Nicole, Clare and Lucy
For giving up so much of their time with helping in the production of this thesis and

especially for making me laugh over the last few months. I would particularly like to thank Nicole, without her help this thesis would still be a pile of untidy papers.

Dr Peter Moore

For the help with the computers and also for the photographs shown in the thesis.

Dino and Gareth

For keeping me constantly amused and being good office companions.

I would also like to thank Bridget, Jeff and Taka for their companionship at various times through the Ph.D, not to mention the support and friendship of Doreen, Leonie, Jinette, Mary and Irene.

Outside of the department I would like to thank;

Nan Davis for her concern and encouragement, and Jenny and Julian Chaudhuri for all their helpful advice. I would also like to thank my family and Mr and Mrs Wilkinson for all their love and support.

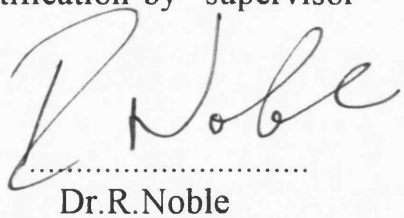
And finally, I must thank Nic for doing so much over the last six months particularly for all his love and attention. His determination, as much as my own, made this thesis possible.

PERSONAL STATEMENT

Except as acknowledged on page 25 and 26, the work in this thesis was performed solely by the candidate and is original.

Julie A. Smith

Certification by supervisor



Dr.R.Noble
(supervisor)

ABBREVIATIONS

ATP	Adenosine triphosphate
Ca^{2+}	Calcium ions
Cl^-	Chloride ions
CO_2	Carbon dioxide
CPAP	Continual positive airway pressure
EMG	Electromyogram
EPSP	Excitatory postsynaptic potential
$P_{ET}CO_2$	End -tidal carbon dioxide
FiO_2	Fractional inspired oxygen
IPSP	Inhibitory postsynaptic potential
K^+	Potassium ions
N_2	Nitrogen
Na^+	Sodium ions
O_2	Oxygen
$PaCO_2$	Arterial partial pressure of carbon dioxide
PaO_2	Arterial partial pressure of oxygen
PEEP	Positive end -expiratory pressure
PIP	Peak inspiratory pressure
PO_2	Tissue oxygen content
REM	Rapid eye movement
SaO_2	Haemoglobin saturation
SO_2	Sulphur dioxide
TEA	Tetraethylammonium
TTX	Tetrodotoxin

1.INTRODUCTION

OVERALL VIEW OF THE INTRODUCTION

The introduction to this thesis has been divided into three sections:

Section 1. presents the evidence that the genioglossus muscle is involved in maintaining the patency of the upper airway. It provides a general background to the activity of the genioglossus muscle and hypoglossal (XIIth cranial nerve); the relationship between the activity of this upper airway muscle and that of the diaphragm is also considered.

Section 2. describes in detail various afferent pathways which influence the activity of the genioglossus muscle and its relationship to the diaphragm. The influence of these factors may have important consequences on the patency of the upper airway.

Section 3. describes the few experiments which have studied the activity of the genioglossus muscle in the neonate. The reasons for the present study have been described in more detail.

1. INTRODUCTION *(SECTION 1.)*

1.1.1 General background

Newborn babies, particularly premature babies, have short periods of time when they stop breathing. These apnoeic incidences, i.e the cessation of airflow for $\geq$ 20seconds, are classified as either central, obstructive or mixed (Mathew, Roberts & Thach, 1982c, Marchal, Bairam & Vert, 1987). Central apnoea is when there is a lack of inspiratory effort and obstructive apnoea occurs when although there is inspiratory effort the airway is obstructed. Mixed apnoea is a combination of the two. Obstructive and mixed apnoeas account for as much as 50% of apnoeas (for references see Mathew et al., 1982c, Marchal et al., 1987).

There is some controversy in the literature as to where in the upper airway occlusion occurs. Airway closure has been reported in the naso-, oro- and hypopharynx and also in the larynx (Reed, Roberts & Thach, 1985). However, in human infants obstructive apnoea is thought to occur mainly at the level of the oropharynx (Reed et al., 1985, Roberts, Reed, Mathew, Menon, Thach, 1985). During inspiration the diaphragm contracts creating a negative intraluminal pressure in the upper airway which, unless adequately opposed, sucks the tongue back against the pharyngeal wall leading to airway closure.

1.1.2 Evidence for neuromuscular mechanisms in the maintenance of upper airway patency

There is now substantial evidence that neuromuscular mechanisms provide airway stability and help in maintaining a patent upper airway during inspiration.

When functional neuromuscular mechanisms are absent, for example in infants studied at postmortem, and the transmural pressure in the upper airway is reduced there is an inward movement of the anterior, posterior and lateral walls (Reed et al., 1985). Measurement of the pressure at which the upper airway closes (upper airway closing pressure) has been used in a number of studies as a measure of upper airway stability (Brouillette & Thach, 1979, Wilson, Thach, Brouillette, Abu-Osba, 1980, Reed et al., 1985, Roberts et al., 1985, Roberts, Reed, Mathew & Thach, 1986, Cohen & Henderson-Smart, 1986 & 1989, Strohl, Wolin, Van Lunteren & Fouke, 1987). In rabbits during occluded inspirations the airway remains patent at intraluminal pressure levels as low as -80cm H_2O. In contrast, closure of the upper airway occurs in animals studied at postmortem at relatively small negative pressures (-6cm H_2O) (Brouillette & Thach, 1979). Similar observations have been reported in newborn infants (Wilson et al., 1980, Reed et al., 1985, Cohen & Henderson-Smart, 1986). These studies provide evidence for the existence of neuromuscular mechanisms which maintain the patency of the extrathoracic airway by opposing negative intraluminal pressure.

Further evidence for such neuromuscular mechanisms is provided by studies investigating the effect of head position on airway patency. In anaesthetized patients and infants studied at postmortem, flexion of the neck (chin towards chest) leads to an obstruction of the upper airway by pushing the tongue against the posterior pharyngeal wall, (Safar, Escarraga & Chang, 1959, Reed et al., 1985). In this position there is an increase in the airway closing pressure and in some cases a positive inflation pressure is actually required to keep the airway open (Wilson et al., 1980, Reed et al., 1985). In contrast, in unanaesthetized adults the airway stability is maintained or actually increased during neck flexion (Shelton & Bosma, 1962). In addition, the airway closing pressure becomes progressively more negative over a number of breaths during either neck flexion or nasal-mask occlusion in both normal and micrognathic infants indicating that there is an improvement in the stability of the

airway (Stark & Thach, 1981, Roberts et al., 1985). The difference between the studies in the anaesthetized and unanaesthetized adult, and the improvement in airway stability in the infant support the hypothesis that there are neuromuscular mechanisms which are initiated during increased negative pressure (Roberts et al., 1985, Stark & Thach, 1981).

1.1.3 Is the genioglossus muscle involved in the maintenance of airway patency?

Several lines of investigation have suggested that the genioglossus muscle plays an important role in maintaining upper airway patency.

The genioglossus muscle is the main protrudor muscle of the tongue. The isometric force of the tongue is proportional to the activity of this muscle (Weiner, Mitra, Salamone, Nochomovitz & Cherniack, 1980). As shown in figure 1, the genioglossus is a fan-shaped muscle that originates on the anterior portion of the mandible and widens out as it extends backward into the tongue (Sauerland & Mitchell, 1975, Lowe, 1981, Van Lunteren & Strohl, 1986). The other extrinsic muscles, i.e the hyoglossus and styloglossus, act primarily as retractors (Sauerland & Mitchell, 1975, Lowe, 1981).

Recent investigations have indicated that the oropharyngeal airway in infants studied at postmortem is enlarged if tension is applied to the genioglossus and geniohyoid tongue muscles, muscles which displace the tongue and hyoid bone (Reed et al., 1985). In addition, the peak integrated electromyographic (EMG) activity of these muscles increase during either nasal occlusion or neck flexion studies in infants and anaesthetized animals (Sauerland & Mitchell, 1975, Brouillette & Thach, 1979, Bonora, Bartlett & Knuth, 1985a, Roberts et al., 1986, Gauda, Miller, Carlo, Difiore, Johnsen & Martin., 1987, Cohen & Henderson-Smart, 1989). Furthermore, the EMG

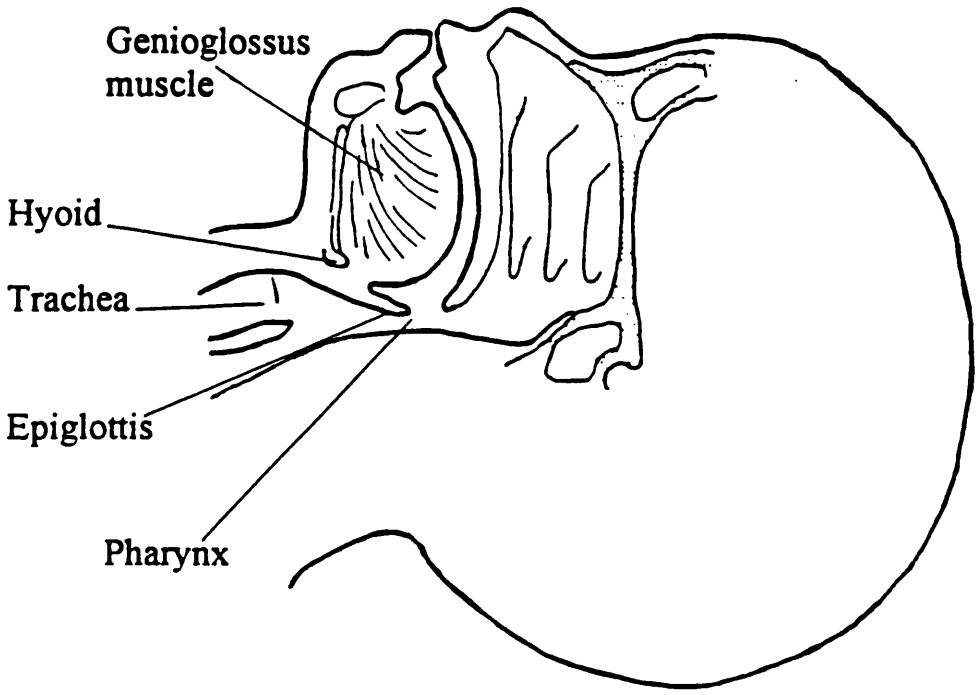


Figure 1. A sketch showing the anatomy of the upper airway in the human neonate. The genioglossus, the main protrudor muscle of the tongue, is fan-shaped muscle that originates in the anterior portion of the mandible and widens out as it extends backward into the tongue.

activity is linearly related to the airway closing pressure (Brouillette & Thach, 1979., Roberts et al., 1986). Electrical stimulation of the genioglossus muscle is found to decrease upper airway resistance with the upper airway becoming dilated and the closing pressure increased (Strohl et al., 1987, Miki, Hida, Shindoh, Kikuchi, Chonan, Taguchi, Inoue & Takishima, 1989b). The genioglossus muscle is therefore believed to play a fundamental role in maintaining the patency and improving the stability of the upper airway (Remmers, DeGroot, Sauerland & Anch, 1978, Brouillette & Thach, 1979, Roberts et al., 1986, Strohl et al., 1987).

Obstruction at the level of the pharynx is believed to be a consequence of a decrease in upper airway muscle tone (Remmers et al., 1978). The contraction of the genioglossus and geniohyoid muscles, in addition to maintaining extrathoracic patency in the face of negative intraluminal pressure, act to re-establish the patency of the oropharyngeal airway following obstruction. A collapsed pharyngeal airway can be re-opened in infants studied at post mortem if tension is applied to the genioglossus and geniohyoid muscles (Reed et al., 1985). Furthermore, periodic airway occlusions, either induced or occurring spontaneously in animals (Brouillette & Thach, 1979), humans suffering from sleep apnoea (Remmers et al., 1978) and in micrognathic infants (Roberts et al., 1986) are alleviated in conjunction with an increase in respiratory - related genioglossus EMG activity. With nasal obstruction in both lambs and adult sheep, inspiratory-related genioglossus activity reaches its peak just prior to the onset of oral breathing (Harding, Buttress, Caddy & Wood, 1987). In addition, electrical stimulation of this muscle reduces the incidence and duration of apnoeic episodes in patients suffering from obstructive sleep apnoea (Miki, Hida, Chonan, Kikuchi & Takishima, 1989a, Miki et al.,1989b).

1.1.4 The activity of the genioglossus muscle

The contraction of the genioglossus muscle increases the oropharyngeal volume by thrusting the tongue forward, drawing it away from the posterior pharyngeal wall (Lowe, 1981).

During each breath the contraction of the genioglossus begins either before or synchronous with the onset of the diaphragm contraction (Brouillette & Thach, 1980, Haxhiu, Van Lunteren, Mitra & Cherniack, 1984, Van Lunteren, Van de Graff, Parker, Mitra, Haxhiu, Strohl & Cherniack, 1984c, Agostoni, Cavagna & Citterio, 1987). As a result, maximum airway patency is obtained while the contraction of the diaphragm is still relatively small. Phasic genioglossus activity therefore plays a crucial role in maintaining a patent pharyngeal airway and allows the diaphragm to draw air into the lungs (Brouillette & Thach, 1980). It is also believed that the force generated by the activity of the genioglossus muscle prevents pharyngeal collapse by opposing the negative intraluminal pressure generated by the contraction of the diaphragm (Brouillette & Thach, 1980, Weiner et al., 1980). The upper airway patency is therefore dependent on a balance between the upper airway muscles and those of the chestwall. It also follows that an increase in negative pharyngeal pressure exceeding the force generated by the genioglossus muscle will promote airway occlusion (Remmers et al., 1978, Brouillette & Thach 1980, Weiner et al., 1980). This may occur under certain conditions such as anaesthesia or sleep which has been shown to depress the recruitment of the genioglossus and geniohyoid muscles (Brouillette & Thach, 1979, Mezzanotte, Tangel & White, 1992).

Electromyographic activity is used as an indication of the activity and performance of a muscle. The EMG activity of the genioglossus muscle is characterized by a continuous tonic discharge with a greater over-lying phasic activity (Sauerland &

Mitchell, 1975, Sauerland & Harper, 1976). The phasic activity, if present, is respiratory related. In cats (Haxhiu et al., 1984), rabbits (Brouillette & Thach, 1979 & 1980) and adult humans (Sauerland & Harper, 1976, Önal, Lopata & O'Connor, 1981) respiratory-related genioglossus muscle activity has been shown to coincide with inspiration and its pattern of activity closely follows inspiratory flow. In adult humans the activity of this muscle also extends into expiration (Sauerland & Mitchell, 1975).

In some reports the respiratory-related activity of the genioglossal muscle or its cranial nerve (the hypoglossal nerve) were recorded during normocapnic normoxia (Brouillette & Thach, 1980, Hwang, St.John & Bartlett, 1983b) whereas in other reports this pattern of activity only became apparent during an increase in chemical drive or resistive loading (Weiner, Mitra, Salamone & Cherniack, 1982, Haxhui et al., 1984, Harding et al., 1987, Parisi, Neubauer, Frank, Edelman & Santiago, 1987, Parisi, Santiago & Edelman, 1988, Weigand, Zwillich & White, 1989, Wood & Harding, 1989, Ukabam, Knuth & Bartlett, 1992). The discrepancies between the various studies may be a result of the experimental procedures used. For example, it is clear that the activity of the genioglossus and its control is highly dependent on head position (Bonora et al., 1985a, Roberts et al., 1986) and anaesthesia (Brouillette & Thach, 1979, Hwang et al., 1983b, St. John & Bledsoe, 1985, see section 1.2.5) .

1.1.5 The hypoglossal nerve

The motor nerve innervating the tongue is the hypoglossal nerve, the XIIth cranial nerve (Brodal, 1981, Lowe, 1981, Van Lunteren & Dick, 1992). This nerve passes between the mylohyoid and hyoglossus muscles before dividing into two branches. The smaller lateral branch innervates the hyoglossus, styloglossus and the inferior longitudinal muscles, whereas the larger medial branch supplies the genioglossus, transverse, and vertical and superior longitudinal muscles (Lowe, 1981). The

hypoglossal nerve also innervates the geniohyoid, sternohyoid and sternothyroid muscles (Van Lunteren & Dick, 1992). The functional significance of whole nerve recordings are therefore complicated by the fact that its nerve fibres supply a range of both intrinsic and extrinsic muscles.

1.1.6 Respiratory-related activity of the hypoglossal nerve

As expected from the recordings of the genioglossus EMG, there is a phasic increase in the discharge frequency of the hypoglossal nerve which coincides with the inspiratory phase of the respiratory cycle (Weiner et al., 1982). In addition, recent intracellular studies have shown that just over 50% of hypoglossal motoneurones recorded either discharge action potentials in rhythmic bursts and/or show a depolarization of the membrane potential which coincides with the central respiratory rhythm (Withington-Wray, Mifflin & Spyer, 1988, Mifflin, 1990, Jiang, Mitchell & Lipski, 1991). The majority of these motoneurones can be classified as inspiratory i.e the onset and cessation of activity coincides with the phasic activity of the phrenic nerve (Hwang, Bartlett & St.John, 1983a, Sica, Cohen, Donnelly & Zhang, 1984, Withington-Wray et al., 1988, Mifflin, 1990, Van Lunteren & Dick, 1992) and towards the end of inspiration these motoneurones are inhibited (Withington-Wray et al., 1988). During expiration the membrane is repolarized but unlike the phrenic motoneurones there is no inhibition (Withington-Wray et al., 1988).

Some motoneurones show a pattern of discharge which is in phase with inspiration but also extends into the early (stage 1) phase of expiration (Hwang et al., 1983a, Mitra & Cherniack, 1983); these are classified as inspiratory - expiratory (I-E) units. In some of these fibres the discharge frequency ceases briefly at the I-E junction (Hwang et al., 1983a, Mitra & Cherniack, 1983).

Although rare and sometimes only recorded during high levels of chemical drive, a few motoneurones display a depolarization or an increase in discharge frequency during expiration (Sumi, 1969b, Hwang et al., 1983a, Withington - Wray et al., 1988, Mifflin, 1990). The depolarization or pattern of discharge of these motoneurones either reaches its peak during the postinspiratory or stage I expiratory phase of the respiratory cycle or remains relatively constant throughout stage I and stage II of expiration. In addition to these patterns of discharge, a small number of motoneurones also display a random pattern of activity with no obvious phasic discharge (Mitra & Cherniack, 1983).

The different phases of activity described above may depend upon the muscles which the hypoglossal motoneurones innervate (see section 1.1.5). This seems unlikely because the majority of the hypoglossal motoneurones recorded by Withington-Wray et al. (1988), regardless of their phase of activation, were located in the ventromedial division of the hypoglossal nucleus. Retrograde labelling with horseradish peroxidase has revealed that although there are slight variations in species the motoneurones in this area generally supply the genioglossus muscle (Uemura-Sumi, Itoh & Mizuno, 1988). Thus it is most likely that the respiratory - related hypoglossal motoneurones described above innervate the genioglossus muscle.

It has been suggested that the phase of respiration in which the hypoglossal motoneurones are active may depend upon the ongoing activity of afferent inputs other than respiratory drive (Hwang et al., 1983a, Withington-Wray et al., 1988). Hwang and colleagues (19833a) found that there were a few hypoglossal motoneurones in adult cats which, during extreme hypercapnia or hypoxia, altered their discharge from an inspiratory pattern of discharge to an inspiratory-expiratory pattern or even a tonic pattern of discharge (see section 1.2.2). These alterations in the phase of discharge during hypercapnia were not, however, recorded in the later study by Withington-Wray

and colleagues (1988). However, in this study the discharge pattern of the inspiratory-related hypoglossal motoneurones could be extended into post-inspiration when the superior laryngeal nerve was stimulated (see section 1.2.3.vi). So although the motoneurones have been classified above according to their activity in relation to the phrenic nerve discharge, this may change.

1.1.7 The hypoglossal nucleus

The distribution of the hypoglossal motoneurones has been determined in a number of species with the use of retrograde axonal transport of horseradish peroxidase (Uemura-Sumi et al., 1988). As previously mentioned there are variations between species, but in general the motoneurones which are carried in the medial branch of the nerve, such as those innervating the genioglossus and geniohyoid muscles, lie in the ventral medial aspect of the nucleus, whereas those carried in the lateral branch, for example the motoneurones innervating the hyoglossus and styloglossus, are located in the dorsal lateral aspect of the nucleus (Uemura-Sumi et al., 1988).

Anatomically, two types of hypoglossal neurones have been identified (Green & Negishi, 1963, Cooper, 1981, Lowe, 1981, Mosfeldt Laursen & Rekling, 1989). The more ventral group are larger than the dorsal group, are multipolar and have numerous dendrites which extend beyond the boundary of the nucleus; these are believed to be motoneurones (Green & Negishi, 1963, Cooper, 1981, Lowe, 1981, Mosfeldt Laursen & Rekling, 1989). The dendrites of these motoneurones are known to spread across the midline into the contralateral hypoglossal nucleus or into the adjacent reticular formation (Cooper, 1981). The second type of neurone have fusiform somata with dendrites that extend only within the boundary of the nucleus and are therefore believed to be interneurones (Green & Negishi, 1963, Cooper, 1981, Lowe, 1981, Mosfeldt Laursen & Rekling, 1989).

In the *in vitro* guinea pig brain slice preparation the two types of cells are distinguished by their electrophysiological properties. The motoneurones have maximal discharge frequencies of 90Hz, resting membrane potentials of approximately -63mV and after-hyperpolarizations with only a single slow phase of hyperpolarization (Mosfeldt Laursen & Rekling, 1989). In contrast, the interneurones are spontaneously active have a maximal discharge frequency of 250 Hz and after-hyperpolarizations with an initial fast phase of hyperpolarization followed by a slow phase (Mosfeldt Laursen & Rekling, 1989).

1.1.8 Electrophysiological properties of hypoglossal motoneurones recorded *in vitro*

Despite the importance of the hypoglossal motoneurones in the maintenance of upper airway stability, there have been relatively few studies on these motoneurones in the intact animal. In recent years, a number of studies have used the *in vitro* brain slice preparation to investigate the membrane properties of hypoglossal motoneurones (Mosfeldt Laursen & Rekling, 1989, Haddad, Donnelly & Getting, 1990, Haddad & Donnelly, 1990, Jiang & Haddad, 1991, Jiang, Agulian & Haddad, 1992a, Jiang, Xia & Haddad, 1992b). These preparations allow a relatively stable recording and control over the perfusion medium (Anderson, 1981).

An understanding of the electrophysiological properties is important when determining the input-output relationship of the motoneurones. As may be expected the action potentials, elicited by short depolarizing current pulses, are generated by a tetrodotoxin (TTX)-sensitive Na^+ current and repolarized by a tetraethylammonium (TEA)-sensitive K^+ current (Mosfeldt Laursen & Rekling, 1989, Haddad et al., 1990). However, in rat hypoglossal motoneurones there is no evidence for the involvement of a Ca^{2+} current in the action potential; Ca^{2+} blockers did not affect the duration of the action potential

or its after-hyperpolarization (Haddad & Donelly, 1990). In contrast, depolarizing currents evoked high threshold calcium spikes in guinea-pig hypoglossal motoneurones in a similar preparation (Mosfeldt Laursen & Rekling, 1989).

The action potentials generated in the hypoglossal motoneurones have clear after-hyperpolarizations. In guinea pigs, the slow phase of the after-hyperpolarization is produced by a Ca^{2+}-dependent K^+ current; the fast phase involves the delayed rectifier (Mosfeldt Laursen & Rekling, 1989). The after-hyperpolarizations are considerably shorter in the hypoglossal motoneurones (35.2ms in the guinea pig and 31ms in the rat) in comparison to the after-hyperpolarizations recorded in dorsal vagal motoneurones (up to 3 secs in guinea pigs) (Yarom, Sigimori & Llinás, 1985, Mosfeldt Laursen & Rekling, 1989, Haddad et al., 1990).

The resting membrane potential differs depending upon the species; in the rat the mean resting membrane potential was -80mV (Haddad et al., 1990) and in the guinea pig it was -63mV (Mosfeldt Laursen & Rekling, 1989). However, both studies showed inward rectification (anomolous rectification), a time dependent increase in conductance with hyperpolarization (Mosfeldt Laursen & Rekling, 1989, Haddad et al., 1990, Van Lunteren & Dick, 1992). In guinea pigs the inward rectification is composed of Ca^{2+} and K^+ currents (Mosfeldt Laursen & Rekling, 1989). These motoneurones also reveal a "sag" during hyperpolarizing and depolarizing pulses due to a caesium-sensitive K^+ current (Mosfeldt Laursen & Rekling, 1989). Hypoglossal motoneurones, in contrast to the dorsal vagal motoneurones and neurones in the ventral and ventrolateral region of the nucleus tractus solitarius (v-NTS), do not possess an A current (Yarom et al., 1985, Haddad & Getting, 1989, Haddad et al., 1990). This is determined by the absence of delayed excitation when the membrane is depolarized following a hyperpolarization. The spike frequency adaption (i.e the slowing of the frequency with time) is also considerably less in the hypoglossal

motoneurones; the spike frequency adaption index (steady state firing divided by peak activity X 100) is 70-90% in hypoglossal motoneurones in comparison with 40% in v-NTS neurons (Haddad & Getting, 1989, Haddad et al., 1990).

The absence of the delayed excitation and the presence of inward rectification will increase the excitability of the hypoglossal motoneurones at the beginning of depolarization. The low level of adaption will also help maintain the excitability of these motoneurones and their response to an excitatory synaptic input throughout a prolonged stimulus time such as inspiration.

1.1.9 The activity of the genioglossus muscle and hypoglossal nerve in relation to ventilation

From a functional point of view it is important that the upper airway muscles contract prior to the contraction of the diaphragm. Indeed this functional demand is reflected in the difference between the inspiratory-related genioglossus EMG and diaphragm patterns (Önal et al., 1981, Patrick, Strohl, Rubin & Altose, 1982, Haxhiu et al., 1984). The inspiratory activity of the diaphragm gradually rises, reaches its peak towards the end of inspiration and then rapidly declines. In contrast the inspiratory activity of the genioglossus muscle increases abruptly, reaches its peak activity early in inspiration and then maintains a plateau throughout before rapidly decreasing. The activity of the two muscles correspond with the different inspiratory discharge patterns displayed by their respective nerves; the phrenic activity augments throughout inspiration until it reaches a peak towards the end, whereas the activity of the hypoglossal nerve rapidly reaches its peak and then gently decreases for the remainder of inspiration (Hwang et al., 1983b, Sica et al., 1984). Although there is a subsequent decrease in hypoglossal discharge frequency the upper airway patency is still maintained. It has been suggested that this is because there there is enough residual genioglossus activity to maintain airway patency (Sica et al., 1984). However, other upper airway muscles, for example

the posterior cricoarytenoid and sternohyoid muscles, also have respiratory-related activity which may help protect the airway from collapsing (Strohl, Wolin, Van Lunteren & Fouke, 1987).

It was proposed (Önal et al., 1981) and later confirmed (Hwang et al., 1983a) that the different inspiratory discharge patterns of the genioglossus muscle and diaphragm are due to the different firing and recruitment patterns of the two motoneurone pools. The majority of phrenic motoneurones are active during normocapnic hyperoxia and can be divided into two neuronal groups differing in their onset time during inspiration. The discharge pattern of both groups of phrenic motoneurones increases gradually reaching a maximum at the end of inspiration (St. John & Bartlett, 1979). In contrast, the majority of hypoglossal motoneurones are only recruited during hypercapnia or hypoxia, and once recruited the discharge frequency rises abruptly to peak levels (Hwang et al., 1983a, Mitra & Cherniack, 1983). Thus, in contrast to the activity of the phrenic motoneurones, the increase in hypoglossal nerve activity associated with chemical stimulation is a consequence of both an increase in discharge frequency and a recruitment of hypoglossal motoneurones which had previously been silent (Hwang et al., 1983a).

A number of studies have revealed that the onset of the hypoglossal discharge is earlier than that of the phrenic (Weiner et al., 1982, Fukuda & Honda, 1982a & b, Sica et al., 1984). However, in contrast to these studies Hwang and colleagues (1983a) were only able to observe an earlier onset of the phasic hypoglossal burst with an increase in ventilatory drive. This discrepancy may be due to variations in methodology, for example the use of anaesthesia (see section 1.2.5) or decerebration (section 1.2.2.viii).

In contrast to recordings of the whole nerve, the onset of single fibre inspiratory burst

activity is variable sometimes preceding and sometimes following the phrenic nerve onset (Sica et al., 1984). This is presumably due to the fact that the activity of the individual fibres summate to produce the discharge of the whole nerve.

Two explanations have been proposed for the difference in the discharge patterns of the phrenic and hypoglossal nerves and single fibres (Hwang et al., 1983a & b, Sica et al., 1984). This may be due to differences in the sensitivity of the two motoneurone pools to a common input, or alternatively specific afferent inputs to the phrenic or hypoglossal motoneurone pools may exist which reflect the different functions of the respective muscles under control.

The immediate and rapid increase in firing rate of the hypoglossal motoneurones together with the slower recruitment of those of the phrenic is appropriate for maintaining airway patency prior to the onset and throughout the course of inspiration. An imbalance in the activity of the cranial nerves supplying the upper airway in comparison to the phrenic nerve activity, in onset, pattern or amplitude could potentially affect the forces generated during inspiration and result in an obstruction of the upper airway (Weiner et al., 1982).

1. INTRODUCTION (*SECTION 2.*)

1.2.1 Afferent inputs which influence the activity of hypoglossal motoneurones

There is now a substantial body of evidence which suggests that the genioglossus muscle is involved in maintaining the patency of the upper airway. It is therefore important to know which factors modulate and influence its activity. Do certain conditions have a differential effect on the activity of the genioglossus muscle in relation to that of the diaphragm? Do the diaphragm and genioglossus muscles share common control mechanisms? Or are there specific afferent inputs to hypoglossal motoneurones? These questions have been addressed in a number of papers (for references see Van Lunteren & Strohl, 1986, Sant'Ambrogio & Mathew, 1988). The following section describes in detail some afferent inputs which are known to influence the activity of hypoglossal motoneurones. The section will consider;

1) the response of the genioglossus muscle and the hypoglossal nerve to increased chemical drive

2) the response to changes in upper airway pressure

3) the response to changes in lung volume

4) the effect of anaesthesia

In each case the mechanisms involved are discussed. The effect that these factors may have on the relationship between the genioglossus and diaphragm muscles has also been considered.

1.2.2 The response of the genioglossus muscle and hypoglossal nerve to increased chemical drive and chemoreceptor input

Obstructive and central apnoeas can result in hypoxia and hypercapnia (Mathew et al., 1982c). It is important to establish how the genioglossus muscle is mediated during these episodes because, as described in the previous section, this muscle helps maintain the patency of the upper airway and influences the duration of the apnoea.

1.2.2.i The effect of chemical stimulation on the genioglossus muscle and its control

In both human adults and a range of animal species, the respiratory-related activity of the genioglossus muscle and the integrated activity of the hypoglossal nerve, recorded from both single fibres and whole nerve preparations, are augmented with increasing levels of chemical stimulation (Brouillette & Thach, 1980, Weiner et al., 1980, Önal et al., 1981, Bruce, Mitra & Cherniack, 1982, Patrick et al., 1982, Weiner et al., 1982, Hwang et al., 1983a & b., Mitra & Cherniack, 1983, St. John, Knuth & Rist, 1984, Haxhiu et al., 1984, St. John & Bledsoe, 1985, Haxhiu, Van Lunteren, Mitra & Cherniack, 1987, Redline & Strohl, 1987, Van Lunteren, Martin, Haxhiu & Carlo, 1989, Hollowell, Bhandary, Funsten & Suratt, 1991). During hypercapnia and/or hypoxia inspiratory motoneurones (the classification refers to the pattern of discharge in relation to the activity of the phrenic activity) are both recruited and increase their discharge frequency (Hwang et al., 1983a, Mitra & Cherniack, 1983, Mifflin, 1990). Furthermore the onset of inspiratory-related hypoglossal activity (recorded in the whole nerve and single fibres) becomes progressively earlier than phrenic nerve discharge (Weiner et al., 1982, Hwang et al., 1983a & b). Stimulation of the carotid sinus nerve, although not equivalent to physiological stimulation of peripheral chemoreceptors, results in a similar increase in respiratory-related hypoglossal discharge (Jiang et al., 1991). Similarly, arterial chemoreceptor stimulation results in

a depolarization of hypoglossal motoneurones at the beginning of inspiration (Mifflin, 1990). This may explain the earlier onset of phasic hypoglossal nerve discharge during increased chemical drive .

In some cases the inspiratory hypoglossal nerve discharge extends into late inspiration (St.John & Bledsoe, 1985) or into the early expiratory phase of the respiratory cycle, occurring after the inspiratory burst has almost ceased (Hwang et al., 1983b, Bruce et al.,1982). With higher levels of chemical drive there is an increase in the level of discharge occurring during this early expiratory burst (Hwang et al., 1983b) which may reflect an increase in the discharge frequency of both expiratory and inspiratory - early expiratory fibres (Hwang et al., 1983a, Mitra & Cherniack, 1983). During high levels of chemical drive some inspiratory fibres extend their discharge into the expiratory phase of the respiratory cycle, thus becoming inspiratory-expiratory fibres (Hwang et al., 1983a). In contrast to the intracellular recordings of Withington-Wray & colleagues (1988), Mifflin (1990) has reported that a few inspiratory hypoglossal motoneurones extend their activity into the postinspiratory phase of the respiratory cycle where the motoneurone had previously been silent. The difference between the results of the two studies may have been due to the fact that the animals in the study by Mifflin (1990) were vagotomized and the removal of this inhibitory afferent input may allow the prolongation of the burst activity into the early expiratory phase of the respiratory cycle during higher levels of chemical stimulation (see section 1.2.4).

Shortly following the increase in phasic activity during chemical stimulation, tonic hypoglossal and genioglossus activity begins to increase (Weiner et al., 1982, Haxhiu et al., 1984 & 1987). Stimulation of the carotid sinus nerve results in a similar increase in tonic hypoglossal discharge (Jiang et al., 1991). Some single fibres have also been shown to have a discharge pattern which is active throughout the respiratory cycle and sometimes during hypercapnia or hypoxia there is a gradual increase in the

activity of these motoneurones (Hwang et al., 1983a, Mitra & Cherniack, 1983). In addition, Hwang et al. (1983a) reported that a few inspiratory motoneurones lose their phasic discharge pattern during extreme levels of hypercapnia or hypoxia and either intermittently or continuously display a discharge pattern which is classified as tonic.

1.2.2.ii The effect of hypoxia and anoxia on the electrophysiological properties of the hypoglossal motoneurones recorded *in vitro* brainstem slices

Hypoglossal motoneurones, recorded in *in vitro* brain stem slice preparations, gradually depolarize during hypoxia (PO_2 15-20 mmHg in the tissue) (Haddad & Donnelly, 1990, Donnelly, Jiang & Haddad, 1992). The membrane input resistance of the hypoglossal motoneurones increases (Haddad & Donnelly, 1990), although Donnelly and colleagues (1992) demonstrated in a more recent study that after 3 mins of hypoxia the input resistance decreases. The mean depolarization of the hypoglossal motoneurones after 5 minutes of hypoxia was approximately 29mV, which is significantly greater than the depolarization recorded in either dorsal vagal motoneurones (ca. 18mV) or hippocampal CA1 neurones (ca. 8mV) (Donnelly et al., 1992). The depolarization of the hypoglossal motoneurones was also at a greater rate than the depolarization of these other neurones (Donnelly et al., 1992). Hypoglossal motoneurones demonstrate a period of enhanced excitability early in the hypoxic period, however the spike frequency subsequently declines and in some motoneurones depolarization blockade occurs (Haddad & Donnelly, 1990, Donnelly et al., 1992).

The early excitability of the hypoglossal motoneurones is essential for the recruitment of the genioglossus muscle fibres and ensuring a patent upper airway at a time when chemical drive is increased. This depolarization of the membrane, together with an increase in central respiratory drive, will ensure an increase in hypoglossal discharge at a time when hypoxia or hypercapnia occur. However if the hypoxia continues the

depolarization blockade will increase the likelihood of airway obstruction.

Anoxia (no oxygen in the tissue) also results in a depolarization of hypoglossal motoneurones (Jiang & Hadddad, 1991, Donnelly et al., 1992), however, the degree of depolarization is greater than that induced by hypoxia and after 5 minutes the excitability of the motoneurones is impaired (Donnelly et al., 1992).

During both anoxia and hypoxia there is an increase in the extracellular and a corresponding decrease in the intracellular K^+ (Haddad & Donnelly, 1990, Jiang & Haddad, 1991, Donnelly et al., 1992, Jiang et al., 1992b); it has therefore been proposed that there is a leakage of intracellular potassium from the neurones or glial cells (Jiang & Haddad, 1991, Jiang et al., 1992b). Although activation of Ca^{2+} - mediated K^+ channels have been proposed as a reason for the extracellular K^+ ions in the hippocampal CA1 region (Leblond & Krnjević, 1989), there is strong evidence that the ATP-sensitive K^+ channels (activated by the depletion of cellular ATP) are involved in the accumulation of extracellular K^+ in the hypoglossal region (Jiang & Haddad, 1991, Jiang et al., 1992b). The activation of either channel limits the level of depolarization which occurs (Jiang & Haddad, 1991, Jiang et al., 1992b). This is considered to be a safe-guard, preventing the depolarization from reaching a level which activates voltage sensitive Ca^{2+} channels; Ca^{2+} influx leads to neuronal injury and cell death (Espinoza & Parer, 1991, Ben-Ari, 1992). Indeed, the hypoglossal motoneurones fail to recover from a 5-6 min period of anoxia when bathed with glibenclamide, a ATP-sensitive K^+ channel blocker (Jiang et al., 1992b).

In addition to the efflux of K^+ ions, Jiang and colleagues (1992a) have shown that during anoxia there is a major influx of Cl^- ions into the hypoglossal neurones and a decrease in extracellular Na^+; the extracellular space decreases by approximately 50% (Jiang et al., 1991, Jiang et al., 1992b). These pathological changes may trigger a

sequence of events which leads to neuronal death. The depolarization, due to the influx of Na^+, will open voltage-dependent Ca^{2+} channels leading to an increase in intracellular Ca^{2+} (Espinoza & Parer, 1991). Ca^{2+} activates phospholipases which break down the cells membrane. As a result lysis, cellular swelling and neuronal death occur. Consequently, the patency of the upper airway will be reduced and the situation aggravated.

1.2.2.iii What role do the peripheral chemoreceptors play in the response of hypoglossal motoneurones to chemical stimulation ?

a) Respiratory-related activity

The amplitude and/or the duration of the respiratory-related membrane depolarizations are increased during selective stimulation of the peripheral chemoreceptors (CO_2 - saturated bicarbonate injection or doxapram) indicating that the peripheral chemoreceptors provide a powerful excitatory input to central drive which generates the phasic hypoglossal activity (Mifflin, 1990).

Thus it may be expected that stimulation of the carotid sinus nerve would provide an excitatory input to these motoneurones. However, electrical stimulation of the carotid sinus nerve evokes both IPSP and EPSP/IPSP sequences in hypoglossal motoneurones (Withington-Wray et al., 1988, Mifflin, 1990). The IPSP activity may be the result of stimulating the baroreceptor afferents as well as chemoreceptor afferents as increases in blood pressure have been shown to hyperpolarize hypoglossal motoneurones (Salamone, Strohl, Weiner, Mitra & Cherniack, 1983, Mifflin, 1990).

b)Tonic activity

The hypoglossal motoneurones were depolarized in *in vivo* adult cats both when the ventilator was turned off and during hypercapnia (7% CO_2) (Mifflin, 1990). A similar depolarization was recorded in an *in vitro* rat brain stem slice preparation and it was therefore suggested that hypoxia has a direct effect on hypoglossal motoneurones (Haddad & Donnelly, 1990).

Although the results of the *in vitro* study indicate that the depolarization may be caused by a direct effect of hypoxia or a mechanism which is independent of the arterial chemoreceptors, the depolarization recorded *in vivo* may be the result of an increase in chemoreceptor activation. The majority of experiments, however, have not been specific in their attempts to study the effect of changes in arterial CO_2 and/or O_2 on the activity of the hypoglossal motoneurones. Increases in arterial end-tidal CO_2 or hypoxia do not distinguish between peripheral and central chemoreceptors or are of a sufficiently short duration to prevent the activation of other afferent inputs such as baroreceptors. Therefore, Mifflin (1990) used selective stimulation of the arterial chemoreceptors by either CO_2-saturated bicarbonate injection or doxapram in order to determine the role of these chemoreceptors in the response of the hypoglossal motoneurones to increased chemical stimulation. The results show that stimulation of arterial chemoreceptors results in a depolarization of the membrane potential, regardless of any accompanying increase in central respiratory drive. It has therefore been proposed that the arterial chemoreceptors set the baseline membrane potential, and it is highly likely that the depolarization recorded during hypercapnia or asphyxia is due to an increase in arterial chemoreceptor discharge (Mifflin, 1990).

1.2.2.iv How does chemical stimulation alter the relationship between the genioglossus and diaphragm muscles?

A number of reports have tried to determine whether the increase in respiratory-related activity of the genioglossus muscle and hypoglossal nerve with increasing hypercapnia or hypoxia, or a combination of the two, is due to the same control mechanism as that mediating the activity of the diaphragm and phrenic nerve. It would seem functionally appropriate for the two motoneurone pools to share a common pathway for chemoreceptor excitation. If the two motoneurone pools do share a common pathway, then it may be expected that the response of genioglossus muscle to chemical stimulation will be proportional to the change in diaphragm activity.

The following section considers how chemical stimulation alters the relationship between the genioglossus muscle and diaphragm.

1.2.2.v The response of humans to steady-state levels of hypoxia and hypercapnia

In adult humans the diaphragm and inspiratory-related genioglossus activity both increase in a linear fashion with increasing chemical drive (Önal et al., 1981, Patrick et al., 1982, Redline et al., 1987, Hollowell et al., 1991). Furthermore, the increase in the activity of the two muscles is proportional (Önal et al., 1981, Patrick et al., 1982). Thus in subjects who respond to increasing chemical drive with small increases in diaphragm EMG also have small genioglossus EMG responses, whereas subjects with large increases in diaphragmatic EMGs also have large genioglossus EMG responses. The similarities in response of the two muscles support the hypothesis that the genioglossus muscle and diaphragm share a common pathway for chemoreceptor excitation (Önal et al., 1981, Redline et al., 1987).

1.2.2.vi The response of animals to steady-state levels of hypoxia and hypercapnia

As previously mentioned, the presence of inspiratory-related hypoglossal nerve, or genioglossus muscle EMG, activity is not always observed in animals during normocapnic normoxia (Weiner et al., 1982, Haxhiu et al., 1984). However, regardless of their initial absence, upon increasing the level of chemical stimulation the peak respiratory-related activities of the hypoglossal nerve and genioglossus muscle are augmented (Bruce et al., 1982, Weiner et al., 1982, Haxhiu et al., 1984).

In contrast to the experiments in humans, the relationship between the genioglossus and diaphragm EMGs in anaesthetized rabbits with increasing levels of chemical stimulation is curvilinear (Brouillette & Thach, 1980). As the level of chemical drive is increased the activity of the diaphragm increases but the activity of the genioglossus muscle remains unchanged; however with higher levels of drive the activity of the genioglossus muscle suddenly increases at a greater rate than that of the diaphragm. A similar curvilinear relationship exists between the hypoglossal and phrenic nerve activity in anaesthetized dogs and cats (Bruce et al., 1982, Weiner et al., 1982). The curvilinear relationship between the activities of the two nerves is due to differential changes in the level of phasic hypoglossal activity; the tonic hypoglossal activity (recorded in both whole nerve and single fibres) increases linearly with phrenic nerve activity during increasing hypercapnia or hypoxia (Weiner et al., 1982, Mitra & Cherniack, 1983). At lower levels of chemical drive the percentage change in phrenic nerve activity is much greater than that of the hypoglossal nerve, however, at higher levels the reverse becomes true and the rate of hypoglossal inspiratory discharge becomes increasingly larger than that of the phrenic (Weiner et al., 1982, Bruce et al., 1982). The hypoglossal activity, both phasic and tonic, continues to rise even when the phrenic nerve activity has reached its maximum (Bruce et al., 1982).

Thus, the force of the tongue contraction, proportional to the genioglossus muscle activity (Weiner et al., 1980), increases during intense chemical stimulation to a greater extent than the contraction of the chest wall muscles. This helps to ensure that the patency of the upper airway is sustained during conditions of high chemical drive. However, the absence, or low level, of phasic discharge of the hypoglossal nerve during lower levels of chemical drive may leave the negative pressure produced by the diaphragm contractions unopposed and, therefore, the airway more susceptible to collapse (Bruce et al., 1982, Weiner et al., 1982).

1.2.2.vii Possible explanations for the disproportionate increase in genioglossus muscle and hypoglossal nerve activity in relation to the diaphragm and phrenic nerve activity during chemical stimulation in animals

As suggested earlier (section 1.1.9) the differences between the discharge pattern of the phrenic and hypoglossal nerves may be due to differences in the sensitivities of the phrenic and hypoglossal motoneurone pools to a common input or, alternatively, specific afferent inputs to one of the two motoneurone pools may exist. These two explanations may also account for the different response of the two nerves to hypoxia. The following section considers both of these explanations in more detail.

Are the different responses of the hypoglossal and phrenic nerves to chemical drive due to differences in the sensitivities of their respective motoneurone pools to a common input?

The difference between the activity of the hypoglossal and phrenic nerves to increasing levels of chemical stimulation may indicate that the respective motoneurone pools differ in their response to a common combined input. This may reflect differences in the properties of the two motoneurone pools, for example; membrane

characteristics, synaptic organisation or modulation of non-respiratory inputs.

The differences between the response of the hypoglossal and phrenic nerves to chemical stimulation may reflect the different recruitment and rates of discharge of the respective motoneurones. As previously mentioned (see 1.1.9), the majority of phrenic motoneurones are already active during normocapnia and therefore only an increase in discharge frequency is recorded with hypercapnia (St. John & Bartlett, 1979). However, the increasing responsiveness of the respiratory-related hypoglossal nerve activity to increasing levels of chemical stimulation may be a consequence of the recruitment of previously silent motoneurones which, once recruited, increase their discharge frequency with further elevations in chemical drive (Hwang et al., 1983a).

The recruitment and rate of discharge of the individual hypoglossal nerve fibres depends upon the thresholds for activation of the motoneurones. For example the inspiratory-expiratory fibres have a higher CO_2 threshold than the inspiratory fibres (Mitra & Cherniack, 1983). However, above an end-tidal level of 7% CO_2 the rate of discharge of the two types of fibre is similar. Although the two types of fibre increase their discharge rate progressively throughout hypoxia (12% inspired oxygen) the discharge frequency of both increases in a curvilinear manner; at lower levels of chemical drive the rate of discharge of both types of motoneurone is relatively low in comparison to the activity of the phrenic nerve but the rate of discharge is greater at higher levels of drive (Mitra & Cherniack, 1983). Intracellular recordings made in *in vitro* brain stem slice preparations have indicated that initially the hypoglossal motoneurones have a low discharge frequency per unit increase in outward current but then the discharge increases rapidly (Haddad et al., 1990). Hence, the curvilinear pattern of discharge of the whole nerve may be a consequence of the differences in the CO_2 threshold levels and the alterations in the discharge frequencies of the different fibre types with increasing chemical drive.

Is there a disproportionate functional input from either the central or peripheral chemoreceptors to the two respective motoneurone pools?

Alternatively, or in addition to the considerations described above, the difference between the activity of the hypoglossal and phrenic nerves to increasing levels of chemical stimulation may reflect a disproportionate functional input from either the central or peripheral chemoreceptors to the two respective motoneurone pools (Bruce et al, 1982). Stimulation of peripheral chemoreceptors, by either nitrogen breathing or cyanide injection, increases the inspiratory-related genioglossus activity to a greater extent than that of the diaphragm activity (Brouillette & Thach, 1980). Similarly decreasing peripheral chemoreceptor drive by oxygen breathing decreases the activity of the genioglossus muscle to a greater extent than that of the diaphragm activity (Brouillette & Thach, 1980). The latter is not observed following denervation of the carotid body (Brouillette & Thach, 1980). This study suggests therefore that the inspiratory-related hypoglossal nerve activity is more dependent on carotid chemoreceptor input than is the activity of the phrenic nerve (Bruce et al., 1982). Both stimulation and inhibition of the carotid chemoreceptors (by chemical means and denervation) have a greater effect on the activity of the hypoglossal in relation to that of the phrenic nerve (Bruce et al., 1982, Van Lunteren, Haxhiu, Mitra & Cherniack, 1984a, Bonora, St. John & Bledsoe, 1985b, St. John & Bledsoe, 1985). Thus, it is possible that afferent input from the peripheral chemoreceptors project disproportionately to the two motoneurone pools.

The increase in the level of genioglossus muscle activity during hypercapnia and its decrease during hypocapnia, despite carotid body-denervation, suggest that central chemoreceptors do influence the level of phasic inspiratory genioglossus discharge (Brouillette & Thach, 1980). However, the result of cooling the intermediate area on the ventral surface of the medulla and decreasing central chemoreceptor input indicates

that phrenic motoneurones are more dependent on input from central chemoreceptors during hypercapnia than are hypoglossal motoneurones (Bruce et al., 1982). As with peripheral chemoreceptors, it may be that the afferent fibres from the central chemoreceptors project in different proportions to the two motoneurone pools.

Despite these studies, the increasing responsiveness of the hypoglossal nerve to high chemical drive cannot be explained on the basis of different functional projections to the two motoneurone pools alone. The balance between the central and peripheral afferent inputs to the hypoglossal motoneurones would also have to alter with increasing chemical drive. This would suggest that the chemoreceptors themselves respond to the stimulus in a curvilinear fashion (Black, McCloskey & Torrance, 1966).

Are there specific afferent inputs to the hypoglossal motoneurone pool?

Experiments which stimulate or inhibit chemoreceptor drive do not exclude the possibility that non-respiratory afferent inputs, modulated by chemoreceptor afferents, may exist which project to the two motoneurone pools unequally. In addition, the chemical stimulus may simultaneously activate the hypoglossal motoneurones directly or through non-respiratory-related mechanisms; either of these will alter the afferent input to the hypoglossal motoneurones in relation to that influencing the discharge of the phrenic nerve.

The response of hypoglossal motoneurones to high chemical drive suggests that higher levels of chemical stimulus are required before the discharge is influenced directly or through non-respiratory mechanisms and therefore, as suggested by St. John & Bledsoe (1985), does not exclude the possibility that the central and peripheral chemoreceptor afferents are distributed equivalently to the two motoneurones. This may also explain the observation that in decerebrate cats the increase in hypoglossal

activity is similar to the corresponding increase in phrenic nerve activity except at high levels of carotid chemoreceptor stimulation. For example, cyanide injections which increase the level of phrenic activity to levels 15% higher than that of the control cause a relatively greater increase in the hypoglossal, whereas lower increases in phrenic nerve activity correspond with a proportionate increase in hypoglossal nerve activity.

The differential response between the hypoglossal and phrenic nerves to increased levels of chemical stimulation may reflect additional activation of the hypoglossal motoneurones by the reticular formation. Increasing the activity of the pontine and medullary reticular formation by electrical stimulation within the brainstem, by stimulating the sciatic nerve or by microinjection of glutamate, provides support for the hypothesis that the activity of hypoglossal motoneurones is more dependent upon processes within the reticular formation than is the bulbospinal-phrenic system (St. John & Bledsoe, 1985, St. John, 1986). During the administration of peripheral chemoreceptor stimulants such as strychnine, cyanide, or doxapram the increase in peak hypoglossal nerve activity is greater than that of the phrenic nerve activity (Bonora et al., 1985b, St. John & Bledsoe, 1985). This occurs in both intact and chemodenervated animals and it has therefore been suggested that the differential changes in the activity of the two nerves are a consequence of the pharmacological actions of these drugs either on the motoneurone pools directly or on other areas within the central nervous system which, in turn, influence the respiratory-related activity of one of the motoneurone pools (St. John & Bledsoe, 1985). It has been proposed that the brainstem reticular formation is a likely possibility as it is well established that doxapram acts upon the reticular system (for references see St. John & Bledsoe 1985).

1.2.2.viii Do the discrepancies between the animal and human studies lie in the methodology? Does anaesthesia or decerebration influence the results obtained?

Not all of the animal studies have recorded a curvilinear relationship between the hypoglossal and phrenic nerves.

In contrast to the previous recordings of Bruce et al. (1982) and Weiner et al. (1982), the increase in the hypoglossal inspiratory discharge recorded by St. John and Bledsoe (1985) during steady-state levels of hypercapnia and hypoxia were proportional to that of the phrenic nerve. The differences between the results may therefore lie in the methodology. Decerebration, used by St. John and Bledsoe (1985), alters the response of the hypoglossal nerve to hypercapnia (Mitra, Prabhakar, Haxhiu & Cherniack, 1986). If the level of decerebration is at the midcollicular level the peak phasic hypoglossal activity is significantly depressed and the $PaCO_2$ at which apnoea occurs (apnoeic point) is increased. On the other hand, if the level of decerebration is further rostral the activity of the hypoglossal nerve is increased and the apnoeic point is unaltered. However, the activity of the phrenic nerve to either type of decerebration is unchanged. Thus, the relationship between the activity of the hypoglossal nerve and that of the phrenic is affected by decerebration (Mitra et al., 1986).

The discrepancies between the previous animal and human investigations may also lie in the use and level of anaesthesia. St. John and Bledsoe (1985) used unanaesthetized decerebrate cats, and their results were similar to the results from studies on unanaesthetized humans (Önal et al., 1981, Patrick et al., 1982). The hypoglossal nerve and genioglossus muscle activity are depressed to a greater extent than that of the phrenic - diaphragm system with anaesthesia (Bruce et al., 1982, Hwang et al., 1983b, Bonora et al., 1985b, St John & Bledsoe, 1985). Following the administration of small

doses of various anaesthetics to decerebrated cats hypoglossal activity responded to an increase in the level of chemical stimulation in a curvilinear manner; only increasing more than phrenic activity at higher levels of chemical drive (Hwang et al., 1983b). This response was very similar to those previously described in anaesthetized animals (Bruce et al., 1982., Weiner et al., 1982). However, in the absence of anaesthesia, a linear increase in hypoglossal inspiratory discharge is recorded (Hwang et al., 1983b), giving support for the studies which claim that the increase in the genioglossus muscle activity is linear (Önal et al., 1981, Patrick et al., 1982, Parisi et al., 1987 & 1988). The effects of anaesthesia on hypoglossal activity have been described in more detail in section 1.2.5.

Is there any evidence therefore that the relationship between the genioglossus muscle and diaphragm during chemical stimulation is curvilinear ?

Some studies have recorded a curvilinear relationship between the genioglossus muscle and the diaphragm, even when the animals were unanaesthetized.

In contrast to the results of St. John and Bledsoe (1985), disproportionate increases in the genioglossus EMG activity in relation to the diaphragm activity were recorded in unanaesthetized intact cats during increased steady - state levels of hypercapnia (Haxhiu et al., 1984 & 1987) and these authors suggest that the differences in the genioglossus and diaphragm responses to chemical stimulation may not be explained totally by the use of anaesthesia (Haxhiu et al., 1984).

However, it has been proposed that the threshold for genioglossus activation may have been interpreted in some studies as a curvilinear response and this may explain the discrepancies between the experiments (Parisi et al., 1988). In awake goats, no respiratory-related genioglossus activity was recorded until the level of $PaCO_2$ had

reached approximately 49mmHg (Parisi et al., 1987) or oxygen saturation had declined to approximately 69% (Parisi et al., 1988). But once the threshold was reached the genioglossus activity continued to increase rapidly with decreasing oxygen or increasing $PaCO_2$ (Parisi et al., 1987 & 1988). The increase in the activity of this muscle under both conditions was linear.

1.2.2.ix The response of the genioglossus muscle and hypoglossal nerve to dynamic responses increases in chemical stimulation in animals

It is not unusual for obstructive apnoea to follow a period of central apnoea (Mathew et al., 1982c). This may be caused by differences in the rate at which the upper airway and respiratory muscles resume ventilatory activity following central apnoea, during which hypoxia and hypercapnia develop. Experiments using steady - state levels of ventilatory drive (Hwang et al., 1983b) or average values of measurements taken periodically during rebreathing tests (Onal et al., 1981) cannot detect differences in the dynamic responses of the motor nerves and muscles to sudden increases in ventilatory drive. Studies have therefore been conducted which have compared the responses of the genioglossus-hypoglossal system to that of the diaphragm-phrenic system during rapid alterations in chemical drive (Haxhiu et al., 1984 & 1987, St. John et al., 1984). Increasing inspired CO_2 in both conscious and sleeping (non-rapid eye movement) cats demonstrates that the transient responses of the genioglossus muscle and diaphragm are dissimilar; whereas the increase in the peak EMG diaphragm activity is rapid and linear, that of the genioglossus is delayed and only begins to increase as a higher level of CO_2 is reached (Haxhiu et al. 1984 & 1987). These studies suggest that the differences in the timing of the two muscles may be due to differences in the CO_2 thresholds; a greater level of CO_2 is required to initiate the phasic genioglossus activity (Haxhiu et al., 1984 & 1987). Although during a sudden increase in chemical drive the diaphragm reaches its new level of activity in advance of the genioglossus,

with the removal of this drive the decline in genioglossus activity is at a greater rate than that of the diaphragm and rapidly reaches its new steady-state level of activity (Haxhiu et al., 1984 & 1987).

In contrast to the results by Haxhiu et al. (1984 & 1987), studies on decerebrated cats have demonstrated that the changes in hypoglossal nerve activity with sudden alterations in chemical drive parallel the response of the phrenic nerve (St. John et al., 1984). The difference between these results and those described above may again reflect differences in the methodology.

1.2.3 Effect of upper airway afferent inputs on the activity of the genioglossus muscle and its control

Upper airway reflexes play an important role in maintaining and/or re-establishing upper airway patency.

Studies have revealed that there are a number of receptors in the upper airway which are sensitive to airflow, changes in temperature and pressure, fluids and carbon dioxide in the upper airway (Sant'Ambrogio, Mathew, Fisher & Sant'Ambrogio, 1983, Hwang, St. John & Bartlet, 1984b, Van Lunteren & Strohl, 1986, Basner, Ringler, Berkowitz, Schwartzstein, Weinberger, Sparrow & Woodrow Weiss, 1990, Nolan, Bradford, O'Regan & McKeogh, 1990, Ukabam et al., 1992, Zhang & Mathew, 1992, for reviews see Sant'Ambrogio & Mathew, 1988). A number of these factors change during normal breathing but particularly during obstructive apnoea.

1.2.3.i Effect of upper airway pressure changes on the activity of the genioglossus muscle and its control

A number of studies have investigated the effect of changes in airway pressure on the activity of the genioglossus muscle and its cranial nerve. These have shown that the activity of this upper airway muscle and its nerve are modulated by pressure changes in the upper airway (review of references see Van Lunteren & Strohl, 1986, Sant'Ambrogio & Mathew, 1988).

Studies, on anaesthetized animals and awake humans, have shown that both the peak and duration of upper airway muscle activity, particularly the genioglossus muscle, increases in a graded manner proportional to the negative upper airway pressure (ranging from -2 to -120 cmH_2O) (Mathew, Abu-Osba & Thach, 1982a, Mathew, Abu-

Osba & Thach, 1982b, Van Lunteren et al., 1984c, Horner, Innes, Holden & Guz, 1991a, Horner, Innes, Murphy & Guz, 1991b, Leiter & Daubenspeck, 1990, Zhang & Mathew, 1992). There is also a substantial increase in the tonic activity of the genioglossus muscle (Mathew et al., 1982a, Leiter & Daubenspeck, 1990). Furthermore, recordings of the hypoglossal nerve confirm an increase in inspiratory-related activity with negative upper airway pressure (Hwang et al., 1984a). In contrast there is a decrease in peak inspiratory flow, inspiratory frequency and diaphragmatic activity (Zhang & Mathew, 1992, Mathew & Farber, 1983, Thach, Menon & Schefft., 1989, Mathew et al., 1982c, Van Lunteren et al., 1984c), and there is an increase in delay between the onset of the genioglossus muscle activity and that of the diaphragm (Van Lunteren et al., 1984c).

The results of positive upper airway pressure experiments are more variable. Positive upper airway pressure decreases genioglossus muscle activity in anaesthetized rabbits (Mathew et al., 1982a), but appears to have no effect on the activity of this muscle or the diaphragm in awake humans (Leiter & Daubenspeck, 1990). The hypoglossal nerve activity is also unaffected by positive upper airway pressure in decerebrate cats; indeed the only change recorded is an increase in its activity at relatively high pressures (+14 & 21 cm H_2O) (Hwang et al., 1984b). The difference between the results of these studies may be due to variation in animal species or differences in preparation. However, it also has to be remembered that the hypoglossal nerve innervates a number of intrinsic and extrinsic muscles of the tongue (see section 1.1.5) and therefore recordings of the activity of the whole nerve may not necessarily reflect only the activity of the genioglossus muscle.

A recent study has demonstrated that although continuous positive airway pressure (CPAP) does not normally affect genioglossus muscle, this muscle is inhibited in

patients suffering from sleep apnoea (Mezzanotte et al., 1992). Thus the degree of inhibition by positive upper airway pressure may depend upon the activity of this muscle under normal resting conditions.

1.2.3.ii Mechanisms mediating the pressure reflex

The response of the genioglossus to negative upper airway pressure occurs in less than 34 seconds in humans suggesting that a reflex pathway is involved (Horner et al., 1991b). This reflex is mediated through receptors located in the mucosa of the upper airway (Mathew et al., 1982b, Van Lunteren et al., 1984c, Horner et al., 1991a). The timing of the response also indicates that it is mediated through mechanoreceptors which sense pressure or deformation of the upper airway, rather than through chemoreceptors (Mathew et al., 1982a, Horner et al., 1991b). Furthermore, this reflex is elicited from either the nasopharyngeal or the laryngopharyngeal areas (Mathew et al., 1982b). Separation of the upper airway further confirms that the reflex can be elicited from superficial receptors located in the mucosa of either the nose or larynx but not the mouth (Van Lunteren et al., 1984c). There is also evidence that subglottic receptors contribute to this pressure reflex (Horner et al., 1991 a & b). The response to negative pressure persists for a number of respiratory cycles and it has been suggested that slowly adapting receptors are involved (Mathew et al., 1982a, Hwang et al., 1984a).

Several sensory nerve inputs have been implicated in the upper airway pressure reflex. Studies using either selective anaesthesia or nerve sectioning have shown that nasal branches of the trigeminal and the internal branches of the superior laryngeal nerves play a significant role in the mediation of these pressure reflexes (Mathew et al., 1982b, Hwang et al., 1984b, Van Lunteren et al., 1984c, Horner et al., 1991a). In

addition, the glossopharyngeal nerve is also thought to play a small role in this response (Hwang et al., 1984b, Horner et al., 1991a). It has been proposed that the glossopharyngeal nerve either inhibits hypoglossal discharge and/or modulates the excitation elicited by the superior laryngeal and trigeminal nerves (Biscoe, Duggan & Lodge, 1972, Hwang et al., 1984b).

1.2.3.iii Importance of airway reflexes in maintaining the patency of the upper airway during normal tidal breathing and obstructive apnoea

Although in the majority of the studies described above the changes in applied upper airway pressure were greater than those recorded during normal tidal breathing, it has been demonstrated that in rabbits the reflex occurs at pressures equivalent to those recorded in spontaneous breathing (-1 to +4 cmH_2O) (Mathew et al., 1982a). The increase in genioglossus muscle activity, as negative intraluminal pressure increases in inspiration, will therefore stiffen the walls of the upper airway and prevent its collapse.

In contrast to what may be expected, the genioglossus muscle activity recorded in patients who suffer from obstructive sleep apnoeas is greater than that recorded in healthy individuals (Jeffries, Brouillette & Hunt, 1984, Mezzanotte et al., 1992). However this may be explained by the fact that patients suffering from apnoeas commonly have smaller airways than normal individuals, a greater resistance to air flow and therefore greater negative intraluminal pressures during inspiration. The greater genioglossus activity in these patients may therefore be elicited by the negative pressure reflex (Mezzanotte et al., 1992).

Airway occlusion techniques have suggested that sub-atmospheric pressure in the

upper airway does not play an important role in regulating upper airway muscle activity in normal sleeping adults (Kuna & Smickley, 1988). However, in contrast, a separate study has shown that topical oropharyngeal anaesthesia results in the development of obstructive apnoeas during sleep (McNicholas, Coffey, McDonnell, O'Regan & Fitzgerald, 1987). Although the apnoeas were not as extreme as those recorded in patients suffering from obstructive sleep apnoea and no alteration in oxygen saturation was recorded, this study does indicate that a defect in the pressure reflex mechanism may contribute to the development of airway obstructions during sleep.

If airway obstruction does occur, the generation of negative pressure leads to an increase in genioglossus muscle activity which will help to re-establish the patency of the upper airway.

1.2.3.iv Response of the genioglossus muscle and hypoglossal nerve to changes in temperature and/or airflow

The pressure reflex is not the only reflex which is thought to be involved in maintaining the patency of the airway.

Decreasing the temperature of the air inhaled through the nose to 8-15°C increases genioglossus activity in awake humans (Basner et al., 1990). It has been proposed that there are receptors located in the nose which are stimulated by airflow and/or temperature (Basner, Simon, Schwartzstein, Weinberger & Woodrow Weiss, 1989). In addition, it has been shown that the level of genioglossus activity is altered by the route of breathing; bypassing the nasal route by either tracheotomy or mouth breathing significantly decreases the activity of the genioglossus muscle (Mathew et al., 1982,

Basner et al., 1989).

Cold air flowing through the larynx is also known to alter the activity of laryngeal receptors with afferents in the superior laryngeal nerves. The phrenic nerve discharge is inhibited when the air flowing to the larynx is cooled (8-16°C) and in some cases apnoea occurs (Ukabam et al., 1992). The response of the hypoglossal nerve to the cooling of the air varied from animal to animal; in some cats the nerve activity increased whereas in others it was reduced (Ukabam et al., 1992). Both responses are mediated by the superior laryngeal nerve and therefore it has been suggested that the differences may have been due to the stimulation of different laryngeal receptors (Ukabam et al., 1992).

1.2.3.v Effect of upper airway chemoreflexes on breathing

Chemoreceptors in the upper airway play a major role in airway reflexes. A number of studies on both mature animals and neonatal humans have demonstrated that small volumes of fluid squirted into the upper airway result in a number of responses which include swallowing, central apnoea, airway obstruction, and occasionally prolonged apnoea and coughing (Lee, Stoll & Downing, 1977, Lucier, Storey & Sessle, 1979, Davies, Koenig & Thach et al., 1988, Davies, Koenig & Thach, 1989, Lawson, Richter, Czyzyk-Krzeska, Bischoff & Rudeshill, 1991). The strength and occurrence of these responses are dependent upon the solution used, e.g a greater incidence of apnoea occurs following an infusion of water or sodium bicarbonate rather than saline (Davies et al., 1988). This reflex is therefore elicited mainly by the stimulation of chemoreceptors, although activation of mechanoreceptors by pressure changes cannot be excluded (Lee et al., 1977, Davies et al., 1988).

The superior laryngeal nerve is the main afferent pathway of this reflex (Lee et al.,

1977, Lawson et al., 1991) and direct stimulation of this nerve evokes a similar inhibition of breathing or phrenic nerve discharge (Lee et al., 1977, Lucier et al., 1979, Lawson, 1982, Van Lunteren et al., 1984c, Lawson et al., 1991). After the stimulus has ceased, there is a persistent period of apnoea followed by prolonged respiratory depression (Lawson, 1982, Jiang et al, 1991); the duration of this poststimulus inhibition is influenced by the duration of the stimulus (Lawson, 1982).

1.2.3.vi Effect of superior laryngeal nerve stimulation on the activity of the genioglossus muscle and its control

As stimulation of the superior laryngeal nerve has been shown to induce apnoea, the effect of this nerve on the activity of the genioglossus muscle and hypoglossal nerve has been investigated in a number of studies.

The superior laryngeal - hypoglossal nerve reflex in adult cats has been studied in detail by both stimulating receptors in the larynx or by electrically stimulating the nerve (Sumi, 1969b, Withington-Wray et al., 1988, Jiang et al., 1991). Stimulation of the superior laryngeal nerve by a short stimulus train (5s, 50 Hz, 0.1-0.3V), in contrast to its effect on phrenic discharge, immediately increases both the tonic and respiratory-related activity of the hypoglossal nerve (Jiang et al., 1991).

The profound effect of this reflex on respiratory-related hypoglossal activity has also been confirmed by a number of intracellular recordings (Withington-Wray et al., 1988, Jiang et al., 1991). By categorizing the hypoglossal motoneurones according to their pattern of discharge, Withington-Wray et al. (1988) revealed that there is an increase in the postinspiratory activity in motoneurones with inspiratory/early-expiratory- or expiratory- related patterns of discharge and an extension of discharge into the

postinspiratory phase in inspiratory-related motoneurones. Furthermore, these studies in adults have revealed that augmentation of hypoglossal activity, as with the inhibition of the phrenic nerve, is prolonged for several seconds after the stimulus is removed (Withington-Wray et al., 1988, Jiang et al., 1991).

Withington-Wray and colleagues (1988) demonstrated that the resting membrane potential of hypoglossal motoneurones are transiently hyperpolarized upon superior laryngeal nerve stimulation (0.5 and 5Hz, 30s) or water injection. However, Jiang and colleagues (1991) recorded a depolarization in a few motoneurones (50Hz, 3-5s, 0.1-0.3v) but in the majority the resting membrane potential was unaffected.

A short single stimulus to the superior laryngeal nerve evokes an ipsilateral excitation and bilateral inhibition (duration 20-30msec) of the hypoglossal nerve (Sica et al., 1984) and complex patterns of both inhibitory and excitatory postsynaptic potentials are elicited in the hypoglossal motoneurones (Sumi, 1969b, Mifflin, Spyer & Withington-Wray, 1986, Withington-Wray et al., 1988). The problem with electrical stimulation of the whole nerve is that a number of fibres are being activated. Therefore the combination of inhibitory and excitatory postsynaptic potentials elicited by a single shock or the variety of responses evoked by the short trains of stimuli reflect the afferent fibres which are stimulated; this will obviously be determined by the intensity and duration of the stimulus.

Single fibre recordings have been used to determine the response of single receptors to pressure changes in the upper airway and have shown that this nerve contains two groups of fibres which are opposite in their response to negative upper airway pressure (Hwang et al., 1984a). The effect that the two types of fibre have on the hypoglossal motoneurones remains to be determined.

1.2.3.vii Effect of CO_2- sensitive reflexes in the upper airway on the activity of the genioglossus muscle

A few studies have recently shown that CO_2 in the isolated larynx stimulates the activity of the genioglossus muscle and hypoglossal nerve (Bradford, Nolan, McKeogh, Bannon and O'Regan, 1990, Nolan et al., 1990, Bartlett, Knuth & Leiter, 1992). In contrast, the peak phrenic nerve activity decreases when the concentration of intralaryngeal CO_2 is increased (Bartlett et al., 1992). The response of both the phrenic and hypoglossal nerve to intralarngeal CO_2 is dose-related and, as with the pressure reflex, the afferent fibres are carried in the superior laryngeal nerve. The responses are abolished after the superior laryngeal nerve is sectioned bilaterally (Nolan et al., 1992, Bartlett et al., 1992).

Bradford and colleagues (1990) tested the response of single afferent superior laryngeal nerve fibres to changes in CO_2 concentrations in the isolated, *in situ*, upper airway. The results of this study showed that the fibres which respond to negative intraluminal pressure were also excited by elevations in the concentration of CO_2 in the upper airway. In contrast, the fibres which respond to positive intraluminal pressure were inhibited during the same procedure. From this, it was suggested that changes in CO_2, in addition to changes in upper airway pressure, mediate genioglossus muscle activity through a reflex mechanism. This reflex is clearly advantageous in situations where CO_2 increases, for example sleep or obstructive apnoeas. During apnoea, the level of CO_2 will increase. This will increase the activity of the genioglossus muscle activity, thereby dilating the airway and restoring pharyngeal patency. However, further studies are required to establish the specific mechanisms involved.

1.2.4 The influence of pulmonary stretch receptors on the control of the genioglossus muscle

A number of excitatory and inhibitory reflexes elicited by receptors in the lungs have been shown to occur. The lung contains a variety of receptors including, pulmonary stretch receptors, irritant receptors and J-receptors (Iscoe, 1992). However, the importance of the pulmonary stretch receptors in the pattern of respiration has been the focus of a number of studies and their role in the termination of inspiration and prolongation of expiration is well defined (Breuer & Hering, 1970, von Euler, 1986). In addition, a number of studies have shown that the activity of the upper airway muscles is also modulated by vagal afferent inputs from stretch receptors in the lung (Cohen, 1975, Brouillette & Thach, 1980, Fukuda & Honda, 1982a, Sica et al., 1984, Van Lunteren, Strohl, Parker, Bruce, Van de Graff & Cherniack, 1984b, Kuna, 1986, Bartlett & St.John, 1988).

1.2.4.i What effect does the vagal volume mediated feed-back have on the activity of the genioglossus muscle and its control?

The inhibitory component of the reflex

Lung inflation greatly reduces or even abolishes phasic inspiratory genioglossus muscle and hypoglossal nerve activity (Brouillette & Thach, 1980., Weiner et al., 1982., Kuna, 1987). That this response is vagally mediated is confirmed by the finding that following vagotomy lung inflation manoeuvres have no effect on the discharge of the hypoglossal nerve or the activity of the genioglossus muscle (Brouillette & Thach, 1980, Van Lunteren et al., 1984b, Bartlett & St.John, 1988).

If the airway is either occluded (at the end of expiration), inflation prevented or the animal vagotomized there is an increase in the duration and peak activity of the genioglossus muscle and its cranial nerve (Brouillette & Thach, 1980, Weiner et al., 1982, Fukuda & Honda, 1982b, Sica et al., 1984, Van Lunteren et al., 1984b, Kuna, 1986 & 1987, Agostoni et al., 1987., Bartlett & St. John, 1988). Furthermore, the discharge of the individual hypoglossal fibres changes from a decrementing pattern, where the hypoglossal activity reaches a peak synchronous with the onset of the phrenic activity and then declines for the remainder of inspiration, to an augmenting pattern where the activity of the nerve continues to increase throughout the course of inspiration (Sica et al., 1984). In addition, there is a recruitment of some hypoglossal inspiratory fibres and a prolongation of the activity of others into early expiration (Sica et al., 1984). Overall, the discharge of the whole nerve changes from a decrementing to an augmenting pattern (Sica et al., 1984).

1.2.4.ii How does the activity of the genioglossus muscle and hypoglossal compare with the effect of this reflex on the diaphragm and phrenic nerve activity?

Although the muscles of the upper airway and the diaphragm are both inhibited by vagally - mediated volume-related feedback mechanisms, there are some important differences in the effects of this reflex on the two muscles. As well as occurring at significantly smaller volumes (Van Lunteren et al., 1984b, Kuna, 1986), the vagally mediated feedback has an earlier and more profound inhibition on the genioglossus muscle and hypoglossal nerve activity than it does on the diaphragm (Brouillette & Thach, 1980, Sica et al., 1984, Van Lunteren et al., 1984b, Kuna, 1986, Agostoni et al., 1987, Bartlett & St.John, 1988). In fact, in some airway occlusion (end-expiration) studies, the phrenic nerve discharge showed no change in its rate of rise exhibiting

only an increase in its duration (Cohen, 1975). The differences in the response of the genioglossus muscle and that of the diaphragm may be due to either a lower threshold level of hypoglossal motoneurones for this inhibitory afferent input or a vagally mediated mechanism to these motoneurones which is independent of the inflation reflex previously described.

The effect of the volume feedback mechanism on the activity of the phrenic nerve is time dependent such that greater volumes are required to achieve inhibition of these motoneurones at the beginning of inspiration (Clark & von Euler, 1972, Younes, Remmers & Baker, 1978, Kuna, 1987). In contrast, the volume associated with the inhibition of hypoglossal motoneurones is time independent (Kuna, 1986). This time-independent volume threshold for genioglossus activity ensures that the upper airway patency is maintained in situations where lung inflation is compromised. Once the volume threshold is reached and inhibition has begun, progressively greater volumes are required to produce further inhibition of the hypoglossal motoneurones (Kuna, 1986). In contrast, partial inhibition of the phrenic activity increases its susceptibility to additional inhibition (Younes et al., 1978). These differences may explain the gradual termination of the genioglossus muscle and hypoglossal nerve activity in comparison to the relatively abrupt termination of the diaphragm and phrenic nerve activity (Cohen, 1975, Kuna, 1986, Agostoni et al., 1987).

The participation of the genioglossus muscle in the vagally-mediated volume related feedback is an important protective mechanism. If pharyngeal airway obstruction occurs there is a reduction in the pulmonary afferent inputs and an increase in the activity of the hypoglossal motoneurones; consequently the genioglossus muscle activity increases, the airway is enlarged and the patency re-established. The greater and earlier effect that the removal of this inhibition has on the genioglossus muscle

ensures that the upper airway is patent prior to contraction of the diaphragm and therefore the obstruction is not exacerbated.

1.2.4.iii The facilitatory component of the vagal reflex

Although inhibition of the inspiratory-related upper airway muscle activity is more prominent, vagally mediated volume related facilitation has occasionally been observed (Fukuda & Honda, 1982b, Van Lunteren et al., 1984b, Agostoni et al., 1987). By blocking the slowly adapting receptors in the bronchi with SO_2 or by occluding the airway, the time to peak of the genioglossus muscle is delayed suggesting that afferent inputs from stretch receptors in the lung are involved in the initial rapid rate of rise of this muscle at the beginning of inspiration (Van Lunteren et al., 1984., Agostoni et al., 1987). Facilitation is less commonly and less consistently recorded in the genioglossus muscle than in the diapragm (Van Lunteren et al., 1984b). However, this may be due to the greater sensitivity of hypoglossal motoneurones to anaesthesia (Brouillette & Thach, 1980, see section 1.2.5).

1.2.4.iv Possible mechanisms which may determine the effect of volume feedback on the genioglossus muscle activity

The two types of response recorded, i.e facilitation and inhibition, suggest that either there are two types of stretch receptors in the lungs and/or trachea which differ with respect to their threshold and central connections, or alternatively that the input from the stretch receptors is the same but that there are two groups of brainstem neurons with different thresholds (Agostoni et al., 1987, review Euler, 1986).

It is thought that the stretch receptors in the bronchi facilitate genioglossus activity at

end-expiratory volumes but inhibit it at larger volumes, such as those experienced during inspiration, whereas the tracheal input inhibits the onset of the genioglossus inspiratory-related activity in addition to reducing its duration (Agostoni et al., 1987). The relative contribution of these two groups of receptors to the volume feedback mechanisms will determine the overall response of the genioglossus muscle.

1.2.5 Effect of anaesthesia on the hypoglossal nerve-genioglossus muscle system

1.2.5.i The effect of anaesthesia on airway patency

Upper airway obstruction is produced or aggravated in patients during sedation or anaesthesia (Safar et al., 1959). Radiographic studies indicate that during these incidents the tongue is retracted against the posterior oropharyngeal wall (Safar et al., 1959). Particular attention has focused on the genioglossus muscle because of its known involvement in the maintenance of upper airway patency (Brouillette & Thach, 1979) and its possible role in the prevention and relief of apnoea (Remmers et al., 1978).

1.2.5.ii The effect of anaesthesia on the genioglossus muscle and its control

Anaesthesia depresses the activity of the genioglossus muscle and hypoglossal nerve.

Brouillette and Thach (1979) were the first to demonstrate the depressive effect of anaesthesia on the activity of the genioglossus muscle. In rabbits, relatively deep levels of anaesthesia (66mg/kg pentobarbital) during airway occlusion induce a closure of the extrathoracic airway. The anaesthesia - induced closure of the airway for a given tracheal pressure is characterized by a reduction in genioglossus muscle activity, relative to its activity during lighter levels of anaesthesia (33mg/kg pentobarbital) (Brouillette & Thach, 1979). Halothane is known to depress the respiratory - related activity of both the genioglossus muscle and hypoglossal nerve in intact and decerebrated cats (Hwang et al., 1983b, Nishinio, Shirahata, Yonezawa & Honda, 1984, Nishinio, Kohchi, Yonezawa & Honda, 1985, Masuda, Ito, Haji & Takeda, 1989, Ochiai, Guthrie & Motoyama, 1989). Other anaesthetics and sedatives, for example, chloralose, pentobarbital and diazepam, are also known to have a depressive

action on the respiratory-related activity of the hypoglossal nerve, even when sub-anaesthetic doses are used (Hwang et al., 1983b, Nishino et al. 1984 & 1985, Bonora et al., 1985b, Masuda et al., 1989).

1.2.5.iii How does the effect of anaesthesia on the control of the genioglossus muscle compare with the effect on diaphragm activity?

Diaphragm EMG and inspiratory-related phrenic nerve activity (recorded at the same time as the genioglossus activity or hypoglossal nerve discharge respectively) indicate that there is a similar depression of the ventilatory system (Hwang et al., 1983b, Nishino et al., 1984 & 1985., Bennett & St. John, 1985, Ochiai et al., 1989, Masuda et al., 1989). However, in general, the anaesthesia - induced depression of the hypoglossal - genioglossus system is more pronounced than that of the phrenic and diaphragm (Brouillette & Thach, 1979, Hwang et al. 1983b, Nishino et al., 1984 & 1985, Masuda et al., 1989, Ochiai et al., 1989). Although both systems are affected by anaesthesia in a dose-related manner, the phasic activity of the genioglossus muscle and hypoglossal nerve decreases at a greater rate and disappears much earlier that of the diaphragm and phrenic nerve (Nishino et al., 1984, Ochiai et al, 1989). The differential depression of the genioglossus muscle and diaphragm suggest that the mechanisms mediating the respiratory activities of the two nerves are in part mediated by different neural pathways (Nishino et al., 1984). The findings of these studies also indicate that the mechanisms responsible for the activation of hypoglossal motoneurones are more sensitive to depression by anaesthesia and sedatives than are those of the phrenic system (Hwang et al., 1983b, Ochiai et al., 1989).

1.2.5.iv The effect of alcohol on upper airway patency and the control of the genioglossus muscle

Alcohol ingestion is associated with an increase in the duration and frequency of apnoea in both healthy individuals (Taasan, Block, Boysen & Wynne, 1981) and patients who suffer from the disorder known as sleep - induced upper airway obstruction (Issa & Sullivan, 1982). The severity of this disorder is closely correlated with alcohol consumption. Alcohol induces obstructive sleep apnoea in subjects who otherwise display only benign chronic snoring (Issa & Sullivan, 1982). Although the underlying mechanisms behind this phenomenon are unclear, the tendency for snoring (an indication of respiratory disturbance during sleep) and obstructions of the upper airway to occur following alcohol consumption is thought to be the result of decreased oropharyngeal muscle tone (Issa & Sullivan, 1982).

As with the selective depression of the genioglossus activity associated with anaesthesia, alcohol ingestion induces a selective depression of the respiratory-related peak genioglossus activity in both awake humans and cats (Bonora, Shields, Knuth, Bartlett & St. John, 1984, Krol, Knuth & Bartlett, 1984). Either a bolus dose or continuous infusion of alcohol depresses the hypoglossal nerve activity in decerebrated cats in a dose-dependent fashion, whereas the phrenic nerve activity is usually unchanged or increases slightly (Bonora et al., 1984). The decrease in the genioglossus muscle response to chemical stimulation with anaesthesia (Hwang et al., 1983b) is also observed following alcohol infusion and again the ventilatory or diaphragm response is not significantly altered (Krol et al., 1984, Bonora et al., 1984). From the similarities in the response of the genioglossus muscle to anaesthesia, sedatives and alcohol, it has been suggested that the mechanism mediating the selective depression of the respiratory-related hypoglossal activity is a general mechanism and not specific to any one of these drugs (Bonora et al., 1984).

Both the exacerbation of sleep apnoea by alcohol and the obstruction of the upper airway associated with anaesthesia may therefore be a consequence of a selective depression of the inspiratory-related genioglossus activity without a corresponding decrease in the force of the diaphragm contraction. The negative pressure generated by the contraction of the diaphragm will suck the flaccid tongue back against the oropharyngeal wall thus obstructing the upper airway.

1.2.5.v Possible mechanisms for the selective depression of hypoglossal motoneurones by anaesthesia and alcohol

The mechanisms underlying the selective depression of the hypoglossal nerve are unclear and it is very likely that there will be variations according to the species and the type of anaesthetic used (Nishino et al., 1984, Masuda et al., 1989). Nevertheless, various studies have provided some general ideas regarding the mechanisms by which anaesthesia, sedatives and alcohol may influence the respiratory-related hypoglossal activity.

Similar results in decerebrated cats both before and after carotid sinus nerve section imply that the selective reduction in activity is probably not a result of the actions of alcohol (Bonora et al., 1984), sedatives (Bonora et al., 1985b) or anaesthetics (Hwang et al., 1983b, Masuda et al., 1989) on carotid chemoreceptors or higher central nervous system structures in this species. Although the selective depression of the hypoglossal nerve induced with halothane was less following denervation of the vagus and carotid sinus nerve the response was still present (Masuda et al., 1989). This suggests that although halothane acts upon both peripheral receptors and central respiratory drive to hypoglossal motoneurones, the selective depression is probably a consequence of its action upon central mechanisms (Masuda et al., 1989). A central mechanism was previously suggested as the likely site for the action of halothane (Bennett & St. John,

1984). This study, again in cats, demonstrated that peripheral neural pathways entering the spinal cord below T1 are unnecessary for the selective depression of the hypoglossal nerve by halothane (Bennett & St.John, 1984). Together, the results of these studies suggest that the selective depression of the neural pathway mediating the phasic hypoglossal nerve activity is occurring within the brain stem, although they do not exclude other areas from modulating the degree of selective depression (Bennett & St.John, 1984). The reticular activating system is one area within the brain stem which has been implicated in this mechanism (Bonora et al., 1985b).

1.2.5.vi The effect of anaesthesia on the relationship between hypoglossal and phrenic nerve activity

As a consequence of the differential activities of the hypoglossal and phrenic nerves to anaesthesia, the relation between the two nerves will be different in anaesthetized animals in comparison to unanesthetized animals.

One such example of this is shown with the response of the two nerves to chemical stimulation (see section 1.2.2.vi.) In the anaesthetized animal a curvilinear relationship between the relative activities of the hypoglossal and phrenic nerve exists, whereas, in the unanesthetized animal a linear relationship is recorded (Hwang et al., 1983b). Anaesthesia has differential actions on the responses of the two nerves at different levels of chemical drive; except at severe levels of chemical drive, the response of the hypoglossal is depressed to a greater extent than that of the phrenic nerve (Bruce et al., 1982, Hwang et al., 1983b). The curvilinear relationship between the hypoglossal and phrenic activities during chemical stimulation in the anaesthetized animal may therefore reflect the differential depression of the two nerves by anaesthesia (Hwang et al., 1983b).

Anaesthesia therefore needs to be taken into consideration when interpreting the results from an anaesthetized preparation.

1.INTRODUCTION (SECTION 3.)

1.3.1 Genioglossus muscle and its control in the neonate

In contrast to the large number of studies in the adult, relatively few studies have investigated the activity of the genioglossus muscle and its control in the neonate. However the obvious importance of the genioglossus muscle in the maintenance of airway patency in the adult and the high incidence of obstructive apnoeas in the newborn infant highlight the obvious need for more information on the control of this upper airway muscle during development.

1.3.2 Genioglossus muscle in the neonate during normal tidal breathing

In neonates, the phasic respiratory-related activity of the genioglossus muscle and hypoglossal nerve during normoxia are episodic, i.e. occurring at variable intervals with long periods of inactivity (Roberts et al., 1986, Bruce, 1986, Gauda et al., 1987, Gauda, Miller, Carlo, DiFiore & Martin, 1989, Harding et al., 1987, Carlo, Martin & Difiore, 1988, Sica, Steele, Gandhi & Prasad, 1988, Cohen & Henderson-Smart, 1989, Watchko, Klesh, O'Day, Weiss & Guthrie, 1989, Carlo & Difiore, 1990).

A number of factors have been shown to influence the degree of phasic activity in the genioglossus muscle, some of which have already been described for the adult (see section 1.2). The absence of genioglossal EMG activity may reflect a high CO_2 threshold for this muscle, for example the threshold in the human infant is around 48 mmHg (Carlo et al., 1988, Carlo & Difiore, 1990). In some cases the absence of phasic genioglossus muscle activity may be explained by an alteration in the route of breathing; when the infant changes from nasal to oral breathing there is a decrease in

the activity of the genioglossus muscle (Roberts et al., 1986). In addition sudden absences of phasic genioglossus EMG activity may reflect a change in the head position or posture of the infant which are known to alter genioglossus activity (Roberts et al., 1986). Although Roberts et al. (1986) were able to correlate a number of these silent genioglossus periods to a change in posture or mouth opening there are still some periods which remain unexplained. These absences of inspiratory-related genioglossus muscle and hypoglossal nerve activity may be related to sleep state; the newborn infant spends the majority of its sleep time in REM, a state in which the phasic activity of the genioglossus muscle is often absent in the adult (Sauerland & Harper, 1976).

Regardless of the underlying reasons for the lack of phasic genioglossus activity, the results from these studies suggest that genioglossus muscle activity is not essential for maintaining the patency of the upper airway during normal tidal breathing (Roberts et al., 1986, Gauda et al., 1989).

1.3.3 A neuromuscular mechanism for the maintenance of upper airway patency in the neonate

A number of studies have suggested that the genioglossus muscle plays an important role in maintaining the patency of the upper airway in infants during situations in which the airway patency may be compromised.

As described previously nasal airway occlusion, producing a greater negative intraluminal pressure, is used as an indication of the stability of the upper airway. In contrast to the adult, nasal occlusion in the neonate frequently leads to airway closure (Roberts et al., 1986, Cohen & Henderson - Smart, 1989). The pressure at which the airway closes is directly correlated with the amplitude of the genioglossus EMG

activity (Roberts et al., 1986). In each case the associated increase in amplitude of the phasic genioglossus muscle activity is less than the activity recorded during occlusions of similar length but without closure (Cohen & Henderson - Smart, 1989). Thus, as in the adult the genioglossus muscle is thought to play an important role in maintaining the patency of the upper airway in neonates during situations in which the patency is compromised.

Rhythmic contractions of the genioglossus muscle, in addition to other upper airway muscles, increase the stiffness of the upper airway and prevent upper airway collapse due to negative intraluminal pressure (generated by the contraction of the diaphragm) (see section 1.1.4). However, spontaneous or induced head flexion frequently leads to airway obstruction in neonates, more so than in adults (Shelton & Bosma, 1962, Stark & Thach, 1981, Roberts et al., 1986) and, in addition, the newborn lamb in comparison to the adult sheep is slower in its response to nasal obstruction (Harding et al., 1987, Wood & Harding, 1989). The greater vulnerability of the infant to airway obstruction may be due to either its sleep state or a lack of maturation both of which may delay the onset and efficiency of mechanisms which maintain airway patency (Roberts et al., 1986).

In infants who suffer from mixed or obstructive apnoeas the ability to maintain pharyngeal patency is compromised. The reasons for this are still unclear. In a recent study, Gauda and colleagues (1989) demonstrated that in premature infants, despite relatively low levels in the amplitude of the diaphragm activity (indicating a decrease in drive to the diaphragm), obstruction of the upper airway frequently occurs. Their study revealed that in these infants there is very little increase in the activity of the genioglossus EMG; genioglossus activity only increases significantly when the patency of the airway is re-established. Thus obstruction in these cases may be due to lack of genioglossus muscle recruitment and therefore a lack of pharyngeal tone.

1.3.4 How is the patency of the airway re-established?

A number of studies have indicated that, as in the adult, the genioglossus muscle plays an important role in re-establishing the patency of the upper airway in the newborn infant when obstruction occurs.

Although the airway in neonates is sometimes obstructed with neck flexion, the airway is re-opened and flow usually resumed within 1 to 3 inspiratory efforts (Stark & Thach, 1981). During airway obstruction induced by neck flexion there is a progressive increase in phasic and tonic genioglossus EMG activity until the airway patency is finally resumed (Roberts et al. 1986). In addition, genioglossus muscle activity is elicited with end-expiratory airway occlusion (Roberts et al. 1986, Gauda et al., 1987 & 1989, Cohen & Henderson - Smart, 1989). With each inspiratory effort there is an increase in the recruitment of the genioglossus muscle (Gauda et al., 1987 & 1989) and once elicited there is a progressive breath-by-breath augmentation of its phasic activity (Roberts et al., 1986, Cohen & Henderson - Smart., 1989). Thus in all these studies the recruitment of genioglossus activity corresponds with a re-opening of the airway. This adds support for the idea that the activity of this muscle is important in re-establishing the patency of the airway during obstruction.

In premature infants who suffer from apnoea, augmentation of genioglossus muscle during airway occlusions is significantly less than that recorded in premature infants who are non-sufferers (Gauda et al., 1987). This may reflect the inability of the apnoeic infant to recruit dilating muscles of the upper airway during spontaneous airway obstructions which will obviously increase the duration of the obstruction.

1.3.5 The onset of firing and pattern of discharge of the hypoglossal nerve during development

Studies on fetal sheep in late gestation (112-140 days, where term is 147 days) have indicated that although genioglossus EMG activity can be recorded during periods of fetal breathing movements it is rarely synchronous with the activity of the diaphragm (Johnston, Gunn & Gluckman, 1986). This would suggest that the neural mechanisms for the activation of inspiratory-related genioglossus activity develop later in gestation.

In the neonate, as with the adult, when the genioglossus activity is recorded it is associated with the inspiratory phase of respiration (Harding et al., 1987, Wood & Harding,1989). However, in contrast to the studies in adult humans or animals, the genioglossus EMG does not precede the diaphragm EMG or the oesophageal pressure deflection (an indication of inspiratory effort). Recordings of the phrenic and hypoglossal discharge in brain stem-spinal cord preparations of newborn rats indicate that the onset of the phrenic nerve discharge precedes that of the hypoglossal nerve (Morin, Monteau & Hilaire, 1992). In addition, and again in contrast to the studies in the adult, the pattern of discharge of the phrenic reaches its peak prior to that of the hypoglossal nerve (Morin et al., 1992).

The differences between the adult and neonate in the onset of firing and discharge pattern may either be due to the lack of afferent inputs to the brain stem in the neonatal preparation or alternatively it may be an indication that there is an immaturity in the control of the upper airway in the neonate.

1.3.6 Are there differences between the hypoglossal motoneurones of the neonate and those of the adult?

An important question is whether the hypoglossal motoneurones in neonates are

comparable to those in adults. *In vitro* brain stem slice preparations have been used to compare the biophysical properties of hypoglossal motoneurones of adult and neonatal rats (Haddad & Donnelly, 1990, Haddad et al., 1990). Intracellular recordings have indicated that the electrophysiological properties of these motoneurones develop postnatally. The newborn hypoglossal motoneurones have significantly lower resting membrane potentials (-73mV), lower rheobases (0.7nA) and higher input resistances (28MΩ) than those recorded in adult slices (-80mV, 2.1nA and 21MΩ respectively) (Haddad et al., 1990). As explained in this report, these differences may be due to the larger soma size and dendritic arborization of the adult motoneurones. However, the action potentials and after-hyperpolarizations recorded in the newborn hypoglossal motoneurones were of longer duration than those recorded in the adult and this may be due to developmental changes occurring at the membrane level (Haddad et al., 1990).

Dynamic biophysical properties of the hypoglossal motoneurones are also different in the neonate in comparison to the adult. Although both display inward rectification, observed as a decrease in the magnitude of the voltage change during hyperpolarizing current pulses, in the neonatal hypoglossal motoneurones the inward rectification is significantly less (Haddad et al., 1990). Repetitive firing also results in a widening of the action potentials generated in the neonatal hypoglossal motoneurones which does not occur in the hypoglossal motoneurones of the adult (Haddad et al., 1990).

The presence of inward rectification and shorter after-hyperpolarizations in the adult motoneurones will increase the excitability of these motoneurones at the start of depolarization, as may occur during hypoxia or hypercapnia (Haddad & Donnelly, 1990, Mifflin, 1990), and thus ensure that the upper airway muscles are activated prior to the diaphragm. The longer after-hyperpolarization and relatively low levels of inward rectification in the neonatal motoneurones may compromise the degree of

excitability of these motoneurones and thus the timing and force of genioglossus muscle contraction.

1.3.7 Factors which influence the activity of the hypoglossal nerve in neonates

It is important to know which factors determine and influence the activity of the genioglossus muscle in neonates. There is however little information regarding the effect of afferent inputs on the activity of the hypoglossal nerve in early life.

1.3.7.i The effect of the vagal afferent input on the activity of the hypoglossal motoneurones in neonates

In neonates the vagal afferent input from the pulmonary stretch receptors has a profound depressant influence on phasic hypoglossal motoneurone discharge.

In contrast to the adult (Sica et al., 1984), the respiratory-related activity of the hypoglossal nerve could not usually be recorded in intact piglets or kittens until a bilateral vagotomy had been performed (Bruce, 1986, Sica et al., 1988). However, in contrast to the phrenic which shows a prolongation of inspiration, following vagotomy, the hypoglossal nerve activity is elevated throughout the inspiratory cycle. (Bruce, 1986). Furthermore, during end-expiratory airway occlusion tests in newborn infants both the duration and the peak amplitude of the upper airway muscles increases whereas there is only an increase in the duration of the diaphragm EMG (Carlo, Miller & Martin, 1985). Thus it appears that, in comparison to the phrenic, lung inflation and hence increased vagal input has a greater depressive effect on the activity of hypoglossal motoneurones.

Even following vagotomy in piglets it is not always possible to record spontaneous

inspiratory hypoglossal activity (Sica et al., 1988), although it is clearly observed in vagotomized kittens (Bruce, 1986). If spontaneous inspiratory hypoglossal activity is recorded in the piglet, its pattern of discharge is decrementing (Sica et al., 1988). In contrast, in adult cats during end-expiratory airway occlusion, when the vagal input is prevented, the pattern of discharge of the hypoglossal nerve becomes augmenting (Sica et al., 1984). Although the difference between the two studies may be due to species variation, it has been proposed that the results are due to a difference in maturation and that in neonates the hypoglossal motoneurones are poorly modulated by central inspiratory drive (Sica et al., 1988).

1.3.7.ii The effect of behavioural state on the control of genioglossus muscle activity in neonates

In studies in fetal sheep genioglossus EMG activity is present for relatively long periods prior to electrocortical differentiation (Johnston et al., 1986). However, towards the end of gestation (112-140 days, term is 147 days) genioglossus activity, as with breathing movements, is only recorded during periods of low voltage electrocortical activity. During high voltage electrocortical activity there is an absence of both phasic and tonic genioglossus EMG activity (Johnston et al., 1986).

In neonatal piglets the spontaneous inspiratory discharges of the hypoglossal nerve are elicited with a change in EEG activity from high voltage low frequency (delta) waves to low voltage, high frequency (theta) waves (Sica et al., 1988). Thus in addition to the removal of the inhibitory vagal input, in some piglets facilitatory influences are also required before modulation by the central pattern generator can be observed. The occurrence of inspiratory hypoglossal activity with a spontaneous change in EEG indicates that there is a possible cortico- and/or intra-bulbar reticular facilitatory influence on these motoneurones (Sica et al., 1988).

1.3.8 The effect of chemical stimulation on the recruitment and activity of the genioglossus muscle

As with the adult, phasic inspiratory genioglossus muscle activity increases in the majority of neonates with increases in respiratory drive (Watchko et al., 1989, Martin, Van Lunteren, Haxhiu & Carlo, 1990). Studies in vagotomized neonates during chemical stimulation also demonstrate that the phasic hypoglossal discharge is augmented (Bruce, 1986, Sica et al., 1988). In addition, and as previously shown in adult humans (Sauerland & Mitchell, 1975), there is an increase in genioglossus activation during the expiratory phase of newborn kittens during hypoxia (Watchko et al., 1989). Together with an increase in the activity of other upper airway muscles (Martin et al., 1990), airway patency will be maintained in both phases of the respiratory cycle when ventilation is increased.

1.3.8.i How does the response to chemical stimulation develop?

There have to date been only a few studies which have investigated the effect of maturation on the response of the genioglossus muscle to increased chemical drive.

In neonatal kittens, genioglossus muscle recruitment during chemical stimulation increases with postnatal age (Watchko et al., 1989). During hypercapnia the actual number of one month old kittens which recruited genioglossus activity was significantly less than the number of two month old kittens (Watchko et al., 1989). Although during acute hypoxia (13% & 10% O_2) the number of kittens which recruited genioglossus muscle activity was comparable in the two age groups, in the younger kittens fewer breaths were associated with genioglossus activity (Watchko et al.,1989). In addition, although the older group of kittens were able to sustain the genioglossus recruitment throughout hypoxia, only half of the one month old kittens were able to sustain this activity throughout the test. The results of this study suggest

that either there is an immaturity in the control of the genioglossus muscle in the neonatal period or alternatively genioglossus muscle recruitment is unnecessary for maintaining the patency of the upper airway in the first few weeks of live (Watchko et al., 1989). There is evidence in both the neonate and the adult that other upper airway muscles such as the alae nasi and posterior cricoarytenoid, are recruited during chemical stimulation (Haxhiu et al., 1984, Martin et al., 1990) and it is conceivable that activity of these muscles may be effective in maintaining airflow in the airway during chemical stimulation in the neonate. Alternatively, there may be an immaturity in the control of the genioglossus muscle within the neonatal period which, under certain conditions, may be detrimental to the health and survival of the animal.

1.3.8.ii The response of the genioglossus muscle to hypoxia in neonates. Is the response comparable to the biphasic ventilatory response?

The ventilatory response to hypoxia in the neonate has been well characterised in both the human infant and a variety of newborn animals (for references see Henderson-Smart, 1984, Rigatto, 1984, Neubauer, Melton & Edelman, 1990). During the first one to two minutes of hypoxia there is an increase in ventilation followed by a return after a few minutes to levels approaching baseline. Although there is a similar biphasic response to sustained acute hypoxia in adults this is not as pronounced and has a slower time course than the "biphasic" ventilatory response recorded in the neonate (Georgopoulos, Walker & Anthonisen, 1989, Van Lunteren et al., 1989, Neubauer et al., 1990).

The underlying reasons for the respiratory depression during hypoxia are not clearly understood, but a number of mechanisms have been proposed including a reduction in pulmonary compliance (La Framboise, Standaert, Woodrum & Guthrie, 1981, La Framboise, Guthrie, Standaert & Woodrum, 1983) and a reduction in metabolism

(Grunstein et al., 1981). However more recently a number of studies have attributed the decrease in ventilation during hypoxia in neonates to a central mechanism (Lawson & Long, 1983, Blanco, Hanson, Johnson & Rigatto, 1984, Martin-Body & Johnston, 1988). It was originally believed that the fall in ventilation was due to a general depression of the respiratory centers (Cross et al., 1954), but more recent studies suggest that the fall in ventilation in the neonate is due to an inhibitory mechanism sited in or above the upper pons (Martin-Body & Johnston,1988, Hanson & Williams, 1989). This is discussed in more detail in appendix A.

If an inhibitory mechanism to hypoxia exists and it is shared by the processes controlling the diaphragm and genioglossus muscles, it might be expected that in neonates the respiratory-related activity of the genioglossus muscle may also respond to hypoxia in a similar biphasic manner. In adults the genioglossus muscle does respond to poikilocapnic hypoxia with a biphasic response which follows the same time course as the diaphragm (Van Lunteren et al., 1989). However, there have only been a few studies which have investigated the effect of hypoxia on the genioglossus muscle and its control in the neonate and the results of these studies are contradictory and therefore inconclusive.

As described above the genioglossus EMG activity in a few kittens (< one-month) respond to hypoxia (10 & 13% O_2) in a manner which is analogous to the biphasic ventilatory response (Watchko et al., 1989). This study provides support for the idea that the hypoglossal and phrenic motoneurones are, at least partially, under similar control mechanisms. In agreement with this study, there was a close correlation between the responses of the phrenic and hypoglossal nerves during hypoxia in neonatal piglets (4-6 days) (Sica et al., 1988). This close correlation was observed regardless of whether the phrenic response to hypoxia was biphasic or sustained and therefore adds supports to the suggestion that there is a common modulation of the

two motoneurone pools (Sica et al., 1988).

In contrast to these reports, an increased level of genioglossus muscle EMG activity was sustained throughout the hypoxic period in piglets (2-17 days) despite the fact that the EMG activity of the diaphragm returned to prehypoxic levels by ten minutes of hypoxia (Martin et al., 1990).

Bruce (1986) recorded only a transient increase in hypoglossal and phrenic discharge during hypoxia in neonatal kittens, but the initial excitatory response of the hypoglossal nerve was more sustained than that of the phrenic nerve. Therefore, although there is an indication that the two motoneurones share similar control mechanisms, this study also suggests that there are afferent inputs, stimulated during hypoxia which affect the activity of the hypoglossal motoneurones without influencing that of the phrenic.

The effect of hypoxia on the individual motoneurones in the neonate have not been recorded. The present study was designed therefore to investigate the effects of hypoxia on the activity of hypoglossal motoneurones in neonatal kittens in an in vivo preparation and to try and establish a) whether these motoneurones respond in a biphasic manner and b) whether this is due to an inhibitory mechanism.

1.3.8.iii The response of hypoglossal motoneurones in neonates to hypoxia or anoxia. A comparison with the response of hypoglossal motoneurones in adults

Any differences between the response of the adult and neonatal genioglossus activity during hypoxia may be due to the different intrinsic properties of their respective motoneurones.

In vitro brain stem slice preparations have been used to compare the electrophysiological responses of the adult and neonatal hypoglossal motoneurones to hypoxia (PO_2 15-20mmHg in tissue for 5 mins, Haddad & Donnelly, 1990) or anoxia (no oxygen in the tissue) (Jiang et al., 1992b). These investigations show that there are fundamental differences between the responses of the adult hypoglossal motoneurones and those of the neonate (less than 2 weeks) to oxygen deprivation.

Hypoxia

During hypoxia, the neonatal hypoglossal motoneurones are depolarized. However, the degree of depolarization is three times lower than that recorded in the adult motoneurones (Haddad & Donnelly, 1990). At the beginning of hypoxia there is an increase in the peak and steady-state frequencies of discharge in both adult and neonatal motoneurones but again the response is significantly smaller in the motoneurones from neonates (Haddad & Donnelly, 1990).

In contrast to the hypoglossal motoneurones in the adult, in the neonatal motoneurones there are no changes in input resistance and no significant increase in the extracellular K^+ ions during oxygen deprivation (Haddad & Donnelly, 1990, Jiang et al., 1992b). The lack of extracellular K^+ ion changes may be explained by the relative lack of ATP-sensitive K^+ channels in the neonatal hypoglossal nucleus which have been implicated in the leakage of the K^+ ions from the hypoglossal motoneurones in the adult (Jiang et al., 1992).

Anoxia

A number of studies have indicated that neonates are more tolerant than adults to periods of anoxia (Neubauer et al., 1990, Bureau, Carroll & Canet, 1988). This may be explained by differences in ionic homeostasis between the neonatal and adult motoneurones (Ben-Ari, 1992). During anoxia the cells are depolarized as a

consequence of a influx of Na^+ ions. As described in section 1.2.2ii, the depolarization byactivating voltage-gated Ca^{2+}channels leads to membrane damage, lysis and neuronal death (Espinoza & Parer, 1991). During anoxia in brain stem slices there is an influx of Cl^- ions and a decrease in extracellular Na^+ ions in both adult and neonatal hypoglossal motoneurones (Jiang et al., 1992a). However, the Cl^- and Na^+ fluxes in the neonatal motoneurones are significantly less than those recorded in the adult; the decrease in the extracellular Cl^- is seven times greater in the adult (Jiang et al., 1992a).

1.3.8.iv How do hypoglossal motoneurones behave in mild levels of hypoxia?

There have been no studies to date which have investigated the effect of mild levels of hypoxia on the activity of hypoglossal motoneurones in neonates.

In the present study we have chosen to look at the effects of mild levels of hypoxaemia. These levels were chosen because they might be experienced by, and involved in, chemoreflex responses in the newborn. For example, it is known that the biphasic ventilatory response (see appendix A.) occurs at levels of hypoxia similar to the levels chosen in the present study. We also wanted to avoid more severe levels of hypoxia which would be expected to produce a substantial metabolic breakdown.

Hypoxia might be experienced in adults in circumstances such as high altitude where the oxygen in the air is low, or in circumstances where the lung is diseased and gaseous exchange is poor; but in the neonate episodes of mild levels of hypoxaemia are a frequent occurence (Mathew et al.,1982c, Poets et al., 1991). It is therefore important to establish how the hypoglossal motoneurones respond to these levels of hypoxia. The activity of these motoneurones will determine the activity of the genioglossus muscle and therefore the patency and stability of the upper airway. If these motoneurones are inhibited during mild levels of hypoxia, the airway may

become obstructed and more severe levels of hypoxia develop. Indeed, it is common for obstructive apnoeas to follow periods of central apnoea, during which hypoxia and hypercapnia develop (Milner & Greenough, 1988).

This study therefore investigated the effect of mild levels of hypoxia on the activity of hypoglossal motoneurones in neonates. The main aim of this study was to establish whether such mild levels of hypoxaemia both excite and inhibit the activity of the hypoglossal motoneurones

1.3.8.v How do hypoglossal motoneurones respond to hypoxia when recorded in neonates in an *in vivo* preparation?

All of the studies to date which have investigated the activity of the hypoglossal motoneurones in the neonate have used the *in vitro* preparation. This preparation can be useful as it provides relatively stable intracellular recordings, a controlled environment and no anaesthesia. However, the slice procedure destroys a number of afferent inputs, including peripheral chemoreceptors, and also lacks modulating influences such as neuromodulators and hormones. Its physiological significance has therefore been disputed (Andersen, 1981).

Notwithstanding the difficulties associated with an in vivo preparation, this study has used this type of preparation to obtain a better understanding of the response of hypoglossal motoneurones to hypoxia in the neonate.

1.3.9 THE AIMS OF THIS STUDY

This study was undertaken to investigate the effect of mild levels of hypoxaemia on the activity of hypoglossal motoneurones in neonates in an *in vivo* preparation.

The aims of this study were;

1) to establish a viable neonatal *in vivo* preparation in which hypoglossal motoneurones can be recorded.

2) to determine whether hypoglossal motoneurones in neonates respond to mild levels of hypoxaemia with an increase or decrease in discharge frequency.

and 3) if there is a decrease in the discharge frequency of the motoneurones; to establish whether this is due to an inhibitory mechanism

2. MATERIALS AND METHODS

2.1 ANIMALS

Experiments were performed on 101 kittens of either sex. These were aged between 10 and 28 days old and weighed between 200 and 650g. All the kittens were from established colonies. At the onset of the work considerable effort had to be put into developing a procedure to establish a physiologically stable preparation. The following procedures, which have been discussed in more detail in section 4.12, were found to succeed and recordings were made from 31 kittens. These were aged between 13 and 27 days old; 20 kittens were $\leq$ 21 days old. To ensure an adequate weight gain each litter was restricted to four kittens. The weight ranged from between 279g and 610g (mean 408g). All of the kittens were housed with their mother until the day of experimentation.

All experiments were licensed by the Home Office in accordance with the Animals (Scientific Procedures) Act, 1986.

2.2 SURGICAL PREPARATION

The animals were sedated with an intramuscular (i.m.) injection of ketamine (15-30mg/kg) (Ketalar, Parke-Davis, U.K). Anaesthesia was then induced and maintained for the initial part of the preparation with 1-2% halothane (May & Baker). The halothane was inhaled through a face mask taped over the animals head. The tracheal area and inner side of a rear leg were shaved and ca. 1 ml of lignocaine (Xylotox, Willows Francis Veterinary) was administered sub-cutaneously to both areas prior to

surgery. 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).

Following the tracheotomy, the animals were artificially ventilated, initially with oxygen enriched air (FiO_2 0.3-0.45), using positive pressure ventilation (Infant Star ventilator). The mean tracheal pressure was maintained between 3 and 4 mbars. The ventilator was typically set with a frequency of 12 breaths per minute, an inspiratory duration of 0.92 seconds and a peak inspiratory pressure (PIP) of 13 mbars, but these settings were adjusted depending on the results of the blood gas analysis (see below).

A femoral artery was cannulated (Portex, intravenous cannulae, size 2FG stretched by hand to a narrower diameter) for the monitoring of blood pressure (Harvard Apparatus Pressure transducer). The blood pressure transducer was calibrated prior to each experiment with a sphygmomanometer. Blood samples ($\leq$ 0.2mls, not greater than 1ml in total per experiment) were taken for arterial blood gas analysis (Instrumentation Laboratory 1302). The femoral vein was also cannulated (Portex, intravenous cannulae, size 2 FG stretched to a narrower diameter). Both the femoral arterial and venous cannulae were filled with heparinised saline (10 units/ml, C.P Pharmaceuticals Ltd, U.K). The solutions used in these experiments have been described in more detail in section 2.5.

The halothane was switched off and a bolus dose of α-chloralose (50mg/kg, SIGMA. Chemical Co.) was administered i.v; further doses were subsequently administered as required (25mg/kg,i.v.). The level of anaesthesia was assessed by the degree of pupilary constriction, stability of the blood pressure and absence of flexor reflexes and muscle tone in the absence of gallamine. A bolus dose of glucose (1-2mls, 5% in 0.9% saline, i.v) was administered. The venous line was then attached to either a gravity-feed system (Baxter CO334) or a pressure pump (World Precision Instruments Ltd.)

and glucose constantly infused at a rate of ca. 0.7 mls/hour.

Gas samples were taken through a narrow diameter tube attached to a 2.5mm paediatric/neonatal airway adaptor (Ohmeda) and end-tidal carbon dioxide levels ($P_{ET}CO_2$) and fractional inspired oxygen (FiO_2) levels were continuosly monitored (Ohmeda 5250 Respiratory Gas Monitor). The respiratory gas monitor was calibrated with a sample gas (Scott Medical Products) prior to each experiment. Deviations of blood gases and pH from the expected physiological levels ($PaCO_2$ 35-40mmHg, pH 7.4 and $PaO_2 \geq 80$mmHg) were corrected by adjusting ventilation and/or by infusions of sodium bicarbonate (8.4% w/v, i.v). The volume of sodium bicarbonate administered was calculated from the following equation (a formula based on the Henderson-Hasselbach equation);

sodium bicarbonate mls / kg = base deficit x weight (kg) x 0.3

(Cooke, 1979)

The haemoglobin saturation (SaO_2) was monitored using a pulse oximeter (Ohmeda 5250 RGM) with the probe attached to the glabrous skin of the hind paw. The dark pigment of the skin often made it impossible to obtain a reliable signal and this was therefore only recorded in a few cases. In one animal the SaO_2 was measured as the FiO_2 was reduced systematically (section 2.4).

The rectal temperature was maintained at ca. 38°C using a homeothermic blanket (Harvard) and lamps. For some of the younger animals the rectal temperature could only be maintained by stopping the experiment and wrapping the animal completely in the blanket for short periods of time.

2.2.1 Exposure of the hypoglossal nerve

With the animal in the supine position, an incision of ca. 2 cm was made towards the larynx, extending the incision made for the tracheotomy and following the line of muscle insertion. The transverse jugular vein was identified and in the majority of cases was ligated with two pieces of braided silk thread (U.S grade 6.0, Pearsall), ca. 0.25cm apart; the vein was cut between the two threads. A length of the hypoglossal nerve (approximately 1cm) was exposed by dissecting it free from the surrounding tissue (Figs. 2 & 3). Braided silk thread (U.S grade 5.0, Pearsall) was loosely placed around the right branch of the nerve, care being taken not to pull and damage it (Fig. 3). This marker was then retrieved through an incision made between the digastric and the masseter muscle of the animal. A second thread (U.S grade 2.0, Pearsall) was loosely tied around the digastric muscle and anterior facial vein. By slightly pulling this second thread to one side, the hypoglossal nerve could be observed easily when the animal was turned later to the prone position. The protrusion of the tongue in response to stimulation verified that the nerve was the hypoglossal.

The animal was placed in the prone position and its head fixed in a stereotaxic head holder. The body was suspended by a vertebral clamp. The brain stem was exposed by an occipital craniotomy (Fig. 4).

At the end of recording the animal was killed with the administration of a large dose of pentobarbitone sodium (200mg, i.v, Euthatal, May & Baker).

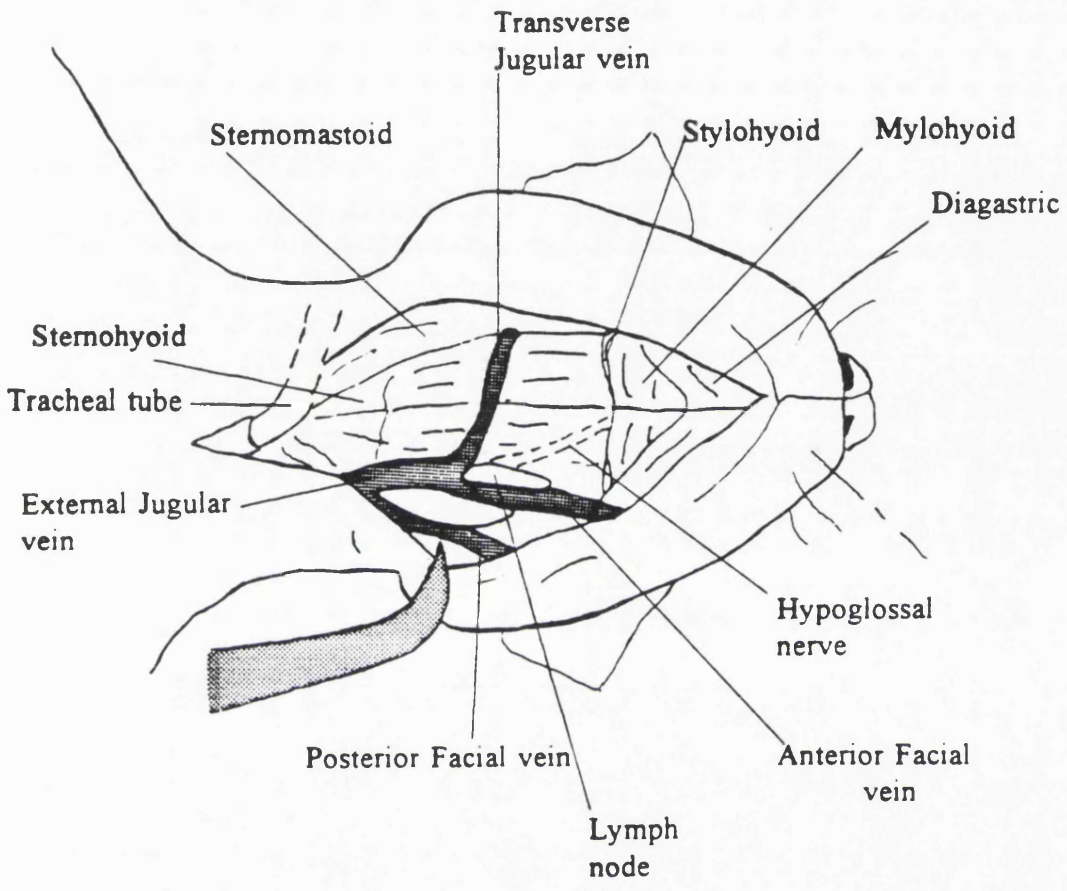


Figure 2. The ventral view of the neck and head of a neonatal kitten showing the position of the hypoglossal nerve.

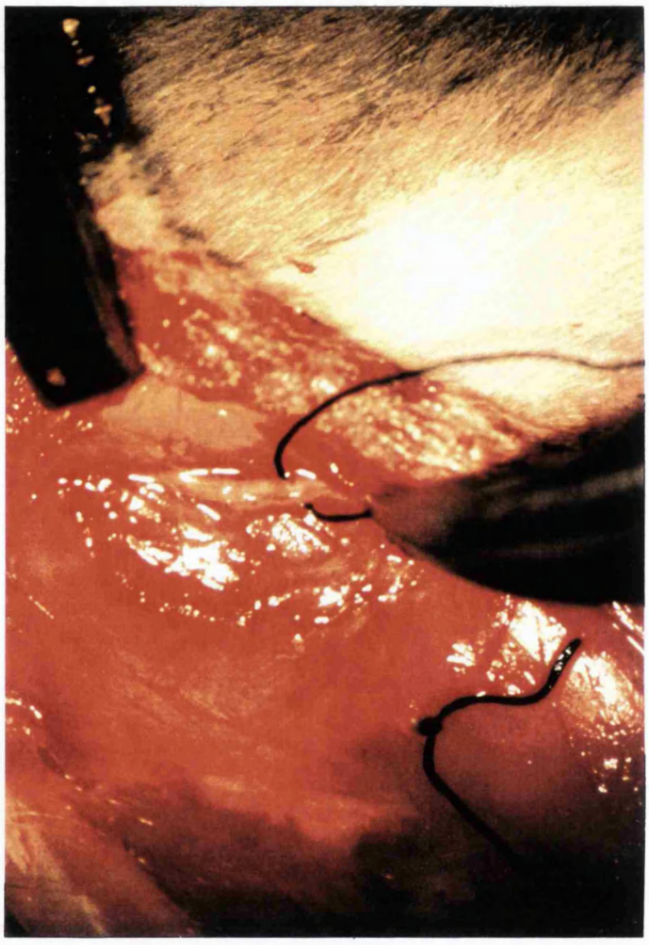


Figure 3. Photograph showing the hypoglossal nerve dissected free from the tissue. Note the thread placed loosely around the nerve which allows for the nerve to be retrieved when the animal is placed in the prone position.

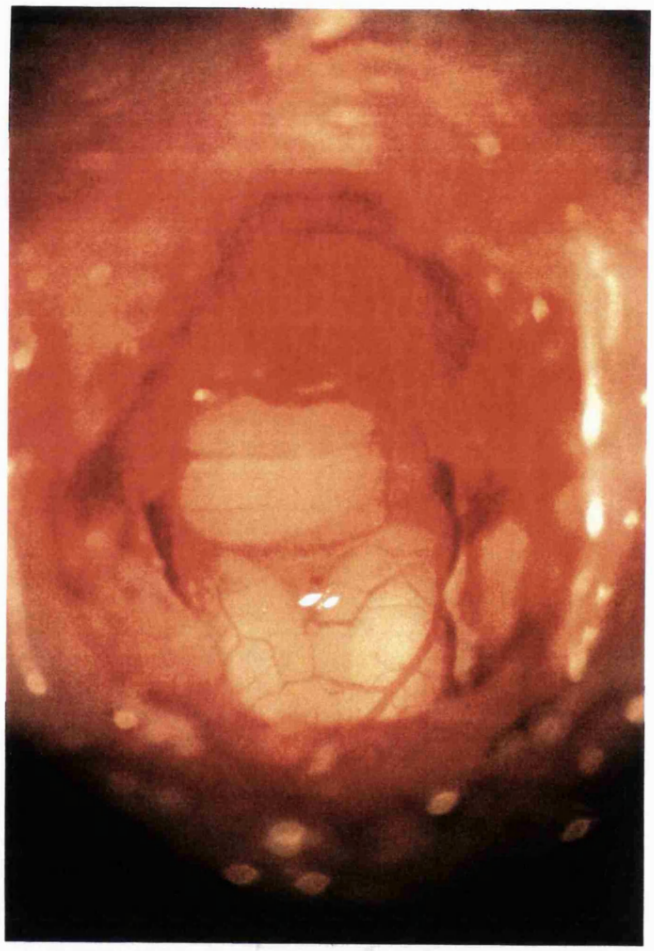


Figure 4. Photograph showing the obex of the medulla oblongata and the cerebellum in a neonatal kitten. This part of the brain stem was exposed by an occipital cranitotomy.

2.3 ELECTROPHYSIOLOGICAL RECORDING

2.3.1 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 prior to the experiment. The electrodes were filled with either 3M potassium chloride or 3M potassium citrate. The impedence of each electrode was measured by passing a constant current pulse (10nA) and using a bridging current (Axoclamp, Axon Instruments) to balance off the voltage drop across the electrode. In addition to this, the shape of each series of electrodes was viewed under a microscope (magnification x10). The electrodes used in the experiment fulfilled two criteria; firstly the impedence across the tip ranged from between 60 and 90 mΩ, and secondly, the shank was smoothly tapered and less than 0.5cm in length. If the electrodes were too long they buckled upon insertion and failed to penetrate the brainstem. The electrodes were stored overnight in humidified air preventing the precipitation of the electrolyte. Often the impedence of the electrodes was less in the tissue than when originally made; this may have been due to the electrodes breaking as they penetrated through the tissue.

2.3.2 Stability

The procedure was conducted on a nitrogen-suspended antivibration table, thus preventing floor movements from affecting the recording.

One of the main problems faced in the experiment was the respiratory movement of the animal and the blood presure-related pulsations of the cerebellum and brainstem. Three procedures were employed to overcome this problem:

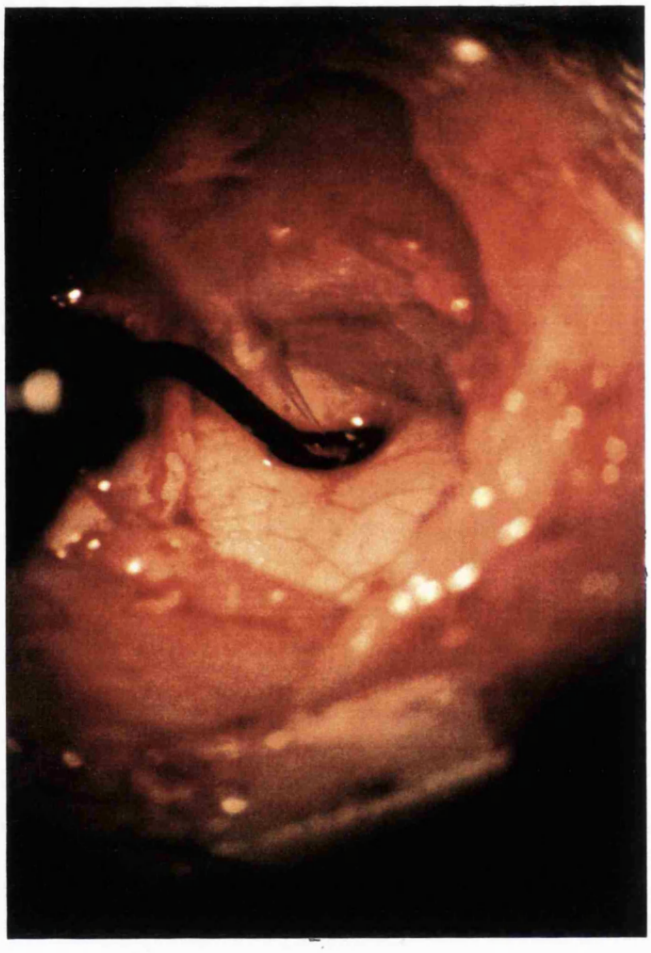


Figure 5. Photograph showing the electrode penetrating the brainstem. Note the horse-shoe shaped foot which was used to help maintain stability of the brainstem during recording. The foot was placed just rostral to the obex.

a) As used in previous studies (Lipski, 1981), a small metal pressure foot was created which could be gently manipulated down onto the surface of the medulla (Fig. 5). The slight pressure helps to isolate the recording area from the pulsations of the surrounding brainstem and cerebellum. The use of a foot, however, creates further difficulties in that it may cut off circulation to this area and damage the integrity of the cells. This problem was avoided by both removing the pressure foot between recordings and by altering the shape of the foot in contact with the medulla to an imcomplete circle (a horse-shoe). To achieve this shape, the eye of a round-bodied, stainless steel surgical needle (Arnolds Veterinary Products Ltd, size 17) was bent over a hot flame and then rapidly cooled. In addition, the foot was usually placed just rostral to the obex where there were no surface blood vessels.

b) To reduce the respiratory-related body movement a bilateral thoracotomy was performed. An incision of ca. 1cm in length was made on either side of the body between the 12th and 13th ribs. The skin, fat and intercostal muscle were pulled away from the incision with toothed artery forceps exposing the lung. A positive end-expiratory pressure (PEEP, 1-2 mbars) was applied to prevent lung collapse.

c) Before starting a recording session the animals were paralyzed with gallamine triethiodide (Flaxedil, May & Baker) This was administered as a bolus dose (7mg/kg, i.v.) as required. Its effects were allowed to wear off so that the depth of anaesthesia could be assessed.

In the initial experiments retractors were used to separate the occipital muscles when exposing the brainstem. However, the brainstem was found to be more stable without these and in the majority of experiments the retractors were not used.

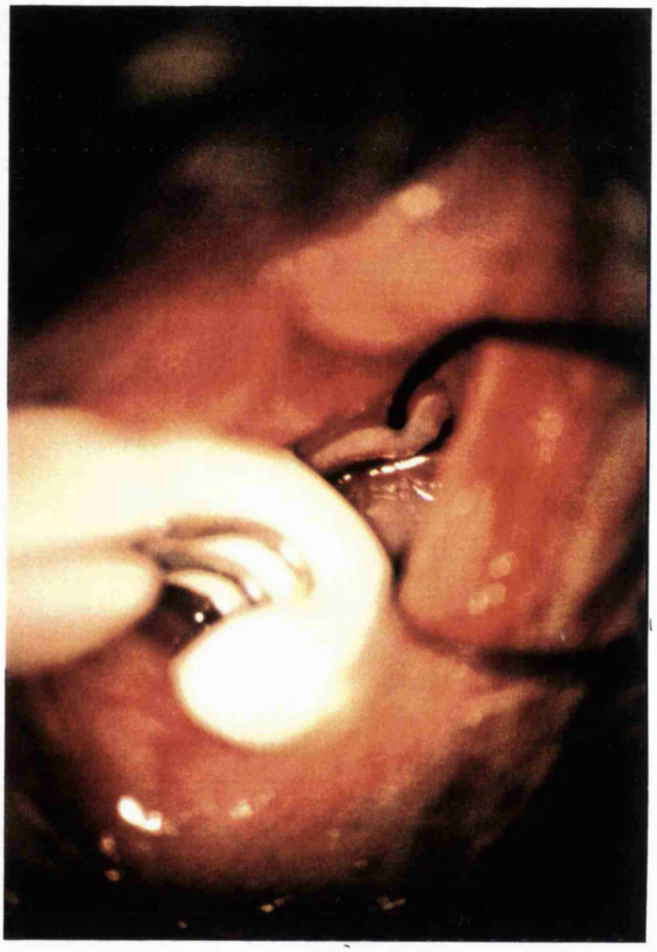


Figure 6. Photograph showing the hypoglossal nerve, with the thread attached, just prior to placement on the platinum bipolar electrode. The kitten is in the prone position

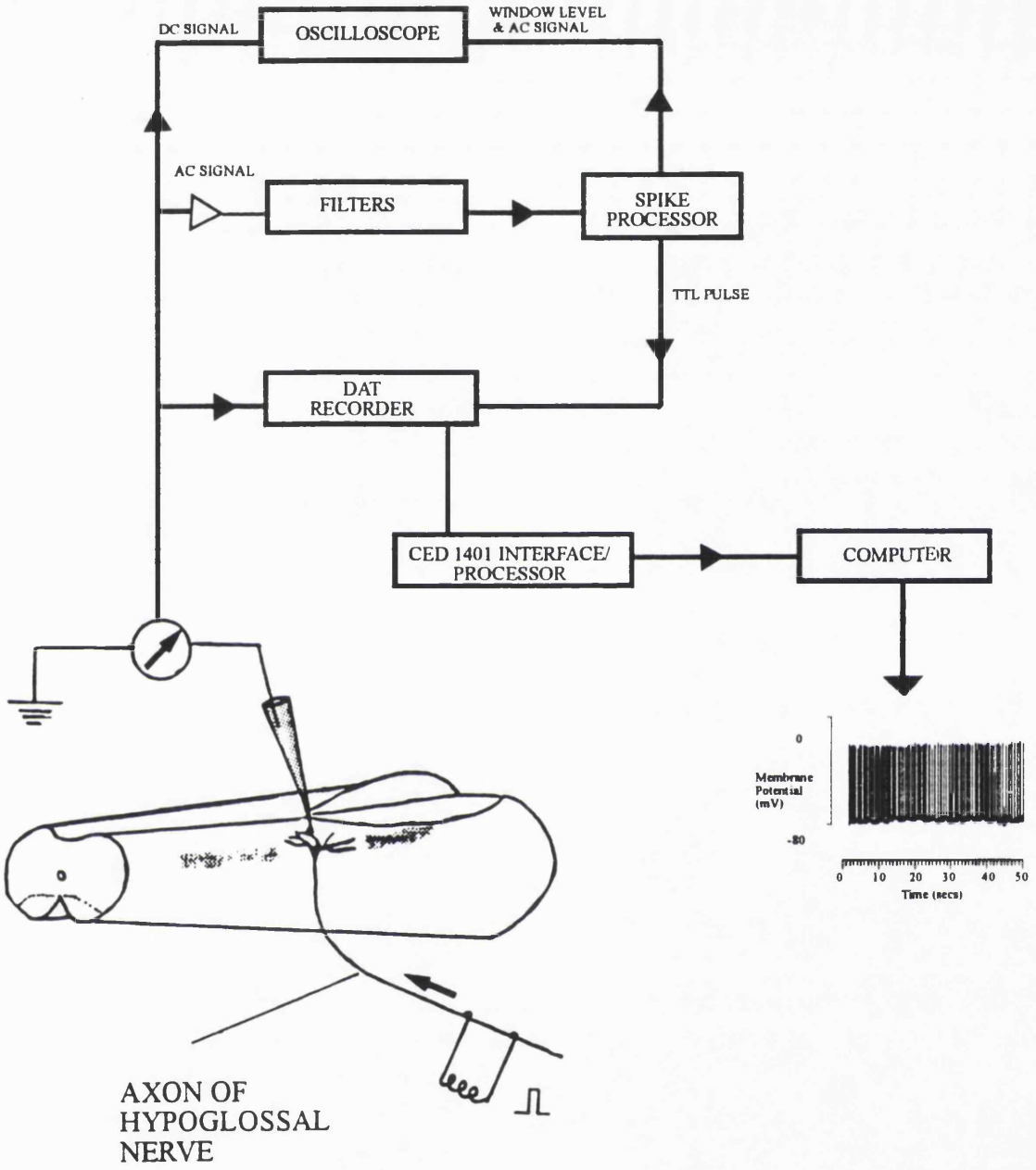


Figure 7. Schematic representation of the recording set up.

2.3.3 Identification of the hypoglossal motoneurones

The hypoglossal nerve was placed on a bipolar platinum stimulating electrode (Fig. 6). A computor controlled stepping motor microdrive (Significat, Digitimer, with Epson HX-20 computer) was used to move the electrode down in 2 or 20 μm steps penetrating the medulla between approximately 0.5mm rostral and caudal of the obex (Fig. 5). In the majority of cases the recordings were slightly rostral to the obex; further caudal to this the arachnoid and pia mater made it difficult to penetrate the brainstem without breaking the electrode. Hypoglossal motoneurones were identified by antidromic stimulation of the hypoglossal nerve (1-6 mA pulse every 600msec, duration 0.1 msec). Antidromic activation of the motoneurones fulfilled the standard criteria described by Lipski (1981); a) activation was at a constant latency, b) the frequency of the action potential followed changes in stimulus frequency and c) orthodromic collision could be observed.

2.3.4 Processing of the signal

The electrode was mounted in a unity gain head stage (Axon Instruments HS-2) and the signal led into an electrometer (Axoclamp, Axon Instruments) with capacitance neutralization and bridge balance facilities. Figure 7 shows a schematic diagram which helps to explain how the signal was processed. The unprocessed DC signal was led directly to the oscilloscope. The signal was also fed through a series of AC amplifiers. The total amplification of the AC signal was x1000. The AC signal was then passed through a series of filters with low (800Hz) and high (6kHz) pass characteristics. The filtered signal was processed using a window discriminator (Spike Processor, Digitimer) to produce standard TTL pulses. The spike processor had a multiplex output so that 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. Both the AC and DC signals were recorded and stored on digital audio tape (Bio-logic, Digital Tape Recorder TR1800).

2.4 EXPERIMENTAL PROTOCOL

Extracellular or intracellular recordings of hypoglossal motoneurones were made. The discharge frequency and/or membrane potential were recorded for a control period of normoxia (FiO_2 0.21-0.23) followed by a test period of mild isocapnic hypoxia. Hypoxia was produced by decreasing the level of inhaled oxygen and substituting it with nitrogen. The FiO_2 during hypoxia ranged from between 0.14 and 0.19, and was maintained at this level for a period of up to 10 minutes for extracellular recordings and 4 minutes for intracellular recordings. Blood gas analysis showed that this typically reduced PaO_2 to between 37 and 60 mmHg. Because blood samples could not be taken during the recording of the motoneurone without compromising stability, the test was repeated and blood gases determined.

For one animal the haemoglobin saturation level was measured systematically at different levels of FiO_2 using the pulse oximeter attached to the hind paw. The results are shown as an oxygen saturation curve (Fig. 8). At an FiO_2 level of 0.16 (the FiO_2 used in the majority of the experiments) the SaO_2 fell to 83%; substantial changes in SaO_2 were only recorded at FiO_2 levels below 0.14. Blood samples were taken at a FiO_2 level of 0.22 (PaO_2 113mmHg) and 0.17 (PaO_2 78mmHg). Upon return to normoxia the SaO_2 returned to its pre-hypoxic level (ca.96%).

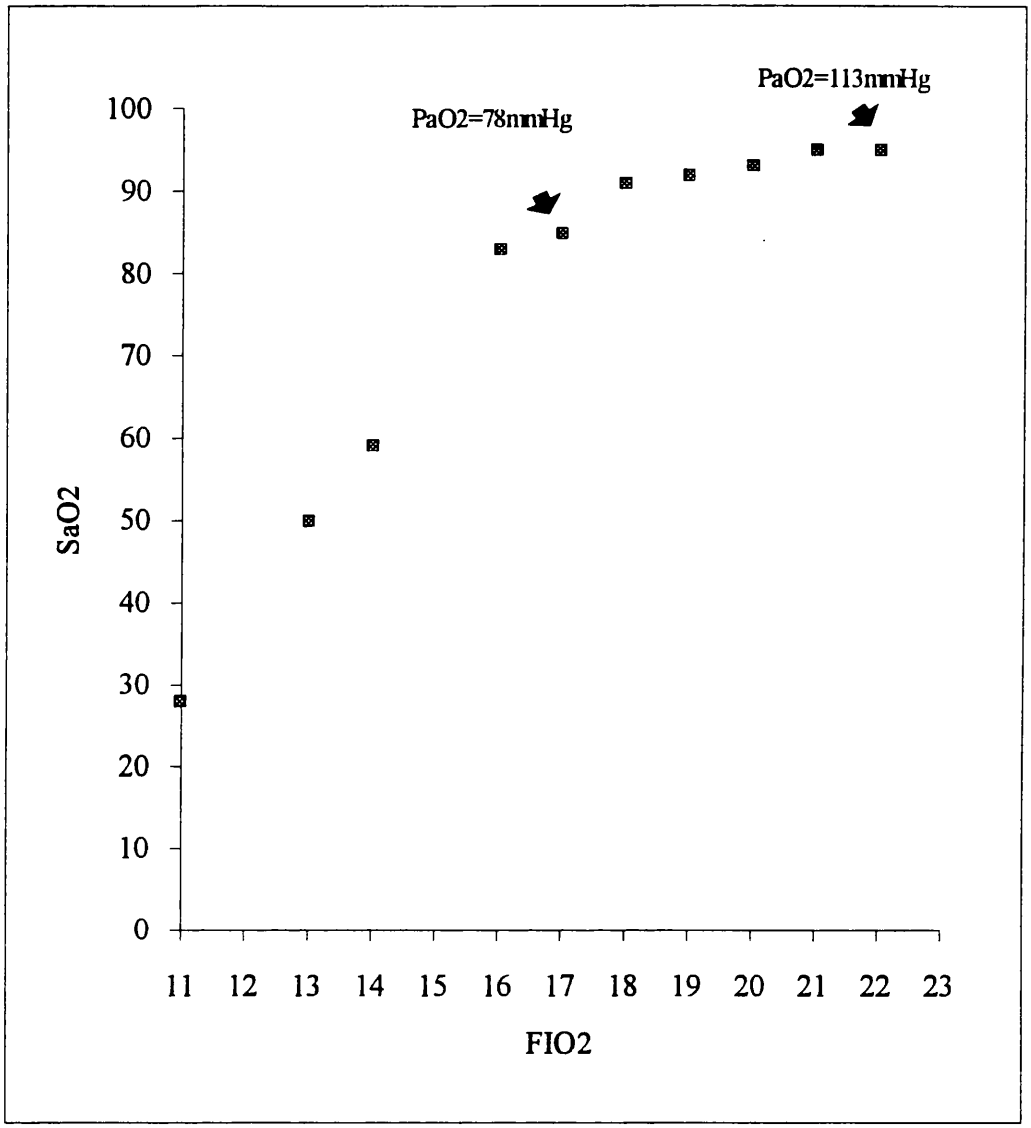


Figure 8. An oxygen dissociation curve for a kitten aged 18 days old and weighing 200g. Saturation (SaO_2) measured with a pulse oximeter (Ohmeda 5250) is plotted against fractional inspired oxygen (FiO_2) measured with a respiratory gas monitor (Ohmeda 5250). Blood samples were taken at an FiO_2 of 0.22 and 0.17 and revealed that the PaO_2 drops from 113mmHg to 78mmHg. In the majority of recordings the FiO_2 was reduced to 16%.

2.4.1 EXTRACELLULAR RECORDINGS

2.4.1.i The criteria for an extracelluar recording

A test was conducted only if the signal-to-noise ratio of the antidromic action potential could easily be discriminated. Examples are shown in figures 10, 11 and 12 in the following chapter.

2.4.1.ii Experimental Protocol

A period of normoxia was recorded for a minimum of 1 minute, followed by a period of hypoxia. The duration of the hypoxic period ranged from between 2 and 10 minutes (4.8 ± 0.26 mins, mean $\pm$ SEM,). FiO_2 during hypoxia ranged from between 0.14 and 0.18. The level of FiO_2 was then returned to normoxia.

2.4.1.iii Analysis

The TTL pulses were processed through a CED 1401 computer interface and analysed using a script program, written in the laboratory by Dr. R. Noble, to run with Spike2 software (Cambridge Electronic design, U.K). The moving time averages of discharge frequency and the mean discharge frequency over successive minutes were calculated. In the former case, the frequency is calculated over a period of time, for example 10 seconds, occuring prior to and including each event (action potential). Because it is responsive to event by event changes it is more sensitive to patterns of discharge than the mean discharge frequency over successive minutes.

2.4.1.iv Statistical Analysis

For each hypoglossal motoneurone, the 60 second period prior to hypoxia was used as the control. 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 using the unpaired Student's *t*-test (two-tailed, $P \leq 0.05$). In order to determine whether the response was sustained, the Student's *t*-test was also used to compare the discharge frequency in the last minute of hypoxia with the discharge frequency in the control period (two-tailed, $P \leq 0.05$); the Bonferroni correction was used to correct for the accumulation of error with multiple *t* tests (Glantz & Slinker, 1990).

2.4.2 INTRACELLULAR RECORDINGS

2.4.2.i The criteria for an intracellular recording

A hypoxic test was performed if the recording was relatively stable and the membrane potential was greater than -50mV. However, two motoneurones were tested with hypoxia despite the fact that their membrane potentials were only -36mV and -43mV. Both recordings were relatively stable and have therefore been included in the results.

2.4.2.ii Experimental Protocol

a) A period of normoxia was recorded followed by a period of hypoxia (up to 4 minutes). FiO_2 during hypoxia ranged from between 0.14 and 0.19. Where possible, i.e the recording was still stable, the oxygen level was returned to the normoxic level.

b) A few motoneurones were recorded during a period of hyperoxia. The FiO_2 was then decreased to normoxia.

c) Changes in input resistance were measured in some hypoglossal motoneurones by recording the voltage changes produced by constant hyperpolarizing current pulses (-1 nA, 2msec) passed through the intracellular microelectrode. The voltage across the electrode resistance was balanced prior to cell penetration using the bridge balance facility of the electrometer. The input resistance was measured during both normoxia and hypoxia.

2.4.2.iii Analysis

The intracellular recordings were processed using a CED 1401 computer interface and analysed using a script program, written in the laboratory by Dr. R. Noble, to run with Spike2 software (Cambridge Electronic design, U.K).

To measure the action potential characteristics, the script program differentiated the DC recording of the membrane potential to obtain dv/dt. It then used set dv/dt values to determine the onset and the termination of the fast components of the action potential. The action potential was displayed on a visual display unit and cursors automatically placed at the measured time of onset and termination (Fig. 9). In this way the processing could be monitored. The peak amplitude was also measured by the program. The time-to peak and the duration were measured as the time between the respective cursors. Triggered averages of the action potential were also obtained using Spike2 software.

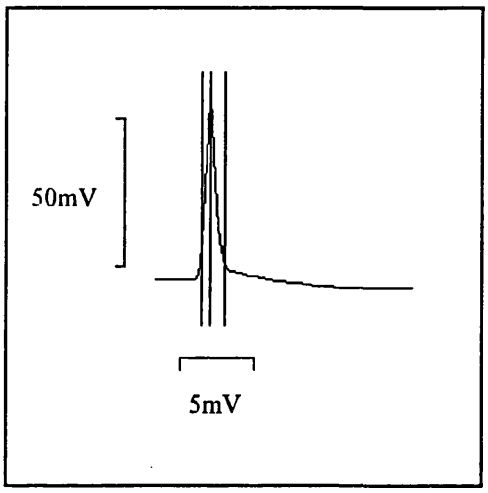


Figure 9. This shows the cursors placed at the onset, peak and termination of an action potential recorded with an intracellular electrode. The difference in their duration gives the duration and time to peak of the action potential.

2.4.2.iv Statistical analysis

The duration and amplitude of the rhythmic EPSP activity during hypoxia was compared to the corresponding activity during hypoxia and tested for significance using the unpaired two-tailed Student's *t* test ($P \leq 0.05$). However, because in some cases there were relatively few rhythmic EPSPs, during either the control or hypoxic period, these could not be tested for normality. The significance of any changes in this activity during hypoxia was therefore confirmed using the non-parametric Mann Whitney U test ($P \leq 0.05$). The results were only considered to be significant if the null hypothesis was rejected with both statistical tests.

In cases where the FiO_2 was returned to normoxia, the changes in rhythmic EPSP activity were tested for significance using the Student's unpaired *t* test with Bonferroni correction (Glantz & Slinker, 1990).

Throughout the results section, where applicable values will be expressed as mean ± the standard error of the mean (SEM)

2.5 SOLUTIONS AND CALIBRATIONS

Saline (0.9%, w/v)

9g of sodium chloride (Sigma Chemical Co, U.K) was dissloved in 1 litre of distilled water.

Heparin (10 units/ ml, C.P Pharmaceuticals Ltd, U,K)

1ml of heparin (5000 units per ml) was diluted with 9 mls of saline. 1ml of this solution was diluted further with 49 mls of 0.9% saline.

α-chloralose (50mg/kg)

0.5g of α-chloralose (Sigma Chemical Co, U.K) was stirred with 100mls of 0.9% saline and heated in a waterbath until all of the chloralose had disolved. The temperature of the waterbath was set at 70°C. The solution was allowed to cool to approximately 35°C before the appropriate volume for the weight of the animal was administered.

Gallamine triethiodide (7mg/Kg)

0.3mls of flaxedil (40mg/ml,May & Baker) were diluted with 1 ml of 0.9% saline and the volume, adjusted for the weight of the animal, administered.

The respiratory gas monitor was calibrated with a sample gas (Scott Medical products) prior to each experiment. The composition of the calibration gas was 6% CO_2, 4%

halocarbon-22, 40% N_20 and 50% O_2.

On the day of each experiment the blood pressure transducer was calibrated with a mercury manometer.

3. RESULTS

Ninety-one hypoglossal motoneurones were recorded in 31 kittens, 41 with extracellular and 50 with intracellular electrodes.

AN OVERALL VIEW OF THE RESULTS

The results have been divided into three sections.

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

Section 2 presents the results from intracellular recordings showing the effects of mild levels of arterial hypoxia on the membrane potential. The results then describe preliminary studies which examined the changes in membrane input resistance which occur during hypoxia. In addition, this section presents the results from motoneurones recorded during hyperoxia and as the FiO_2 was decreased to normoxia.

Section 3 looks at the action potential profile and the changes which occur during mild levels of hypoxaemia.

The results are summarized at the end of each section

3. 1 DISCHARGE FREQUENCY

In normoxia the hypoglossal motoneurones exhibited an irregular pattern of discharge with frequencies ranging from 0.00 to 14.42 $\pm$ 1.45 impulses per second. The discharge frequency for the majority of motoneurones was less than 2 impulses per second.

3.1.1 Effect of hypoxia on discharge frequency

The effect of hypoxia on discharge frequency was determined for 41 hypoglossal motoneurones recorded with extracellular microelectrodes in 18 kittens. The motoneurones were grouped according to their response to hypoxia and have been summarized in table 1. A two-tailed, unpaired Student's *t*-test ($P \leq 0.05$) was used to test the significance of the results using the criteria outlined in section 2.4.1.iv.

3.1.1.i Hypoglossal motoneurones which reponded to hypoxia with a sustained increase in discharge frequency

The discharge frequency increased in 14 hypoglossal motoneurones and this increase was sustained throughout the hypoxic period. The discharge frequency of these motoneurones during normoxia ranged from between 0.0 and 14.42 $\pm$ 1.45 impulses per second (Fig. 14). The maximum increase in discharge frequency was recorded within 2 to 5 mintues of reducing the FiO_2. The peak discharge frequency of the motoneurones during hypoxia ranged from between 2.0 $\pm$ 1.54 and 46.05 $\pm$ 2.21 impulses per second.

Two examples of hypoglossal motoneurones which responded to hypoxia in this way

	SUSTAINED INCREASE	TRANSIENT INCREASE	DECREASE	NO EFFECT	TOTAL
NUMBER OF MOTONEURONES	14 (34%)	11 (26%)	8 (5) (20%)	8 (20%)	41

Table 1. A summary of the responses of the hypoglossal motoneurones recorded in 18 neonatal kittens (13 to 27 days old) to mild levels of isocapnic hypoxia (FiO_2 0.14 to 0.18). The number in brackets are motoneurones which were inhibited during hypoxia but increased their discharge frequency in the post hypoxic period (Student's *t* test with Bonferroni correction).

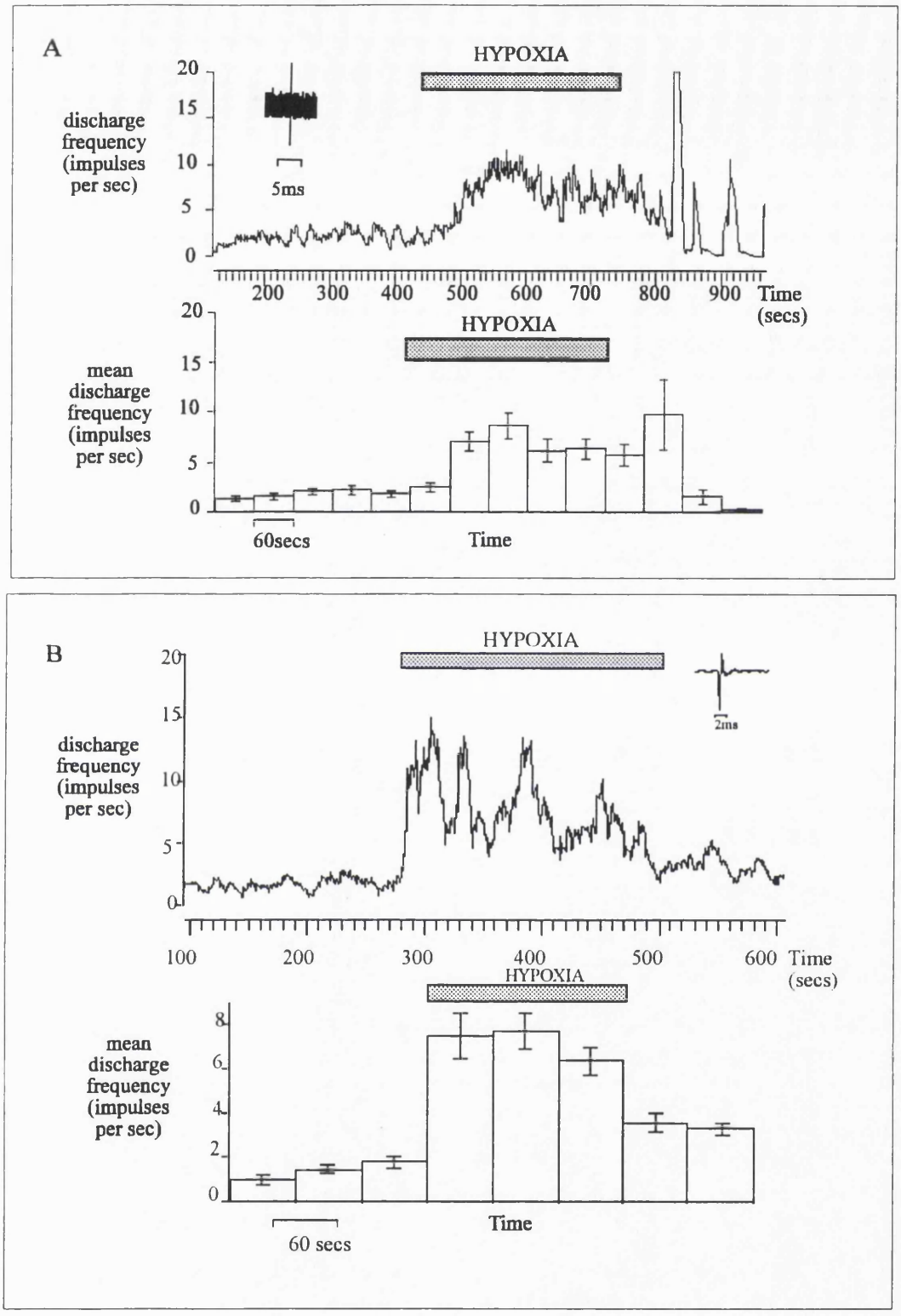


Figure 10. Two examples of hypoglossal motoneurones which responded to hypoxia (stippled bars, FiO_2 0.16 and 0.17 respectively) with a sustained increase in discharge frequency. The response is represented as a moving time average (top trace in each case, sampling time 10 secs) and discharge frequency averaged (mean $\pm$ SEM) over each successive minute (bottom trace in each case). The recordings were extracellular and insets show antidromic action potentials. The kittens were aged 25 and 26 days old respectively and weighed 610 and 390g .

are shown in figure 10. In one motoneurone, shown in figure 10a, the discharge frequency increased significantly ($P \leq 0.001$) from 1.35 ± 0.29 to 8.68 ± 1.24 impulses per second within 3 minutes of changing to hypoxia and although this decreased slightly to 6.43 ± 1.01 impulses per second by the last minute of hypoxia, this was still significantly greater ($P \leq 0.001$) than the discharge frequency recorded during the control period. The discharge frequency did not return to the control level until the FiO_2 had returned to normoxia. In the second motoneurone, shown in figure 10b, the discharge frequency increased from 0.98 ± 0.21 to 7.68 ± 0.77 impulses per second within 2 minutes of decreasing the FiO_2, and again, although there was a slight decrease in the discharge frequency after another minute of hypoxia, the level of discharge frequency (6.32 ± 0.65 impulses per second) was still significantly greater ($P \leq 0.001$) than the discharge frequency recorded during the control period.

3.1.1.ii Hypoglossal motoneurones which reponded to hypoxia with a transient increase in discharge frequency

Eleven motoneurones did not sustain the increase in discharge frequency for the duration of hypoxia, i.e by the last minute of hypoxia the level of discharge had returned to, or fallen below, the level recorded during the control period. These were classified as being transient. The discharge frequency of these motoneurones during normoxia ranged from between 0.07 ± 0.04 and 12.90 ± 1.52 impulses per second (Fig. 14). The discharge frequency of this group initially increased, reaching a maximum within 2 to 6 minutes of reducing the oxygen in the inspired air, but subsequently decreased to the prehypoxic level despite continuing hypoxia. In some motoneurones the decrease was to a level lower than that previously recorded during the control period. The peak discharge frequency during the initial response to hypoxia ranged from between 4.78 ± 1.09 and 85.63 ± 7.23 impulses per second.

Two examples of motoneurones which responded to hypoxia with only a transient

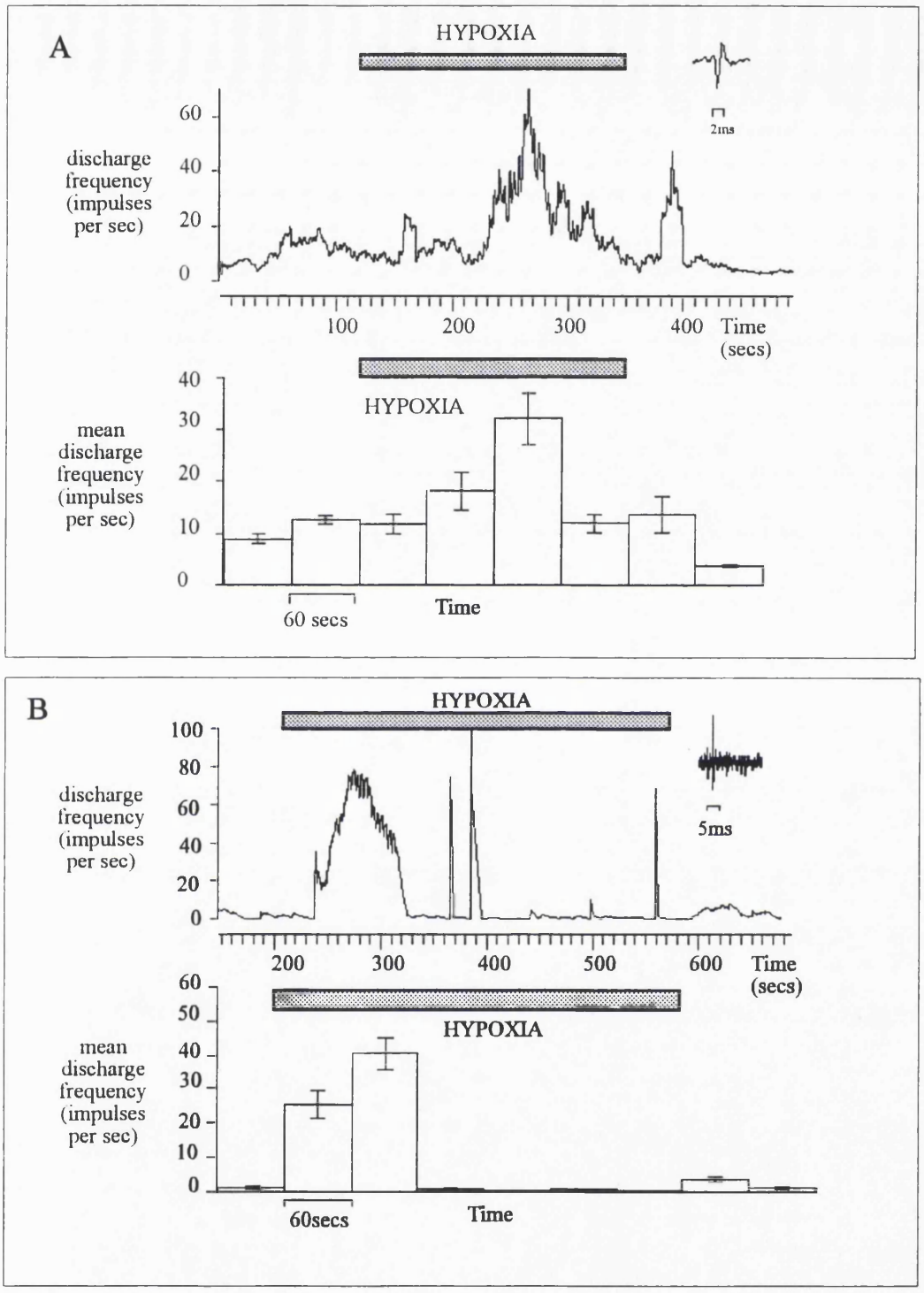


Figure 11. Two examples of hypoglossal motoneurones which responded to hypoxia (stippled bars, FiO_2 0.18 and 0.17 respectively) with only a transient increase in discharge frequency. The response is represented as a moving time average (top trace in each case, sampling time 10 secs) and discharge frequency averaged (mean + SEM) over each successive minute (bottom trace in each case). The recordings were extracellular and insets show antidromic action potentials. The kittens were aged 19 and 16 days old respectively and weighed 315 and 310g. Note that the motoneurone in A showed an increase in discharge frequency upon return to normoxia

increase in discharge frequency are shown in figure 11. In the first example, shown in figure 11a, the discharge frequency increased significantly ($P \leq 0.001$) from 8.98 ± 1.02 to 32.10 ± 4.89 impulses per second within 3 minutes of reducing the FiO_2 level. However, a minute later the discharge frequency was reduced to 11.98 ± 1.78 impulses per second which was not significantly different from the level of discharge frequency recorded in the control period. The discharge frequency of the second motoneurone, shown in figure 11b, increased significantly ($P \leq 0.001$) from 1.22 ± 0.25 to 40.52 ± 4.54 impulses per second within 2 minutes of changing the inspired air to hypoxia. However, within the next minute the discharge frequency had fallen to 0.88 ± 0.33 impulses per second, and in the last minute before returning to normoxia the discharge frequency was 0.40 ± 0.11 impulses per second. This was significantly lower ($P \leq 0.01$) than the discharge frequency recorded during the control period.

3.1.1.iii Hypoglossal motoneurones which reponded to hypoxia with a decrease in discharge frequency

Eight motoneurones showed a decrease in discharge frequency during hypoxia without showing an initial increase. The discharge frequency of these motoneurones during normoxia ranged from between 0.33 ± 0.12 and 12.08 ± 1.73 impulses per second (Fig. 14). Figure 12 shows two examples of motoneurones which responded to hypoxia in this way. The motoneurone in figure 12a had a discharge frequency during normoxia of 4.27 ± 0.79 impulses per second but after a minute of hypoxia this decreased significantly ($P \leq 0.001$) to a level of 0.32 ± 0.09 impulses per second. Similarly the discharge frequency of the motoneurone in figure 12b decreased significantly from 2.00 ± 0.72 to 0.10 ± 0.04 impulses per second ($P \leq 0.01$) within 3 minutes of reducing the FiO_2 level. However, when the FiO_2 was again returned to normoxia the discharge frequency of this motoneurone increased to 11.92 ± 1.83 impulses per second. This was significantly greater than the discharge frequency recorded in the control period ($P \leq 0.001$). Five of these motoneurones showed a

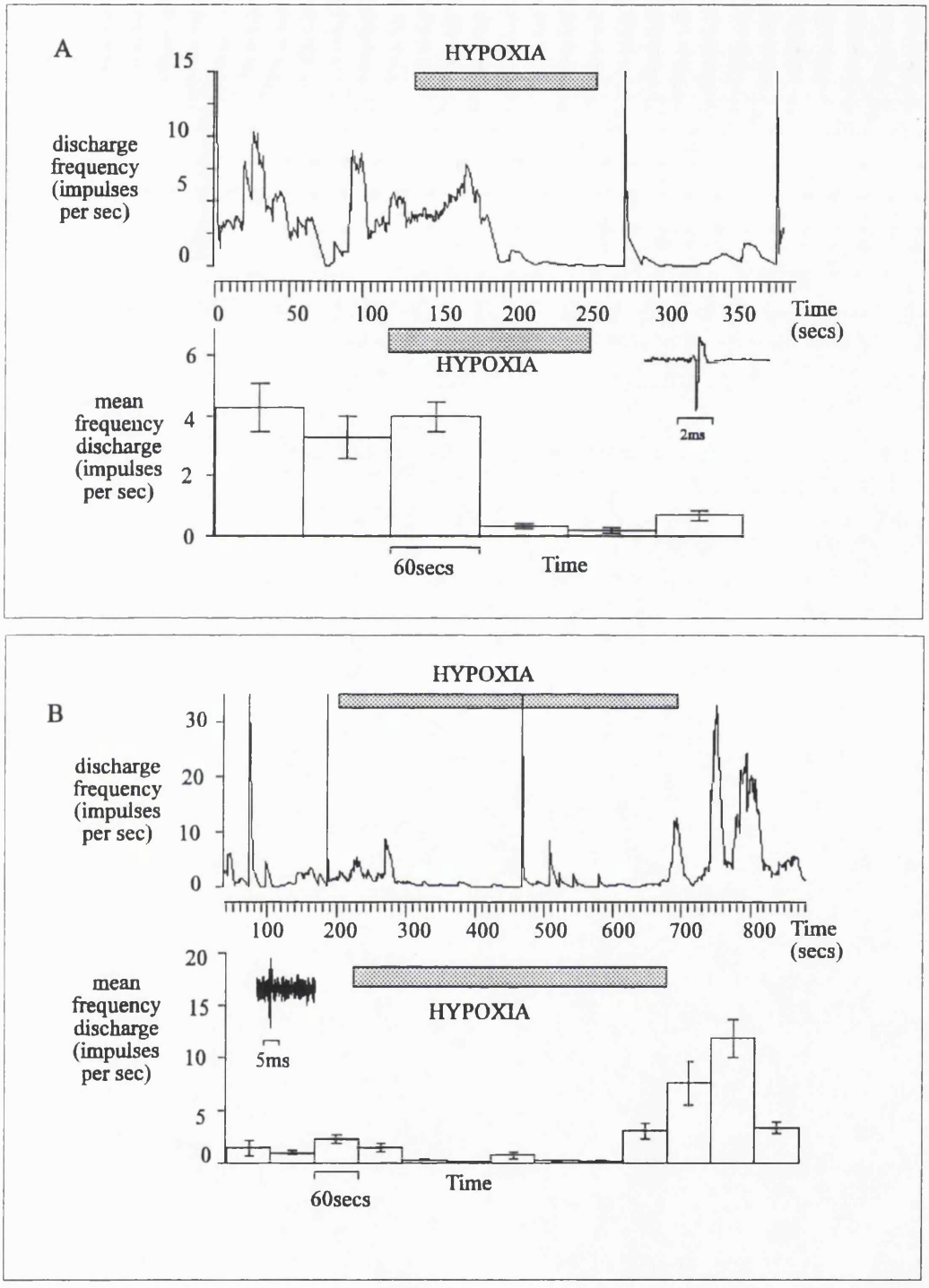


Figure 12. Two examples of hypoglossal motoneurones which responded to hypoxia (stippled bars, FiO_2 0.17 and 0.15-0.14 respectively) with a decrease in discharge frequency. In both cases the discharge frequency was reduced through out the hypoxic period. In the motoneurone in B there was an increase in discharge frequency in the posthypoxic period (not observed in A). Each response is represented as a moving time average (top trace, sampling time 10 secs) and discharge frequency averaged (mean + SEM) over each successive minute (bottom trace). The recordings were extracellular and insets show antidromic action potentials. The kittens were aged 16 and 27 days old respectively and weighed 279 and 585g. Note that in both cases during hypoxia the motoneurones were still able to generate action potentials and could be antidromically activated.

significant increase ($P \leq 0.01$) in discharge frequency in the immediate period after hypoxia when compared to the discharge frequency during the control period.

During the decrease in both the transient and inhibited groups, the motoneurones were still able to generate action potentials when antidromically activated.

3.1.1.iv Hypoglossal motoneurones which did not alter their discharge frequency during hypoxia

Eight motoneurones showed no alteration in discharge frequency during hypoxia (FiO_2 0.18-0.16 for 4 to 8 minutes). The discharge frequency of these motoneurones ranged between 0.12 ± 0.07 to 2.4 ± 0.3 impulses per second (Fig. 14). In 4 of these motoneurones the FiO_2 was reduced further, reaching 0.14 in one motoneurone. However, there were still no changes in the discharge frequency of these motoneurones.

3.1.1.v Influence of the discharge frequency during normoxia on the hypoglossal motoneurones response to hypoxaemia

The discharge frequency of the majority of the hypoglossal motoneurones during normoxia was less than 2 impulses per second (Fig. 13). For those motoneurones which responded to hypoxia with an increase or decrease in discharge frequency, the range of discharge frequencies during normoxia were similar (Fig. 14). However, as described above section 1.3.1.iv, motoneurones which showed no obvious change in discharge frequency during hypoxia displayed a relatively low level of discharge, ranging from 0.12 ± 0.07 to 2.4 ± 0.3 impulses per second.

During hypoxia the discharge frequency of the majority (83%) of motoneurones recorded was less than 10 impulses per second (Fig. 13). There was a significant

correlation (r= 0.66, $P < 0.001$) between the discharge frequency during normoxia and the discharge frequency during hypoxia.

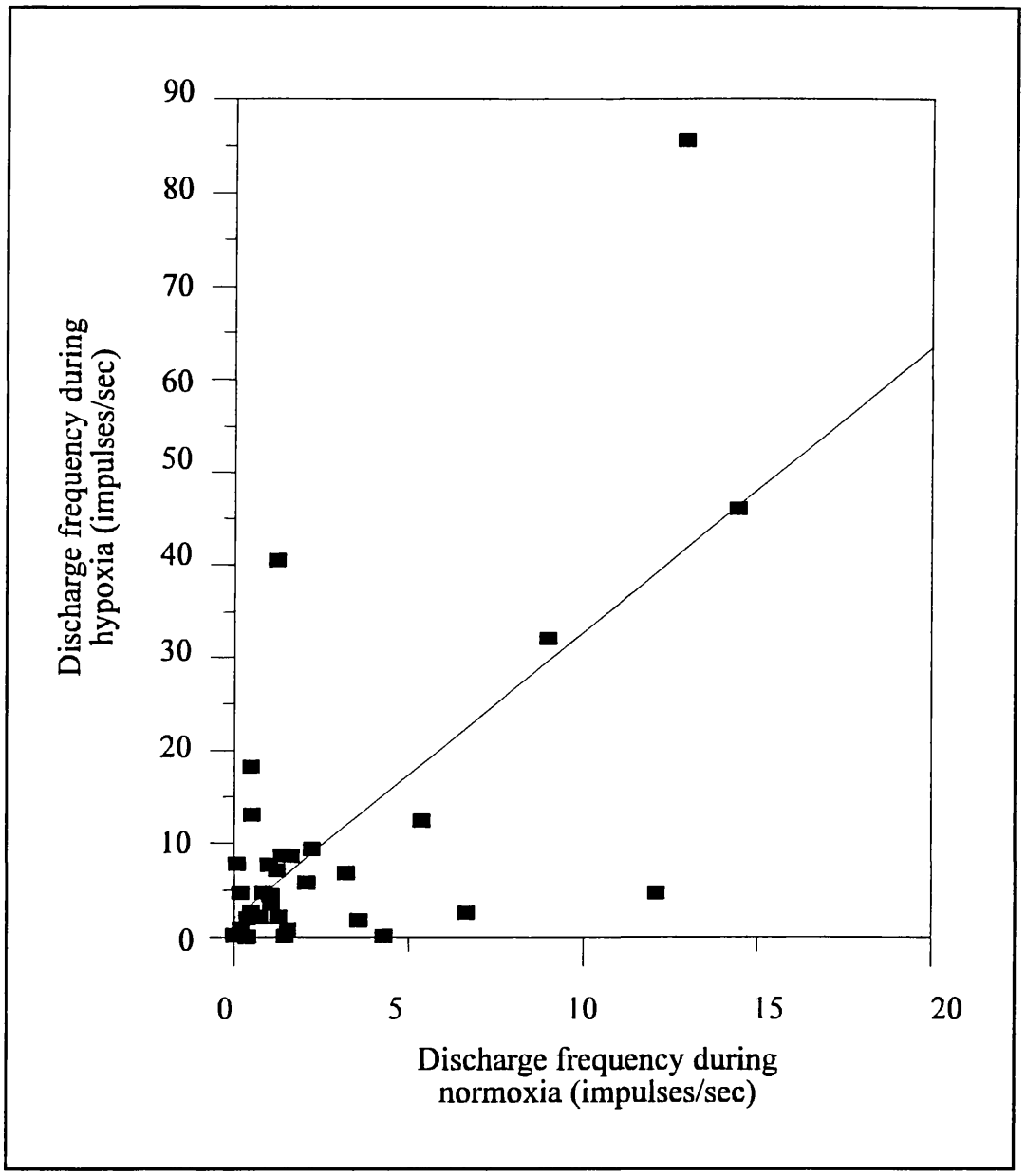
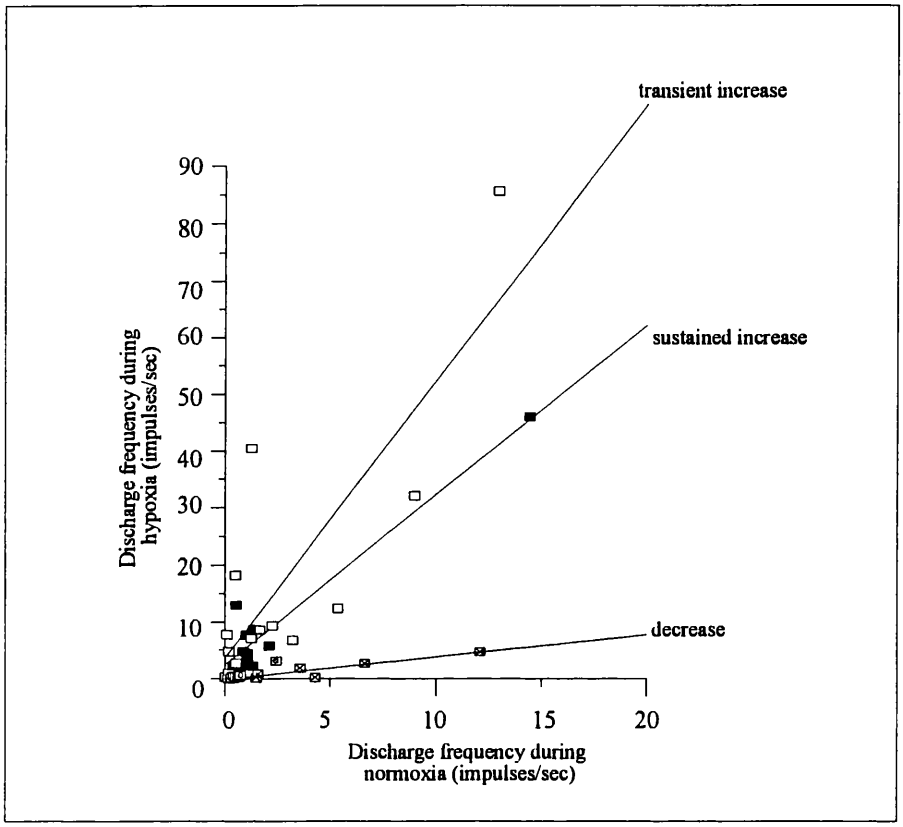


Figure 13. A graph showing the mean discharge frequencies (impulses per second), recorded with extracellular electrodes, of the hypoglossal motoneurones during hypoxia as a function of their mean discharge frequencies (impulses per second) during normoxia. There was a significant correlation between the two factors (r= 0.66, $P<0.001$). The equation of the line of best fit is y = 1.39 + 3.01x.



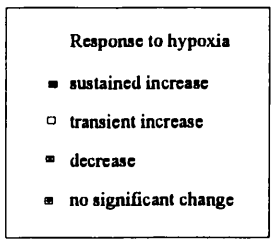


Figure 14. A graph showing the mean discharge frequencies (impulses per second), recorded with extracellular electrodes, of the hypoglossal motoneurones during hypoxia as a function of their mean discharge frequencies (impulses per second) during normoxia. The hypoglossal motoneurones have been represented by symbols according to their response to hypoxia and this is shown in the key. The lines are those of best fit for the motoneurones which responded to hypoxia with a transient increase, a sustained increase and a decrease in discharge frequency, respectively. For each response characteristic, there was a significant correlation ($P<0.01$) between the discharge frequency during hypoxia and that recorded during normoxia. The correlation coefficients were 0.79, 0.95 and 0.93 for the transient increase, sustained increase and decrease in discharge frequency, respectively. The equations of the lines were; $y=5.1+4.7x$, $y=2.2+3.02x$ and $y=-0.18+0.4x$, respectively.

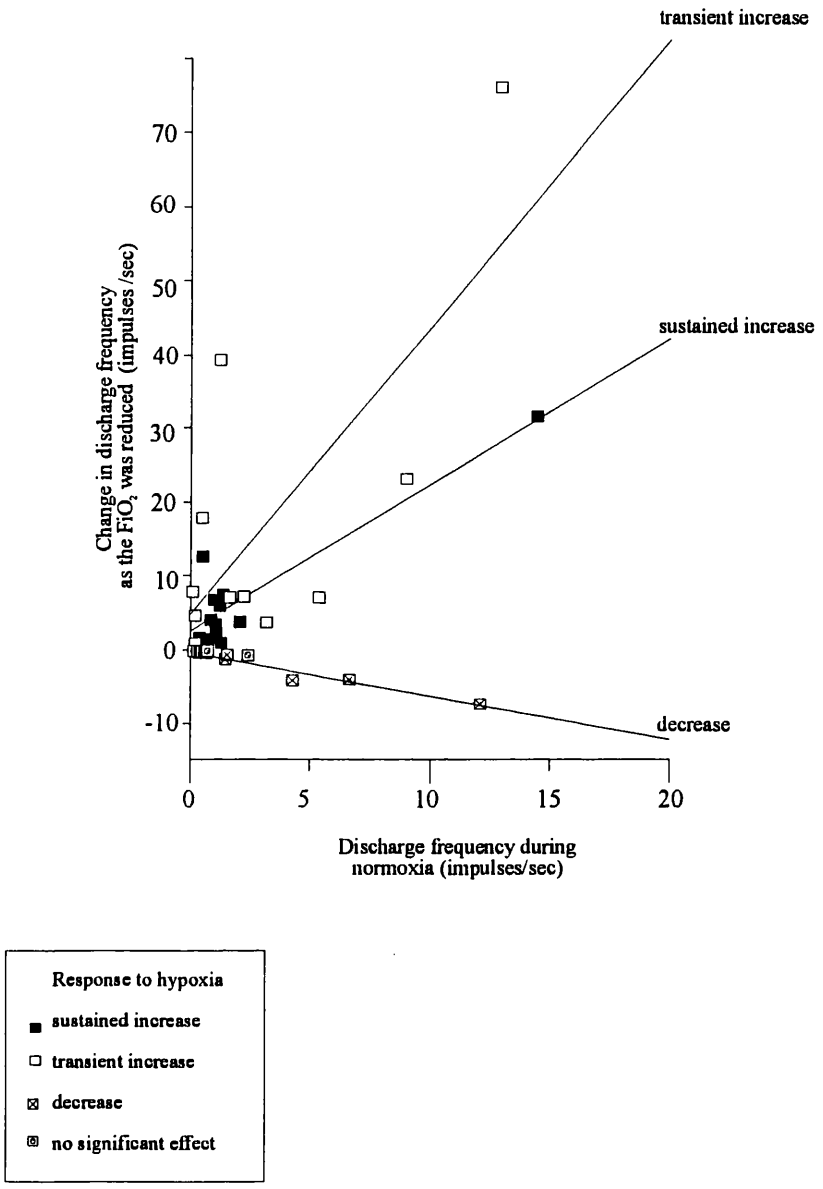


Figure 15. A graph showing the change in mean discharge frequencies (impulses per second), recorded with extracellular electrodes, of the hypoglossal motoneurones as the FiO2 was reduced, plotted as a function of their mean discharge frequencies (impulses per second) during normoxia. The hypoglossal motoneurones have been represented with symbols according to their response to hypoxia and this is shown in the key. The lines are those of best fit for the motoneurones which responded to hypoxia with a transient increase, a sustained increase and a decrease in discharge frequency, respectively. For each response characteristic, there was a significant correlation ($P<0.01$) between the change in discharge frequency during hypoxia and that recorded during normoxia. The correlation coefficients were 0.72, 0.91 and 0.97 for the transient increase, sustained increase and decrease in discharge frequency respectively. The equations of the lines were; $y=4.8+ 3.9x$, $y=2.5+2.0x$, $y=-0.3-0.6x$, respectively.

When the motoneurones were grouped according to their response to hypoxia there was a significant correlation ($P< 0.01$) between the discharge frequency during normoxia and the discharge frequency of the motoneurones during hypoxia (Fig. 14). The correlation coefficients are given in the figure legend. The majority of motoneurones with a discharge frequency of greater than 10 impulses per second during hypoxia responded with a transient increase in discharge frequency, i.e by the end of hypoxia the level of discharge had returned to, or below the level recorded during the control period.

Figure 15 shows the change in discharge frequency of the motoneurones when the FiO_2 was reduced, as a function of the discharge frequency during normoxia. As the graph shows, when the motoneurones were considered according to their response to hypoxia, i.e sustained increase, transient increase and decrease in discharge frequency, there was a significant correlation ($P < 0.01$) between the change in discharge frequency during hypoxia and the discharge frequency of the motoneurones during normoxia. The individual correlation coefficients are given in the legend of figure 15. Again the majority of the motoneurones with the greatest change (> 10 impulses per second) in discharge frequency during hypoxia exhibited a transient response.

3.1.1.vi Are the different reponses recorded due to the variation in the level of hypoxia used?

Although different levels of hypoxia were used, ranging from between 0.14 and 0.18, at each level of hypoxia hypoglossal motoneurones could be recorded which showed any one of the responses described above. For example, with an FiO_2 of 0.17; 5 motoneurones showed a sustained increase, 3 showed a transient increase, 4 showed a decrease and 3 showed no change in discharge frequency. This is shown in both figures 16 and 17.

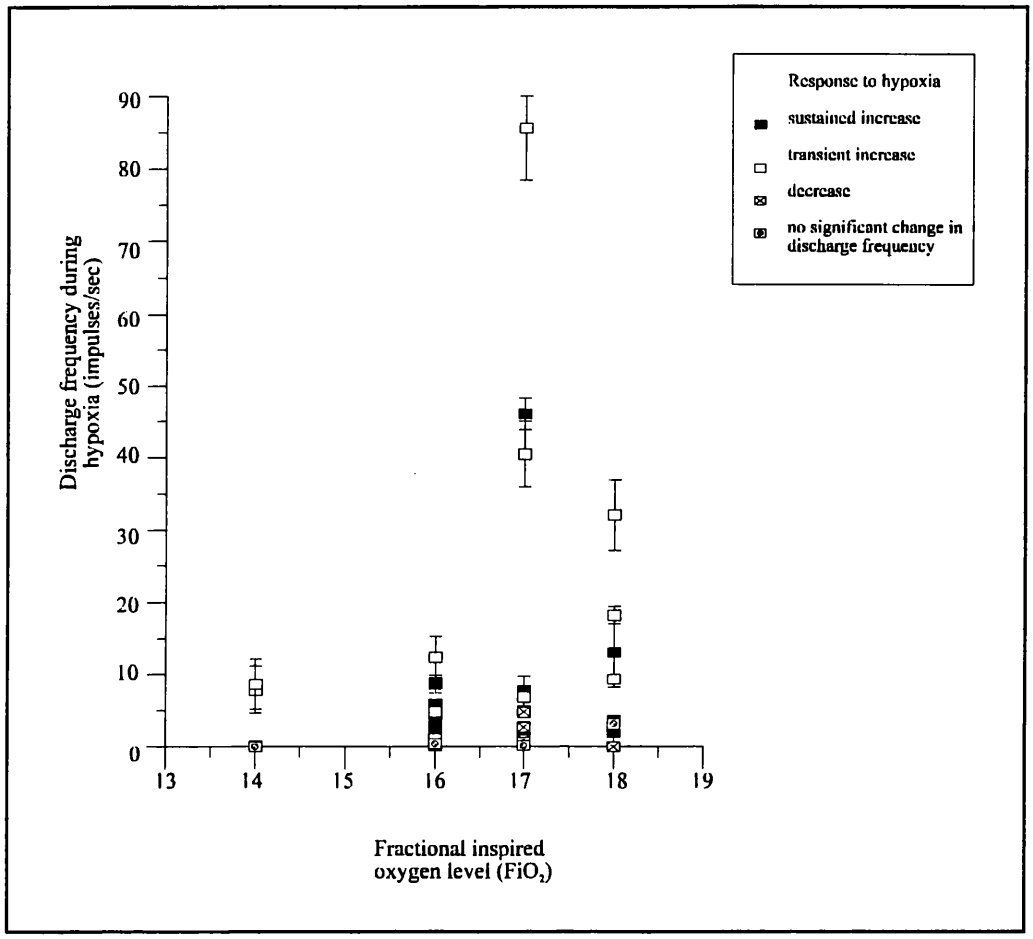


Figure 16. A graph showing the mean discharge frequencies (impulses per second) of hypoglossal motoneurones during hypoxia as a function of the fractional inspired oxygen level (FiO_2). The response characteristics to hypoxia are represented with different symbols shown in the key. There was no correlation between the two factors when the motoneurones were considered according to their response characterisitics (r= 0.32, 0.28 & 0.22) for the transient increase, sustained increase and decrease respectively.

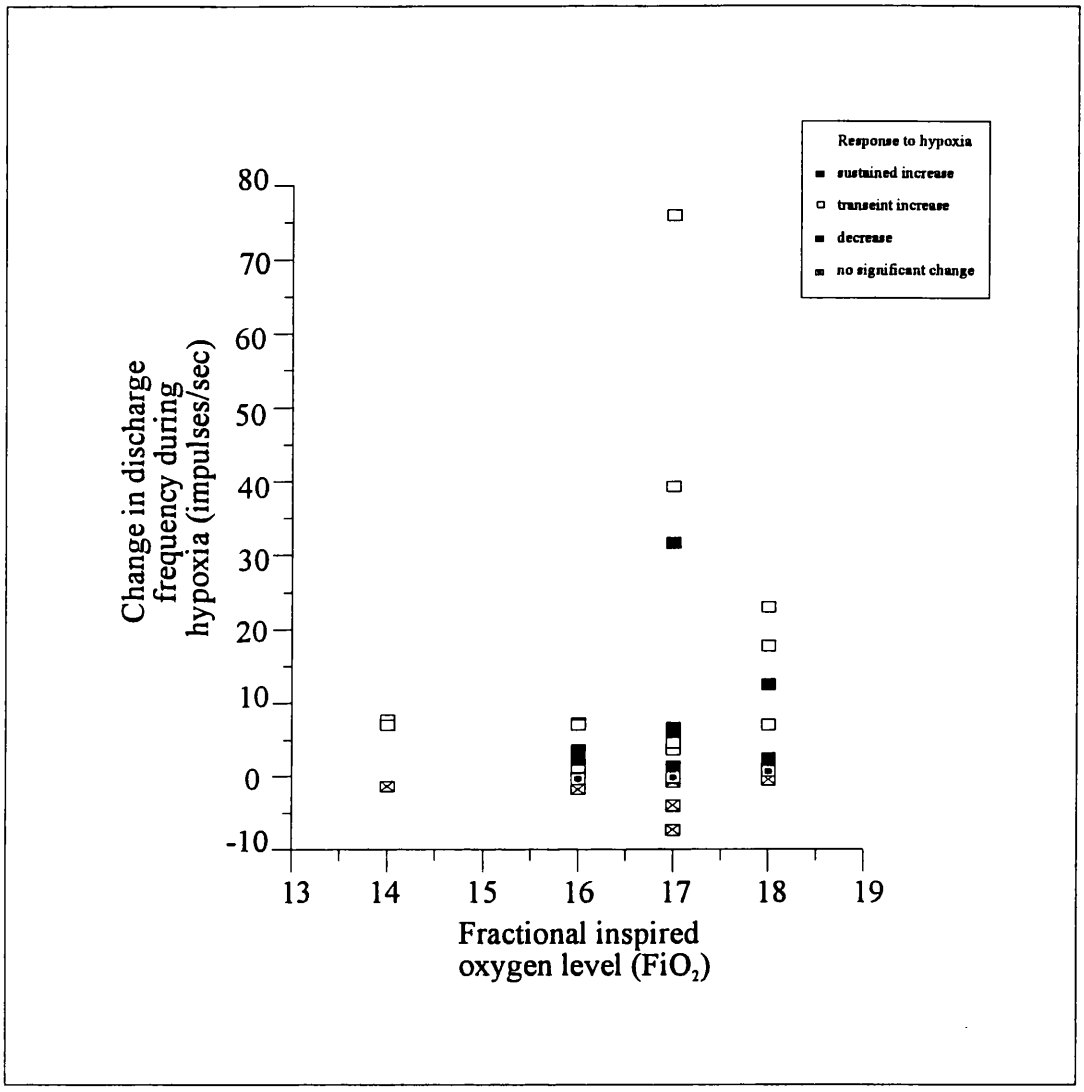


Figure 17. A graph showing the change in mean discharge frequencies of hypoglossal motoneurones during hypoxia (impulses per second) as a function of the fractional inspired oxygen level (FiO_2). The response characteristics to hypoxia have been represented with different symbols shown in the key. There was no correlation between the two factors when the motoneurones were considered as a group ($r = 0.14$, $P>0.05$) or when the motoneurones were considered according to their response characteristics ($r= 0.24$, 0.14 & 0.2) for the transient increase, sustained increase and decrease, respectively.

When the motoneurones were considered according to their response characteristics, for each group there was no correlation between the level of FiO_2 and either the discharge frequency during hypoxia (Fig. 16), or the change in discharge frequency as the FiO_2 was reduced (Fig. 17). The correlation values are given in the figure legends.

3.1.2 Latency of antidromic action potentials

The latency of the antidromic action potentials for the hypoglossal motoneurones recorded with extracellular electrodes ranged from between 0.6 and 2.8 msecs. However, for each hypoglossal motoneurone there was no change in the latency when challenged with hypoxia. The response of the hypoglossal motoneurones to hypoxia occurred irrespective of latency i.e when grouped according to their response to hypoxia the range of latencies of the groups were similar.

3.1.3 Patterns of discharge in normoxia and hypoxia

During normoxia, only 2 of the 41 motoneurones showed any clear rhythmic activity (determined by observing the traces). In the first motoneurone (Fig. 10a) there was an increase in discharge frequency during hypoxia (FiO_2 0.16). In figure 18a the discharge of this motoneurone is represented as a gated pulse generated by the spike processor. During hypoxia the frequency of the bursts of action potentials increased from 12 to 14 bursts per minute. The duration of the bursts and the mean number of action potentials per burst significantly increased from 0.65 ± 0.08 to 1.20 ± 0.16 seconds (two-tailed, unpaired Student's *t* test $P \leq 0.05$) and from 7.17 ± 1.20 to 24.47 ± 4.73 impulses per second ($P \leq 0.001$), respectively (Fig. 18b). In addition to the changes in phasic discharge there was an increase in irregular tonic activity.

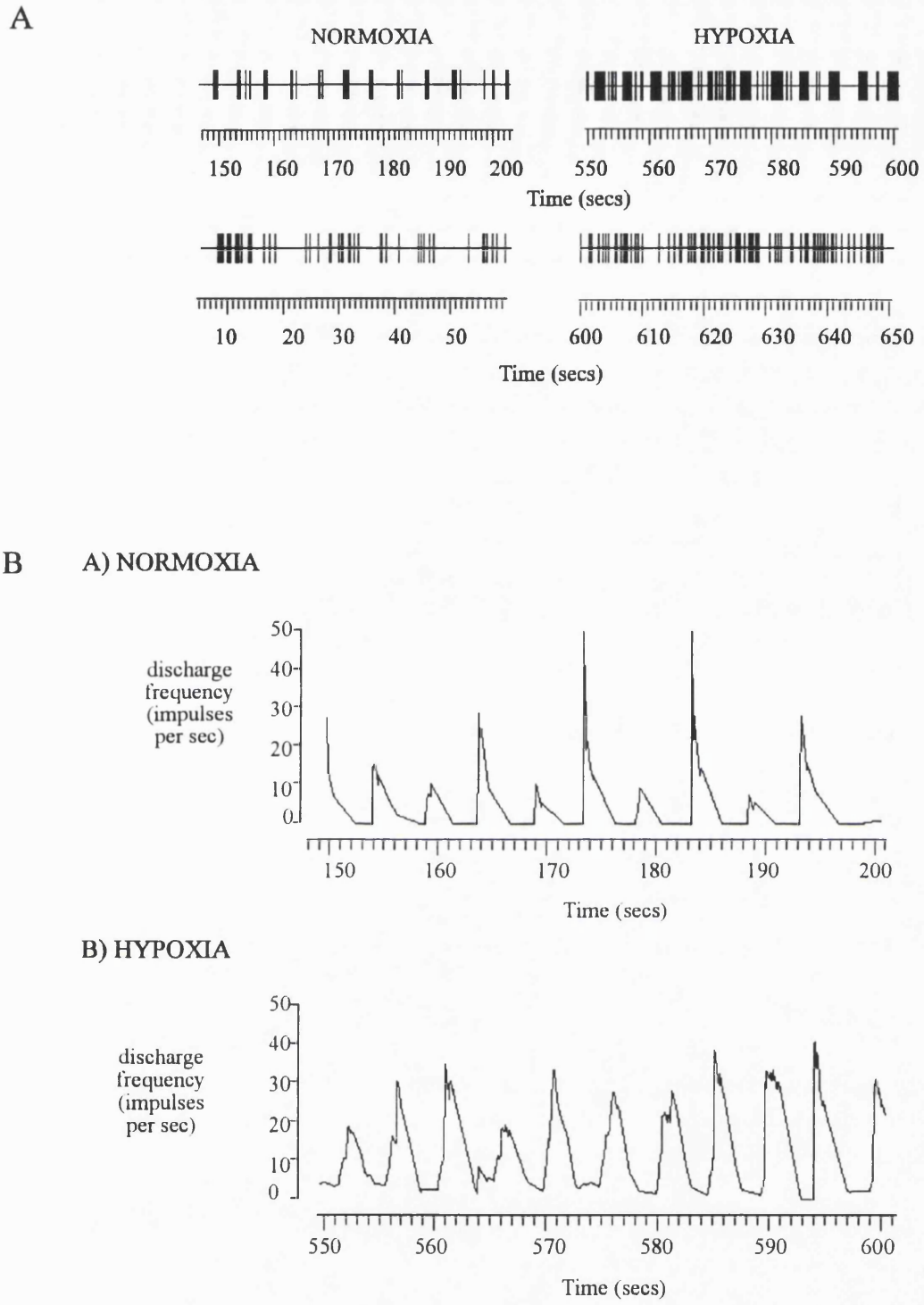


Figure 18. A) Patterns of discharge of two hypoglossal motoneurones during normoxia and hypoxia. In each case the motoneurones were recorded extracellularly and the discharge is represented as a gated pulse generated by a spike processor. In the top trace the discharge was rhythmic during normoxia (left) and this activity was increased in both duration and frequency during hypoxia (FiO2 0.16, right). The bottom trace in A shows a motoneurone which increases its discharge frequency during hypoxia (FiO2 0.16) but with no obvious rhythmic activity. B) shows the moving time average (sampling time was 3 secs) of the recording shown in the top trace of A) during both normoxia and hypoxia.

In the second motoneurone there was a transient increase in discharge frequency during hypoxia (FiO_2 0.18). The discharge frequency has again been represented as a gated pulse generated by the spike processor (Fig. 19). During the first 2 minutes of hypoxia, as discharge frequency increased, the individual phasic bursts of activity became indistinguishable. However, within the next minute the discharge frequency declined and distinct phasic bursts of discharge were again recorded. When the phasic bursts of activity in this latter period of hypoxia were compared with those recorded in the control period there was no significant difference in frequency (16 bursts per minute) or duration of the bursts (0.87 ± 0.11 during normoxia compared to 1.17 ± 0.17 seconds during hypoxia). Although the number of action potentials per burst did increase slightly from 8.46 ± 0.92 to 11.21 ± 1.88 impulses per second this was not statistically significant.

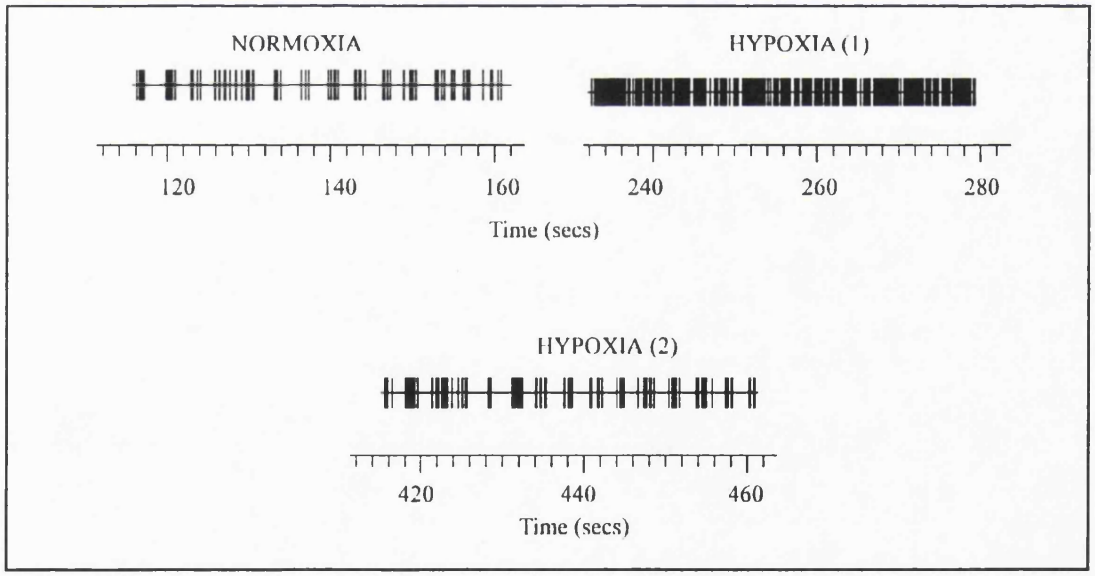


Figure 19. Pattern of discharge of a hypoglossal motoneurone during normoxia, early hypoxia (1) and late hypoxia (2). The hypoglossal motoneurone which responded to hypoxia (FiO_2 0.18) with a transient excitation. The motoneurone was recorded extracellularly and is represented as a gated pulse. The discharge was rhythmic during normoxia but during early hypoxia there was an increase in discharge frequency and the bursts became indistinguishable. However, after a further minute the discharge frequency decreased and again rhythmic bursts could be distinguished. The duration of the bursts (secs) and the number of action potentials

per burst (impulses/sec) were not significantly different from those recorded in the control period.

Three additional motoneurones showed phasic activity during hypoxia although this pattern of discharge was not clearly distinguishable during normoxia. The discharge of one of these motoneurones, shown in figure 11b, is shown in figure 20 as a gated pulse generated by the spike processor. This diagram clearly shows that the motoneurone has a phasic pattern of discharge during the initial part of hypoxia which was not clear during normoxia.

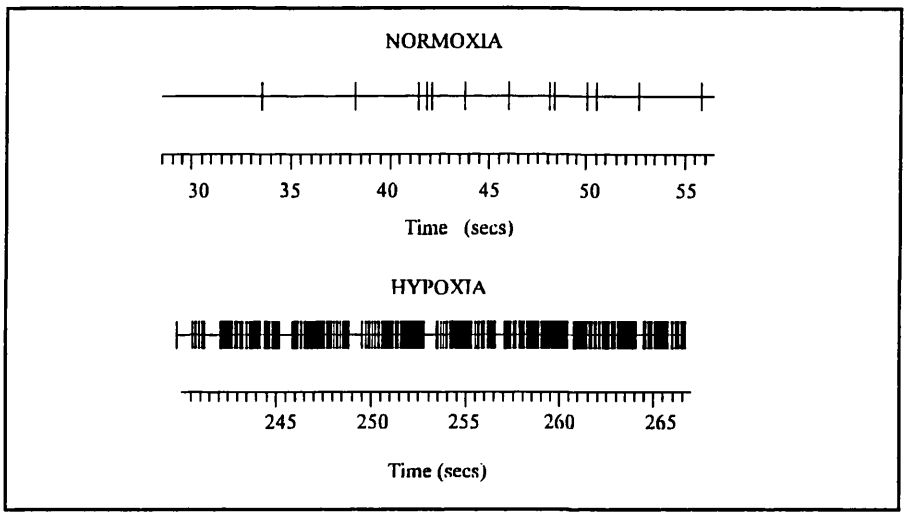


Figure 20. Pattern of discharge of a motoneurone recorded extracellularly during normoxia (upper trace) and hypoxia (lower trace). The discharge is represented as a gated pulse generated by a spike processor. The discharge frequency during normoxia was 1.2 ± 0.25 (mean ± SEM) impulses per second and no distinguishable rhythmic activity could be observed in the trace. However, during hypoxia (FiO_2 0.17) the discharge frequency increased to 40.5 ± 4.54 impulses per second and distinct bursts of activity could be distinguished.

The majority of motoneurones (n=36) had no obvious phasic pattern of discharge and the changes in frequency during hypoxia were attributed to an increase or decrease in

irregular tonic activity. An example is shown in figure 18.

3.1.4 Effect of age and end-tidal CO_2 on the discharge frequency and the response of the hypoglossal motoneurones to hypoxia

Although further studies are required to look specifically at the effect of age and the level of end-tidal CO_2 on the discharge frequency and the response of the hypoglossal motoneurones to hypoxia, the effects that these parameters may have on the results of the present study have been considered.

3.1.4.i The effect of age on the discharge frequency and the response of the hypoglossal motoneurones to hypoxia

There was no correlation ($r < 0.001$) between the age of the animal on the day of recording and the level of discharge frequency of the hypoglossal motoneurones during normoxia (Fig. 21).

The hypoglossal motoneurones were divided into two groups, according to the age of the kitten in which they were recorded ($\leq$ 21 days and > 21 days). Twenty one days was chosen as the age at which to divide the group because it is around this time that neonatal kittens respond to hypoxia (approx. 5 mins) with a maintained increase in ventilation (see appendix A.). The results are summarized in table 2. There was no significant difference in the responses of the hypoglossal motoneurones to mild levels of hypoxia recorded in the two groups (chi-squared $P \geq 0.05$). This is also shown in figure 22.

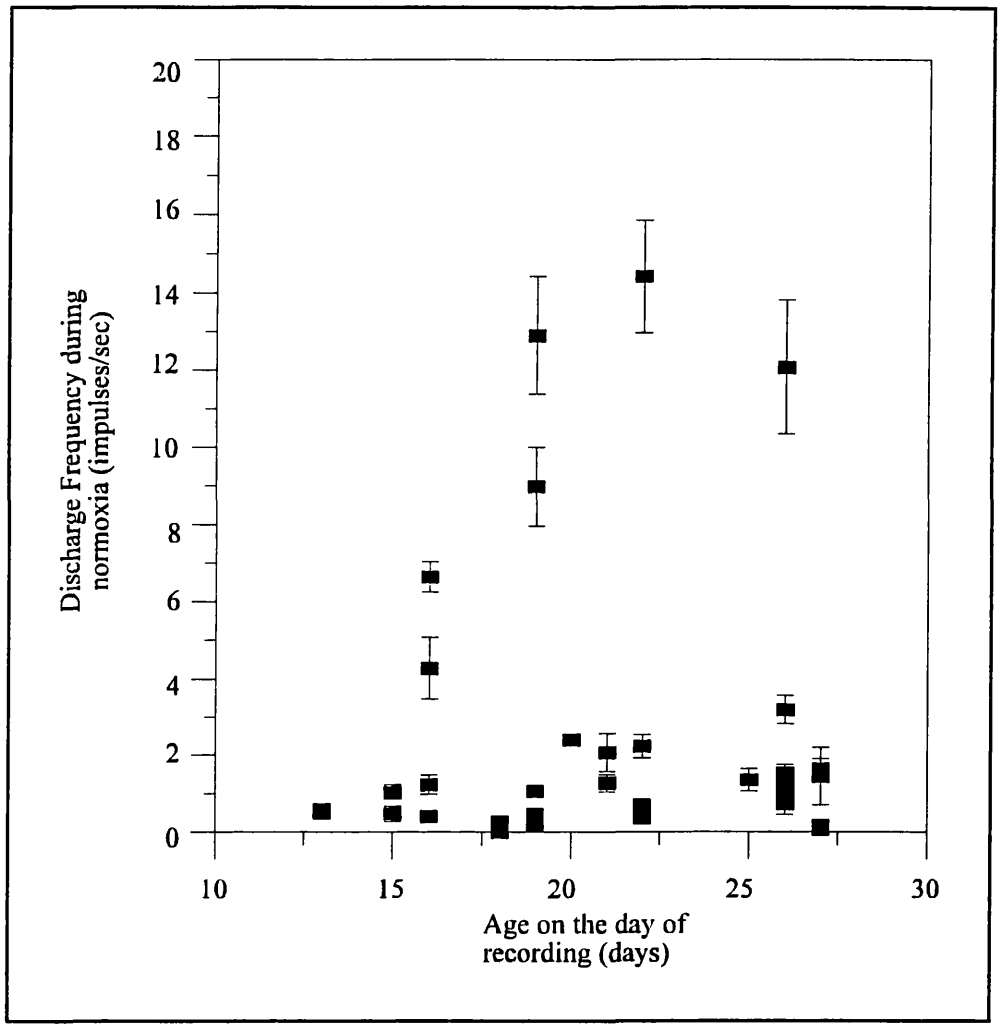


Figure 21. A graph showing the mean discharge frequencies (impulses per second) of hypoglossal motoneurones during normoxia against the age of the animal on the day of the recording. There was no significant correlation between the two factors ($r<0.001$).

	SUSTAINED INCREASE	TRANSIENT INCREASE	DECREASE	NO EFFECT	TOTAL
< 21 DAYS	7	5	3 (1)	7	22
> 21 DAYS	7	6	5 (4)	1	19
TOTAL	14	11	8	8	41

Table 2. A summary of the responses of the hypoglossal motoneurones recorded in 18 neonatal kittens (13 to 27 days old) to mild levels of isocapnic hypoxia (FiO_2 0.14 to 0.18). The kittens have been divided into two groups according to their age (< 21 and > 21 days) on the day of the recording. The number in brackets are motoneurones which were inhibited during hypoxia but increased their discharge frequency in the post hypoxic period (Student's *t* test with Bonferroni correction).

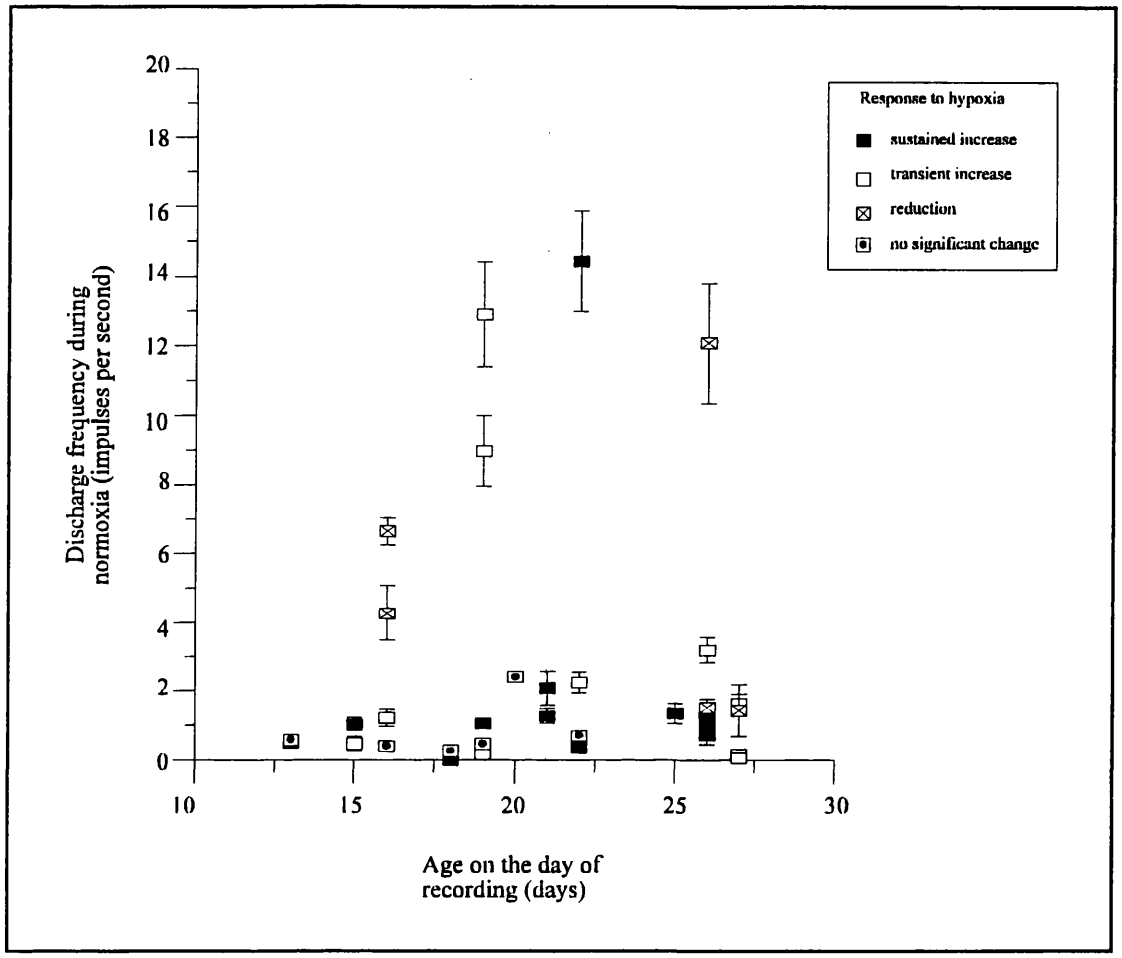


Figure 22. A graph showing the mean discharge frequencies of hypoglossal motoneurones during normoxia (impulses per second) against the age of the kittens on the day of the recording. The response characteristics to hypoxia have been represented with different symbols shown in the key. There was no significant correlation between the discharge frequency of the motoneurones during normoxia and the age of the kittens on the day of recording when the motoneurones were grouped according to their response to hypoxia (r=0.22, 0.24 & 0.008 for the transient increase, sustained increase & decrease respectively).

Figure 23 below shows that the change in discharge frequency (impulses per second) during hypoxia did not correlate (r =0.14, P>0.05) with the age of the kitten on the day of recording. This graph again shows that the response to hypoxia (i.e sustained

increase, decrease etc.) recorded with the extracellular electrodes occurred in kittens regardless of their age.

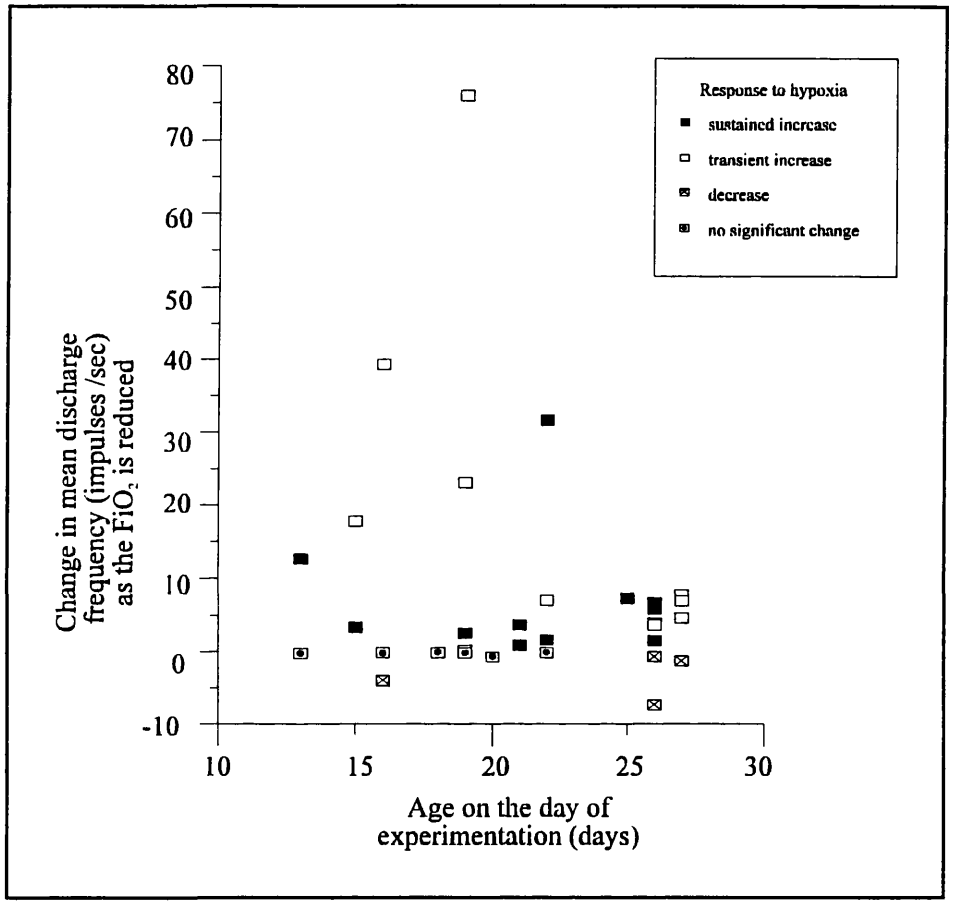


Figure 23. A graph showing the change in mean discharge frequencies (impulses per second) of hypoglossal motoneurones during normoxia against the age of the kittens on the day of the recording. The response characteristics to hypoxia have been represented with different symbols shown in the key. For the hypoglossal motoneurones which responded to hypoxia with a sustained excitation or inhibition, there was no correlation (r=0.10 & 0.22, respectively) between the two factors. For the motoneurones which responded to hypoxia with a transient excitation the equation of the line of best fit was y=72.8-2.5x and the correlation coefficient was 0.52. This was just significant (P=0.05).

It was possible to record more than one type of response in any one kitten, for example, in one kitten hypoglossal motoneurones were recorded which responded to

hypoxia with a sustained increase and others responded with a transient increase in discharge frequency. Futhermore, there was no pattern to the order with which these responses were recorded.

3.1.4.ii The effect of end-tidal CO_2 on the discharge frequency and the response of the hypoglossal motoneurones to hypoxia

Although for each test the $P_{ET}CO_2$ was constant, this varied between tests. For this group of motoneurones recorded with extracellular electrodes, the end-tidal CO_2 ranged from 31 to 56 mmHg (41.8 ± 0.82 mmHg).

When the discharge frequency of the hypoglossal motoneurones during normoxia, taken as a group, were plotted against the level of $P_{ET}CO_2$ in each case, there was no correlation ($r = 0.19$, $P > 0.05$) between the two factors (Fig. 24).

However, when the change in discharge frequency (impulses per second) of the motoneurones as the FiO_2 as a group, was considered as a function of the $P_{ET}CO_2$ level (mmHg), although there was only a weak correlation between these two factors ($r = 0.36$), this was found to be significant ($P<0.05$). This is shown in figure25.

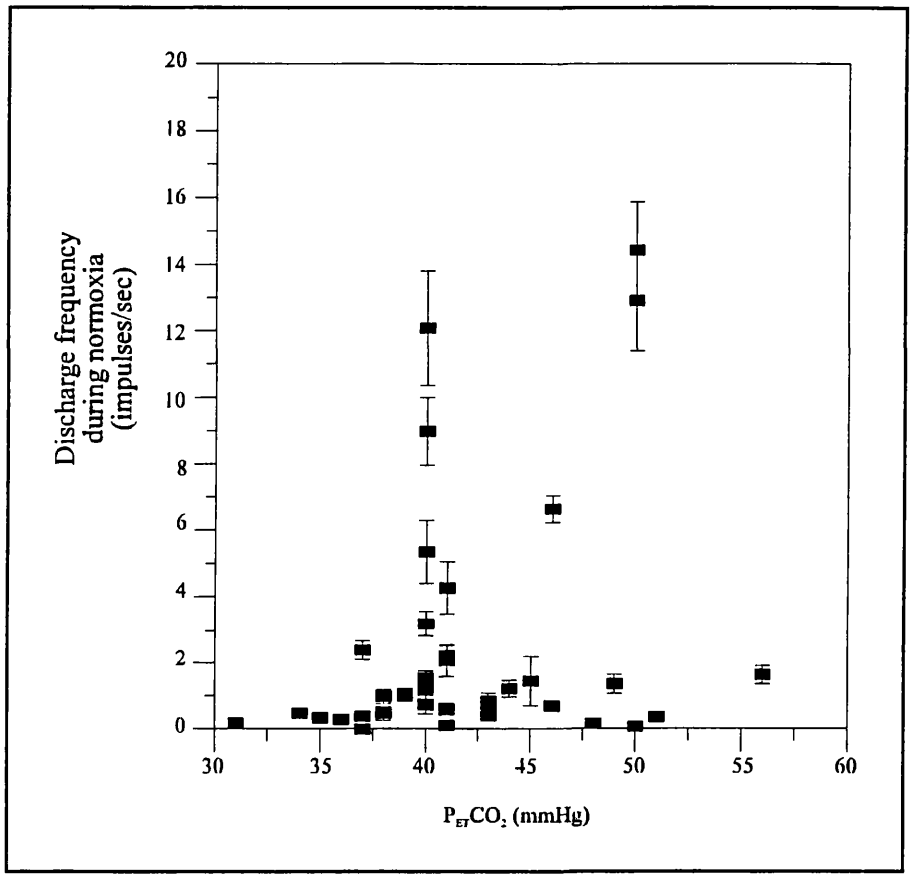


Figure 24. A graph showing the mean discharge frequencies (impulses per second), recorded with extracellular electrodes, of hypoglossal motoneurones during normoxia as a function of the end-tidal carbon dioxide ($P_{ET}CO_2$) level measured with a respiratory gas monitor (Ohmeda) during each recording. There was no significant correlation between these two factors ($r = 0.19$, $P > 0.05$).

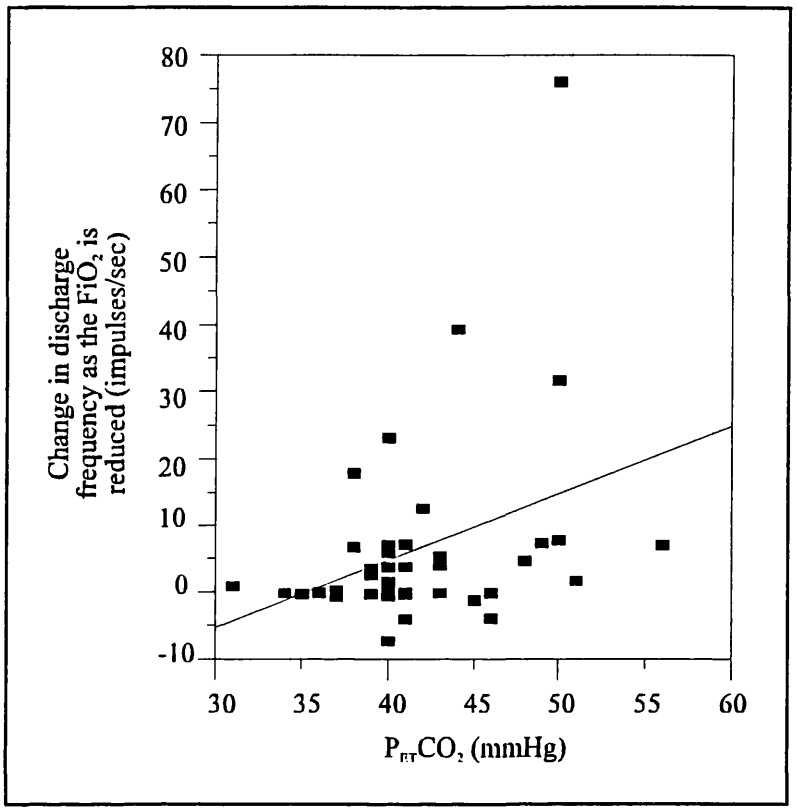


Figure 25. A graph showing the change in mean discharge frequencies (impulses per second) of hypoglossal motoneurones (recorded with extracellular electrodes), when the FiO_2 was reduced, as a function of the end-tidal carbon dioxide ($P_{ET}CO_2$) level measured with a respiratory gas monitor (Ohmeda) during each recording. Although the correlation coefficient was only 0.36 this was found to be significant ($P < 0.05$).

When the motoneurones were considered according to their response to hypoxia there was no correlation between the change in discharge frequency (impulses per second) during hypoxia and the $P_{ET}CO_2$ level (mmHg) (Fig. 26). This was true regardless of whether the motoneurones which responded with a transient or sustained excitation were considered separately or together.

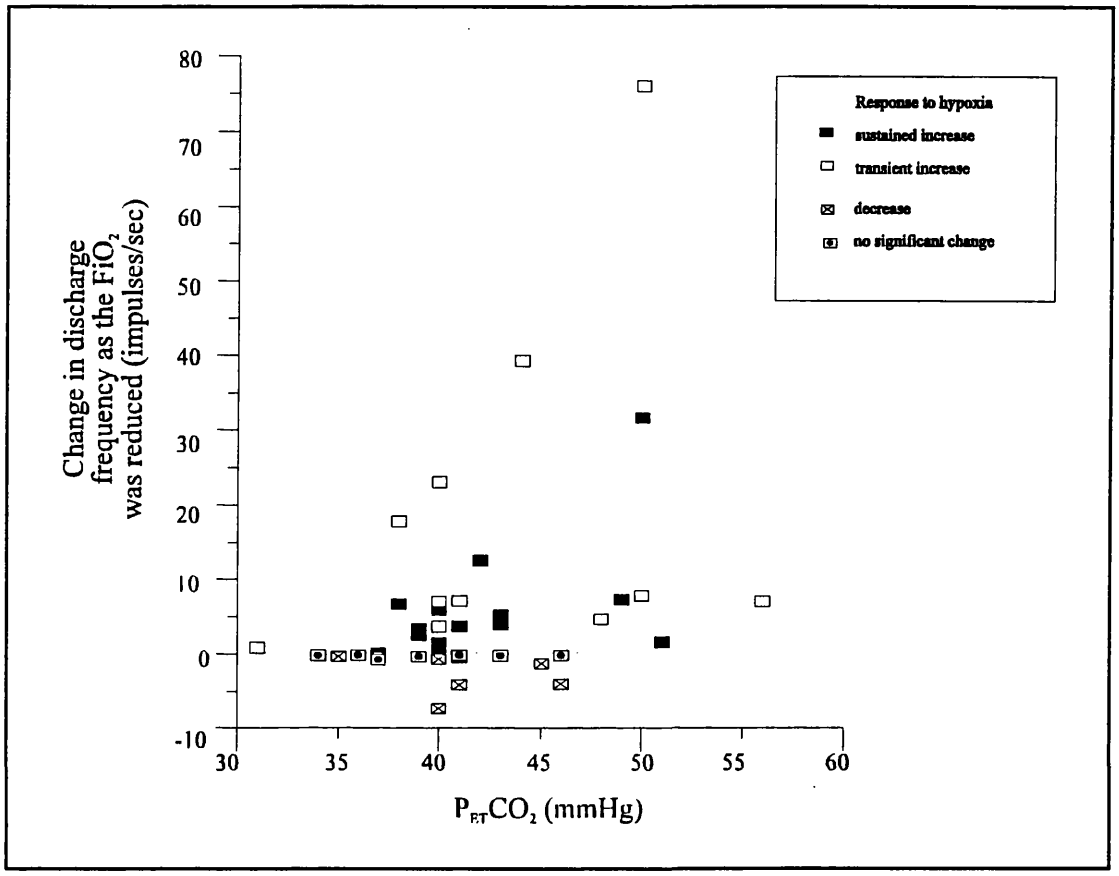


Figure 26. A graph showing the change in mean discharge frequencies (impulses per second) of hypoglossal motoneurones when the FiO_2 was reduced during normoxia as a function of the end-tidal carbon dioxide ($P_{ET}CO_2$) level measured with a respiratory gas monitor (Ohmeda). The response characteristics to hypoxia have been represented with different symbols shown in the key. There was no correlation between the end-tidal CO_2 level during normoxia and the response of the hypoglossal motoneurones to hypoxia when considered according to their different response characterisitics (r= 0.27, 0.5 & 0.34).

3.1.5 SUMMARY OF SECTION 1

This section has shown that hypoglossal motoneurones in neonatal kittens respond to even small changes in FiO_2.

Of the 41 hypoglossal motoneurones which were tested with hypoxia;

1) 14 showed a sustained increase
2) 11 showed a transient increase
3) 8 showed a decrease
4) and 8 showed no change in discharge frequency.

Only 2 motoneurones showed phasic patterns of discharge during normoxia although in a further 3 motoneurones phasic patterns of discharge became clear during hypoxia.

3.2 MEMBRANE POTENTIALS

3.2.1 Introduction

This section of the study presents the results of the intracellular recordings investigating the effect of mild levels of hypoxaemia on the membrane potentials of hypoglossal motoneurones in neonatal kittens. Intracellular recordings were made of 50 hypoglossal motoneurones in 20 kittens. The effect of hypoxia was recorded in 21 of these motoneurones. Twenty-five motoneurones were recorded only during normoxia and the FiO_2 was not reduced to hypoxia. A further 4 motoneurones were recorded during hyperoxia and in 2 of these the FiO_2 was then reduced to normoxia.

3.2.2 Resting membrane potential during normoxia

The resting membrane potential of the 48 motoneurones recorded during normoxia ranged from between -36 and -71 mV. The average resting membrane potential of these motoneurones was -53 ± 1.50 mV.

3.2.3 Effects of hypoxia on membrane potential

The resting membrane potentials of the 21 motoneurones which were recorded during hypoxia again ranged from between -36 and -71mV during normoxia (Fig 27). The average resting membrane potential was -58 ± 2.11 mV. Blood samples revealed that the PaO_2 ranged from between 70 and 113 mmHg (79.5 ± 4.9 mmHg) during normoxia and fell to between 37 and 64 mmHg (47.2 ± 3.8 mmHg) during hypoxia (FiO_2 0.15-0.17). All of the kittens were acidotic (pH 7.18-7.24). There was no

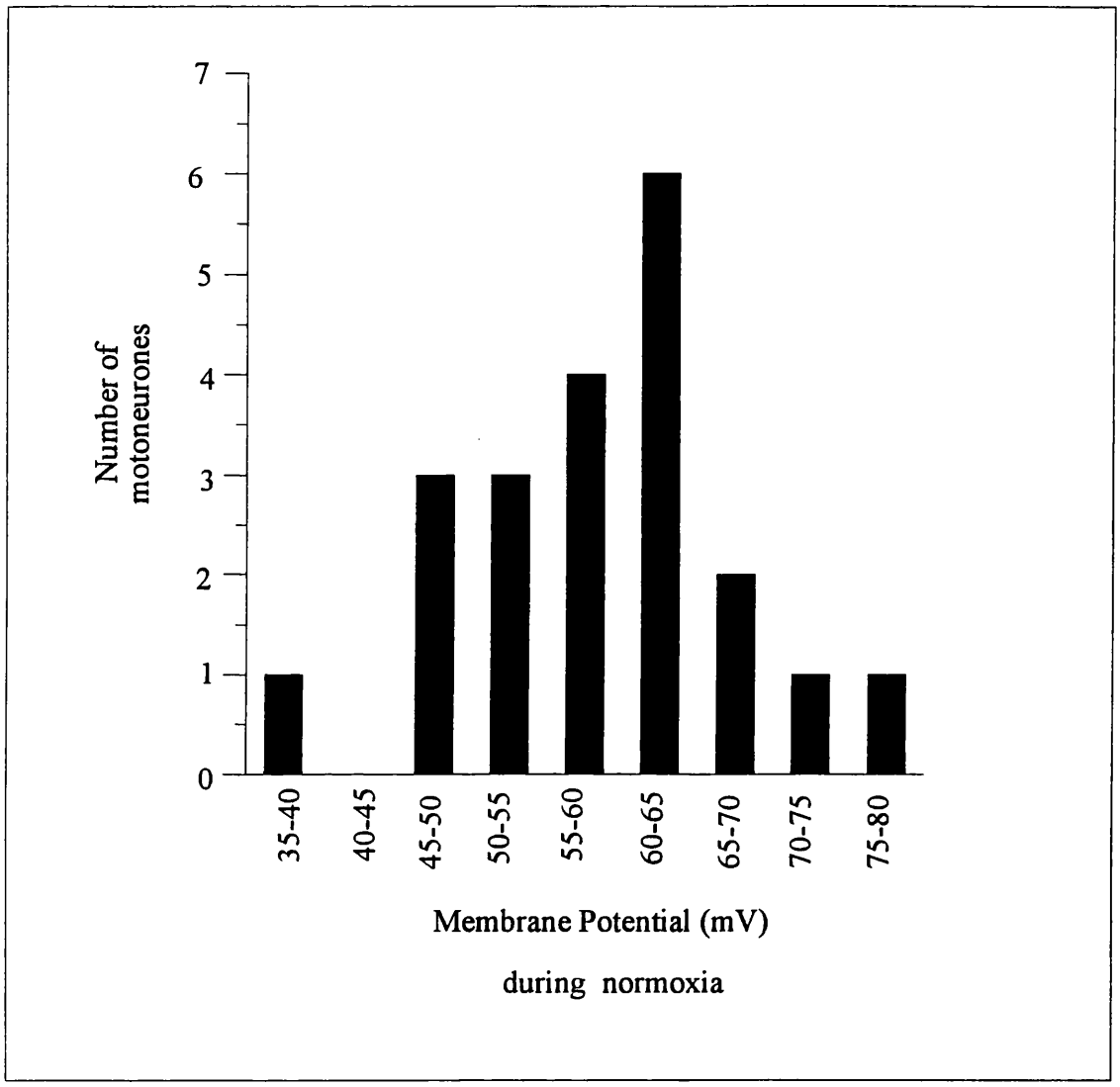


Figure 27. A bar chart showing the distribution of the resting membrane potentials (mV) recorded during normoxia. The data are those of the 21 hypoglossal motoneurones which were later tested with hypoxia.

difference in the arterial pH during hypoxia compared to normoxia. Tables showing the blood gas values for the individual recordings during normoxia and hypoxia are shown in the respective figures.

Sixteen out of the 21 hypoglossal motoneurones showed a gradual decrease in membrane potential with the onset of hypoxia. The average depolarization was 15 ± 1.39mV. An example of a hypoglossal motoneurone which responded to hypoxia in this way is shown in figure 28. The resting membrane potential of this motoneurone during normoxia was approximately -68mV and this depolarized by approximately 16mV during hypoxia. When the hypoxic test was repeated after the recording, and the FiO_2 was again reduced to 0.15, the PaO_2 fell from 90 to 47mmHg.

Two of the 16 motoneurones subsequently repolarized despite continued hypoxia. The membrane potential of these motoneurones during normoxia were -61 and -62 mV. Figure 29 shows the first of these motoneurones. Although initially during hypoxia the motoneurone depolarized gradually to -38mV, after a further minute of hypoxia the membrane repolarized to approximately -51mV.

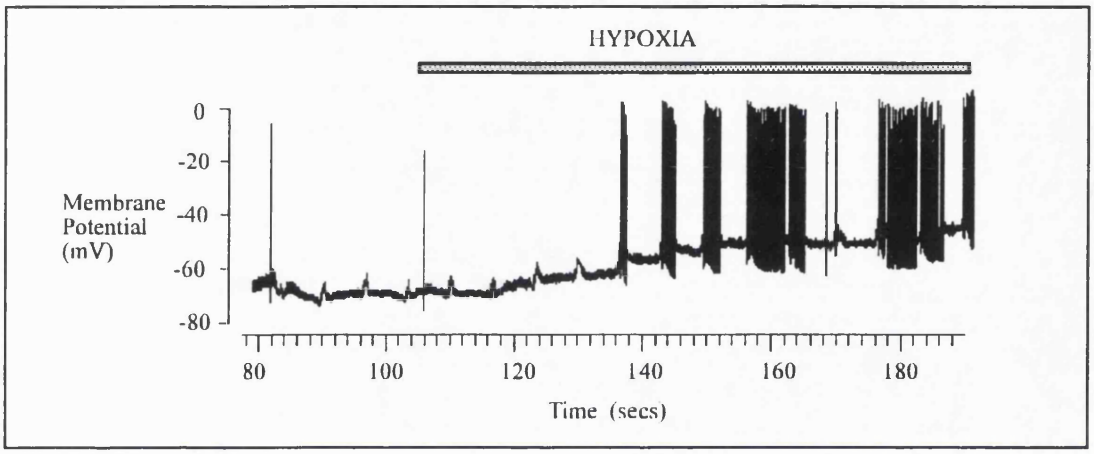


Figure 28. Intracellular recording showing a depolarization of a hypoglossal motoneurone during hypoxia (stippled bar). In this case the resting membrane potential during normoxia was -68mV; this depolarized by approximately 16 mV during hypoxia (FiO_2 0.15). Note the presence of small rhythmic EPSPs which reached threshold with the gradual depolarization. The table below shows the PaO_2, $PaCO_2$ and pH during normoxia and hypoxia (FiO_2 0.15). The blood samples were taken shortly after the recording of the motoneurone had been completed in a repeat of the test. The kitten in which this was recorded was aged 23 days old and weighed 527g.

	NORMOXIA	HYPOXIA (FIO2 0.15)
PaO2 (mmHg)	90	47
PaCO2 (mmHg)	34	38
pH	7.24	7.22

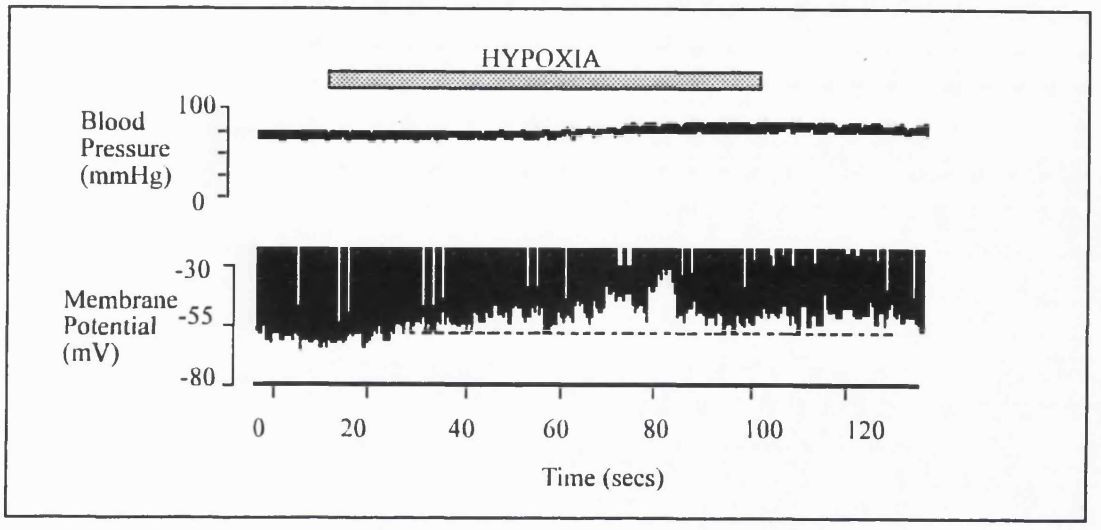


Figure 29. Intracellular recording showing an example of a hypoglossal motoneurone which responded to hypoxia with a transient depolarization. With the onset of hypoxia (FiO_2 0.17-0.16) there was a gradual depolarization of approximately 23mV (from the normoxic level of approx. -61mV) followed by a repolarization to approximately -51mV. The motoneurone was antidromically stimulated (1/600 msec) throughout the recording. The action potentials have been truncated. This figure also shows the blood pressure trace, which was approx. 70mmHg during normoxia. Towards the end of hypoxia the mean arterial blood pressure increased to 77mmHg. A blood sample was taken during hypoxia (FiO_2 0.16), in a repeat of the test, after the recording had been completed. The PaO_2 was 60mmHg, $PaCO_2$ was 37mmHg and pH was 7.11. The kitten in which this was recorded was aged 20 days old and weighed 400g.

A further 4 motoneurones were hyperpolarized during hypoxia by 12 ± 5.12mV. An example of a hypoglossal motoneurone which responded to hypoxia in this manner is shown in figure 30. During hypoxia the membrane of this motoneurone hyperpolarized from approximately -57mV to -80mV.

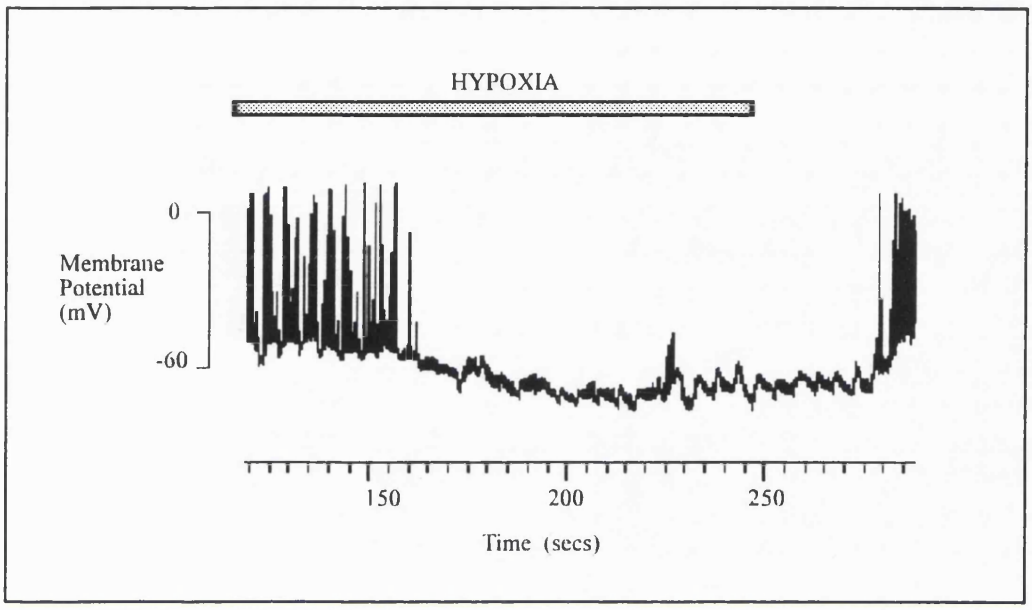


Figure 30. Intracellular recording showing an example of a hypoglossal motoneurone which was hyperpolarized during hypoxia (FiO_2 0.19). The membrane hyperpolarized from -57mV to approx. -80mV without any initial depolarization. A blood sample was taken during normoxia, shortly before the recording was taken. The PaO_2 was 99mmHg (FiO_2 0.23), the $PaCO_2$ was 36 and the pH was 7.2. The kitten in which this was recorded was aged 18 days old and weighed 390g.

In another motoneurone the hypoxic challenge was repeated twice and on both occasions the membrane hyperpolarized. Figure 31 shows the recording of this hypoglossal motoneurone. On the first occasion the FiO_2 was reduced to between 0.17 and 0.16 and the membrane was hyperpolarized by approximately 19mV (Fig. 31a). After approximately 14 minutes the test was repeated and the FiO_2 was again reduced to 0.17. Again the motoneurone hyperpolarized, this time by approximately 6mV (Fig. 31b).

One motoneurone showed no change in membrane potential when the FiO_2 was reduced to 0.17. The recording of this hypoglossal motoneurone is shown in figure 33.

The results from the intracellular recordings are summarized in table 3.

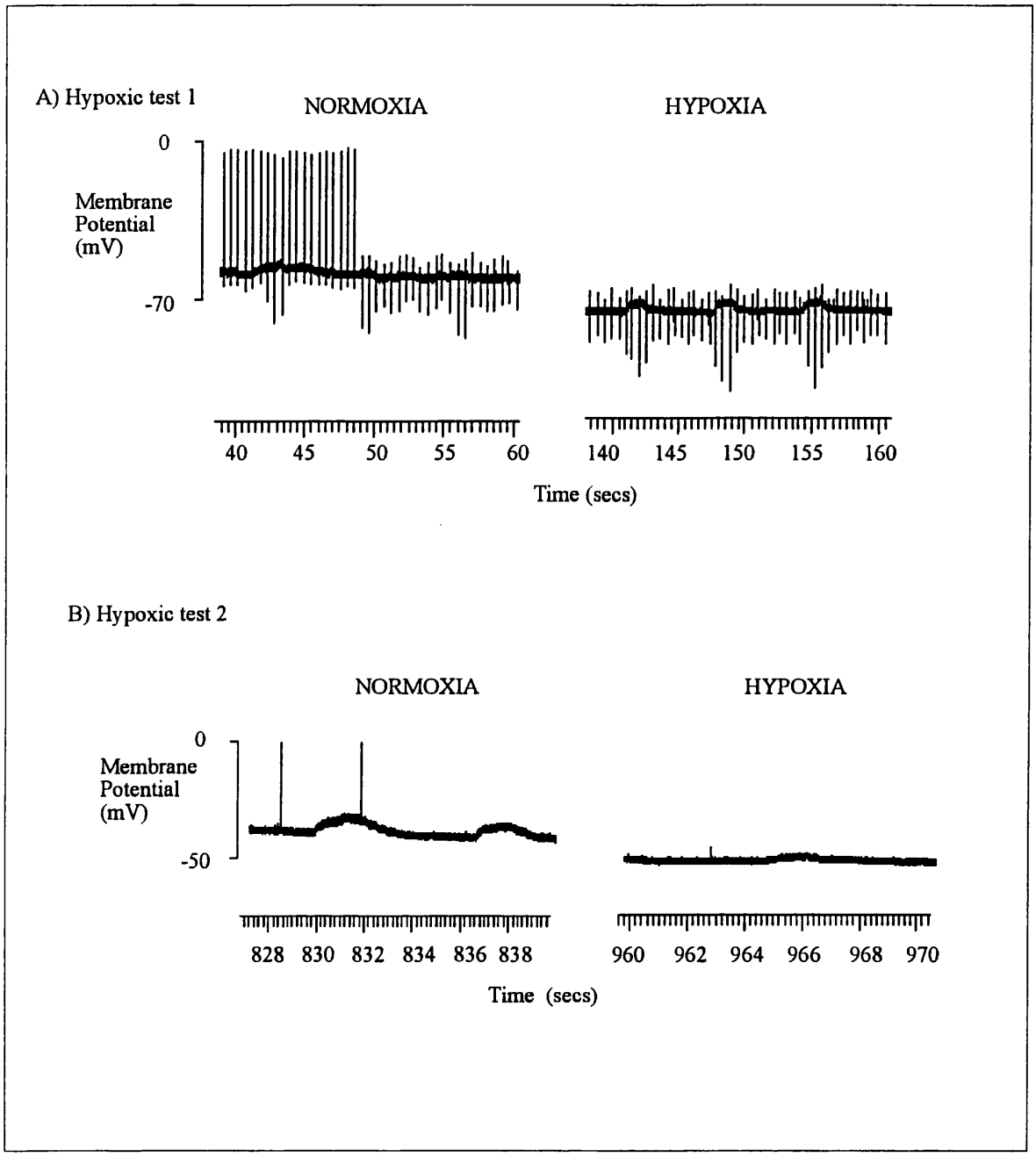


Figure 31. Intracellular recording of one hypoglossal motoneurone showing the effects of hypoxia on rhythmic EPSP activity. Two hypoxic tests were performed on this motoneurone. During the first hypoxic test (A, FiO_2 0.17-0.16) the membrane was hyperpolarized (by approx. 19 mV) and rhythmic EPSPs increased in amplitude. Short hyperpolarizing current pulses (2 msecs, -1nA) were passed through the intracellular electrode. Note the increase in input resistance during rhythmic EPSPs. Both the input resistance associated with the resting membrane potential and the rhythmic EPSPs increased during hypoxia. Upon return to normoxia the EPSPs are still clearly distinguishable approximately 14 minutes later (B, normoxia). During a second hypoxic challenge (FiO_2 0.18-0.17) the membrane is again hyperpolarized (by approx. 6 mV) but on this occasion the amplitude of EPSPs decreased.

	SUSTAINED DEPOLARIZATION	TRANSIENT DEPOLARIZATION	HYPERPOLARIZATION	NO EFFECT	TOTAL
NUMBER OF MOTONEURONES	**14 (67%)**	**2 (9%)**	**4 (19%)**	**1 (5%)**	**21**

Table 3. A summary of the changes in membrane potentials of the hypoglossal motoneurones, recorded with intracellular electrodes, as the FiO_2 was reduced to between 0.14 and 0.19. Twenty-one hypoglossal motoneurones were recorded in 13 neonatal kittens (13 to 26 days old).

3.2.4 Effects of hypoxia on rhythmic excitatory postsynaptic potentials (EPSP)

Only 10 of the 48 hypoglossal motoneurones recorded during normoxia displayed rhythmic excitatory postsynaptic potential (EPSP) activity. Six were tested with hypoxia. In one hypoglossal motoneurone the generation of action potentials during hypoxia made it difficult to measure such EPSPs accurately. The recording of this motoneurone is shown in figure 28. An unpaired, two-tailed Student's *t* test was used to determine the significance of the changes in both the amplitude and duration of the rhythmic EPSP activity in the remaining 5 motoneurones during hypoxia. However, in some cases there were only a few rhythmic EPSPs, in either the test or control period, and the significance was therefore confirmed using the non-parametric Mann Whitney U test (section 2.4.2.iv).

The effect of hypoxia on the rhythmic EPSP activity was variable:

In 2 motoneurones there was a significant increase in the amplitude ($P \leq 0.001$) and duration ($P \leq 0.05$) of the EPSP activity during hypoxia. In the first motoneurone, shown in figure 32, there was an increase in the amplitude of the EPSP activity during hypoxia, from 7.5 ± 0.37 to 15.1 ± 0.8 mV, and an increase in duration, from 1.0 ± 0.13 to 1.9 ± 0.29 seconds. In the second motoneurone, the amplitude and duration of the rhythmic EPSP activity increased, from 6.1 ± 0.65 to 25.2 ± 0.69 mV and from 1.5 ± 0.16 to 2.0 ± 0.09 seconds respectively (Fig. 33). In both of these hypoglossal motoneurones the EPSP activity reached threshold and generated action potentials.

In one motoneurone, shown in figure 34, the amplitude of the rhythmic EPSP activity decreased significantly ($P \leq 0.001$) during the hypoxic challenge from 3.2 ± 0.13 to

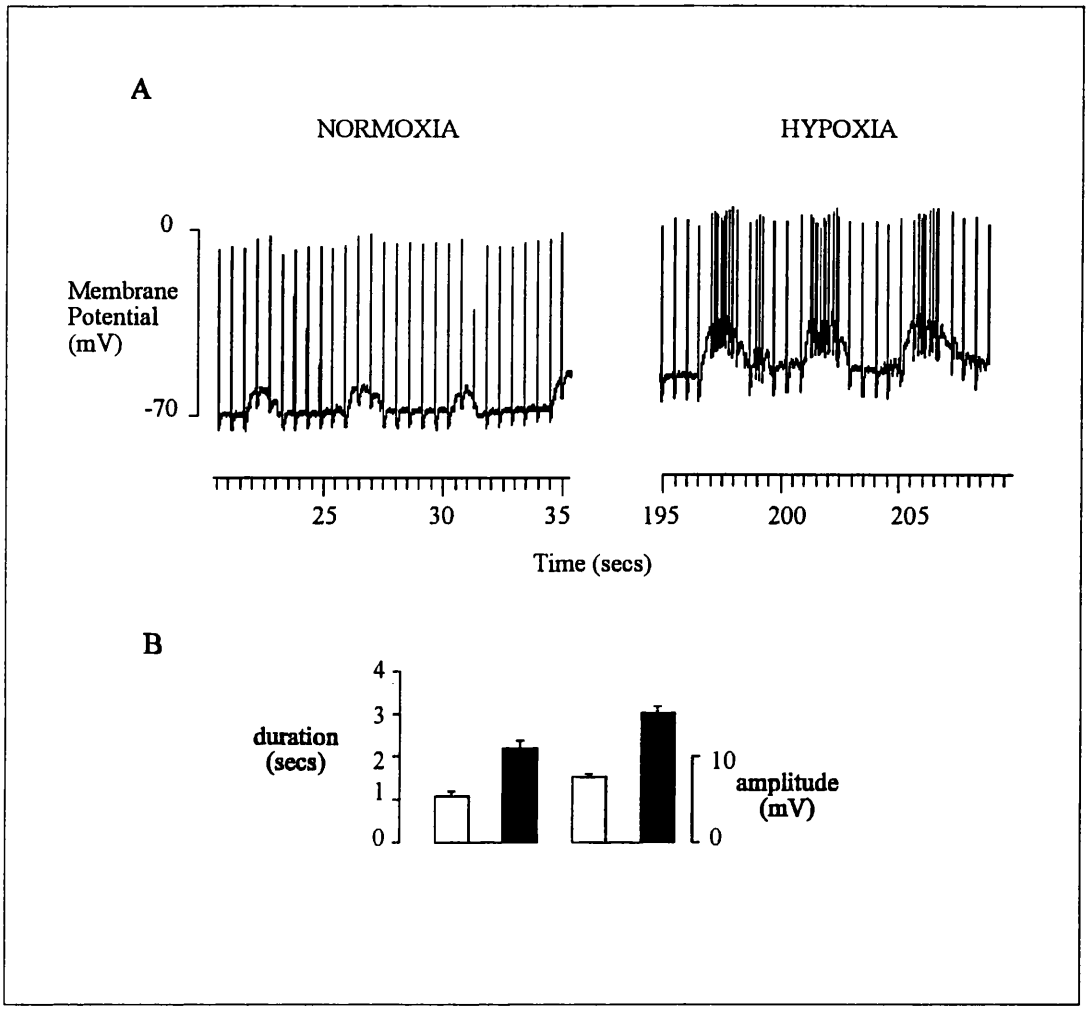


Figure 32. Intracellular recording of one hypoglossal motoneurone showing the effects of hypoxia on the rhythmic EPSPs. A) shows that as the membrane is depolarized from approximately -71 to -51mV during hypoxia (FiO2 0.18) and that there was an increase in the duration and amplitude of the rhythmic EPSPs (Student's t test $P < 0.05$). Note that the EPSPs reach threshold and bursts of action potentials were generated. The average duration and amplitude of the EPSPs for this hypoglossal motoneurone during normoxia (□) and hypoxia (■) are shown as a bar chart in B.

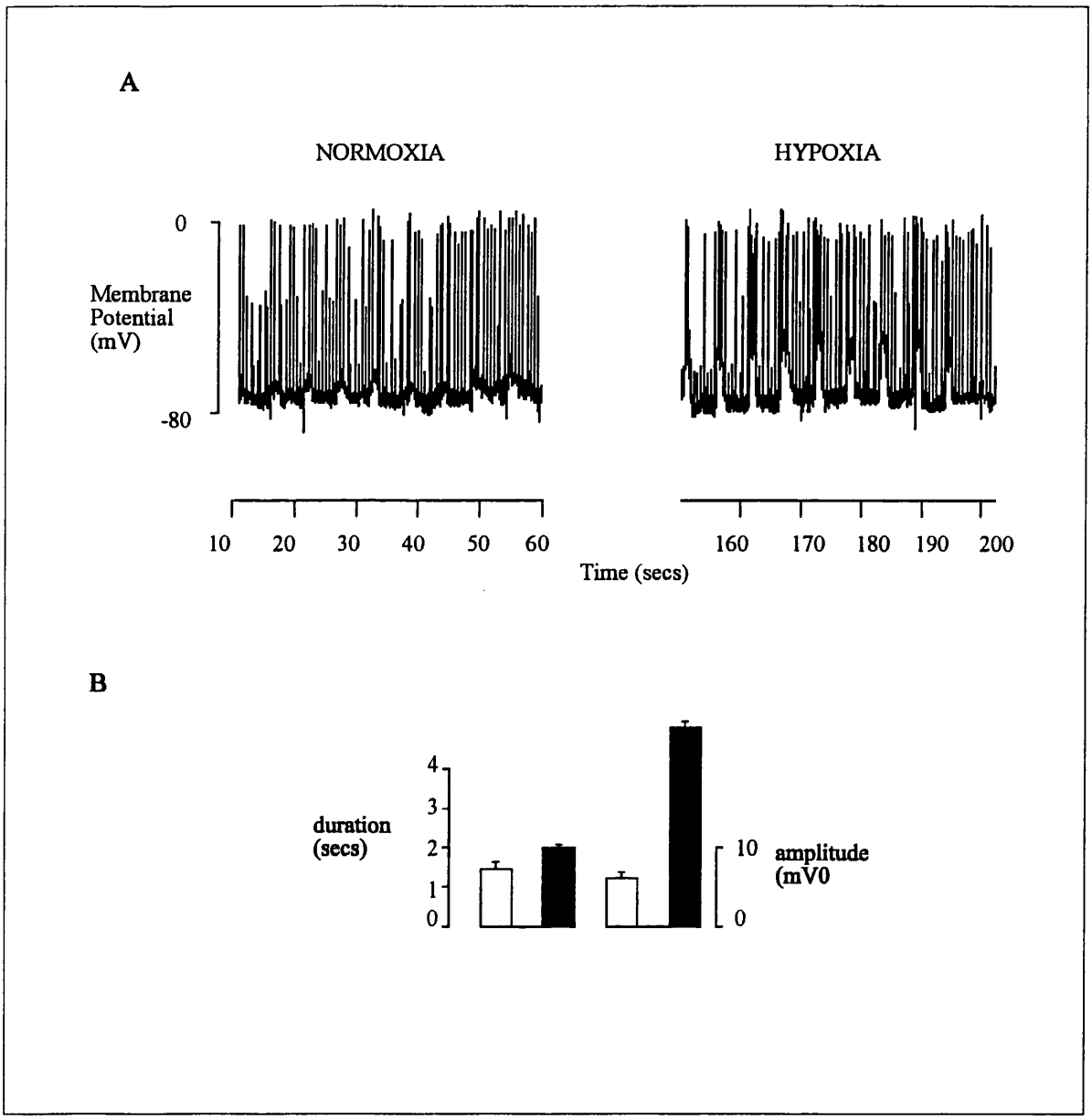


Figure 33. Intracellular recording of a hypoglossal motoneurone showing the effects of hypoxia on the rhythmic EPSPs. A) shows that although the resting membrane potential does not obviously alter during hypoxia (FiO_2 0.17), remaining at approx. -78mV, there is an increase in the duration and amplitude of the rhythmic EPSPs (Student's t test $P < 0.05$). The EPSPs reach threshold and bursts of action potentials are generated. Note that the motoneurone was antidromically activated (1/600msec) throughout the recording and that the antidromic action potential fails to invade the soma on occasions, when only the IS spike is recorded during both normoxia and hypoxia. The average duration and amplitude of the EPSPs for this hypoglossal motoneurone during normoxia (□) and hypoxia (■) are shown as a bar chart in B).

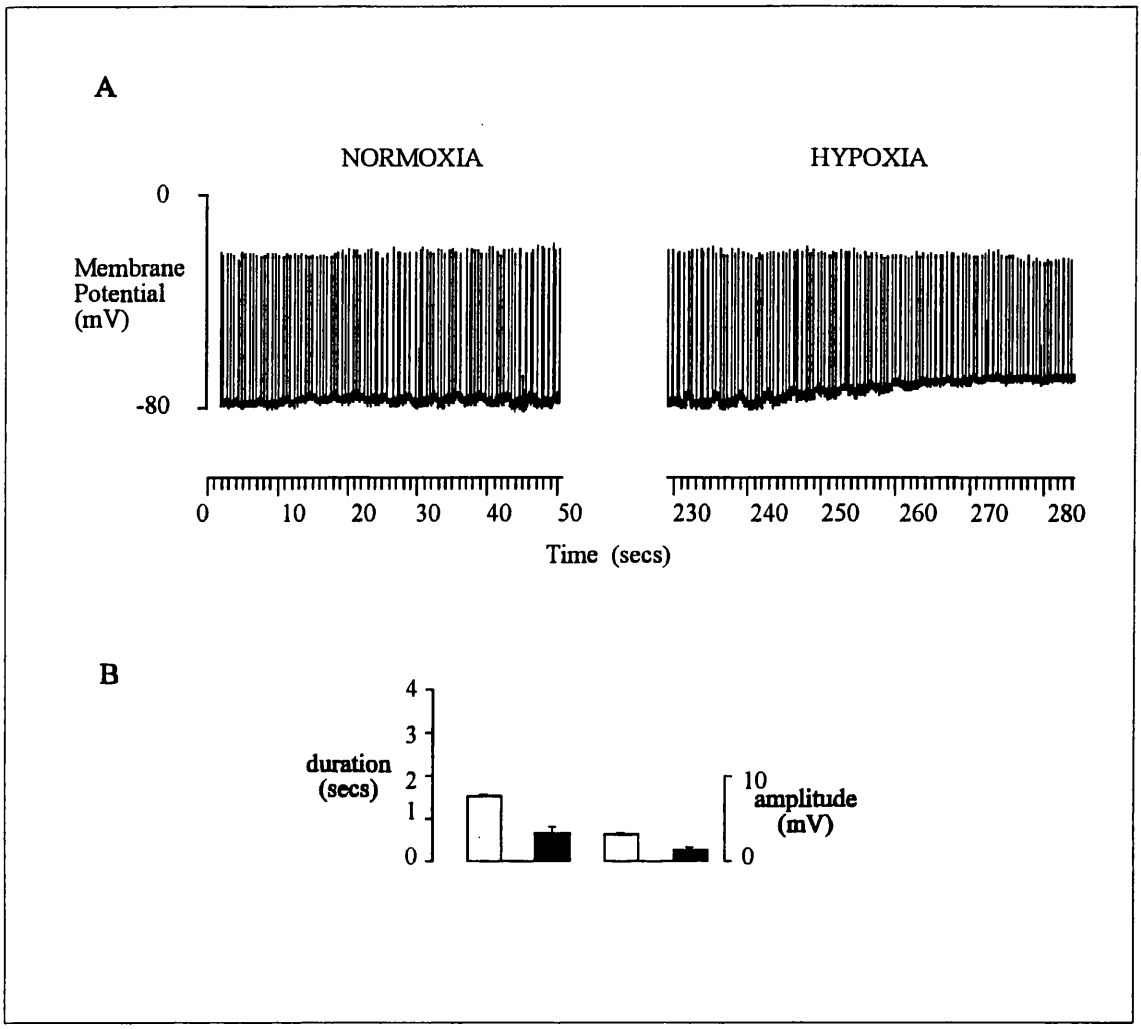


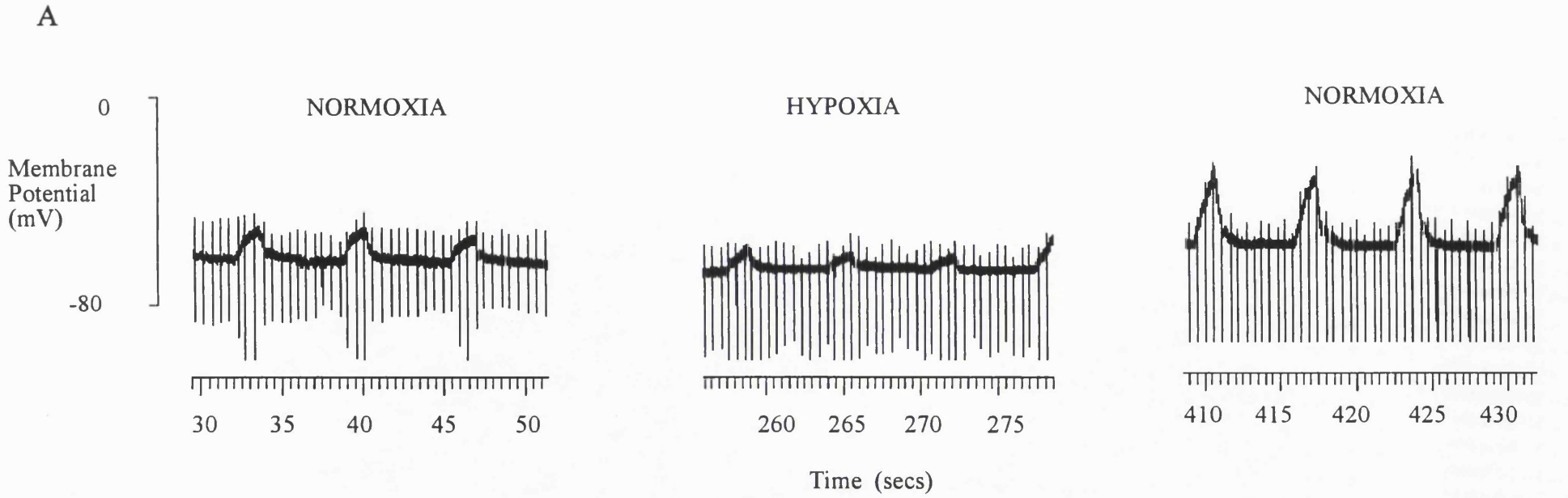
Figure 34. Intracellular recording of a hypoglossal motoneurone showing the effects of hypoxia on rhythmic EPSPs. A) shows that as the membrane is depolarized from approximately -58 to -45mV during hypoxia (FiO_2 0.16) and that there is a decrease in the duration and amplitude of the rhythmic EPSP activity (Student's t test $P < 0.05$). The average duration and amplitude of the EPSPs for this hypoglossal motoneurone during normoxia (□) and hypoxia (■) are shown as a bar chart in B.

1.6 ± 0.17 mV. The duration of the rhythmic EPSP activity also decreased significantly ($P \leq 0.01$) from 1.5 ± 0.04 to 0.8 ± 0.05 seconds.

Although the amplitude of the rhythmic EPSP activity decreased in another motoneurone (Fig. 35) from 9.4 ± 0.61 to 7.7 ± 0.36 mV this was not significant with the Student's *t* test with Bonferroni correction. However, when the FiO_2 was returned to normoxia the amplitude of the EPSP activity increased significantly to 28 ± 0.82mV ($P < 0.001$). The increase in the duration of the rhythmic EPSPs during hypoxia was not significant, but again when the FiO_2 was returned to normoxia there was an increase in the duration of the EPSP activity to 2.4 ± 0.07 seconds ($P < 0.001$).

In the remaining motoneurone the membrane depolarized from -52 to -41mV during hypoxia (FiO_2 0.15) but there there was no significant change in either the duration or amplitude of the rhythmic EPSP activity.

In addition to these motoneurones, EPSP activity appeared in a further 2 motoneurones during hypoxia. In one motoneurone, shown in figure 36, EPSP activity appeared initially during hypoxia (FiO_2 0.17) but as the FiO_2 approached 0.15 this activity disappeared. In the second motoneurone the hypoxic test was repeated twice, approximately 14 minutes apart. In the first test the EPSP activity appeared during hypoxia (Fig. 31a) and was still present upon return to normoxia (see section 3.2.8). However, in the second test on the same motoneurone the EPSP activity (still present following the first hypoxic challenge) decreased in both amplitude and duration ($P \leq 0.01$) (Fig. 31b). The amplitude of the rhythmic EPSP activity decreased from 6.5 ± 0.82 to 2.6 ± 0.24 mV and the duration from 2.8 ± 0.13 to 1.9 ± 0.09 seconds. However, when the FiO_2 was returned to normoxia after the second test the amplitude and duration of the EPSP activity increased to 5.42 ± 0.17 mV and 3.1 ± 0.04 seconds



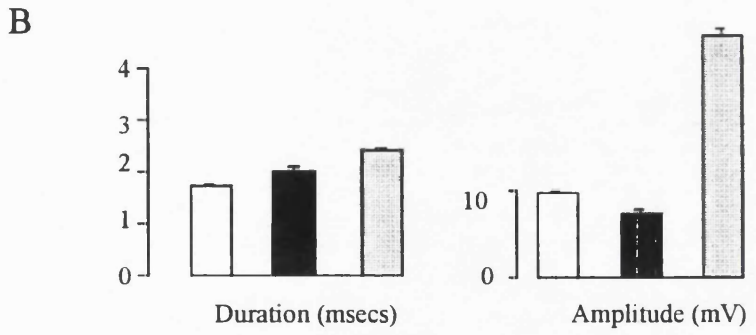


Figure 35. Intracellular recording of a hypoglossal motoneurone showing the effects of hypoxia on the rhythmic EPSP activity. A) shows that as the membrane is hyperpolarized from approximately -60 to -63mV during hypoxia (FiO_2 0.16) and there was a decrease in the EPSP amplitude although this was not significant with the Student's t test $P < 0.05$ (with Bonferroni correction). Note that short hyperpolarizing current pulses (2msecs, -1nA) were passed through the electrode and there was an increase in input resistance associated with the rhythmic EPSPs. During hypoxia the input resistance increased (see figure 44). As the FiO_2 was returned to normoxia, the amplitude of the EPSPs significantly increased ($P<0.001$) and the membrane returned to the level recorded during the control period. Note, however, that the membrane input resistance did not decrease. The average duration and amplitude of the EPSP for this hypoglossal motoneurone during normoxia (▒), hypoxia (□) and upon return to normoxia (■) are shown as a bar chart in B). A blood sample was taken in a repeat of the test, shortly after this recording had been completed. The PaO_2 was 64 mmHg, $PaC0_2$ was 29mmHg and pH was 7.13.

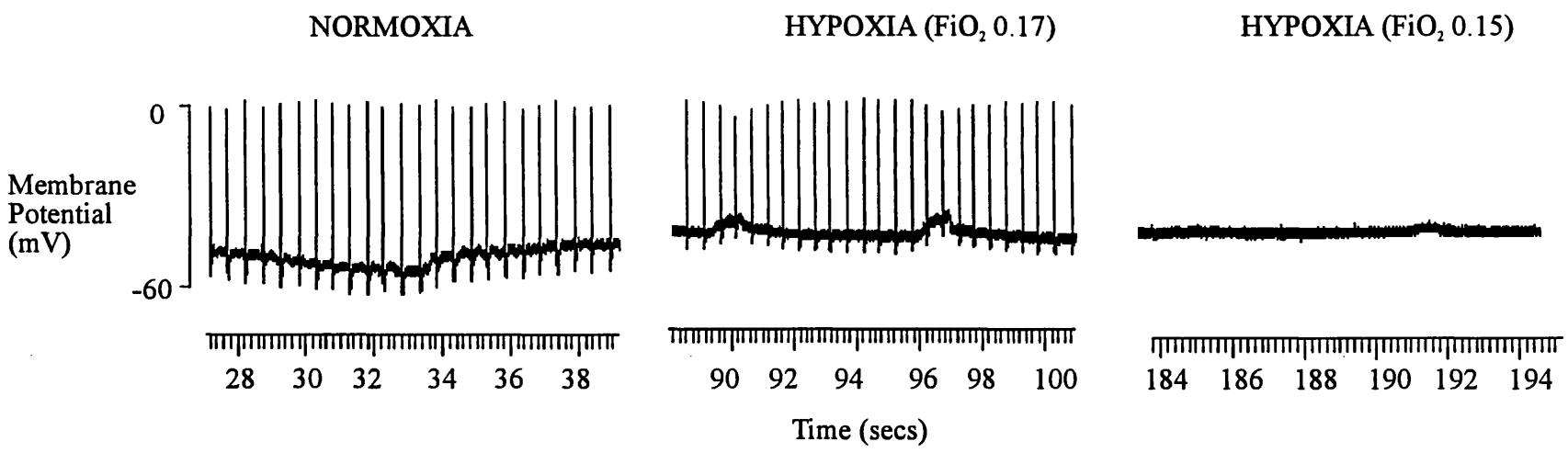
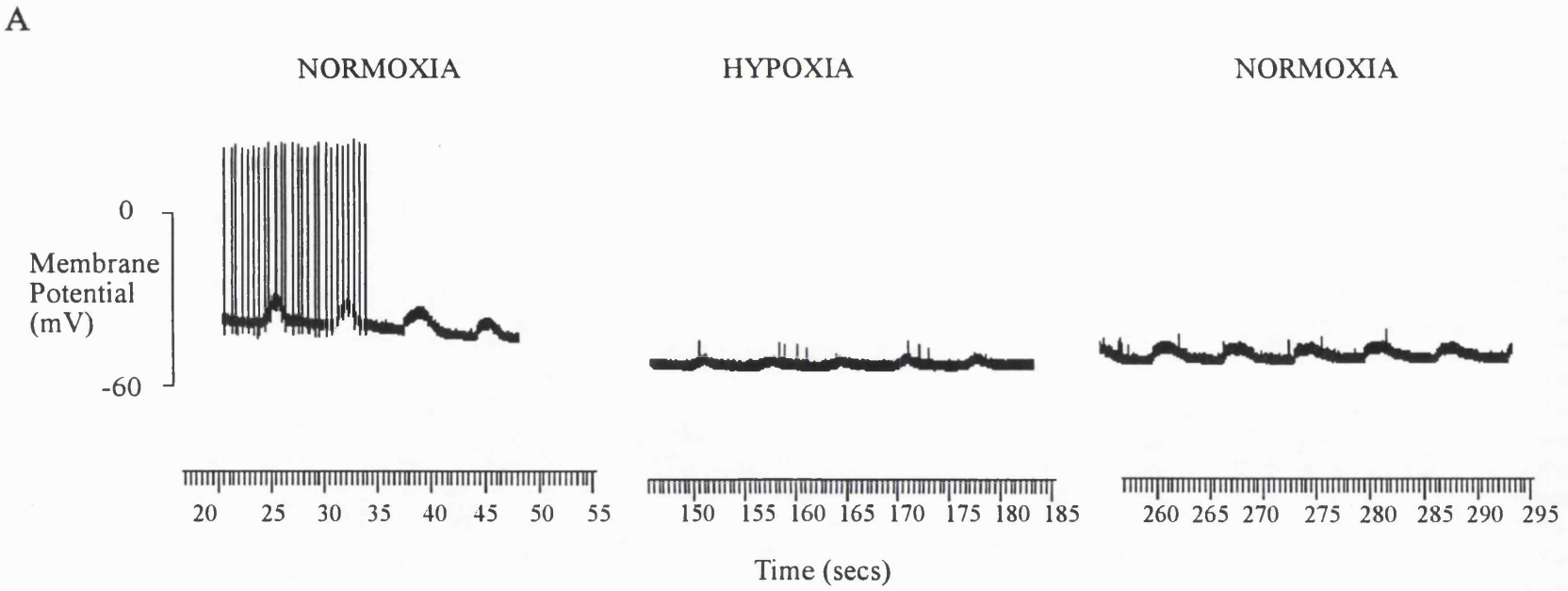


Figure 36. Intracellular recording of one hypoglossal motoneurone showing the effects of hypoxia on rhythmic EPSPs. During hypoxia (FiO_2 0.17) the membrane depolarized from approx. -59 to -49mV and rhythmic EPSPs become apparent. However, as the FiO_2 level reached 0.15 (approximately 1 minute later) the EPSP activity decreases and eventually disappeared. The kitten in which this was recorded was aged 17 days old and weighed 410g.



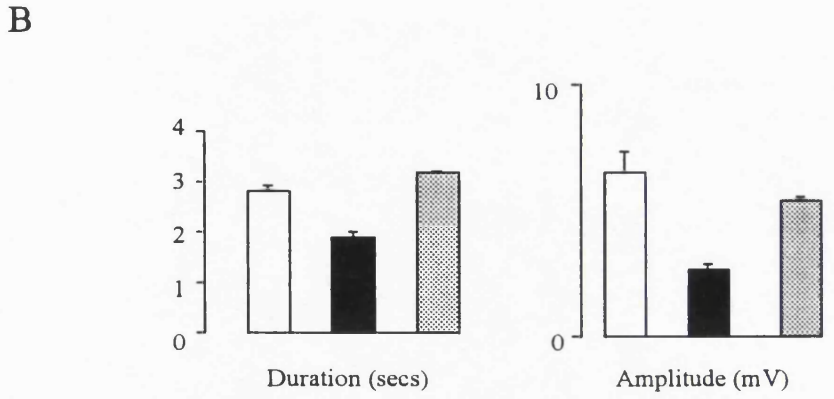


Figure 37. Intracellular recording of a hypoglossal motoneurone showing the effect of hypoxia (FiO_2 0.17) and the return to normoxia on rhythmic EPSP activity. During hypoxia the membrane was hyperpolarized, by approximately 6mV, and there was a significant decrease in the duration and amplitude of the rhythmic EPSPs (Student's t test $P < 0.01$, with Bonferroni correction). Upon return to normoxia the amplitude and duration of the EPSPs were not significantly different from those recorded in the control period. The average duration and amplitude of the EPSP for this hypoglossal motoneurone during normoxia (▭), hypoxia (■) and upon return to normoxia (▩) are shown as a bar chart in B). This was the second hypoxic test completed on this motoneurone; the first hypoxic test is shown in figure 31.

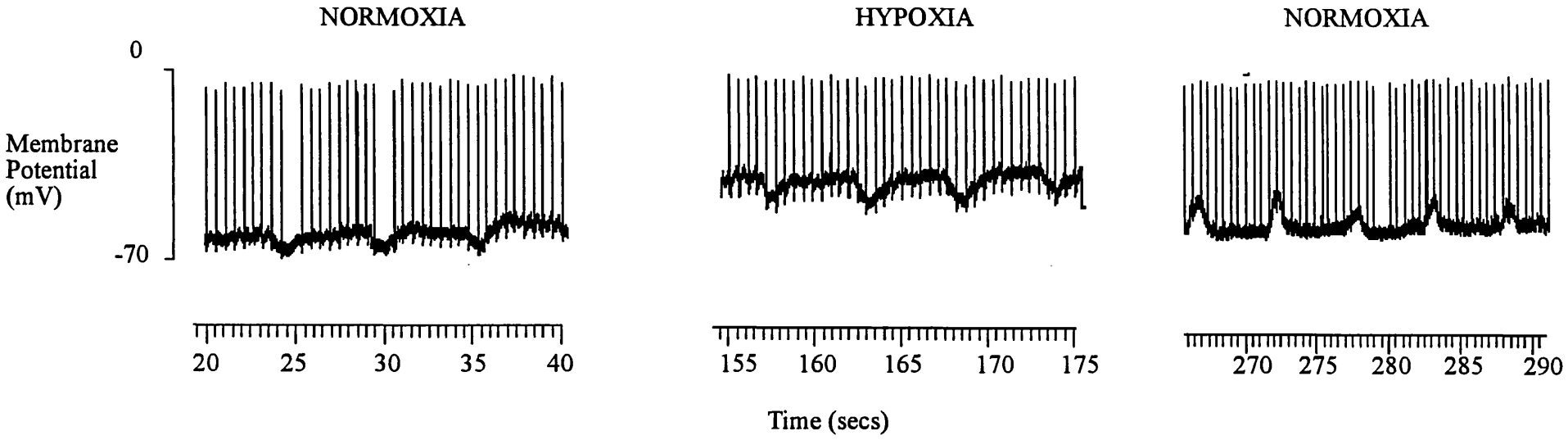


Figure 38. Intracellular recording of one hypoglossal motoneurone showing the effect of hypoxia and returning to normoxia on rhythmic EPSPs. The membrane depolarized from approx. -63 to -40mV during hypoxia (FiO_2 0.17), but repolarized when the FiO_2 was returned to normoxia. EPSP activity was recorded in the post-hypoxic period when the FiO_2 was returned to normoxia.

respectively. This is shown in figure 37. When compared to the rhythmic EPSPs recorded in the control period (recorded before reducing the FiO_2 for the second time) there was no significant difference in either the amplitude or duration of the EPSP activity.

EPSP activity also appeared in 2 other motoneurones after the hypoxic test when the FiO_2 had returned to normoxia. The recordings of these motoneurones are shown in figures 38 and 39 and are discussed in more detail in section 3.2.8. During hypoxia (FiO_2 0.17), the resting membrane of the motoneurone shown in figure 38 depolarized from -63 to -40mV but no rhythmic EPSPs were recorded. However, as the FiO_2 was returned to normoxia rhythmic EPSP activity appeared. The second motoneurone hyperpolarized from -67 to -71mV during hypoxia (FiO_2 0.15). Again no rhythmic EPSPs were recorded until the level of FiO_2 had returned to normoxia. This is shown in figure 39.

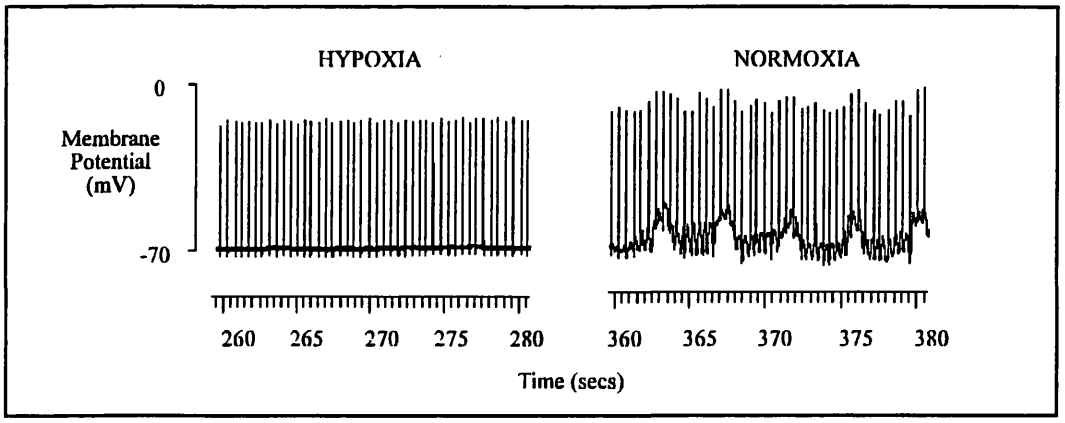


Figure 39. Intracellular recording of a hypoglossal motoneurone showing the effect of hypoxia and returning to normoxia on rhythmic EPSPs. EPSP activity was recorded in a period of post-hypoxic normoxia. The motoneurone had previously hyperpolarized from approximately -67 to -71mV during hypoxia (FiO_2 0.15).

Rhythmic EPSPs were also recorded in one motoneurone when the FiO_2 level was decreased from hyperoxia (FiO_2 0.44) to normoxia. This is shown in figure 46b and

is discussed in more detail in section 3.2.9.

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

3.2.5.i Is the change in membrane potential during hypoxia dependent upon the initial resting membrane potential?

Figure 40a 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 no correlation between these two factors (r = 0.02). However, when the motoneurones which depolarized during hypoxia were considered separately (Fig. 40b) there was a significant correlation (r = 0.66, $P<0.05$) between the resting membrane recorded during normoxia and the change in membrane potential during hypoxia.

3.2.5.ii Effect of resting membrane potential during normoxia on the presence of rhythmic EPSP activity

There was no relationship between the resting membrane potential of the hypoglossal motoneurones during normoxia and the presence of EPSP activity. The membrane potential of the motoneurones which had EPSP activity during normoxia covered a wide range (between -45 and -71mV, mean ± SEM, -60 ± 3.12mV). The increase in rhythmic EPSP activity or its appearance during or following hypoxia was independent of the changes in membrane potential; figures 31a, 32 and 33 show recordings of motoneurones in which the EPSP activity increased during hypoxia, yet the membrane in each case was hyperpolarized, depolarized or unaltered respectively. In addition, figure 31 shows a motoneurone which was tested with hypoxia twice and,

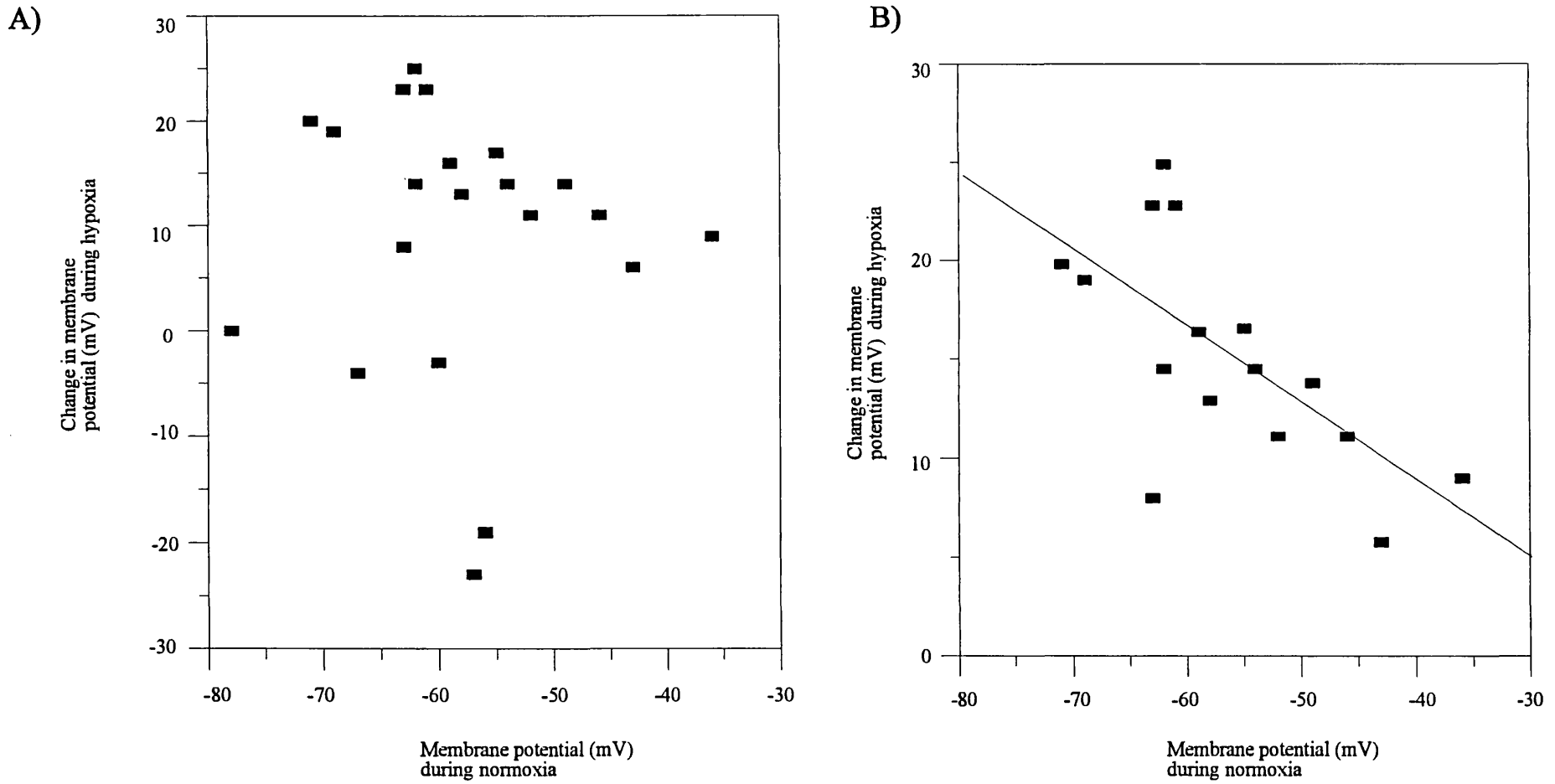


Figure 40. Two graphs showing the change in resting membrane potentials (mV) of the hypoglossal motoneurones during hypoxia as a function of the membrane potentials recorded during normoxia. A) shows all of the motoneurones tested with hypoxia, whereas B) shows only those that were depolarized, at least, initially during hypoxia. Taking the group of motoneurones together (A), there was no correlation ($r=0.02$) between the two factors. But there was a significant correlation ($r=0.66$, $P<0.05$) 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 ($y=-7.0 -0.4x$) has been drawn on the graph.

although the membrane was hyperpolarized each time, rhythmic EPSP activity appeared on the first but decreased on the second occasion.

3.2.5.iii The effect of age on the response of the hypoglossal motoneurones to hypoxia

There was no correlation ($r < 0.001$) between the membrane potential during normoxia and the age of the kittens on the day of recording (Fig. 41).

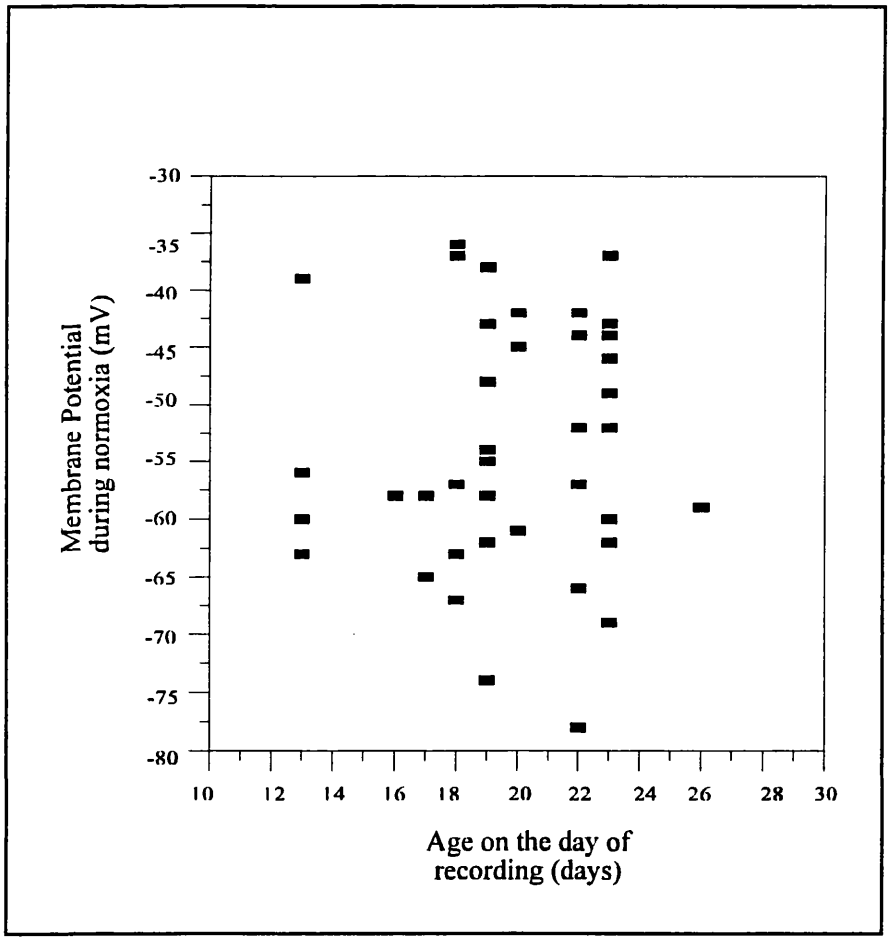


Figure 41. A graph showing the resting membrane potentials of hypoglossal motoneurones (mV) during normoxia against the age of the kittens on the day of recording. There was no significant correlation ($r<0.001$) between the two factors.

The motoneurones were divided into two groups according to the age of the kitten on the day of recording (≤ 21 and > 21 days old). The hypoglossal motoneurones which responded to hypoxia with an initial depolarization and subsequent repolarization, or hyperpolarization of the membrane were recorded in the younger group of kittens (Table 4). This is also shown figure 42. However, there was no correlation between the change in membrane potential during hypoxia and age (r = 0.39, $P>0.05$).

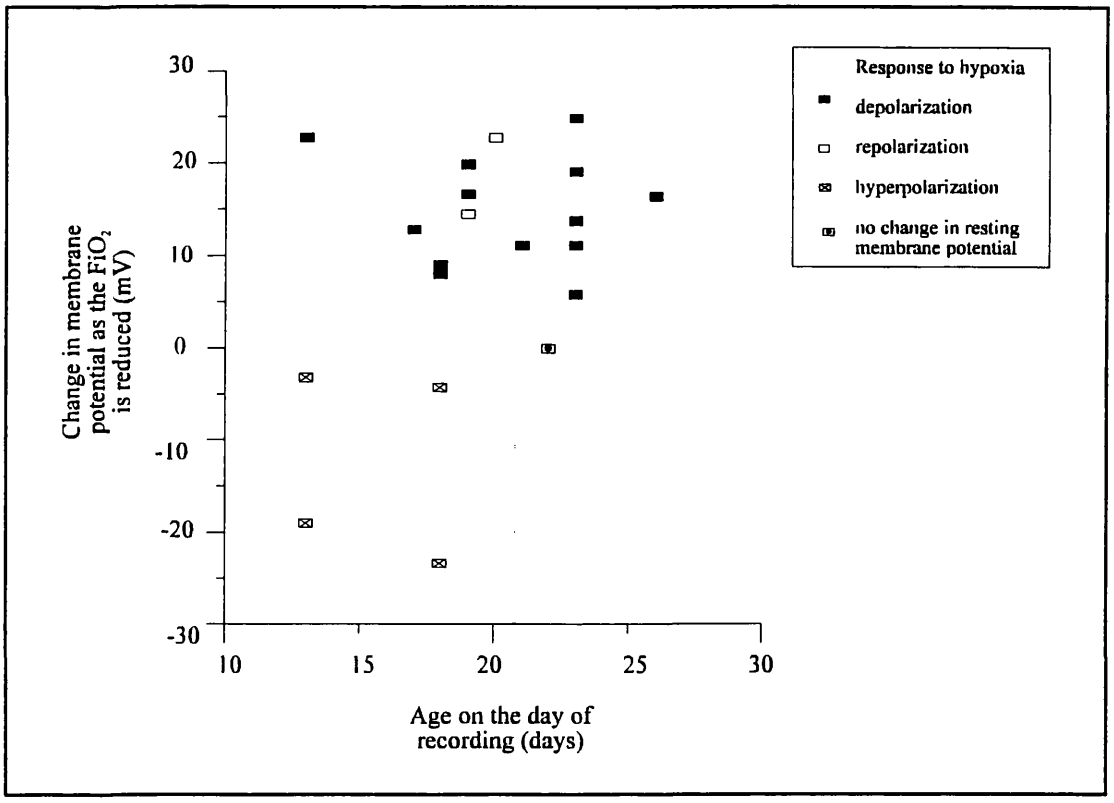


Figure 42. A graph showing the change in resting membrane potentials (mV) of hypoglossal motoneurones when the FiO_2 was reduced against the age of the kittens on the day of recording. Taking the hypoglossal motoneurones as a group, there was no correlation between these two factors (r = 0.39, $P>0.05$). Various symbols have been used for the hypoglossal motoneurones depending upon their response to hypoxia. Note that the motoneurones which showed only an initial depolarization and subsequently repolarized or were hyperpolarized during hypoxia were recorded in the younger kittens (≤ 21 days).

	DEPOLARIZATION	HYPERPOLARIZATION	TRANSIENT DEPOLARIZATION	NO EFFECT	TOTAL
> 21 DAYS	6	0	0	1	7
≤ 21 DAYS	8	4	2	0	14
TOTAL	14	4	2	1	21

Table 4. A summary of the changes in membrane potentials of the hypoglossal motoneurones, recorded with intracellular electrodes, to mild levels of isocapnic hypoxia (FiO_2 0.14 to 0.19) recorded in 21 hypoglossal motoneurones in 13 neonatal kittens (13 to 26 days old). The kittens have been divided into two groups according to their age on the day of recording (≤ 21 and > 21 days).

The hypoglossal motoneurones with rhythmic EPSP activity were recorded in kittens aged between 13 and 26 days old, suggesting that rhythmic EPSP activity was not dependent upon age. However, not enough motoneurones with rhythmic EPSP activity were tested to be able to establish whether a relationship existed between age and the changes in this activity during hypoxia.

3.2.5.iv 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 between 29 and 46 mmHg (36.9 ± 1.41 mmHg).

In the present study there was no significant correlation (r = 0.35, P>0.05) between the level of $PaCO_2$ during normoxia and the membrane potential of the motoneurones when taken as a group (Fig. 43).

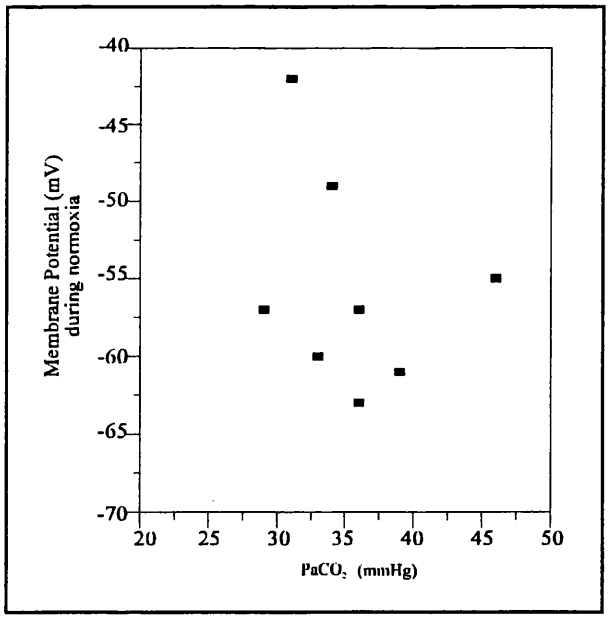


Figure 43. A graph showing the resting membrane potentials (mV) during normoxia as a function of the $PaCO_2$ measured from blood samples taken shortly after each recordings. There was no significant correlation between the two factors (r = 0.35, P>0.05).

However, not enough blood samples could be taken to establish whether there was a relationship between the arterial CO_2 level and the response of the motoneurones to hypoxia.

3.2.6 Changes in blood pressure during hypoxia

The blood pressure was 50 ± 6.3mmHg during normoxia. In the majority of kittens there was either an increase (9 ± 4.41mmHg) or a decrease (11 ± 2.52mmHg) in the blood pressure during hypoxia. Larger changes in blood pressure occured at more severe levels of hypoxia (FiO_2 0.14) and under these conditions it was usually impossible to maintain cell penetration due to loss of stability.

Figure 29 shows an example of a recording in which the blood pressure increased during hypoxia (FiO_2 0.17-0.16) In this case the membrane depolarized gradually with the onset of hypoxia but during this initial 60 seconds blood pressure remained unchanged at 70 mmHg. Mean arterial blood pressure then increased to 77 mmHg and during this period the level of depolarization was reduced. Thus, in this case, there was an initial depolarization during the first minute of hypoxia followed by a repolarization. The onset of the initial depolarization occurred without a change in arterial pressure.

3.2.7 Changes in input resistance

Changes in input resistance were tested in 2 motoneurones, both of which were hyperpolarized during hypoxia. In both cases the hyperpolarization was asssociated with an increase in input resistance. Figure 44a shows the recording from one of these motoneurones. The rhythmic EPSP activity was associated with an increase in the transmembrane voltage drop generated by the constant current pulse, indicating an

increase in input resistance. During hypoxia there was an increase in the input resistance related with both the rhythmic EPSP activity and that of the resting membrane potential when compared to normoxia (Fig. 44b).

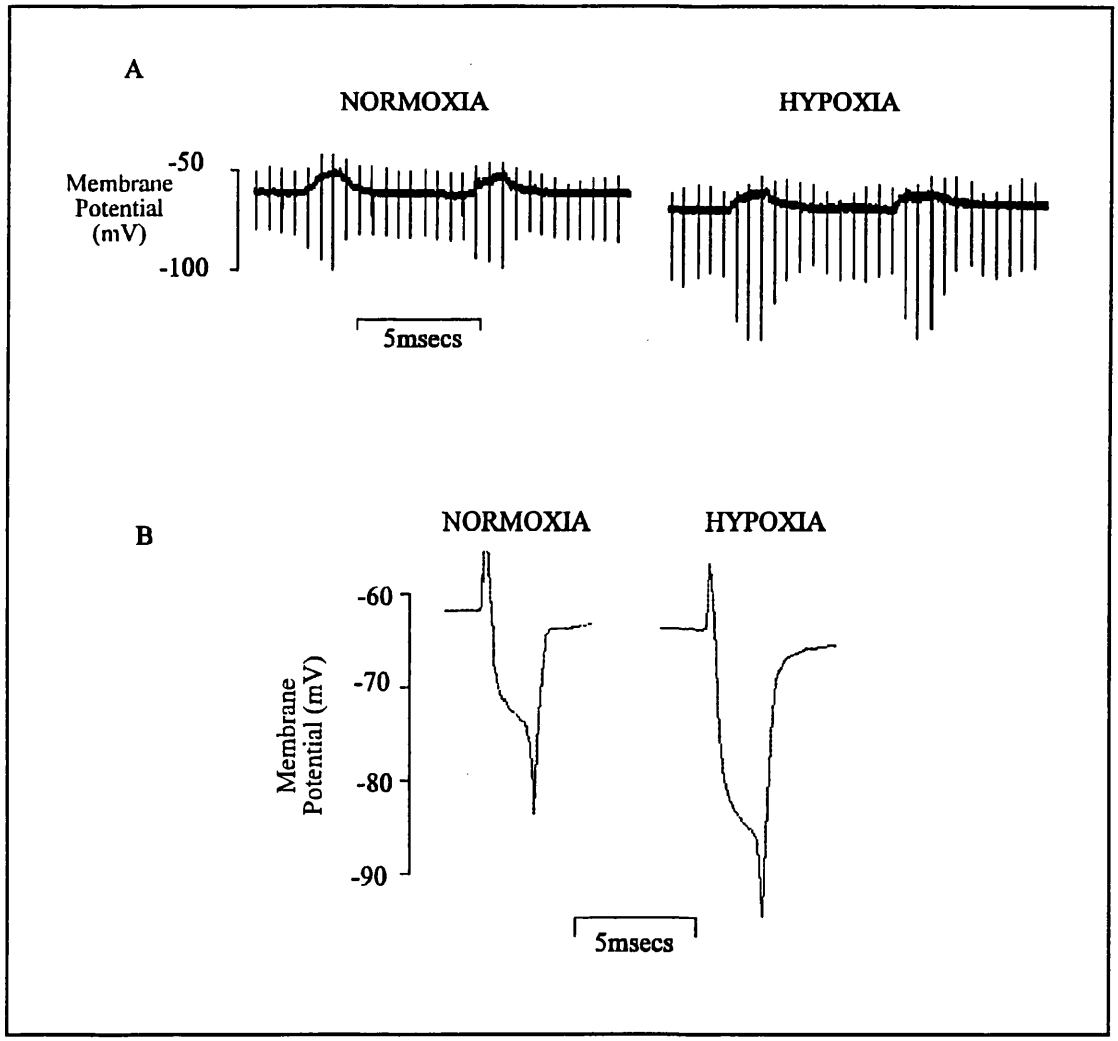


Figure 44. Effects of systemic hypoxia on hypoglossal membrane input resistance. Short hyperpolarizing current pulses (2msecs, -1nA) were passed through the intracellular electrode. This motoneurone hyperpolarized from approximately -60 to -63mV during hypoxia (FiO_2 0.17-0.16). Note the increase in input resistance during the rhythmic EPSPs and the increase in input resistance during hypoxia (FiO_2 0.16). The average transmembrane voltage drop during the hyperpolarizing current pulses (excluding voltage changes during EPSPs) are shown in B. The PaO_2 during hypoxia was approximately

64mmHg, $PaCO_2$ was 29mmHg and pH 7.13.

3.2.8 Returning to normoxia

Five hypoglossal motoneurones were maintained for a long enough period of time to return to normoxia following the hypoxic challenge. Two of these motoneurones were depolarized during hypoxia but repolarized when the FiO_2 was returned to normoxia. One of these motoneurones is shown in figure 38 page 166. During hypoxia the membrane was depolarized by approximately 23mV, from a resting membrane potential of -63 mV, but when the level of FiO_2 was returned to normoxia the membrane began to repolarize. As described in section 3.2.4, the recording also shows that rhythmic EPSP activity appeared when normoxia was reached.

The other 3 motoneurones were hyperpolarized during hypoxia and their responses have all been described earlier in sections 3.2.3 and 3.2.4. As the FiO_2 level was returned to normoxia there were changes in both the resting membrane potential and rhythmic EPSP activity:

The membrane potential recorded from one of these motoneurones is shown in figure 35 (page163). In this case the membrane was only slightly hyperpolarized during hypoxia (FiO_2 0.16) by approximately 3mV; as the FiO_2 level was returned to normoxia the membrane repolarized gradually to approximately -60mV, reaching the membrane potential previously recorded in the control period. As already discussed in section 3.2.4, the amplitude of the rhythmic EPSP activity recorded in this motoneurone was reduced during hypoxia but increased, to a level which was greater than that recorded in the control period, as the FiO_2 was returned to normoxia. Thus the amplitude of this EPSP activity was greater after the hypoxic challenge than

during the control period (see bar chart Fig. 35b).

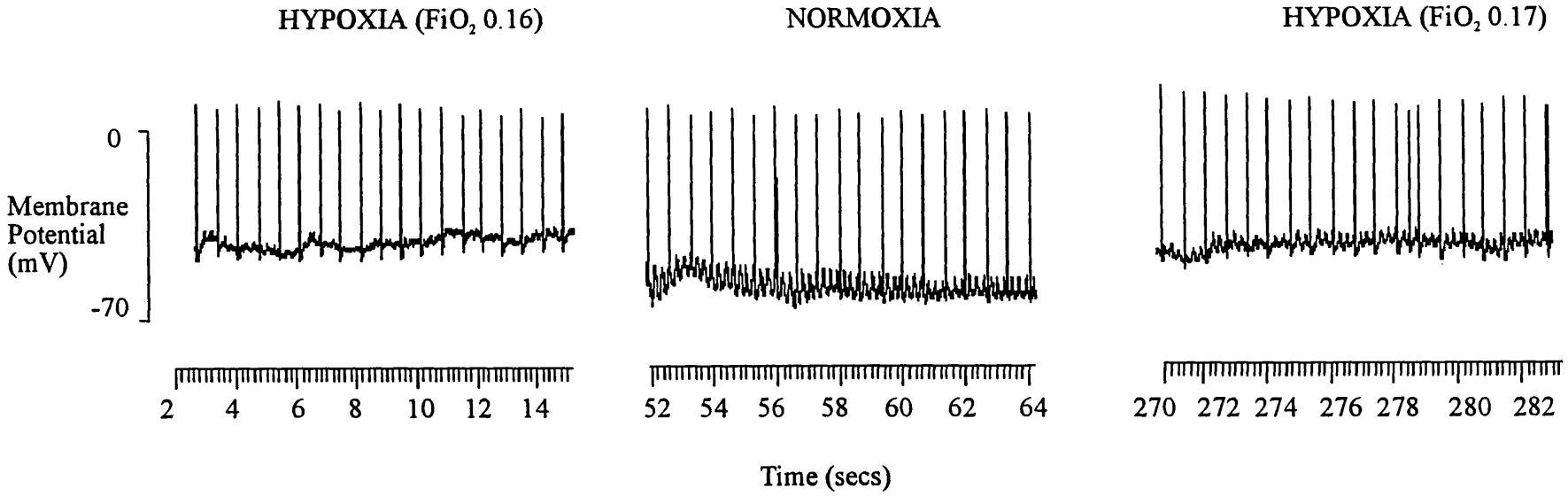
The second motoneurone hyperpolarized by approximately 4 mv during hypoxia from a resting membrane potential of approximately -67mV during normoxia. The recording, shown in figure 39 (page 167), shows that upon return to normoxia there was an onset of rhythmic EPSP activity which had not previously been present during the control period or during hypoxia. This has been discussed in section 3.2.4.

The third hypoglossal motoneurone, which was successfully recorded for a long enough period of time to return to normoxia, is shown in figure 31 (page 156). Two tests were completed and on both occasions the membrane was hyperpolarized during hypoxia. These have been described in detail in section 3.2.3. Between the first and second test, the FiO_2 was returned to normoxia and the membrane potential had returned to the control value.

An additional 2 motoneurones were penetrated during hypoxia (FiO_2 0.16) and the resting membrane potentials recorded were approximately -45 and -50mV. The FiO_2 was increased to the normoxic level and both motoneurones were hyperpolarized (by 18 and 12mV respectively) to -63 and -62mV. The recording of the one motoneurone is shown in figure 45. In both cases the recording was maintained during normoxia and a hypoxic test was completed. Both motoneurones were depolarized (by 17 and 15mV respectively) although the second of these motoneurones subsequently repolarized to -59mV after approximately 2 minutes of hypoxia.

3.2.9 Changing from hyperoxia to normoxia

Four hypoglossal motoneurones were penetrated during hyperoxia (FiO_2 0.42, 0.26, 0.44 and 0.34). The resting membrane potentials during hyperoxia were approximately



	NORMOXIA	HYPOXIA
PaO_2 (mmHg)	70	43
$PaCO_2$ (mmHg)	46	39
pH	7.2	7.2

Figure 45. An example of an intracellular recording of a hypoglossal motoneurone which was penetrated during hypoxia (FiO_2 0.16). The motoneurone was antidromically stimulated throughout (1/600 msec). The membrane was hyperpolarized from approx. -45 to -63mV as the FiO_2 was increased to normoxia. When the FiO_2 was again reduced, this time to 0.17, the membrane depolarized by approximately 17mV reaching a membrane potential of -39mV. Blood samples were taken shortly after the recording during a repeat of the test. The table shows the PaO_2, $PaCO_2$ and pH during normoxia and hypoxia (FiO_2 0.16).

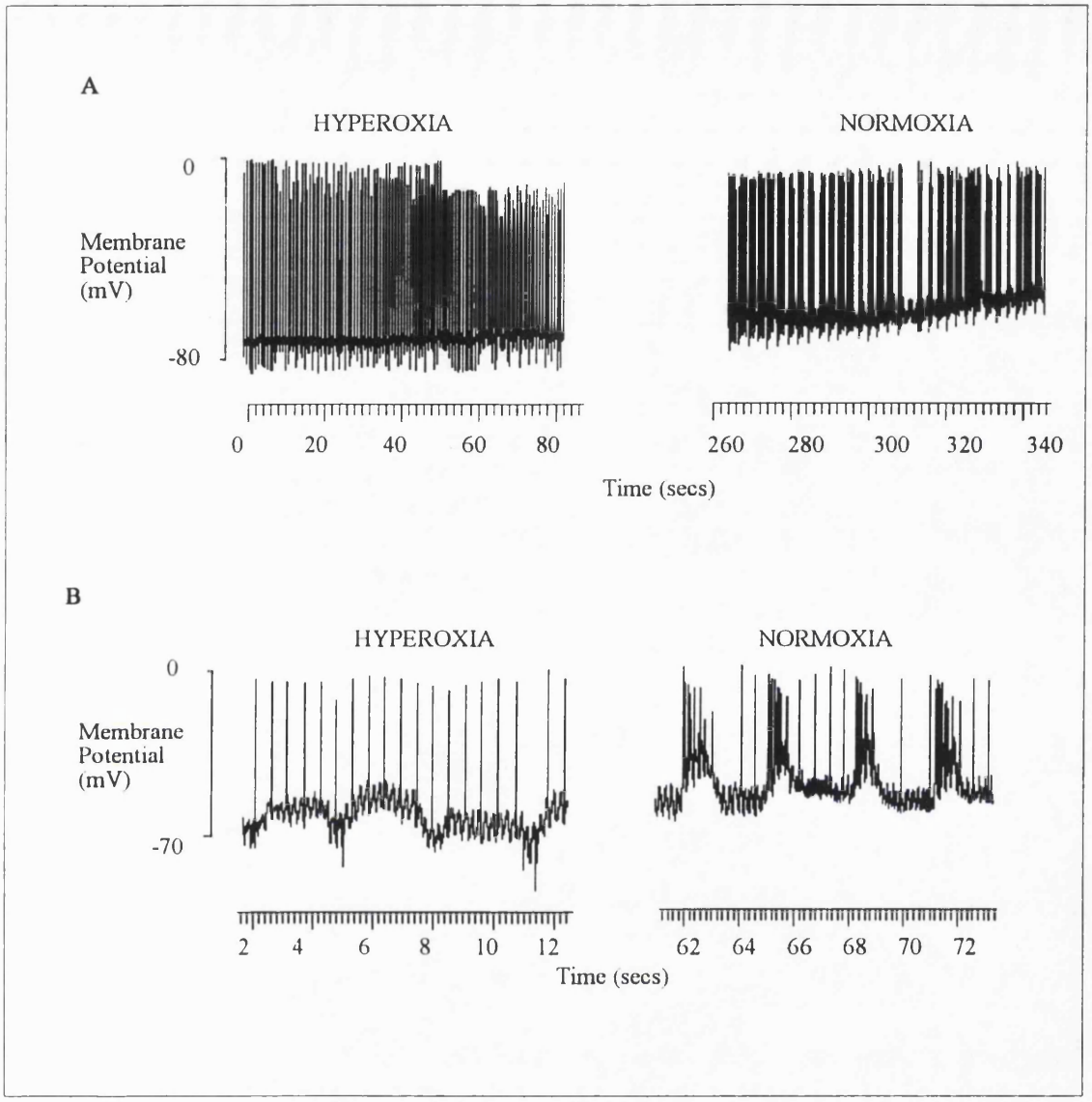


Figure 46. Intracellular recordings of two hypoglossal motoneurones showing the effect of changing the FiO2 from hyperoxia to normoxia. The resting membrane potential of the first motoneurone (A) had a membrane potential of -71 mV during hyperoxia (FiO2 0.34). During normoxia the membrane depolarized to approximately -64mV. Note that also during normoxia the pattern of discharge of the hypoglossal motoneurone became rhythmic. In the second motoneurone, shown in B), the membrane potential depolarized to -64mV. Note that during normoxia rhythmic EPSPs appeared reaching threshold and generating bursts of action potentials.

-44, -50, -68 and -71mV respectively. Recordings of the latter two motoneurones are shown in figure 46. In both cases the FiO_2 was reduced to the normoxic level and the membrane depolarized (by 7 and 4mV respectively). Both began firing spontaneously as the membrane depolarized. The recording in figure 46a shows that this motoneurone generated phasic bursts of spontaneous discharge as the FiO_2 level reached normoxia. The second motoneurone, shown in figure 46b, had rhythmic EPSP activity during normoxia which reached threshold and generated bursts of action potentials.

3.2.10 SUMMARY OF SECTION 2

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

Of the 21 hypoglossal motoneurones tested with hypoxia;

1) 14 showed a sustained depolarization
2) 2 showed a depolarization which repolarized despite the fact that hypoxia was continued
3) 4 were hyperpolarized
4) 1 showed no change in membrane potential

10 motoneurones displayed rhythmic EPSP activity of which 6 were tested with hypoxia;

1) 2 showed an increase in amplitude during hypoxia
2) 1 showed a decrease in the amplitude of the EPSP activity
3) 2 showed no change in the EPSP activity
4) and in 1 the rhythmic EPSP activity could not be measured because during hypoxia the motoneurone generated action potentials.

The changes in rhythmic EPSP activity were independent of changes in resting membrane potential.

3.3 ACTION POTENTIAL CHARACTERISTICS

3.3.1 Introduction

The third part of the results section investigates the effect of mild levels of hypoxaemia on the action potential profile.

3.3.2 Orthodromic action potentials

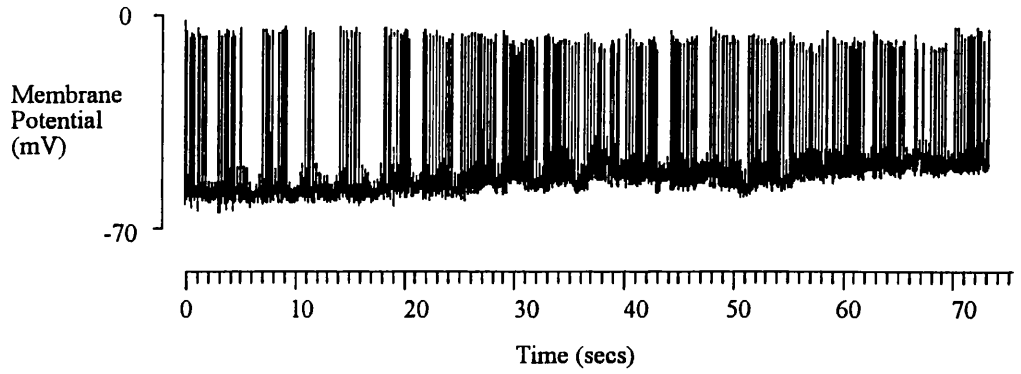
Of the 50 hypoglossal motoneurones which were recorded with intracellular electrodes, orthodromic discharge was recorded in 16 motoneurones during normoxia. Eight of these recordings had phasic bursts of activity. Two examples are shown in figure 47.

The orthodromic action potential characteristics from 10 hypoglossal motoneurones have been analysed. The mean amplitude of the action potentials ranged from between 41.6 $\pm$ 0.14 and 54.8 $\pm$ 0.61mV. The mean duration of the action potentials ranged from between 1.3 $\pm$ 0.01 and 2.11 $\pm$ 0.03 msec with the mean time to peak ranging from between 0.4 $\pm$ 0.01 and 0.8 $\pm$ 0.01 msec. The motoneurones all had action potentials with distinct after-hyperpolarizations; the mean amplitude of the after-hyperpolarizations ranged from between 3.6 $\pm$ 0.81 and 10.6 $\pm$ 0.09mV. Triggered averages of the action potentials recorded from two hypoglossal motoneurones are shown in figure 48. Both show distinct after-hyperpolarizations.

3.3.3 The effect of hypoxia on the orthodromic action potential characteristics

Spontaneous activity was recorded in 4 of the 21 hypoglossal motoneurones tested

A)



B)

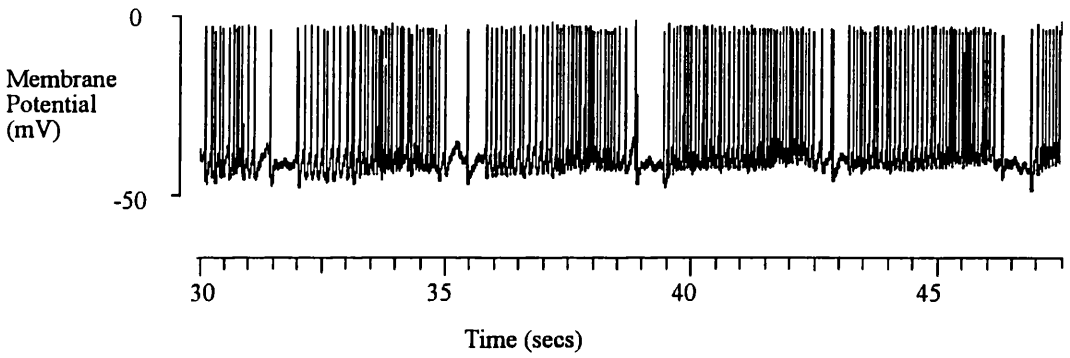


Figure 47. Intracellular recording of two hypoglossal motoneurones which fired spontaneously during normoxia in phasic bursts. The motoneurone in A) had a membrane potential of approx. -58mV whereas that in B) had a membrane potential of approx. -48 mV. A blood sample was taken during normoxia shortly before the recording in B) was obtained. The PaO_2 was 98 mmHg, $PaCO_2$ was 46mmHg and pH was 7.2. The kittens were aged 16 and 19 days old respectively and weighed 310 and 415g.

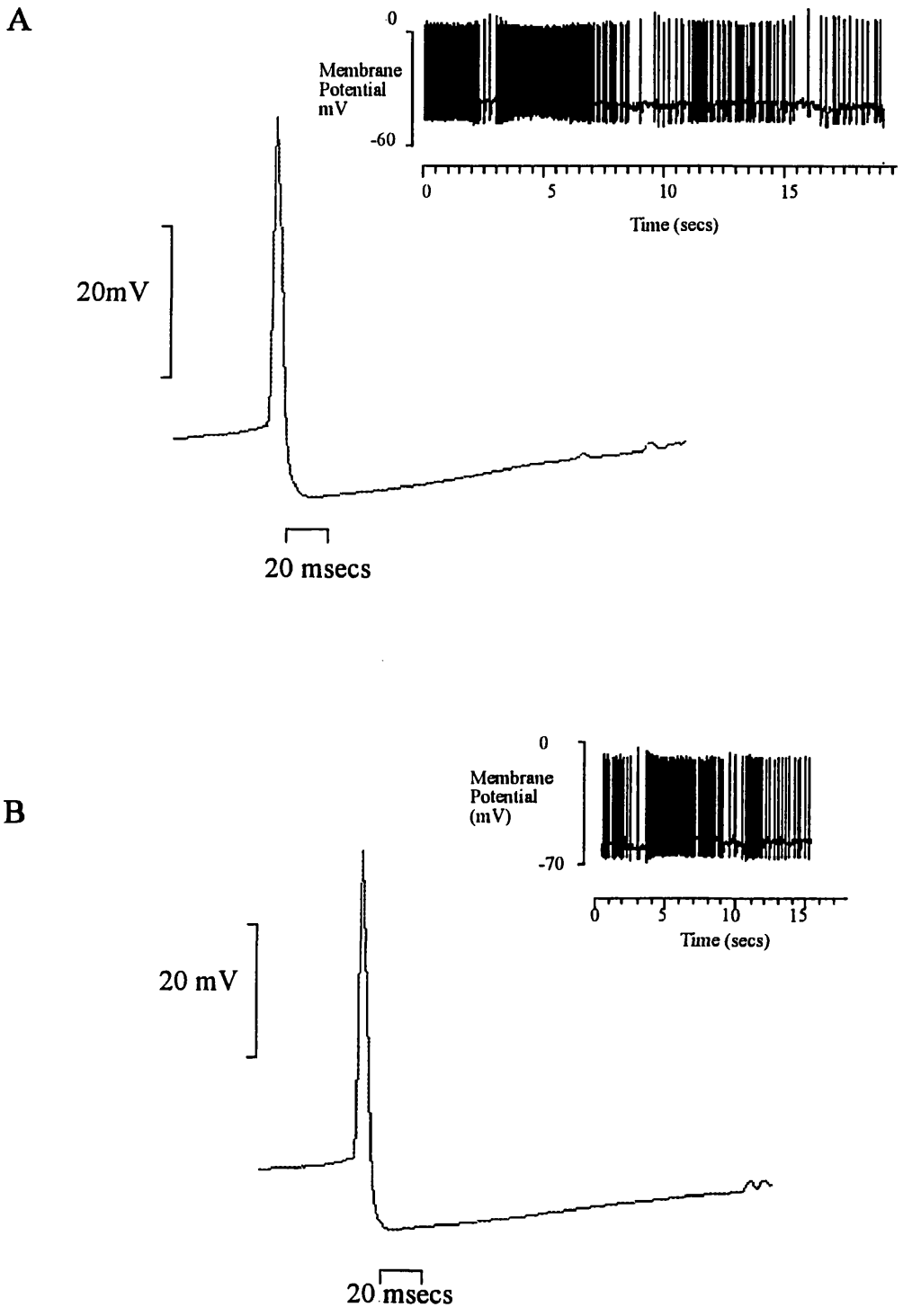


Figure 48. Action potential profiles, shown as triggered averages (> 260 sweeps), recorded in two hypoglossal motoneurones during normoxia. Note the distinct after-hyperpolarization in each case. The insets show the trace of recording from which the action potentials were measured.

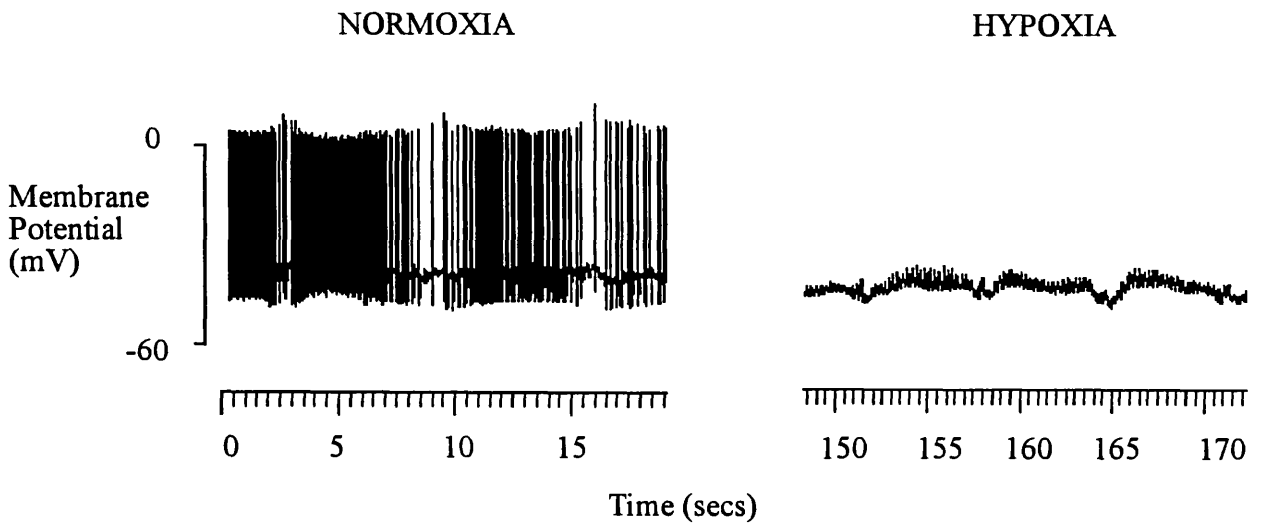


Figure 49. Intracellular recording of a hypoglossal motoneurone which fired spontaneously during normoxia but failed to show any orthodromic activity during hypoxia (FiO_2 0.16). The kitten was aged 23 days old and weighed 527g.

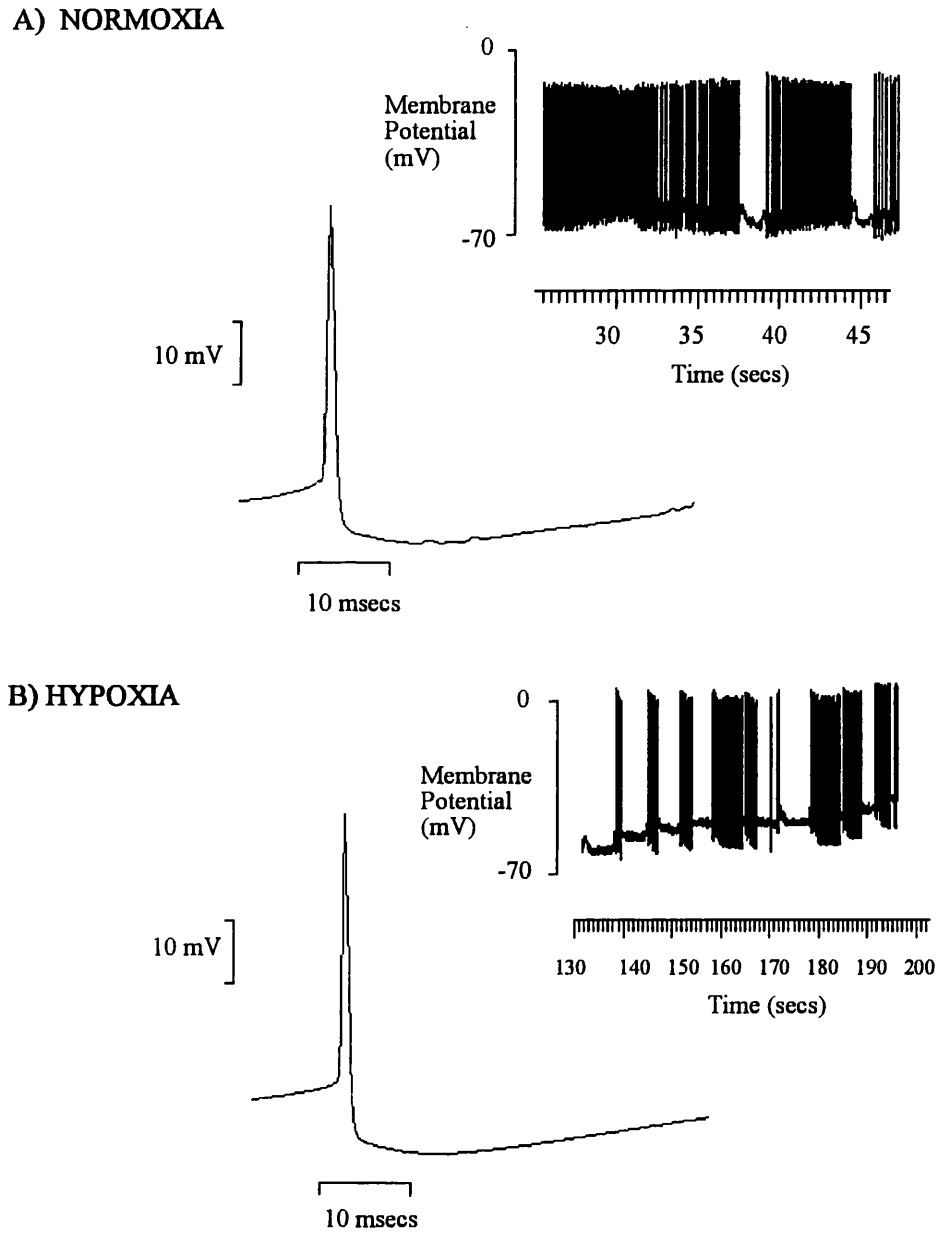


Figure 50. Intracellular recording of a hypoglossal motoneurone showing the effect of hypoxia (FiO_2 0.14) on othodromic action potentials. The action potentials recorded during normoxia (A) and hypoxia (B) are shown as triggered averages (> 290 sweeps). There was no difference in the action potential profile during hypoxia. The insets show the trace of recording from which the action potentials were measured. Note that the motoneurone had bursts of action potentials and that the membrane depolarized during hypoxia (B). This recording is also shown in figure 28.

with hypoxia. In all 4 the activity was phasic during normoxia. The mean action potential characteristics for each motoneurone were determined from a section of the recording which included a minimum of 260 action potentials. The mean amplitude of the action potentials ranged from between 41.6 $\pm$ 0.14 and 44.8 $\pm$ 0.15mV. The mean duration of the action potentials ranged from between 1.8 $\pm$ 0.01 and 1.9 $\pm$ 0.01msec with the mean time to peak ranging from between 0.7 $\pm$ 0.02 and 1.9 $\pm$ 0.01 msec. The mean amplitude of the after-hyperpolarizations ranged from between 4.4 $\pm$ 0.09 and 8.8 $\pm$ 0.01mV.

Three of the four motoneurones did not fire spontaneously during hypoxia. One of these is shown in figure 49. In the fourth motoneurone there was very little difference in the mean duration (1.8 $\pm$ 0.00 ms) and mean time to peak (0.7 $\pm$ 0.01 ms) of the action potentials recorded during normoxia in comparison to those recorded during hypoxia (1.8 ms $\pm$ 0.01 and 0.6 $\pm$ 0.01 respectively). Triggered averages of the action potentials show that the action potential profiles are similar under the two conditions (Fig. 50).

An additional 5 motoneurones, which showed no othodromic discharge during normoxia, began firing spontaneously during hypoxia; all were depolarized and/or showed an increase in rhythmic EPSP activity. Examples are shown in figures 32 and 33 of the last section (page 158 & 159). In two of these, the spontaneous activity was observed only when the FiO_2 levels approached 0.14 and the recording was lost soon after. In a further motoneurone, which hyperpolarized during hypoxia, spontaneous activity was recorded during the posthypoxic period as the membrane repolarized. However, because the motoneurones had been antidromically stimulated (1 every 600 msecs) it was difficult to obtain an accurate measure of the mean orthodromic action potential characteristics.

3.3.4 Antidromic action potentials

The mean antidromic action potential characteristics for 20 hypoglossal motoneurones were determined from a section of recording during normoxia which included only antidromic action potentials (Fig. 51). The mean amplitude of the antidromic action potentials ranged from 31.6 ± 0.96 to 65.2 ± 0.33mV. The mean duration of these action potentials ranged from 1.6 ± 0.02 to 2.64 ± 0.03 ms, with mean time to peak ranging from 0.6 ± 0.01 to 1.1 ± 0.02ms. The longer durations were those of antidromic impulses with variation in initial segment-soma dendritic (IS-SD) spike separation (Fig. 52a). In the majority (n=16) of motoneurones the action potentials had distinct after-hyperpolarizations with mean amplitudes ranging from 0.9 ± 0.23 to 9.9 ± 0.45mV (Fig. 51a).

3.3.5 The effect of hypoxia on antidromic action potential characteristics

Of the 21 hypoglossal motoneurones tested with hypoxia, 14 generated action potentials which were mainly those evoked by antidromic stimulation of the nerve. The mean amplitude of these antidromic action potentials generated by these motoneurones during normoxia ranged from between 38.6 ± 0.3 and 61.4 ± 0.34mV, a mean duration between 1.6 ± 0.03 and 2.64 ± 0.03 msecs and the mean time to peak between 0.6 ± 0.01 and 1.1 ± 0.02 ms. The majority (n=10) of these motoneurones generated action potentials with distinct after-hyperpolarizations during normoxia (Fig. 51a); the amplitudes ranged from between 0.9 ± 0.23 and 8.0 ± 0.16mV. No after-hyperpolarizations were recorded in the remaining 4 motoneurones during the control period (Fig. 51b).

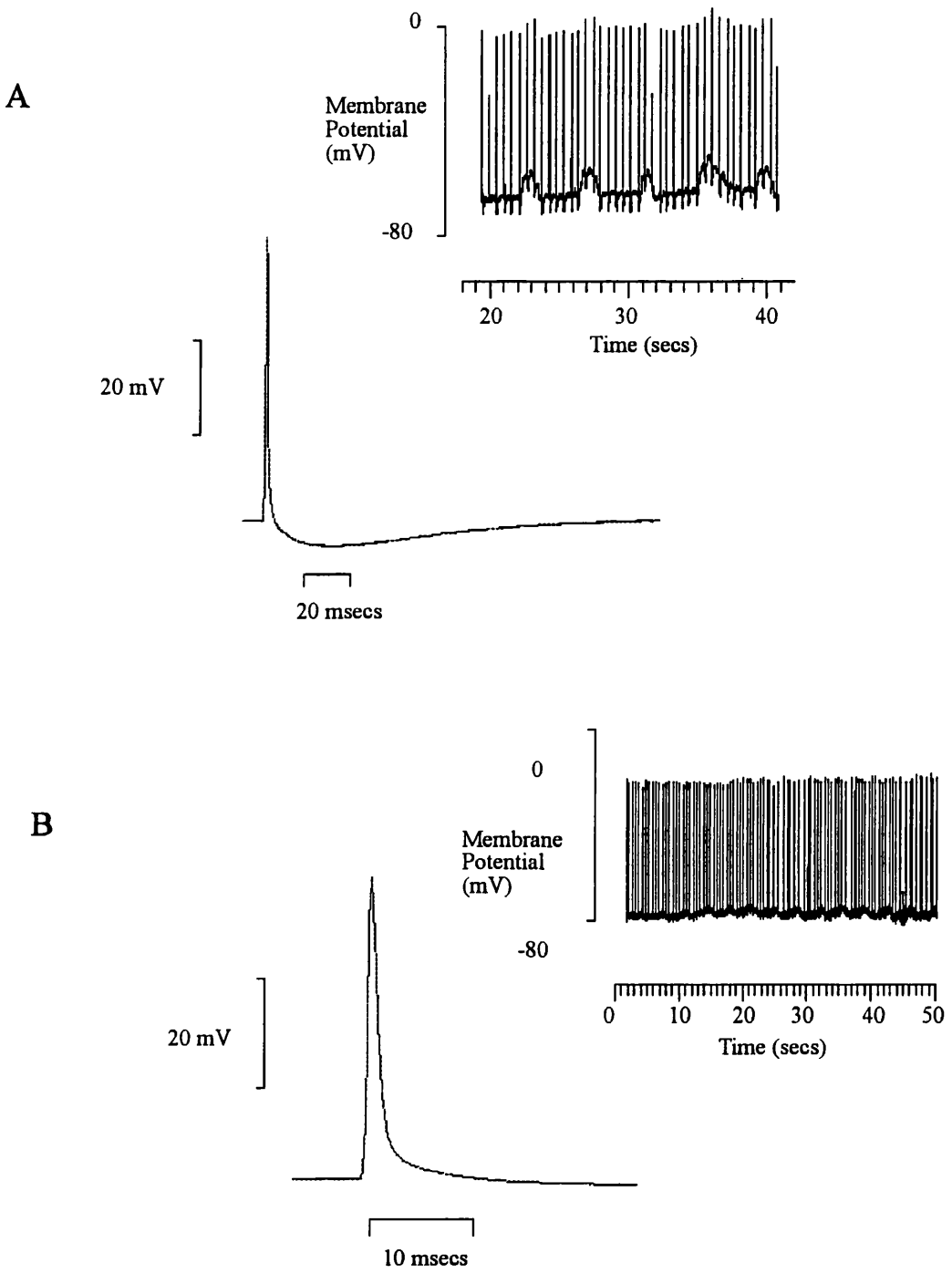


Figure 51. Intracellular recording of two hypoglossal motoneurones showing the antidromic action potentials (1/600 msecs) as triggered averages. The insets show the trace of recordings from which the action potentials were measured. The action potentials shown in A had clear after-hyperpolarizations which were not present in B.

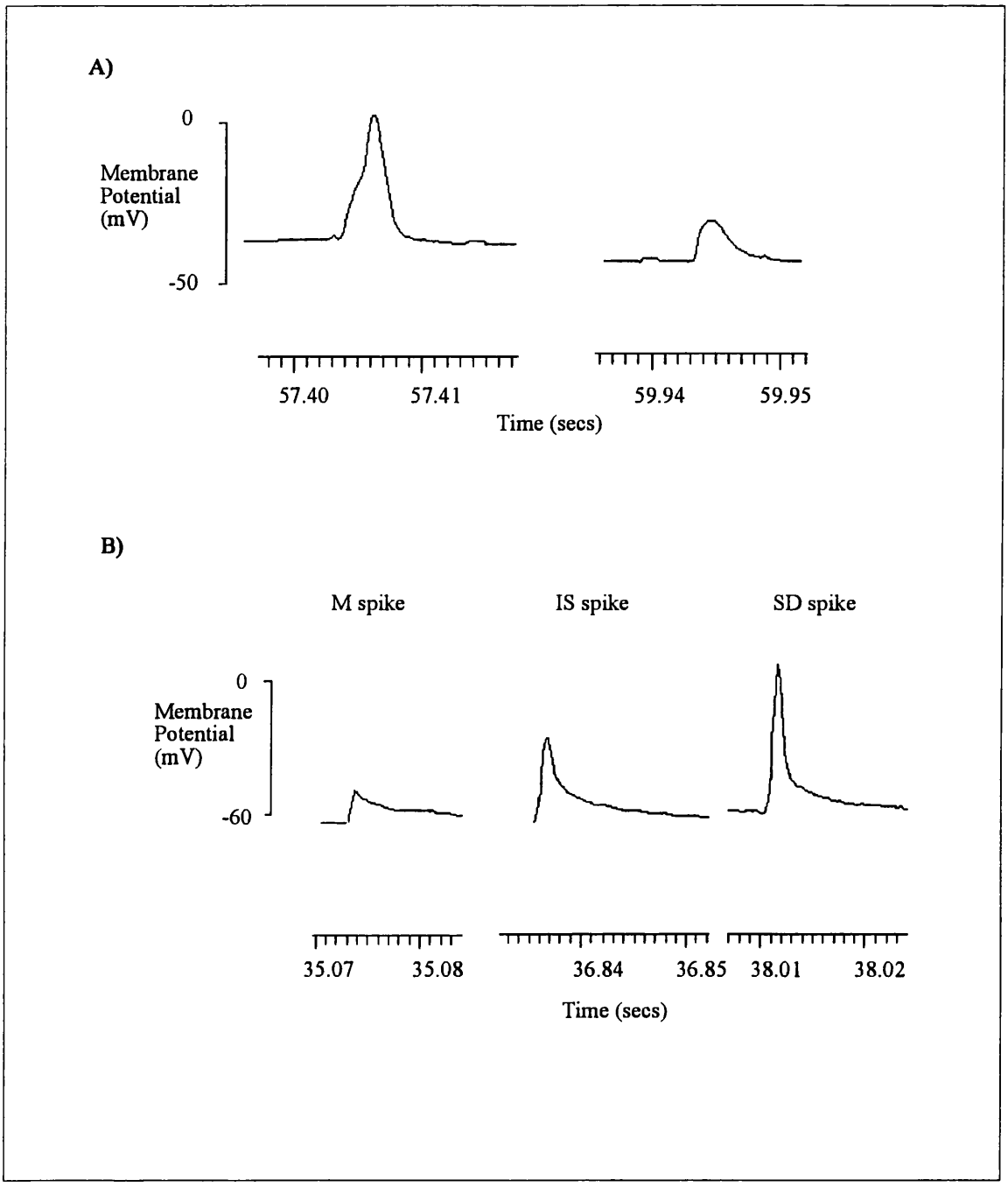
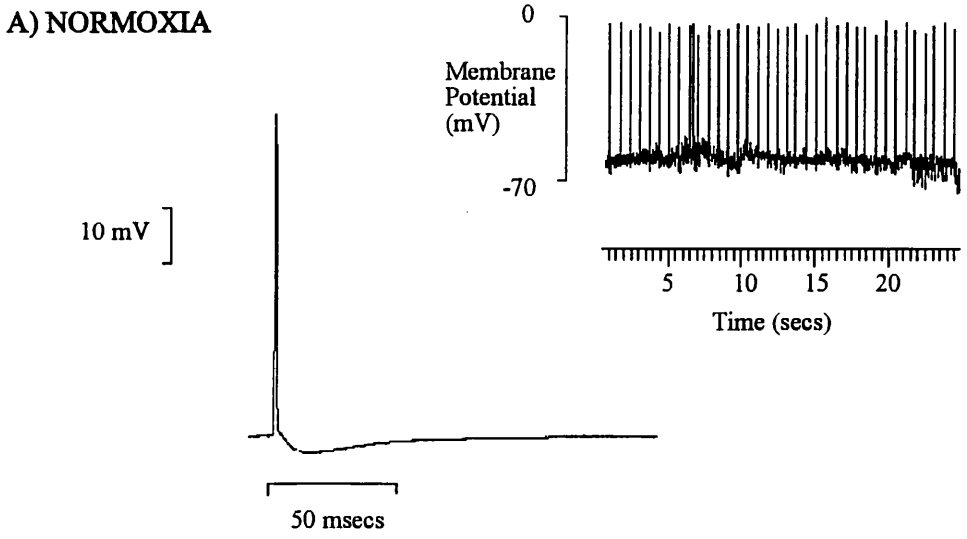


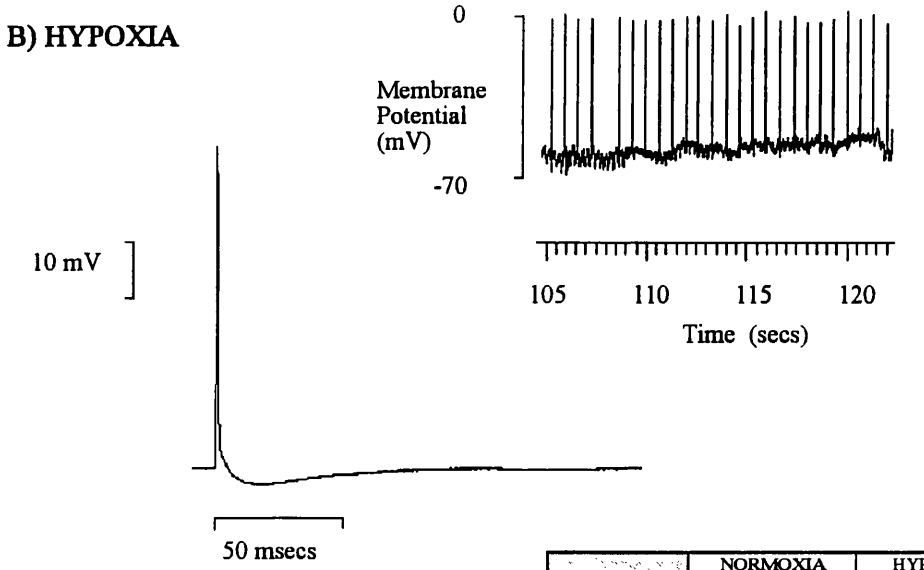
Figure 52. Action potentials showing initial segment-soma dendritic (IS-SD) separation. The action potentials in A) were recorded during normoxia; no hypoxic test was completed on this motoneurone. The action potentials in B) were recorded during hypoxia (FiO_2 0.17) and show the M, IS and SD spike.

In one motoneurone during hypoxia the antidromic action potential failed to invade the soma (FiO_2 0.14). Triggered averages of the action potentials generated by the remaining 13 motoneurones were used to establish if hypoxia at the level used in this study had any effects on the mean action potential profile. Nine of these motoneurones depolarized during hypoxia, 2 repolarized, 1 hyperpolarized and 1 showed no change in membrane potential.

Triggered averages of the antidromic action potentials show that for the majority (n=8) of hypoglossal motoneurones analysed, there was no obvious change in the action potential profile when the FiO_2 was reduced to the hypoxic level. Figure 53 shows the mean triggered average of the action potentials from one motoneurone during normoxia and hypoxia (a and b respectively) and clearly shows that the action potential profile does not alter. Seven of these motoneurones were depolarized during hypoxia whereas the eighth showed no change in membrane potential (see section 3.2.3). Triggered averages of the remaining 2 motoneurones which depolarized during hypoxia revealed a change in the shape of the after-hyperpolarization, with a faster time to peak and a faster decay (Fig. 54). Both of these motoneurones were recorded in the same kitten.

The action potentials generated by one of the motoneurones which repolarized during hypoxia, following an initial depolarization, is shown in figure 55. During the initial depolarization there was no obvious change in the action potential profile (Fig. 55a), however, during the repolarization there was a delay in the time to peak of the after-hyperpolarization and an increase in its duration (Fig. 55b). The second motoneurone, which also repolarized during hypoxia, generated action potentials with no clear after-hyperpolarizations during normoxia and continued to do so during hypoxia (FiO_2 0.17-0.16).





	NORMOXIA	HYPOXIA (FIO, 0.17)
PaO_2 (mmHg)	73	49
$PaCO_2$ (mmHg)	36	39
pH	7.2	7.2

Figure 53. Antidromic action potentials (1/600msec), shown as triggered averages, recorded in a hypoglossal motoneurone during normoxia (A) and hypoxia (B, FiO_2 0.17). The insets show the trace of the recording from which the action potentials were measured. The membrane was depolarized from -63 to -53mV when the FiO_2 was reduced. Note that the action potential profile during hypoxia is the same as that in normoxia. Blood samples were taken during normoxia and hypoxia (FiO_2 0.17), shortly after the completion of this recording, in a repeat of the test. The table shows the PaO_2, $PaCO_2$ and pH.

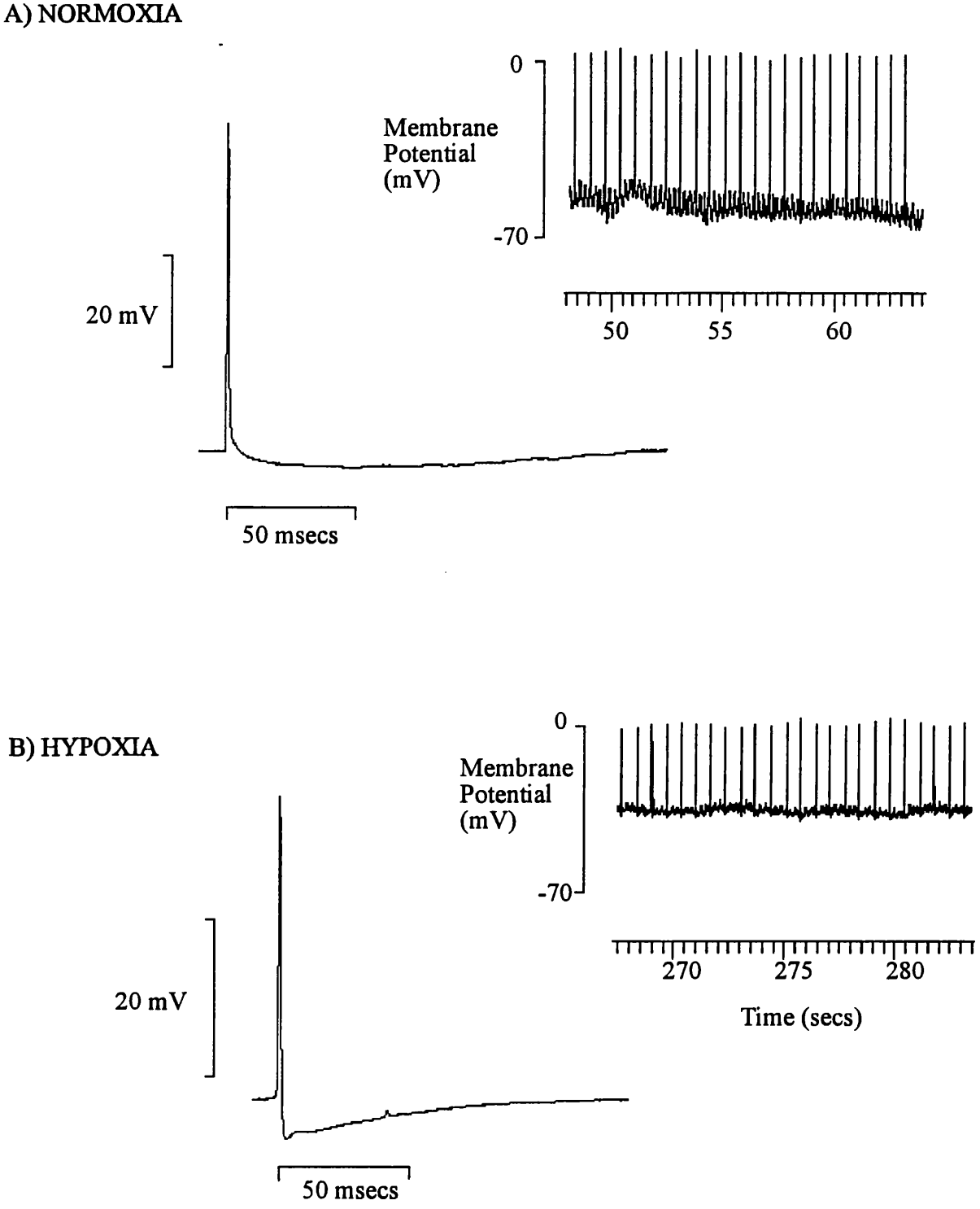


Figure 54. Antidromic action potentials (1/600msec), shown as triggered averages, recorded in a hypoglossal motoneurone during normoxia (A) and hypoxia (B, FiO_2 0.17). The insets show the trace of the recording from which the action potentials were measured. During hypoxia the membrane was depolarized from approx. -63 to -39mV. The after- hyperpolarization has a faster time to peak and a faster decay during hypoxia than that occurring during normoxia. Blood samples were taken during normoxia and hypoxia, shortly following the completion of the recording, in a repeat of the test (see table in figure 45). The PaO_2 fell from 73 to 49 mmHg as the FiO_2 was reduced

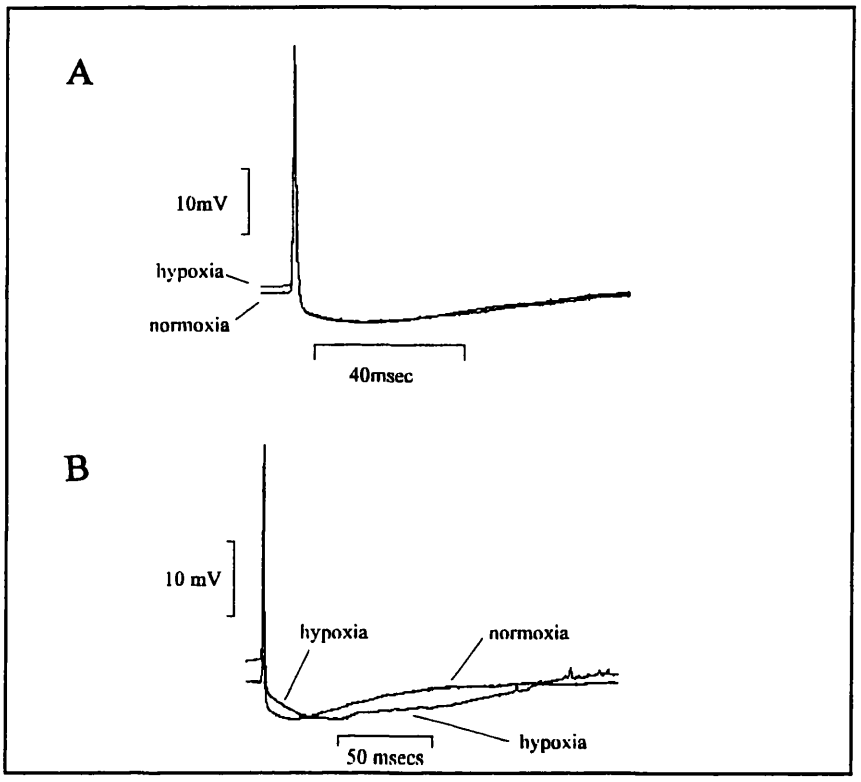


Figure 55. Examples of antidromic action potentials, shown as triggered averages, recorded in a hypoglossal motoneurone which was transiently depolarized during hypoxia (FiO_2 0.16). In each case, the average action potentials during normoxia and hypoxia have been superimposed. The hypoxic period in A) was taken as the membrane depolarized from approx. -61mV to -38mV and that the hypoxic period in B) was taken as the membrane repolarized reaching approx. -51mV. Note that in B the time to peak and duration of the after-hyperpolarizations were increased during hypoxia. A blood sample taken during hypoxia (FiO_2 0.16) revealed that the PaO_2 was 60mmHg.

Only two motoneurones which were hyperpolarized during hypoxia were antidromically stimulated throughout the recording. In one of these, previously shown in figure 30 (page 154), the antidromic action potentials failed to invade the soma, however, in the second, there was a delay in the time to peak of the after-hyperpolarization and an increase in its duration during hypoxia. The triggered average of the action potentials recorded from this motoneurone are shown in figure 56.

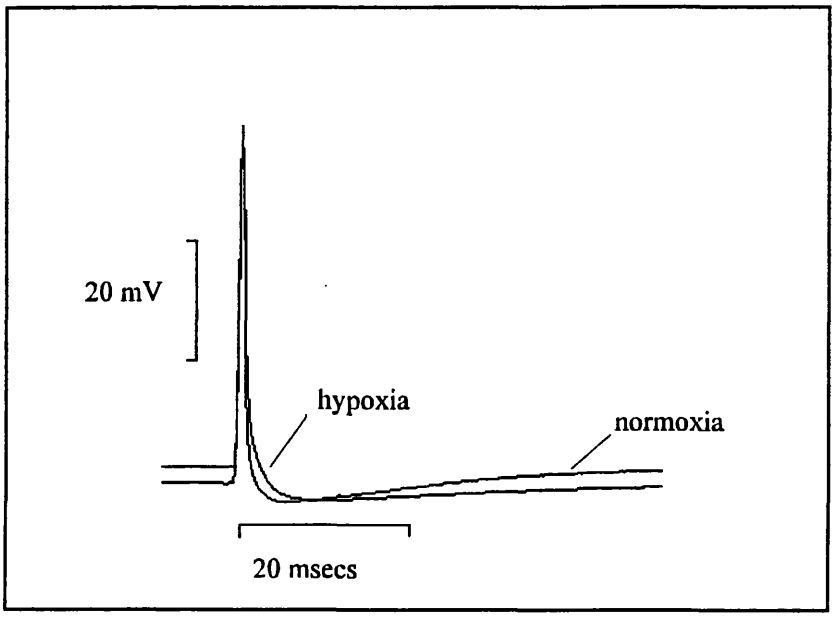


Figure 56. Examples of antidromic action potentials, shown as triggered averages, recorded in a hypoglossal motoneurone which was hyperpolarized from approximately -67 to -71mV during hypoxia (FiO_2 0.15). The average action potentials during normoxia and hypoxia have been superimposed. Note that the time to peak and duration of the after-hyperpolarizations are increased during hypoxia.

One hypoglossal motoneurone generated antidromic action potentials which occasionally throughout the recording showed IS-SD spike separation (Fig. 33, page 159). An additional two motoneurones during hypoxia (FiO_2 0.17) also occasionally showed IS-SD separation as the membrane depolarized (Figure 52b).

3.3.6 SUMMARY OF SECTION 3

In the majority of hypoglossal motoneurones there was no change in the action potential profile as the FiO_2 was reduced to hypoxia.

However, in 2 motoneurones which depolarized during hypoxia there was a faster time to peak and a faster decay of the after-hyperpolarization.

In one motoneurone which hyperpolarized during hypoxia there was a delay in the time to peak and an increase in the duration of the after-hyperpolarization.

Similarly in one motoneurone which repolarized during hypoxia, although there was no change in the after-hypeprolarization during the initial depolarization, as the membrane repolarized there was a delay in the time to peak and an increase in the duration of the after-hypeprolarization.

4.DISCUSSION

4.1 A summary of the results of this thesis

This study has addressed the hypothesis that the decrease in genioglossus muscle activity during hypoxia in neonates is due to an inhibition of hypoglossal motoneurones.

To my knowledge, this is the first study which has investigated the effect of hypoxaemia on hypoglossal motoneurones in neonates in an *in vivo* neonatal preparation. The results of this study show that the output of these motoneurones is affected by even mild levels of hypoxaemia. Furthermore, this study has shown that although some of these motoneurones respond with an increase in discharge frequency, for a large proportion there is either a decrease or the increase is only transient.

To address further the hypothesis that the decrease in discharge frequency is due to inhibitory mechanisms, I have recorded the membrane potential of hypoglossal motoneurones during similar levels of arterial hypoxia. The results show that, although the membrane depolarized in the majority of hypoglossal motoneurones, at least some were either hyperpolarized or unable to maintain the initial depolarization for the period of hypoxia. These changes parallel the changes in discharge frequency. This study therefore shows that in neonatal kittens the activity of hypoglossal motoneurones is altered during mild levels of hypoxaemia by changes in membrane potential.

Another important result of this study was that hypoxaemia affected rhythmic EPSPs independently of its effect on the resting membrane potential. This suggests that hypoxaemia has an effect on hypoglossal output through a mechanism which is independent of, or in addition to, that exerted through respiratory rhythm.

Taking the results of the extracellular and intracellular recordings together, this study provides clear evidence that with even mild levels of hypoxaemia, changes in hypoglossal motoneurone activity in neonates are mediated by inhibitory, as well as excitatory, mechanisms.

4.2 Overall view of the discussion

In this chapter, I will discuss the possible mechanisms underlying both the excitation and inhibition of the hypoglossal motoneurones during mild levels of hypoxaemia. In addition, I will consider the changes in rhythmic EPSP activity recorded during hypoxia and will again discuss some possible mechanisms underlying these changes. Towards the end of the chapter, I will discuss the *in vivo* preparation and some factors which need to be taken into consideration when interpreting the results. Finally, I will outline the possible clinical relevance of these results and make some suggestions for future studies.

4.3 Levels of hypoxia

There are several reasons for considering that the levels of hypoxia used in this study were mild.

The partial pressure of the arterial blood (PaO_2) fell during the decrease in FiO_2 (0.16-0.18) to 47.2 ± 3.8 mmHg (mean ± SEM). At similar levels of hypoxaemia, the haemoglobin oxygen saturation (SaO_2) was reduced by no more than 15% (section 2.4). From graphs presented in a recent report levels of SaO_2 and PaO_2, similar to those used in the present study, did not substantially change the oxygen saturation of the haemoglobin circulating in the brain tissue of neonatal puppies, when measured with double beam spectroscopy, and were considered, therefore, to be mild (Nioka,

Chance, Smith, Mayevsky, Eilly, Alter & Asakura, 1990). Thus in the present study, the small changes in SaO_2 would not be expected to alter substantially the oxygen available to the tissue and have also been described as mild.

A number of studies, both on adults and neonates, have shown that there is an increase in cerebral blood flow during hypoxaemia which maintains oxygen delivery to the brain tissue (Cohen, Alexander, Smith, Reivich & Wollman, 1967, Jóhannsson & Siesjö, 1975, Gardiner, 1980, Massik, Jones, Miyabe, Tang, Hudak, Koehler & Traystman, 1989, Laudignon, Beharry, Rex & Aranda, 1990, Suguihara, Bancalari & Hehre, 1990). Indeed, the levels of hypoxia have to be severe before there is a decrease in cerebral O_2 consumption (Jóhannsson & Siesjö, 1975, Gardiner, 1980). For example, in adults rats, the cerebral metabolic rate for oxygen was maintained even at levels as low as 22 mmHg (Jóhannsson & Siesjö, 1975). Furthermore, changes in levels of ATP or ADP in brain tissue were only been recorded when PaO_2 was substantially reduced; in rats the PaO_2 had to fall below 25mmHg (Siesjö & Nilsson, 1971) and in dogs less than 30mmHg (Hilberman, Harihara Subramanian, Haselgrove, Cone, Egan, Gyulai & Chance, 1984). In a more recent study, the oxygen saturation of the haemoglobin circulating in the brain tissue of neonatal dogs had to fall below 20% before there was any significant fall in the phosphocreatine-inorganic phosphate ratio, an indication of cellular energy metabolism (Nioka et al., 1990).

4.4 Effects of hypoxia on the discharge frequency of the hypoglossal motoneurones in neonatal kittens

The results of the present study show that, although 34% of the hypoglossal motoneurones recorded with extracellular electrodes exhibited a sustained increase in

discharge frequency, for a substantial proportion (46%) the change during these mild levels of arterial hypoxia was either only a transient increase or a decrease in discharge frequency.

The results of the present study would explain why the recruitment of the genioglossus muscle, or the increase in discharge frequency of the hypoglossal nerve, during even mild levels of hypoxia is not always sustained in neonates (Bruce, 1986, Sica et al., 1988, Watchko et al., 1989). Although the activity of both the muscle and its cranial nerve increase during the early stages of hypoxia, as with the phrenic nerve, this activity subsequently decreases.

4.5 Effects of hypoxia on the membrane potential of hypoglossal motoneurones in neonatal kittens

If the changes in discharge frequency of the hypoglossal motoneurones recorded with extracellular electrodes are due to changes in membrane potential, it would be expected that at least some of the hypoglossal motoneurones would be hyperpolarized, or repolarized after an initial depolarization, during similar levels of hypoxaemia. This was indeed found to be the case; for although most of the hypoglossal motoneurones were depolarized, at least some subsequently repolarized or were hyperpolarized. The results of this study therefore support the hypothesis that the response of the hypoglossal motoneurones to hypoxia are mediated by inhibitory as well as excitatory mechanisms.

The levels of hypoxaemia used in this study did not depress the ability of the motoneurones to generate action potentials; in the majority of cases the motoneurones, recorded with either extracellular or intracellular electrodes, could still be antidromically activated. In addition, there was no evidence that the subsequent decline

in discharge frequency during these levels of arterial hypoxia was due to depolarization blockade. We are, however, unable to say how the motoneurones would respond during longer periods or more severe levels of hypoxia.

This thesis has therefore provided strong evidence that the decrease in discharge frequency of the hypoglossal motoneurones during mild levels of hypoxaemia is due to changes in membrane potential.

4.6 Possible mechanisms which may be involved in the response of the hypoglossal motoneurones to mild levels of hypoxaemia

A distinctive feature of the results of this study was that different response characteristics could be recorded from hypoglossal motoneurones in the same preparation. Thus, in the same kitten, as the FiO_2 was reduced some hypoglossal motoneurones were excited, some transiently excited and some inhibited. This may reflect different subpopulations of motoneurones. However, this is not the only possible explanation for the different responses recorded.

There are a number of possible mechanisms which may explain or be involved in the different responses of the hypoglossal motoneurones to hypoxaemia. 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) a central inhibitory mechanism,
6) a direct effect of hypoxia on the motoneurones
7) the age of the kittens.

In the following section each of these possible mechanisms will be considered.

4.6.1 Variations in the degree of hypoxia

The response of the hypoglossal motoneurones may vary depending upon the degree of hypoxia. Thus with mild levels of hypoxia hypoglossal motoneurones may be inhibited and at more severe levels they may be excited, or *vice versa*. However, in the present study, at any level of FiO_2, motoneurones could be recorded with the different response characteristics (see Fig. 16, page 134). 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 hypoglossal motoneurone 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 hypoxic tests, the blood samples showed that PaO_2 levels varied. 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 different response characteristics could be recorded in the same kitten and that the sequence in which they were recorded was not consistent. For example, in one preparation the first motoneurone recorded may have responded to hypoxaemia with a transient excitation and the second motoneurone with a sustained excitation, in another experiment it may have been the other way around.

Another consideration is the levels of PO_2 experienced by the individual

motoneurones. This will vary 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 (see section 4.6.6), the different response characteristics may reflect the PO_2 experienced by each motoneurone as a result of differences in their position within the tissue.

4.6.2 Changes in chemoreceptor discharge

The most likely explanation for the excitation of the hypoglossal motoneurones, 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 hypoglossal motoneurones (Mifflin, 1990).

Several studies have shown, using single or few fibre preparations, that the discharge of the carotid sinus nerve in anaesthetized kittens increases during hypoxia (Blanco et al., 1984, Marchal, Bairam, Haouzi, Crance, Di Giulio, Vert & Lahiri, 1992, Mulligan, 1991, Morray, Bennet, Noble & Hanson, 1992). At 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). 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 excitation of the hypoglossal motoneurones was due to an increase in the discharge of the peripheral chemoreceptors, then it is also conceivable that the inhibitory responses recorded were due to a decrease in peripheral chemoreceptor

activity. However, there is some controversy in the literature as to the response of the chemoreceptors to hypoxia in neonates (Schwieler, 1968, Blanco et al., 1984, Carroll & Bureau, 1987, Marchal et al., 1992, Morray et al., 1992). In some studies the increase in carotid chemoreceptor discharge was sustained throughout a 5 to 10 minute period of hypoxia when the FiO_2 was reduced to between 0.06 and 0.12 (Schwieler, 1968, Blanco et al., 1984), or the PaO_2 level reduced to 40 to 50mmHg (Morray et al., 1992). In contrast, another study has suggested that there is a decrease in peripheral chemoreceptor function over a period of 18 minutes of hypoxia (PaO_2 41mmHg) in 2-3 day old lambs (Carroll & Bureau, 1987). In agreement with this latter study, Marchal and colleagues (1992) recently demonstrated that although 13 kittens showed a sustained increase in chemoreceptor discharge during hypoxia (approximately 7% O_2 for 2-3 mins), 11 kittens did not sustain the increase in discharge throughout the period of hypoxia. In these kittens the carotid chemoreceptor discharge, which in normoxia was approximately 4 impulses per second increased to approximately 16 impulses per second but then declined to 11 impulses per second. However this level of discharge is still substantially elevated in comparison to the control level.

If the peripheral chemoreceptor discharge was not maintained in the kittens in the present study during the mild levels of hypoxaemia, it may explain why some of the motoneurones were unable to sustain an increase in activity and others were inhibited.

However, if the changes in hypoglossal motoneurone activity are to be fully explained by changes in chemoreceptor activity, this hypothesis would have to account for the fact that the different responses, i.e 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, this has been shown to be the case in the single fibre study by Marchal and colleagues (1992), some fibres sustained the increase in

discharge frequency whereas in others, after the initial increase, the discharge frequency declined. If there is a differential distribution of the types of fibres involved in the hypoglossal system it might explain the different response characteristics recorded, i.e the excitation and the transient excitation. However, because peripheral chemoreceptor discharge is still elevated above control it is more difficult to see how it could produce the effect on those hypoglossal motoneurones which were simply inhibited.

4.6.3 Changes in lung compliance

The inhibition of the hypoglossal motoneurones during hypoxia in the present study may be due to an increase in vagal afferent input. The kittens in the present study were mechanically ventilated with positive pressure and therefore did not exclude changes in volume and lung compliance from occurring during hypoxia. Although contradictory results have been reported on the effect of hypoxia on dynamic lung compliance and pulmonary resistance in the newborn (La Framboise et al., 1981 & 1983 La Framboise & Woodrum 1985, Côté, Yunis, Blanchard, Mortola & Bureau, 1988, Blanco et al., 1984), functional residual capacity was found to increase. An increase in functional residual capacity would increase the volume in the lungs at the end of expiration and, by increasing stretch receptor activity, would increase vagal afferent input. Although the discharge frequency of the pulmonary stretch receptor afferents is low in kittens of less than two weeks old, when compared to the discharge frequency of older kittens, they still fire continually in response to a maintained stimulus (Schwieler, 1968). Furthermore, vagal afferent input has a profound depressant effect on the activity of the hypoglossal nerve (Kuna, 1986, Bartlett & St.John, 1988). Thus changes in lung dynamics during hypoxia might be expected to alter the activity of the hypoglossal motoneurones. However, preliminary results from

vagotomized kittens (from studies in the laboratory since the completion of the present study) suggest that the hypoglossal motoneurones are still inhibited during hypoxia (Li & Noble, personal communication).

4.6.4 Changes in cerebral blood flow

Another possibility is that the transient responses and the inhibitions recorded in the hypoglossal motoneurones during hypoxaemia are due to vascular vasodilation, an increase in cerebral blood flow and as a consequence CO_2 wash-out. Lee and Milhorn (1975) suggested that central depression of ventilation during moderate levels of hypoxia (PaO_2 45-55mmHg) is due to an increase in brain blood flow and as a consequence a ventral medullary alkalosis. In support of this idea, medullary alkalosis was found to occur in chemodenervated adult cats during mild hypoxaemia (PaO_2 >60mmHg) (Neubauer, Santiago, Posner & Edelman, 1985) 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 effect 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. This would have reduced the stimulation of the central chemoreceptor sites. It is also possible that the CO_2 transport by haemoglobin may have increased under the hypoxic conditions (Haldane effect) again leading to medullary alkalosis. Thus it may be that the mild levels of hypoxaemia induced a ventral medullary alkalosis and this may be the underlying

reason for the repolarization or hyperpolarization, or even the decrease in rhythmic EPSP activity (section 3.2.4), recorded in the present study. The effect of central hypocapnia on the activity of the hypoglossal motoneurones needs to be investigated, particularly in the neonate.

4.6.5 Central Inhibition

Notwithstanding the mechanisms outlined above, another possibility is that the hypoxia, either by a direct action or through the peripheral chemoreceptors, activates central inhibitory mechanisms in the brain stem.

It could be envisaged that the hypoxia influences the output of the hypoglossal motoneurones through pools of both excitatory and inhibitory interneurones. This theoretical model is shown in figure 57. The response characteristics of the hypoglossal output would then depend upon the balance in the activity of the two respective pools. For example, if the activity of the excitatory interneurone pool out-weighs that of the inhibitory interneurones, the overall response of the hypoglossal motoneurones will be an excitation. Similarly if the activity of the inhibitory interneurone pool out-weighs that of the excitatory interneurones, the overall response of the hypoglossal motoneurones will be an inhibition. The interneurone pools may not necessarily be within the hypoglossal nucleus itself, and may involve projections from other brainstem nuclei.

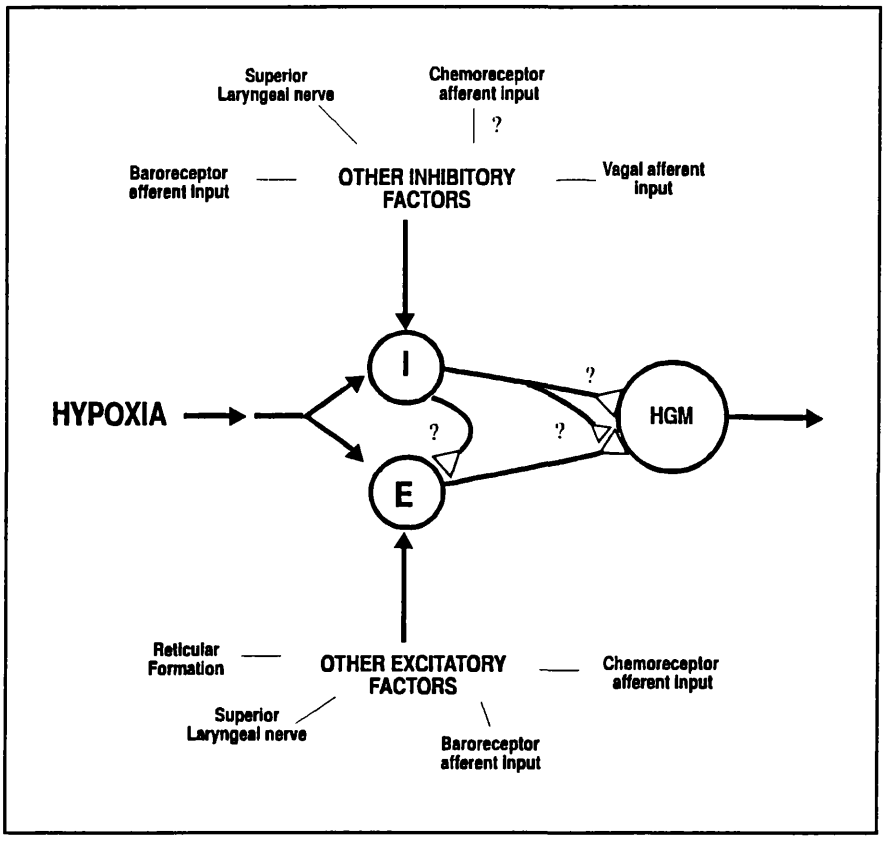


Figure 57. Theoretical model of ways in which mild levels of hypoxaemia may influence hypoglossal motoneurone output. It is proposed that hypoxia excites both excitatory (E) and inhibitory (I) interneurone pools and the output of the hypoglossal motoneurones (HGM) will be determined by their activity and the balance between them. It is further proposed that the activity of each will be influenced by other factors, for example upper airway afferents and feed-back from the lungs, which are known to alter HGM output.

The inhibitory and excitatory neurone pools may have different thresholds and different latencies for activation. It may be that the inhibitory neurones, under certain conditions, have longer latencies or higher thresholds for activation than those of the excitatory neurones; and, as a result, the overall response of the hypoglossal motoneurones is an initial excitation followed by an inhibition i.e a transient response. Thus the mechanism mediating the inhibition, and the mechanism by which some

motoneurones are inhibited after an initial excitation, may be one and the same. It is also envisaged that other factors, some of which have already been described, might influence the activity and excitability of the interneurone pools and therefore alter the balance between the inhibitory and excitatory neurones.

If both excitatory and inhibitory interneurones are simultaneously activated during hypoxia, as suggested by the model, it may explain why when the hypoxia is removed a number of the motoneurones, which were inhibited, showed a rebound excitation. The disinhibition may reflect the removal of inhibitory components revealing underlying excitatory inputs.

In the model I have suggested the possibility that the inhibitory interneurones may, at least in part, inhibit the hypoglossal motoneurones presynaptically. The hyerpolarization during hypoxaemia was associated with an increase in input resistance. A possible explanation for this is that the inhibition of the hypoglossal motoneurones was due to either a removal of an excitatory input or a presynaptic inhibition, with a consequent change in membrane conductance.

This model is speculative; but from the results of the present study it is clear that in neonates both excitatory and inhibitory mechanisms mediate the response of the hypoglossal motoneurones to hypoxia .

In adult cats, electrical stimulation of the reticular formation has been shown to increase both the peak phrenic and hypoglossal nerve activity and in some cases the tonic hypoglossal nerve activity (St. John, 1986). It is therefore possible that the excitation of the hypoglossal motoneurones during hypoxia is due to excitatory projections from the reticular formation

The inhibitory mechanism may be the same or similar to the mechanism which has been implicated in the ventilatory response to hypoxia in neonates, certainly the time course is similar. Initially during hypoxia there is an increase in ventilation, but after only a few minutes this decreases (Henderson-Smart, 1984, Rigatto, 1984, Neubauer et al., 1990). A number of studies, using transections of the brainstem, have attributed the decrease in ventilation during hypoxia in neonates to a central inhibitory mechanism (Martin-Body & Johnston, 1988, Hanson & Williams, 1989). Recent studies suggest that this inhibitory mechanism is at a site in the rostral pons or at a more rostral brainstem location (Coles, Kumar & Noble, 1989, Noble & Williams, 1989, Moore, Parkes, Noble & Hanson, 1991).

If hypoxia inhibits the phrenic motoneurones by eliciting an inhibitory mechanism situated in the rostral pons it is also conceivable that it will influence other pools of motoneurones. Studies, using retrograde transneuronal tracers, have shown that there are projections from pontine nuclei (for example, the Köllicker Fuse nucleus) to the hypoglossal nucleus (Ugolini, Kuypers & Simmons, 1987) and it is therefore important to establish how stimulation of this area affects hypoglossal motoneurones. Inhibitory projections from this area to the hypoglossal motoneurones may mediate the hyperpolarization or repolarization recorded in the present study during hypoxia. Studies are therefore required to investigate the effect of stimulation of areas within the rostral pons on the activity of the hypoglossal nerve in neonates.

4.6.6 The direct effect of hypoxia on the hypoglossal motoneurones

Notwithstanding the above argument, the excitation of the hypoglossal motoneurones may be due the direct effect of hypoxia. In neonatal rat brain stem slices, the hypoglossal motoneurones were depolarized when the oxygen perfused through the chamber was reduced (Haddad & Donnelly, 1990, Haddad et al., 1990). However, in

these *in vitro* studies the hypoglossal motoneurones were only depolarized and showed no repolarization or hyperpolarization. The difference in the techniques suggests that the inhibition recorded in the present study is dependent upon the integrity of the brain stem and cardiovascular respiratory reflexes, perhaps requiring the peripheral chemoreceptor afferent inputs to be intact.

4.6.7 The influence of age on the response of the hypoglossal motoneurones to hypoxia

In the present study on neonates, a large proportion of the hypoglossal motoneurones were only excited transiently or were inhibited. In contrast, in adult cats also studied *in vivo*, the hypoglossal motoneurones all depolarized during chemical stimulation (Mifflin,1990). The difference in the results may reflect the levels of hypoxia; in the latter study, hypoxia was achieved by switching the ventilator off. Nevertheless, it is also possible that the different responses are due to differences in age. The response of the genioglossus muscle to hypoxia has been shown to be different depending upon the age of the kitten; although all the two month old kittens could sustain an increase in genioglossus 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).

In the present study there was no obvious relationship between the age of the animals on the day of recording and the response of the hypoglossal motoneurones to hypoxaemia when recorded with extracellular electrodes. However, it is interesting that all of the hypoglossal motoneurones which repolarized or hyperpolarized were recorded in kittens of less than 21 days old. Further studies are required to determine more specifically the effect of maturation on the output of these motoneurones.

One important question is whether the membrane potentials recorded in neonatal kittens are similar to those recorded in the adult. Hypoglossal motoneurones recorded in *in vitro* neonatal rat brain stem slices have lower resting membrane potentials (-69 ± 9mV, mean ± S.D) than the resting membrane potentials of those recorded in adults (-80 ± 2mV) (Haddad & Donnelly, 1990). It is therefore perhaps not surprising that the average resting membrane potential of the hypoglossal motoneurones in the present study on neonatal kittens was -53mV (-58mV for those tested with hypoxia), lower than the average resting membrane potential recorded (-62mV) in adult cats *in vivo* (Mifflin 1990). The resting membrane potentials of the hypoglossal motoneurones ranged from between -36 to -78mV (Fig 27). This was no different to the range of membrane potentials recorded in *in vivo* studies on adult cats (Green & Negishi, 1963, Sumi, 1969, Withington-Wray et al., 1988).

4.7 Patterns of discharge and rhythmic excitatory postsynaptic (EPSP) activity

In the present study on neonatal kittens just over a fifth of the hypoglossal motoneurones recorded with intracellular microelectrodes had rhythmic EPSPs during normoxia. However, none of these reached action potential threshold and this may explain the lack of phasic discharge recorded in the extracellular studies.

4.7.1 Effect of hypoxaemia on rhythmic EPSPs

The results of this thesis show that there is a recruitment of rhythmic EPSP activity and/or an increase in amplitude and duration of these EPSPs in some hypoglossal motoneurones during mild levels of hypoxaemia. However, there were also a few motoneurones which exhibited a decrease in amplitude and/or duration of the rhythmic EPSP activity and, in one motoneurone, although rhythmic EPSP activity appeared

during hypoxaemia, this activity subsequently disappeared even though hypoxia was continued.

One of the most interesting results of this study was the dissociation between the effect on EPSP activity and the resting membrane potential. This will be discussed in more detail in section 4.8 below.

4.7.2 Respiratory-related activity

The most likely interpretation of the rhythmic EPSPs recorded in the present study is that they were respiratory-related. Recent intracellular studies have shown that in adult cats the phasic discharge of action potentials and/or rhythmic depolarization of the membrane potential (EPSPs) in the hypoglossal motoneurones coincides with central respiratory rhythm (Withington-Wray et al., 1988, Mifflin, 1990). These motoneurones have been categorized into at least three groups according to the phase of respiration in which this activity occurs. Indeed, in these studies the rhythmic EPSPs were always associated with respiration.

It is unlikely that, in the present study, the rhythmic EPSPs were movement artifact; they did not always follow the ventilator and changed during hypoxia. In addition, the input resistance associated with the rhythmic EPSPs increased during hypoxia. So although in the present study the phrenic nerve was not recorded, it seems most likely that the rhythmic EPSPs are respiratory-related.

Further studies are required to confirm both that the rhythmic EPSPs are respiratory-related and also to establish in which phase(s) of the respiratory cycle they occur. The latter may be important when considering the changes in EPSP activity during hypoxaemia .

If the rhythmic EPSP activity is respiratory-related, the absence of this activity in the majority of motoneurones recorded in the present study supports the idea suggested by Sica and colleagues (1988) that hypoglossal motoneurones in neonates are poorly modulated by respiratory drive.

4.8 Changes in rhythmic EPSP activity during hypoxia independent of changes in resting membrane potential

One of the most interesting results in this study was that, although mild hypoxaemia clearly affected both the resting membrane potential and rhythmic EPSP activity, the changes in membrane potential were not always associated with the changes in the rhythmic EPSPs. For example, the membrane of the three motoneurones shown in figures 31a, 32 and 33 were, respectively, hyperpolarized, depolarized and unaltered during hypoxaemia, whereas the amplitude of the rhythmic EPSP activity increased in all three. For those motoneurones which were hyperpolarized during hypoxia the membrane impedance increased (Fig. 44). It would therefore be expected that for any given synaptic current there would be an increase in the voltage drop across the membrane. Thus the increase in the amplitude of the rhythmic EPSPs in the motoneurones which were hyperpolarized, may be explained by the increase in membrane impedance. However this would not explain why in one motoneurone, in which the response to hypoxia was tested twice, the membrane hyperpolarized on both occasions but the rhythmic EPSPs appeared during the first test, remained until the second test, but then, in the second test, the EPSPs declined.

If the rhythmic EPSPs are linked to respiration the results of this study suggest that hypoxia has an effect on hypoglossal output independently of, or in addition to, its effect on respiratory rhythm. The diagram in figure 58 outlines this hypothesis more clearly.

Although it is not possible to determine whether the tonic activity is mediated by respiratory drive, the results of the present study suggest that the changes in tonic activity during mild levels of hypoxaemia are independent of the mechanism generating respiratory rhythm. It would be interesting to see whether these two components could be separated; for example, does specific stimulation of the chemoreceptors affect the response of the membrane potential to mild levels of hypoxaemia independently of any effect on rhythmic EPSP activity?

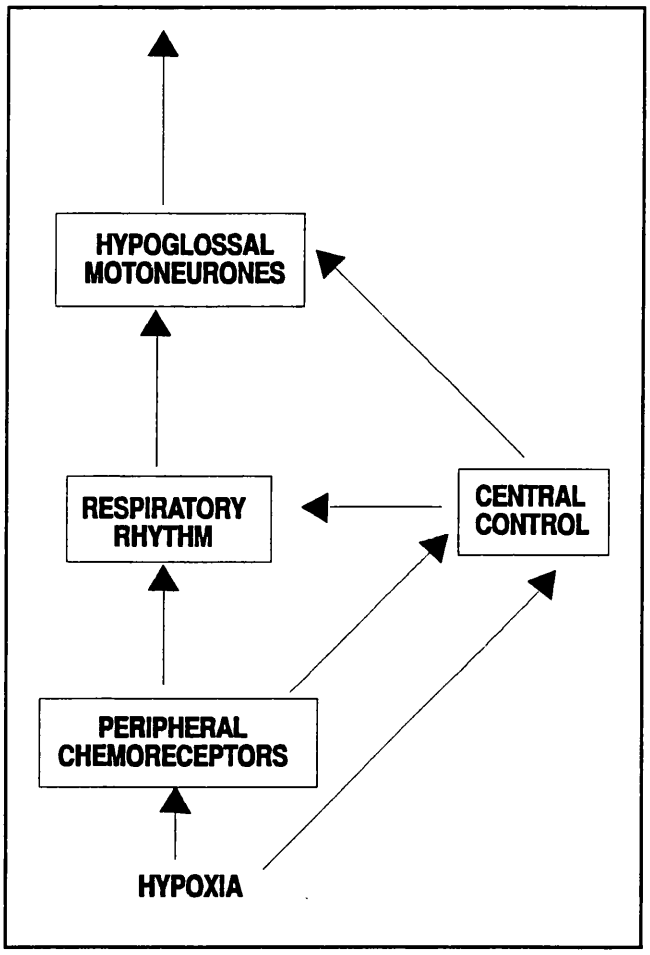


Figure 58. Possible pathways by which mild levels of hypoxaemia may influence hypoglossal motoneurone output. It is proposed that hypoxia has an effect on hypoglossal output by a mechanism which is independent of, or in addition to, its effects exerted

through respiratory rhythm. Hypoxia may act directly or through the peripheral chemoreceptors.

4.9 Possible reasons for lack of rhythmic EPSP activity in the recordings of the hypoglossal motoneurones

Studies in neonates have demonstrated that the rhythmic activity of the genioglossus muscle or hypoglossal nerve is often absent during normal tidal breathing but typically appears during nasal occlusion, hypercapnia or hypoxia (Roberts et al., 1986, Gauda et al., 1987, Carlo et al., 1988 & 1990, Martin et al., 1990). It is therefore perhaps not surprising that in the present study rhythmic EPSP activity occurred in only a few motoneurones during normoxia. However, even during hypoxia no rhythmic EPSPs were recorded in the majority of hypoglossal motoneurones studied.

In an earlier study on adult cats very few of the hypoglossal motoneurones recorded had respiratory-related activity (Sumi, 1969). In contrast, recent intracellular studies on adult cats have shown no difficulty in recording respiratory-modulated hypoglossal motoneurones (Withington-Wray et al., 1988, Mifflin, 1990). However, even in the study by Withington-Wray and colleagues (1988) approximately half of the motoneurones had no obvious phasic activity.

Although it is possible that the sample of hypoglossal motoneurones in the present study were in some way biased towards motoneurones with no obvious rhythmic activity, perhaps due to their size or position, the absence of rhythmic activity in the majority of hypoglossal motoneurones may be due to a number of other factors which need to be considered:

1) The absence of rhythmic activity in the sample of hypoglossal motoneurones may have been due to the mild levels of hypoxia used. In a study on adult goats no respiratory-related genioglossus activity was recorded until oxygen saturation had declined to approximately 69% (Parisi et al., 1988). In the present study, the levels of hypoxia induced only mild levels of hypoxaemia; in the majority of cases the PaO_2 was greater than 43mmHg and SaO_2 decreased by no more than 15% (Fig 8).

2) The levels of CO_2 in the present study may not have been high enough to have reached the threshold level for the rhythmic activity in the majority of the hypoglossal motoneurones recorded. Previous studies have indicated that the CO_2 threshold for activation of the genioglossus muscle is around 47mmHg ($P_{ET}CO_2$) in human infants and 49mmHg ($PaCO_2$) in adult awake goats (Carlo et al., 1988 & 1990, Parisi et al., 1987). In the present study, whilst for each test the $P_{ET}CO_2$ level was constant, between preparations the $P_{ET}CO_2$ levels ranged widely from 31 to 56mmHg and blood samples showed that the $PaCO_2$ ranged from 29 to 46mmHg. Single hypoglossal nerve fibres differ from each other in their sensitivity to CO_2 and the threshold level required to induce respiratory-related activity (Hwang et al., 1983a, Mitra & Cherniack, 1983). Thus, because the majority of motoneurones did not show rhythmic activity, it is possible that higher levels of arterial CO_2 are required to initiate this type of activity in these motoneurones.

The $PaCO_2$ level at which phrenic nerve activity begins, after hyperventilation, increases as the level of halothane anaesthesia is increased (Kurth, Hutchison, Caton & Davenport, 1989). Thus in the present study anaesthesia may have had a similar effect on the CO_2 threshold for activation of the hypoglossal motoneurones and this may explain the absence of rhythmic activity in the sample of motoneurones recorded.

3) Anaesthesia depresses the respiratory-related activity of the hypoglossal nerve in

adult cats even at sub-anaesthetic levels (Hwang et al., 1983b, Nishino et al., 1984 & 1985, Bonora et al., 1985b & Masuda et al., 1989). The absence of rhythmic bursts of activity in the hypoglossal motoneurones recorded in the present study may be therefore be due to the anaesthesia. Nevertheless, in anaesthetized adult cats, some recent studies have recorded hypoglossal motoneurones with respiratory-related activity (Withington-Wray et al., 1988, Mifflin, 1990). It is therefore possible that anaesthesia has a greater inhibitory effect on phasic hypoglossal activity in neonates than it does on adults.

Different types of anaesthetic produce different degrees of depression of the phasic activity of hypoglossal motoneurones (Hwang et al., 1983b, Nishino et al., 1984). The cats in the adult studies were anaesthetized with sodium pentobarbitone whereas in the present study the kittens were anasethetised with α-chloralose. Chloralose strongly depresses ventilation in kittens below 3 weeks of age (Schwieler, 1968) and its use in the present study may explain the absence of rhythmic activity recorded in the majority of hypoglossal motoneurones. In a few kittens sodium pentobarbitone was used as the anaesthetic but it had such a profound depressant effect on the blood pressure that in these kittens no results could be obtained and this anaesthetic was not used in further studies.

4) The lack of rhythmic activity in the majority of the hypoglossal motoneurones recorded in the present study may be explained by the fact that the kittens were not vagotomized. As described in the introduction, vagal afferent input has a profound depressant influence on the phasic discharge of the hypoglossal fibres (section 1.2.4). In kittens (6 to 70 days old) respiratory-related hypoglossal nerve activity was only recorded when the animals had been vagotomized (Bruce, 1986). In addition, Sica et al. (1988) demonstrated that, unless a bilateral vagotomy has been performed, hypoxia (FiO_2 0.15 for 6-8 minutes) does not elicit inspiratory-related discharges in the

hypoglossal nerve in neonatal piglets. The influence of vagal afferent input is thought to be stronger in neonates than in adults and this may explain why respiratory related activity was recorded in intact adult cats (Sica et al., 1984) but only after vagotomy in kittens (Bruce, 1986).

5) The kittens in the present study were tracheotomized; a procedure which has been shown to reduce respiratory-related genioglossus EMG activity in adult rabbits (Mathew et al., 1982a). Bypassing reflexes stimulated by upper airway pressure and flow may therefore have influenced the degree of rhythmic activity expressed by the hypoglossal motoneurones recorded in the present study.

6) Posture and head position are others factors which have been shown to influence the level of phasic activity recorded in the genioglossus muscle and hypoglossal nerve (Bonora et al., 1985a, Roberts et al., 1986). The kittens in the present study had their heads placed firmly into a stereotaxic head holder at a fixed angle and it is possible that this chosen angle may have influenced the degree of rhythmic activity recorded in the hypoglossal motoneurones.

When all of the above factors are taken into consideration it is not surprising that relatively few of the hypoglossal motoneurones recorded in the neonatal kittens in the present study showed rhythmic bursts of discharge or EPSP activity.

4.10 Possible mechanisms involved in the changes of the rhythmic EPSP activity during hypoxaemia

The changes in the rhythmic EPSP activity during hypoxaemia may be due to a number of mechanisms. Similar changes in respiratory-related rhythmic hypoglossal

activity under certain experimental conditions have been recorded in adult cats and these mechanisms may be involved in the changes recorded in the present study. In this section these mechanisms have each been considered.

4.10.1 Stimulation of carotid chemoreceptors

The most likely explanation for the appearance or increase in amplitude and/or duration of the rhythmic EPSP activity during hypoxia in the present study is an increase in chemoreceptor discharge. In adult cats studied *in vivo*, Mifflin (1990) demonstrated that selective stimulation of the carotid body chemoreceptors with CO_2-saline or doxapram increases the amplitude and, occasionally, the duration of the respiratory-related depolarizations.

If the increase in the amplitude of the rhythmic EPSP activity is due to an increase in peripheral chemoreceptor discharge, then it is also possible that the decrease in the rhythmic EPSPs, recorded in a few motoneurones, is due to a corresponding decrease in peripheral chemoreceptor activity. However, as described in section 4.6.2 there is some controversy in the literature as to the response of the peripheral chemoreceptors to hypoxia in neonates (Schwieler, 1968, Blanco et al., 1984, Carroll & Bureau, 1987, Mulligan, 1991, Marchal et al., 1992). If the peripheral chemoreceptor discharge was not maintained in the kittens in the present study during the mild levels of hypoxaemia, it may explain the decrease in rhythmic EPSP activity recorded in some motoneurones.

Further studies are required to investigate the effect of specific chemoreceptor stimulation on the rhythmic EPSP activity recorded in hypoglossal motoneurones in neonates (see section 4.15).

4.10.2 Central depression

Mifflin suggested that the decrease in respiratory-related hypoglossal motoneurone activity may be due to a direct depressant effect of hypoxia. When adult cats were challenged with asphyxia (by turning the ventilator off) the inspiratory-related depolarizations were initially increased (6-10 secs) but within 18 to 24 seconds, as the resting membrane potential depolarized, the respiratory-related activity decreased and eventually disappeared (Mifflin, 1990). However, the levels of hypoxia which would be achieved by switching off the ventilator are obviously extreme and it seems unlikely that similar levels of hypoxia were achieved in the present study where relatively mild levels of hypoxaemia were induced (section 4.3).

4.10.3 Effect of suprapontine structures on the rhythmic activity of the hypoglossal motoneurones

Studies investigating the effect of suprapontine structures on the phasic activity of the hypoglossal nerve have suggested that the cortex may have an inhibitory effect on hypoglossal output, whereas the rostral part of the pons and midbrain structures may have an excitatory influence (Mitra et al., 1986). Following midcollicular decerebration the activity of the hypoglossal nerve is reduced whereas decerebration at a more rostral level or decortication results in an increase in the phasic hypoglossal activity at higher levels of CO_2 (Mitra et al., 1986). Hutt and colleagues (Hutt, Parisi, Santiago & Edelman, 1989) suggested that brain hypoxia (50% carboxyhaemoglobin) increases genioglossus activity in the adult goat by depressing cortical activity and consequently withdrawing tonic suprapontine inhibition of hypoglossal inspiratory activity. This could be due to the removal of a direct inhibitory action of the higher centres on respiratory drive or due to the withdrawal of tonic cortical inhibition of the reticular system. Stimulation of the reticular formation by electrical stimulation, glutamate

injection or sciatic nerve stimulation increases the phasic activity and sometimes the tonic discharge of the hypoglossal nerve (St. John, 1986). Regardless of the mechanism involved, hypoxic cortical depression may be the mechanism underlying the increase in the rhythmic EPSP activity recorded in the hypoglossal motoneurones in the present study. It is also possible that this is the mechanism by which the resting membrane is depolarized during hypoxaemia .

4.11 Effect of hypoxaemia on the action potential profile

In the present study, the mean action potential profile for the majority of hypoglossal motoneurones was unaltered during hypoxia. However, in a few motoneurones there were changes in the time course of the after-hyperpolarization.

The after-hyperpolarization is an important factor determining the maximum firing frequency of the motoneurone for a given input current (Kernell,1965). In this regard, the increased duration of the after-hyperpolarization recorded in hypoxia during both the repolarizing phase of the transient response (Fig. 55b) and during hyperpolarization (Fig. 56), would be expected to reduce the maximum discharge frequency and therefore restrict the excitability of these motoneurones.

In contrast, two motoneurones which were depolarized showed a faster time to peak and a faster decay of the after-hyperpolarization during the arterial hypoxia (Fig. 54).

Thus in the first case not only has the membrane been hyperpolarized during hypoxia, and is therefore further away from threshold, but once the threshold has been reached for a same given input the discharge frequency would be conceivably lower. In the second case, however, not only is the membrane brought closer to threshold, but for the same given input the discharge frequency is potentially greater. It would be

interesting to see whether these changes in after-hyperpolarization are secondary to the changes in resting membrane potential by setting the membrane at different potentials. However, in the majority of motoneurones in the present study there were no changes in the after-hyperpolarization despite a depolarization or hyperpolarization of the membrane.

4.12 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 hypoglossal motoneurones (see section 1.1.8). 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. This is the first study to my knowledge that has recorded these motoneurones in neonates in an *in vivo* preparation. The first aim of this thesis was therefore to establish a physiologically stable preparation which allowed these motoneurones to be recorded. Although this has been described in chapter 2, there are a few factors which I think made this a relatively stable preparation and I have therefore discussed these further.

The kittens had noticeably less chance of survival the longer they were maintained on the halothane anaesthetic and it was crucial that the 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.

Blood gas analysis was extremely important. By assessing the gas tensions, metabolic and respiratory acidosis could be detected and either the ventilator adjusted or sodium bicarbonate administered. However, because of the small blood volume and to avoid

anaemia, the number of blood samples taken was strictly limited. This meant that, realistically, blood samples could not be taken after every recording. Thus in some cases the blood samples were taken approximately every 90 minutes or after 2 recordings. The respiratory gas monitor (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 the arterial blood gas analysis and therefore provided a good indication of gaseous exchange.

All of the kittens were susceptible to non-respiratory acidosis and, although sodium bicarbonate was given, in some cases this could not be corrected fully. A previous study also reported that kittens of less than 3 weeks old were more susceptible to acidosis than older kittens (> 4 weeks old) (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 addition, in adult cats the level of decerebration is known to influence the response of the hypoglossal nerve to chemical stimulation (Mitra et al., 1986) and it would therefore have complicated any results obtained.

Glucose was constantly infused at a rate of 0.7ml per hour and without this the physiological state of the kittens quickly deteriorated. Higher rates of infusion were found to make the animals anaemic.

The pulsation of the cerebellum and brainstem was the biggest problem encountered in these *in vivo* preparations. As described in the method section three procedures were employed to overcome this. These were a) the use of a pressure foot, b) paralysing the animal and c) performing a bilateral thoracotomy. Although other procedures were

tried, these three had the most influence on the stability of the brainstem. However, these procedures had other inherent difficulties which had to be solved. For example, to prevent the lungs collapsing after the bilateral thoracotomy, a positive end-expiratory pressure had to be 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 i.e a peak inspiratory pressure of approximately 13 mbar and a positive end-expiratory pressure of 1-2 mbar. The possible effect of changes in lung compliance during hypoxia have already been discussed in section 4.6.3.

Without a physiologically stable preparation and a relatively stable brainstem, recording was pointless and the experiment terminated.

4.13 Factors which may have influenced the activity of the hypoglossal motoneurones and their response to hypoxia

It is impossible in *in vivo* preparations to maintain the conditions constant between animals. Furthermore, the condition in the same preparation may alter over time. In this section I have considered several factors which may have altered the activity of the hypoglossal motoneurones during normoxia and, in each case, I have considered the influence this may have had on the response of these motoneurones to hypoxaemia.

4.13.1 Anaesthesia

A number of studies have shown that anaesthesia depresses the activity of the hypoglossal nerve (section 1.2.5) and this may explain the low levels of discharge

which were recorded from these motoneurones during normoxia (Fig 13).

4.13.1.i Does anaesthesia influence the response of the hypoglossal motoneurones to hypoxia?

As anaesthesia is known to depress the activity of the genioglossus muscle and hypoglossal nerve, the obvious question is: does it alter the response of the hypoglossal motoneurones to hypoxaemia? Hwang and colleagues (1983b) have shown that 0.5% halothane in decerebrated adult cats decreases the response of the hypoglossal nerve to hypoxia. It is therefore likely that the increase in discharge frequency recorded in some of the hypoglossal motoneurones in the present study, would have been greater if the kittens had not been anaesthetized. However, in contrast to the study of Hwang et al. (1983b), the halothane was switched off at least 2 hours before recording. Nevertheless, the long term effect of halothane on these motoneurones is unknown. Furthermore, the influence of α-chloralose and the paralysing agent, gallamine triethiodide, on the response of the individual hypoglossal motoneurones to hypoxia is uncertain, and studies are required therefore to investigate this further.

Recent studies by my colleagues have shown that the increase in ventilation and phrenic nerve discharge during hypoxia (PaO_2 35 - 45 mmHg) were still 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. 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).

Returning to the hypoglossal motoneurones, although the ventilatory response was not altered in the kittens in the study of Morray et al. (1991) using the same anaesthetic procedure as the one used in the present study, it is possible that the anaesthesia will have altered the response of the hypoglossal motoneurones to hypoxaemia and the inhibition of some of these motoneurones was a result of anaesthesia.

In addition to these considerations, the levels of anaesthesia will alter during the course of an experiment due to its continual metabolic breakdown. However, it is unlikely that the different response characteristics to hypoxia are due to alterations in the level of anaesthesia. The sequence in which the different responses were recorded altered from preparation to preparation. As described earlier (section 3.1.4.i), in one preparation the first motoneurone recorded may have responded to the hypoxaemia with a transient excitation and the second motoneurone with a sustained excitation, in another experiment it may have been the other way around.

4.13.2 Influence of the end-tidal CO_2 on the discharge frequency of the hypoglossal motoneurones during normoxia

Although during each test the $P_{ET}CO_2$ was constant, it did vary between tests. When the discharge frequency, taken as a group, during normoxia, was considered as a function of their respective $P_{ET}CO_2$ levels, there was no correlation between the two factors (Fig. 24). However, 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 (see 1.2.2). 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).

It is therefore possible that the individual hypoglossal motoneurones recorded in the present study had different thresholds for activation. This may explain the graph shown in figure 24. The $P_{ET}CO_2$ may not have been high enough to have reached the threshold for the activation of the majority of hypoglossal motoneurones and therefore the level of discharge frequency of these motoneurones during normoxia was relatively low (<2 impulses per second). When the $P_{ET}CO_2$ was above 39mmHg there were more motoneurones with higher levels of discharge frequency and hence, in comparison to the other motoneurones, these motoneurones may have had relatively low CO_2 thresholds for activation.

4.13.2.i Influence of the end-tidal CO_2 on the response and discharge frequency of the hypoglossal motoneurones during hypoxia

Hypoglossal motoneurones with different response characteristics to hypoxia could be recorded over a wide range of $P_{ET}CO_2$ (Fig 26). Although it is possible that the response of the individual motoneurones to hypoxia may alter as 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 a set level of hypoxia.

Further studies are required to investigate the effect of altering the PCO_2 level on the discharge frequency and the membrane potential of the individual hypoglossal motoneurones, and to determine the effect that this has on their response to hypoxia.

4.13.3 Category of motoneurone recorded

The hypoglossal nerve innervates a number of upper airway muscles, including the hyoglossus and styloglossus (see section 1.1.5) 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 1.4mm from the surface of the medulla, 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 genioglossus muscle, it would be preferable to antidromically stimulate this branch of the nerve.

4.13.4 Blood pressure recordings

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). Morray and colleagues (1992) have reported that in 4 week old kittens the mean arterial blood pressure increased from 59±7 to 82±12mmHg during hypoxaemia (PaO_2 40-50mmHg). As described earlier, although the kittens in this study were older than those used in the present study, they were also anaesthetized with α-chloralose after initial induction with halothane. The levels of hypoxaemia were also similar to those used in the present study.

Although not all of the changes in membrane potential were associated with changes in blood pressure it is possible that in some cases the effect of mild hypoxaemia on the hypoglossal motoneurones may have been exerted through its effect on baroreceptor reflexes.

Elevations in arterial blood pressure reduce the respiratory related phasic discharge of the hypoglossal nerve whereas decreasing arterial blood pressure significantly increases this respiratory-related activity (Salamone et al., 1983). In agreement, hypoglossal motoneurones in adult cats are hyperpolarized and discharge decreased when the arterial blood pressure increases (Mifflin, 1990). In the present study the changes in blood pressure were inconsistent, sometimes increasing and sometimes decreasing during the hypoxic challenge (section 3.2.6), and were not always associated with changes in membrane potential. In the example given in figure 29 on page 154 the membrane potential depolarized gradually with the onset of hypoxia despite the fact that the mean arterial pressure remained unchanged at 70 mmHg. After approximately 60 seconds the mean arterial blood pressure increased gradually to 77 mmHg and during this period the level of depolarization was reduced. Thus, in this case, although there was no change in blood pressure with the depolarization, there was an increase in blood pressure which was associated with the repolarization.

Further studies are required to investigate more systematically the effect of the baroreceptor reflex on the activity of the hypoglossal motoneurones in neonates. It is possible to change the arterial blood pressure by inflating a balloon in the descending aorta (increasing arterial blood pressure) or in the inferior vena cava (decreasing arterial blood pressure) (Salamone et al., 1983), but this may be difficult to do while maintaining a stable intracellular recording of a hypoglossal motoneurone.

The effect that each of the above factors has on the response of the hypoglossal motoneurones to mild hypoxaemia needs to be investigated further. In this thesis, although each of these points has been taken into consideration, no time was available to study these in more detail.

4.14 The clinical relevance and implications of the results of this thesis

As described in the introduction, the genioglossus muscle, innervated by the hypoglossal nerve, plays an important role in maintaining upper airway patency. The results of this study show that inhibitory mechanisms are activated during mild levels of hypoxaemia. If similar inhibition of hypoglossal motoneurones occurs in human babies during apnoeic episodes, the patency of the airway may be compromised and the survival of the infant threatened.

It is common for newborn babies, particularly premature babies, to stop breathing for short periods of time (cessation of airflow for $\geq$ 20 seconds) (Henderson-Smart, 1981). This is known as apnoea (see section 1.1.1). During these apnoeic episodes hypoxia and hypercapnia develop (Mathew et al., 1982c, Poets et al., 1991). The fact that over 50% of the apnoeic episodes in human neonates are mixed, i.e central apnoea followed by obstructive apnoea (Milner & Greenough, 1988, Peliowski & Finer, 1990), may reflect the response of the hypoglossal motoneurones to the hypoxia which develops during central apnoea. This study has shown that even mild levels of hypoxaemia inhibit some hypoglossal motoneurones and as a result would reduce the activity of the genioglossus muscle. As a consequence the airway will become obstructed and the apnoea prolonged.

4.15 Proposals for future work

Now that it is clear that hypoglossal motoneurones in neonates can be both inhibited and excited during even mild levels of hypoxaemia, it is important to establish what mechanisms are involved. Future studies should be directed towards this goal.

Determining the effect of the peripheral chemoreceptors on the activity of the hypoglossal motoneurones in neonates may be the first step.

If the inhibition of the hypoglossal motoneurones is mediated by the peripheral chemoreceptors then once the kittens have been chemodenervated, these motoneurones should not be inhibited during hypoxaemia. The first step in determining the role of the peripheral chemoreceptors in the inhibition of the hypoglossal motoneurones is therefore to compare the response of the motoneurones to hypoxaemia in intact and chemodenervated animals.

On the other hand, if the peripheral chemoreceptors are involved in the inhibition of the hypoglossal motoneurones during hypoxaemia, the inhibition should be augmented when the peripheral chemoreceptors are stimulated specifically. The arterial chemoreceptors can be specifically stimulated by injecting a bolus of CO_2-saline solution into the lingual artery directed at the carotid sinus. In addition, if the peripheral chemoreceptors are inhibited by a bolus injection of saline saturated with 100% O_2, the inhibition produced by the hypoxaemia should be augmented .

Similarly, if the excitation is mediated by the peripheral chemoreceptors, the motoneurones should not be depolarized in the chemodenervated kittens. In addition transient peripheral chemoreceptor stimulation will augment the depolarization and transient inhibition should reduce it.

The same techniques can also be used to establish whether the peripheral chemoreceptors are involved in the response of the rhythmic EPSP activity to hypoxaemia. If the increase in rhythmic EPSPs during hypoxaemia is through stimulation of the peripheral chemoreceptors, infusion of CO_2 into the lingual artery will increase this activity. The interesting question is; can the changes in rhythmic EPSP activity and resting membrane potential which occur during hypoxaemia be separated? For example does stimulation of the peripheral chemoreceptors alter rhythmic EPSPs independently of any effect on the resting membrane potential? Or *vice versa*?

Moving on from this, it is important to establish whether there are inhibitory projections from the rostral pons which influence the activity of the hypoglossal motoneurones. Does stimulation of the area in the rostral pons, previously implicated in the biphasic ventilatory response, inhibit hypoglossal output?

4.16 CONCLUSION

In this study I have investigated the effect of mild levels of hypoxaemia on the activity of hypoglossal motoneurones in an *in vivo* preparation. In order to do this a viable neonatal *in vivo* preparation, which could be maintained for the duration of the experiment, was first established. The effect of mild levels of hypoxaemia were then recorded.

1) This thesis has clearly shown that the effects of mild levels of hypoxaemia on hypoglossal motoneurones in neonatal kittens are mediated by both inhibitory and excitatory mechanisms.

2) The results also suggest that hypoxaemia has an effect on rhythmic EPSPs independently of, or in addition to, its effect on the resting membrane potential.

REFERENCES

Agostoni, E., Cavagna, M. and Citterio, G. Effects of stretch receptors of bronchi or trachea on genioglossus muscle activity. *Respiration Physiology* 67:335-345, 1987.

Andersen, P. Brain slices- A neurobiological tool of increasing usefulness. *Trends in Neuroscience* 3:53-56, 1981.

Bartlett, D.Jr. and St.John, W.M. Influence of lung volume on phrenic, hypoglossal and mylohyoid nerve activities. *Respiration Physiology* 73:97-110, 1988.

Bartlett, D.Jr., Knuth, S.L., Leiter, J.C. Alteration of ventilatory activity by intralaryngeal CO_2 in the cat. *Journal of Physiology* 457:177-185,1992

Basner, R.C., Ringler, J., Berkowitz, S., Schwartzstein, R.M., Weinberger, S.E., Sparrow, D. and Weiss, J.W. Effect of inspired air temperature on genioglossus activity during nose breathing in awake humans. *Journal of Applied Physiology* 69(3):1098-1103, 1990.

Basner, R.C., Simon, P.M., Schwartzstein, R.M., Weinberger, S.E. and Woodrow Weiss, J. Breathing route influences upper-airway muscle activity in awake normal adults. *Journal of Applied Physiology* 66(4):1766-1771, 1989.

Ben-Ari, Y. Effects of anoxia and aglycemia on the adult and immature hippocampus. *Biol. Neonate* 62:225-230, 1992.

Bennett, F.M. and St.John, W.M. Anesthesia selectively reduces hypoglossal nerve activity by actions upon the brain stem. *Pflugers Archives* 401:421-423, 1984.

Biscoe, T.J., Duggan, A.W. and Lodge, D. The inhibition of hypoglossal motoneurones by glossopharyngeal nerve stimulation in the rat. *Journal of Physiology* 226:71P, 1972.

Black, A.M.S., McCloskey, D.I. and Torrance, R.W. The responses of peripheral chemorceptors to sudden changes of hypercapnic and hypoxic stimuli. *Journal of Physiology* 185: 67-68P, 1966.

Blanco, C.E., Hanson, M.A., Johnson, P. and Rigatto, H. Breathing pattern of kittens during hypoxia. *Journal of Applied Physiology* 56(1):12-17, 1984.

Bonora, M., Bartlett, D.Jr. and Knuth, S.L. Changes in upper airway muscle activity related to head position in awake cats. *Respiration Physiology* 60:181-192, 1985a.

Bonora, M., Shields, G.I., Knuth, S.L., Bartlett, D.Jr. and St.John, W.M. Selective depression by ethanol of upper airway respiratory motor activity in cats. *American Review of Respiratory Disease* 130:156-161, 1984.

Bonora, M., St.John, W.M. and Bledsoe, T.A. Differential elevation by protriptyline and depression by diazepam of upper airway respiratory motor activity. *American Review of Respiratory Disease* 131:41-45, 1985b.

Bradford, A., Nolan, P., McKeogh, D., Bannon, C., O'Regan, R.G. The responses of superior laryngeal nerve afferent fibres to laryngeal airway CO_2 concentration in the anaesthetized cat. *Experimental Physiology* 75:267-270, 1990.

Breuer, J. and Hering, E. Self-steering of respiration through the nervus vagus. In: *Ciba Foundation Symposium on Breathing: Hering-Breuer Centenary Symposium*, edited by Porter, R. London: Churchill, 1970, p. 359-364.

Brodal, A. The Cranial Nerves. In: *Neurological Anatomy in Relation to Clinical Medicine*, New York: Oxford University Press, 1981, p. 448-577.

Brouillette, R.T. and Thach, B.T. A neuromuscular mechanism maintaining extrathoracic airway patency. *Journal of Applied Physiology* 46(4):772-779, 1979.

Brouillette, R.T. and Thach, B.T. Control of genioglossus muscle inspiratory activity. *Journal of Applied Physiology* 49(5):801-808, 1980.

Brown, D.L. and Lawson, E.E. Brain stem extracellular fluid pH and respiratory drive during hypoxia in newborn pigs. *Journal of Applied Physiology* 64:1055-1059, 1988.

Bruce, E.N. Hypoglossal and phrenic nerve responses to chemical stimuli in kittens. In: *Neurobiology of the Control of Breathing*, edited by von Euler, C. and Lagercrantz, H. New York: Raven, 1986, p. 75-80.

Bruce, E.N., Mitra, J. and Cherniack, N.S. Central and peripheral chemoreceptor inputs to phrenic and hypoglossal motoneurons. *Journal of Applied Physiology* 53(6):1504-1511, 1982.

Bureau, M.A., Carroll, J.L. and Canet, E. Response of newborn lambs to CO-induced hypoxia. *Journal of Applied Physiology* 64(5):1870-1877, 1988.

Carlo, W.A. and DiFiore, J.M. Respiratory muscle responses to changes in chemoreceptor drive in infants. *Journal of Applied Physiology* 68(3):1041-1047, 1990.

Carlo, W.A., Martin, R.J. and DiFiore, J.M. Differences in CO_2 threshold of respiratory muscles in preterm infants. *Journal of Applied Physiology* 65(6):2434-2439, 1988.

Carlo, W.A., Miller, M.J. and Martin, R.J. Differential response of respiratory muscles to airway occlusion in infants. *Journal of Applied Physiology* 59(3):847-852, 1985.

Carroll, J.L. and Bureau, M.A. Decline in peripheral chemoreceptor excitatory stimulation during acute hypoxia in the lamb. *Journal of Applied Physiology* 63(2):795-802, 1987.

Clark, F.J and Von Euler, C. On the regulation of depth and rate of breathing. *Journal of Physiology* 222:267-295, 1972.

Cohen, G. and Henderson-Smart, D.J. Upper airway stability and apnea during nasal occlusion in newborn infants. *Journal of Applied Physiology* 60(5):1511-1517, 1986.

Cohen, G. and Henderson-Smart, D.J. Upper airway muscle activity during nasal occlusion in newborn babies. *Journal of Applied Physiology* 66(3):1328-1335, 1989.

Cohen, M.I. Phrenic and recurrent laryngeal discharge patterns and the Hering-Breuer reflex. *Am.Journal of Physiology* 228(5):1489-1496, 1975.

Cohen, P.J., Alexander, S.C., Smith, T.C., Reivich, M. and Wollman, H. Effects of hypoxia and normocarbia on cerebral blood flow and metabolism in conscious man. *Journal of Applied Physiology* 23(2):183-189, 1967.

Coles, S.K., Kumar, P. and Noble, R. Pontine sites inhibiting breathing in anaesthetized neonatal lambs. *Journal of Physiology* 409:66, 1989.

Cooke, R.W.I. *A Guide to Ventilator Care of Infants*, England:Vickers Medical, 1979.

Cooper, M.H. The hypoglossal nucleus of the primate: A golgi study. *Neuroscience letters* 21:249-254, 1981.

Côté, A., Yunis, K., Blanchard, P.W., Mortola, J.P. and Bureau, M.A. Dynamics of breathing in the hypoxic awake lamb. *Journal of Applied Physiology* 64(1):354-359,

1988.

Davies, A.M., Koenig, J.S. and Thach, B.T. Upper airway chemoreflex responses to saline and water in preterm infants. *Journal of Applied Physiology* 64(4):1412-1420, 1988.

Davies, A.M., Koenig, J.S. and Thach, B.T. Characteristics of Upper Airway Chemoreflex Prolonged Apnea in Human Infants. *American Review of Respiratory Disease* 139:668-673, 1989.

Davis, G.M.,, Bureau, M.A and Gaultier, CL The sustained ventilatory response to hypoxic challenge in the awake newborn piglet with an intact upper airway. *Respiration Physiology* 71:307-314, 1988.

Donnelly, D.F., Jiang, C. and Haddad, G.G. Comparative responses of brain stem and hippocampal neurones to O_2 deprivation: in vitro intracellular studies. *American Journal of Physiology* 262:L549-L554, 1992.

Espinoza, M.I. and Parer, J.T. Mechanisms of ashyxial brain damage, and possible pharmacologic interventions; in the fetus. *American Journal of Obstetrics and Gynaecology* 164:1582-1291, 1991.

Euler, C.Von. Brainstem mechanisms for generation and control of breathing pattern. In: *Handbook of Physiology. The Respiratory System. Vol.II:Control of Breathing*, edited by Cherniack, N.S. and Widdicombe, J.G. American Physiological Society, 1986, p. 1-68.

Fukuda, Y. and Honda, Y. Differences in respiratory neural activities between vagal (superior laryngeal), hypoglossal, and phrenic nerves in the anesthetized rat. *Japanese Journal of Physiology* 32:387-398, 1982a.

Fukuda, Y. and Honda, Y. Roles of vagal afferents on discharge patterns and CO_2 responsiveness on efferent apnoea larygeal, hypoglossal and phrenic activities in aneasthetized rats. *Japanese Journal of Physiology* 32:689-698, 1982b.

Gardiner, R.M. Cerebral blood flow and oxidative metabolism during hypoxia and asphyxia in the new-born calf and lamb. *Journal of Physiology* 305:357-376, 1980.

Gauda, E.B., Miller, M.J., Carlo, W.A., DiFiore, J.M., Johnsen, D.C. and Martin, R.J. Genioglossus Response to Airway Occlusion in Apneic versus Nonapneic Infants. *Pediatric Research* 22(6):683-687, 1987.

Gauda, E.B., Miller, M.J., Carlo, W.A., DiFiore, J.M. and Martin, R.J. Genioglossus and diaphragm activity during obstructive apnea and airway occlusion in infants. *Pediatric Research* 26(6):583-587, 1989.

Georgopoulos, D., Walker, S. and Anthonisen, N.R. Increased chemoreceptor output and ventilatory response to sustained hypoxia. *Journal of Applied Physiology* 67(3):1157-1163, 1989.

Glantz, S.A. and Slinker, B.K. One-way analysis of variance. In: *Primer of Applied Regression and Analysis of Variance*, New York: McGraw-Hill, Inc., 1990, p. 294-295.

Green, J.D. and Negishi, K. Membrane potentials in hypoglossal motoneurones. *Journal of Neurophysiology* 26:835-856, 1963.

Grunstein, M.M., Hazinski, T.A. and Schleuter, M.A. Respiratory control during hypoxia in newborn rabbits: implied action of endorphins. *Journal of Applied Physiology* 51(1):122-130, 1981.

Haddad, G.G. and Donnelly, D.F. O_2 deprivation induces a major depolarization in brain stem neurons in the adult but not in the neonatal rat. *Journal of Physiology* 429:411-428, 1990.

Haddad, G.G., Donnelly, D.F. and Getting, P.A. Biophysical properties of hypoglossal neurons in vitro: intracellular studies in adult and neonatal rats. *Journal of Applied Physiology* 69(4):1509-1517, 1990.

Haddad, G.G. and Getting, P.A. Repetitive firing properties of neurons in the ventral region of nucleus tractus solitarius. In vitro studies in adult and neonatal rat. *Journal of Neurophysiology* 62(6):1213-1224, 1989.

Hanson, M.A. and Williams, B.A. The effect of decerebration and brain-stem transection on the ventilatory response to acute hypoxia in normoxic and chronically hypoxic newborn rats. *Journal of Physiology* 414:25P, 1989.

Harding, R., Buttress, J.A., Caddy, D.J. and Wood, G.A. Respiratory and upper airway responses to nasal obstruction in awake lambs and ewes. *Respiration Physiology* 68:177-188, 1987.

Haxhiu, M.A., Van Lunteren, E., Mitra, J. and Cherniack, N.S. Responses to chemical stimulation of upper airway muscles and diaphragm in awake cats. *Journal of Applied Physiology* 56(2):397-403, 1984.

Haxhiu, M.A., Van Lunteren, E., Mitra, J. and Cherniack, N.S. Comparison of the response of diaphragm and upper airway dilating muscle activity in sleeping cats. *Respiration Physiology* 70:183-193, 1987.

Henderson-Smart, D.J. The effect of gestational age on the incidence and duration of recurrent apnoea in newborn babies. *Australian Paediatric Journal* 17:273-276, 1981.

Henderson-Smart, D.J. Regulation of breathing rhythm in the newborn. The role of brainstem immaturity and inhibition. *Journal of Developmental Physiology* 6:83-92, 1984.

Hilberman, M., Harihara Subramanian, V., Haselgrove, J., Cone, J.B., Egan, J.W., Gyulai, L. and Chance, B. In vivo time-resolved brain phosphorus nuclear magnetic resonance. *Journal of Cerebral Blood Flow and Metabolism* 4:334-342, 1984.

Hollowell, D.E., Bhandary, P.R., Funsten, A.W. and Suratt, P.M. Respiratory-related recruitment of the masseter: response to hypercapnia and loading. *Journal of Applied Physiology* 70(6):2508-2513, 1991.

Horner, R.L., Innes, J.A., Holden, H.B. and Guz, A. Afferent pathway(s) for pharyngeal dilator reflex to negative pressure in man: a study using upper airway anesthesia. *Journal of Physiology* 436:31-44, 1991.

Horner, R.L., Innes, J.A., Murphy, K. and Guz, A. Evidence for reflex upper airway dilator muscle activation by sudden negative airway pressure in man. *Journal of Physiology* 436:15-29, 1991.

Hutt, D.A., Parisi, R.A., Santiago, T.V. and Edelman, N.H. Brain hypoxia preferentially stimulates genioglossal EMG responses to CO_2. *Journal of Applied Physiology* 66(1):51-56, 1989.

Hwang, J.C., Bartlett, D.Jr. and St.John, W.M. Characterization of respiratory-modulated activities of hypoglossal motoneurons. *Journal of Applied Physiology* 55(3):793-798, 1983a.

Hwang, J.C., St.John, W.M. and Bartlett, D.Jr. Respiratory-related hypoglossal nerve activity: influence of anesthetics. *Journal of Applied Physiology* 55(3):785-792, 1983b.

Hwang, J.C., St.John, W.M. and Bartlett, D.Jr. Receptors responding to changes in upper airway pressure. *Respiration Physiology* 55:355-366, 1984a.

Hwang, J.C., St.John, W.M. and Bartlett, D.Jr. Afferent pathways for hypoglossal and phrenic responses to changes in upper airway pressure. *Respiration Physiology* 55:341-354, 1984b.

Iscoe, S.D. Central Control of the Upper Airway. In: *Respiratory Control and the Upper Airway*, edited by Mathew, O.P. and Sant'Ambrogio, G. New York: Dekker, 1992, p. 125-192.

Issa, F.G. and Sullivan, C.E. Alcohol, snoring and sleep apnea. *Journal of Neurology, Neurosurgery and Psychiatry* 45:353-359, 1982.

Jeffries, B., Brouillette, R.T. and Hunt, C.E. Electromyographic study of some accessory muscles of respiration in children with obstructive sleep apnea. *American Review of Respiratory Disease* 129:696-702, 1984.

Jiang, C., Agulian, S. and Haddad, G.G. Cl^- and Na^+ homeotasis during anoxia in rat hypoglossal neurons: Intracellular and extracellular in vitro studies. *Journal of Physiology* 448:697-708, 1992a.

Jiang, C. and Haddad, G.G. Effect of anoxia on intracellular and extracellular potassium activity in hypoglossal neurones in vitro. *Journal of Neurophysiology* 66(1):103-111, 1991.

Jiang, C., Mitchell, G.S. and Lipski, J. Prolonged augmentation of respiratory discharge in hypoglossal motoneurons following superior laryngeal nerve stimulation. *Brain Research* 538:215-225, 1991.

Jiang, C., Xia, Y. and Haddad, G. Role of ATP-sensitive K^+ channels during anoxia: Major differences between rat (newborn and adult) and turtle neurons. *Journal of Physiology* 448:599-612, 1992b.

Jóhannsson, H. and Siesjö, B.K Cerebral blood flow and oxygen consumption in the rat in hypoxic hypoxia. *Acta Physiologica Scandinavia* 93:269-276, 1975.

Johnston, B.M., Gunn, T.R. and Gluckman, P.D. Genioglossus and alae nasi activity in fetal sheep. *Journal of Developmental Physiology* 8:323-331, 1986.

Kernell, D. The limits of firing frequency in cat lumbosacral motoneurones possessing

different time course of afterhyperpolarization. *Acta Physiologica Scandinavia* 65:87-100, 1965.

Knill, R.L. and Gelb, A.W. Peripheral chemoreceptors during anesthesia: Are the watchdogs sleeping? *The Journal of Anesthesiology* 57(3):151-152, 1982.

Krol, R.C., Knuth, S.L. and Bartlett, D.Jr. Selective reduction of genioglossal muscle activity by alcohol in normal human subjects. *American Review of Respiratory Disease* 129:247-250, 1984.

Kuna, S.A Inhibition of inspiratory upper airway motoneurone activity by phasic volume feedback. *Journal of Applied Physiology* 60(4):1373-1379, 1986.

Kuna, S.T. Interaction of hypercapnia and phasic volume feedback on motor control of the upper airway. *Journal of Applied Physiology* 63(5):1744-1749, 1987.

Kuna, S.T. and Smickley, J. Response of genioglossus muscle activity to nasal airway occlusion in normal sleeping adults. *Journal of Applied Physiology* 64(1):347-353, 1988.

Kurth, C.D., Hutchison, A.A., Caton, D.C. and Davenport, P.W. Maturational and anesthetic effects on apneic thresholds in lambs. *Journal of Applied Physiology* 67(2):643-647, 1989.

La Framboise, W.A., Guthrie, R.D., Standaert, T.A. and Woodrum, D.E. Pulmonary mechanics during the ventilatory response to hypoxemia in the newborn monkey. *Journal of Applied Physiology* 55(3):1008-1014, 1983.

La Framboise, W.A., Standaert, T.A., Woodrum, D.E. and Guthrie, R.D. Occlusion pressures during the ventilatory response to hypoxemia in the newborn monkey. *Journal of Applied Physiology* 51(5):1169-1174, 1981.

La Framboise, W.A. and Woodrum, D.E. Elevated diaphragm electromyogram during neonatal hypoxic ventilatory depression. *Journal of Applied Physiology* 59:1040-1045, 1985.

Laudignon, N., Farri, E., Beharry, K., Rex, J. and Aranda, J.V. Influence of adenosine on cerebral blood flow during hypoxic hypoxia in the newborn piglet. *Journal of Applied Physiology* 68(4):1534-1541, 1990.

Lawson, E.E. Recovery from central apnea: effect of stimulus duration and end-tidal

CO_2 partial pressure. *Journal of Applied Physiology* 53(1):105-109, 1982.

Lawson, E.E., Richter, D.W., Czyzyk-Krzeska, M.F., Bischoff, A. and Rudesill, R.C. Respiratory neuronal activity during apnea and other breathing patterns induced by laryngeal stimulation. *Journal of Applied Physiology* 70(6):2742-2749, 1991.

Leblond, J. and Krnjević, K. Hypoxic changes in hippocampal neurons. *Journal of Neurophysiology* 62(1):1-13, 1989.

Lee, J.C., Stoll, B.J. and Downing, S.E. Properties of the laryngeal chemoreflex in neonatal piglets. *American Journal of Physiology* 233(1):R30-R36, 1977.

Lee, L.Y. and Milhorn Jr, H.T. Central ventilatory responses to O_2 and CO_2 at three levels of carotid chemoreceptor stimulation. *Respiration Physiology* 25:319-333, 1975.

Leiter, J.C. and Daubenspeck, J.A. Selective reflex activation of the genioglossus in humans. *Journal of Applied Physiology* 68(6):2581-2587, 1990.

Lowe, A.A. The neural regulation of tongue movements. *Progessive Neurobiology* 15:295-344, 1981.

Lucier, G.E., Storey, A.T. and Sessle, B.J. Effects of upper respiratory tract stimuli on neonatal respiration: reflex and single neuron analyses in the kitten. *Biology of the Neonate.* 35:82-89, 1979.

Marchal, F., Bairam, A., Haouzi, P., Crance, J.P., Di Guilo, C., Vert, P. and Lahiri, S. Carotid chemoreceptor response to natural stimuli in the newborn kitten. *Respiration Physiology* 87:183-193, 1992.

Marchal, F., Bairam, A. and Vert, P. Neonatal apnoea and apneic syndromes. *Clinical Perinatology* 14(3):509-530, 1987.

Martin, R.J., Bruce, E.N. and Carlo, W.A. Respiratory muscle responses to hypercapnea in preterm infants. In: *Neurobiology of the Control of Breathing*, edited by von Euler, C. and Lagercrantz, H. New York: Raven, 1986,

Martin, R.J., Van Lunteren, E., Haxhiu, M.A. and Carlo, W.A. Upper airway muscle and diaphragm responses to hypoxia in the piglet. *Journal of Applied Physiology* 68(2):672-677, 1990.

Martin-Body, R.L. and Johnston, B.M. Central origin of the hypoxic depression of

breathing in the newborn. *Respiration Physiology* 71:25-32, 1988.

Massik, J., Jones Jr, M.D., Miyabe, M., Tang, Y-L., Hudak, M.L., Koehler, R.C. and Traystman, R.J. Hypercapnia and response of cerebral blood flow to hypoxia in newborn lambs. *Journal of Applied Physiology* 66(3):1065-1070, 1989.

Masuda, A., Ito, Y., Haji, A. and Takeda, R. The influence of halothane and thiopental on respiratory-related nerve activities in decerebrate cats. *Acta Anaesthesiologica Scandinavia* 33:660-665, 1989.

Mathew, O.P and Farber, J.P. Effect of upper airway negative pressure on respiratory timing. *Respiration Physiology* 54:259-268, 1983.

Mathew, O.P., Abu-Osba, Y.K. and Thach, B.T. Genioglossus muscle responses to upper airway pressure changes: afferent pathways. *Journal of Applied Physiology* 52(2):445-450, 1982a.

Mathew, O.P., Abu-Osba, Y.K. and Thach, B.T. Influence of upper airway pressure changes on genioglossus muscle respiratory activity. *Journal of Applied Physiology* 52(2):438-444, 1982b.

Mathew, O.P., Roberts, J.L. and Thach, B.T. Pharyngeal airway obstruction in preterm infants during mixed and obstructive apnea. *Journal of Pediatrics* 100(6):964-968, 1982c.

McNicholas, W.T., Coffey, M., McDonnell, T., O'Regan, R. and Fitzgerald, M.X. Upper airway obstruction during sleep in normal subjects after selective topical oropharyngeal anesthesia. *American Review of Respiratory Disease* 135:1316-1319, 1987.

Mezzanotte, W.S, Tangel, D.J. and White, D.P. Waking genoglossus electromyogram in sleep apnea patients versus normal controls (a neureomuscular compensatory mechanism). *Journal of Clinical Investigation* 89:1571-1579, 1992.

Mezzanotte, W.S, Tangel, D.J. and White, D.P. Waking genioglossal electromyogram in sleep apnea patients versus normal controls (a neuromuscular compensatory mechanism). *Journal of Clinical Investigation* 89:1571-1579, 1992.

Mifflin, S.W. Arterial chemoreceptor input to respiratory hypoglossal motoneurons. *Journal of Applied Physiology* 69(2):700-709, 1990.

Mifflin, S.W., Spyer, K.M. and Withington-Wray, D.J. Respiratory modulation of hypoglossal motoneurones in the cat. *Journal of Physiology* 362:61P, 1986.

Miki, H., Hida, W., Chonan, T., Kikuchi, Y. and Takishima, T. Effects of submental electrical stimulation during sleep on upper airway patency in patients with obstructive sleep apnea. *American Review of Respiratory Disease* 140:1285-1289, 1989a.

Miki, H., Hida, W., Shindoh, C., Kikuchi, Y., Chonan, T., Taguchi, O., Inoue, H. and Takishima, T. Effects of electrical stimulation of the genioglossus on upper airway resistance in anesthetized dogs. *American Review of Respiratory Disease* 140:1279-1284, 1989b.

Milner, A.D. and Greenough, A. Adaptation of the respiratory system. *British Medical Bulletin* 44(4):909-918, 1988.

Mitra, J. and Cherniack, N.S. The effects of hypercapnia and hypoxia on single hypoglossal nerve fiber activity. *Respiration Physiology* 54:55-66, 1983.

Mitra, J., Prabhakar, N.R., Haxhiu, M. and Cherniack, N.S. Comparison of the effects of hypercapnia on phrenic and hypoglossal activity in anesthetized decerebrate and decorticate animals. *Brain Research Bulletin* 17:181-187, 1986.

Moore, P.J., Parkes, M.J., Noble, R. and Hanson, M.A. Reversible blockade of the secondary fall of ventilation during hypoxia in anaesthetized newborn sheep by focal cooling in the brain stem. *Journal of Physiology* 438:242P, 1991.

Morin, D., Monteau, R. and Hilaire, G. Compared effects of serotonin on cervical and hypoglossal inspiratory activities: An in vitro study in the newborn rat. *Journal of Physiology* 451:605-629, 1992.

Morray, J., Bennet, L., Noble, R. and Hanson, M.A. Effect of halothane on the carotid chemoreceptor receptor response to hypoxia in anaesthetized kittens. *Journal of Physiology* 446:425P, 1992.

Morray, J., Noble, R. and Hanson, M.A. The effects of halothane on the ventilatory response to hypoxia in chloralose-anaesthetized kittens. *Journal of Physiology* 438:244P, 1991.

Mosfeldt Laursen, A. and Rekling, J.C. Electrophysiological properties of hypoglossal motoneurones of guinea-pigs studied in vitro. *Neuroscience* 30(3):619-637, 1989.

Mulligan, E.M. Discharge Properties of Carotid Bodies. Developmental Aspects. *Lung Biology in Health and Disease* 53:321-340, 1991.

Neubauer, J.A., Melton, J.E. and Edelman, N.H. Modulation of respiration during brain hypoxia. *Journal of Applied Physiology* 68(2):441-451, 1990.

Neubauer, J.A., Santiago, T.V., Posner, M.A. and Edelman, N.H. Central medullary pH and ventilatory responses to hyperfusion and hypoxia. *Journal of Applied Physiology* 58(5):1659-1668, 1985.

Nioka, S., Chance, B., Smith, D.S., Mayevsky, A., Reilly, M.P. and Asakura, T. Cerebral energy metabolism and oxygen state during hypoxia in neonate and adult dogs. *Pediatric Research* 28(1):54-62, 1990.

Nishino, T., Kohchi, T., Yonezawa, T. and Honda, Y. Responses of recurrent laryngeal, hypoglossal, and phrenic nerves to increasing depths of anesthesia with halothane or enflurane in vagotomized cats. *Anesthesiology* 63:404-409, 1985.

Nishino, T., Shirahata, M., Yonezawa, T. and Honda, Y. Comparison of changes in the hypoglossal and the phrenic nerve activity in response to increasing depth of anesthesia in cats. *Anesthesiology* 60:19-24, 1984.

Noble, R., Williams, B.A, Hanson, M.A. and Smith, J.A. Transient,intense chemoreceptor stimulation does not affect discharge of rostral pontine neurones found to be excited during hypoxia in anaesthetized newborn lambs. *Journal of Physiology* 429:81P, 1990.

Noble, R. and Williams, B.A. Excitation of neurones in the rostral pons during hypoxia in anaesthetized neonatal lambs. *Journal of Physiology* 146P, 1989.

Nolan, P., Bradford, A., O'Regan, R.G., McKeogh, D. The effects of changes in laryngeal airway CO_2 concentration on genioglossus muscle activity in the anaesthetized cat. *Experimental Physiology* 75:271-274, 1990

Ochiai, R., Guthrie, R.D. and Motoyama, E.K. Effects of varying concentrations of halothane on the activity of the genioglossus, intercostals, and diaphragm in cats: an electromyographic study. *Anesthesiology* 70:812-816, 1989.

Önal, E., Lopata, M. and O'Connor, T.D. Diaphragmatic and genioglossal electromyogram responses to CO_2 rebreathing in humans. *Journal of Applied Physiology* 50(5):1052-1055, 1981.

Parisi, R.A., Neubauer, J.A., Frank, M.M., Edelman, N.H. and Santiago, T.V. Correlation between genioglossal and diaphragmatic responses to hypercapnia during sleep. *American Review of Respiratory Disease* 135:378-382, 1987.

Parisi, R.A., Santiago, T.V. and Edelman, N.H. Genioglossal and diaphragmatic EMG reponses to hypoxia during sleep. *American Review of Respiratory Disease* 138:610-616, 1988.

Patrick, G.B., Strohl, K.P., Rubin, S.B. and Altose, M.D. Upper airway and diaphragm muscle responses to chemical stimulation and loading. *Journal of Applied Physiology* 53:1133-1137, 1982.

Peliowski, A. and Finer, N.N. A blinded, randomized, placebo-controlled trial to compare theophylline and doxapram for the treatment of apnea of prematurity. *Pediatrics* 116:648-653, 1990.

Poets, C., Stebbens, V.A. and Southall, D.P. Arterial oxygen saturation and breathing movements during the first year of life. *Journal of Developmental Physiology* 15(6):341-345, 1991.

Redline, S. and Strohl, K.P. Influence of upper airway sensory receptors on respiratory muscle activation in humans. *Journal of Applied Physiology* 63:368-374, 1987.

Reed, W.R., Roberts, J.L. and Thach, B.T. Factors influencing regional patency and configuration of the human infant upper airway. *Journal of Applied Physiology* 58(2):635-644, 1985.

Remmers, J.E., Degrout, W.J., Sauerland, E.K. and Anch, A.M. Pathogenesis of upper airway occlusion during sleep. *Journal of Applied Physiology* 44(6):931-938, 1978.

Rigatto, H. Control of ventilation in the newborn. *Annuual Review of Physiology* 46:661-674, 1984.

Roberts, J.L., Reed, W.R., Mathew, O.P., Menon, A.A. and Thach, B.T. Assessment of pharyngeal airway stability in normal and micrognathic infants. *Journal of Applied Physiology* 58(1):290-299, 1985.

Roberts, J.L., Reed, W.R., Mathew, O.P. and Thach, B.T. Control of respiratory activity of the genioglossus muscle in micrognathic infants. *Journal of Applied Physiology* 61(4):1523-1533, 1986.

Safar, P., Escarraga, L.A. and Chang, F. Upper airway obstruction in the unconscious patient. *Journal of Applied Physiology* 14(5):760-764, 1959.

Salamone, J.A., Strohl, K.P., Weiner, D.M., Mitra, J. and Cherniack, N.S. Cranial and phrenic nerve responses to changes in systemic blood pressure. *Journal of Applied Physiology* 55(1):61-68, 1983.

Sant'Ambrogio, F.B., Sant'Ambrogio, G., Mathew, O.P. and Tsubone, H. Contraction of trachealis muscle and activity of tracheal stretch receptors. *Respiration Physiology* 71:343-353, 1988.

Sant'Ambrogio, G. and Mathew, O.P. Control of upper airway muscles. *News in Physiological Science* 3:167-172, 1988.

Sant'Ambrogio, G., Mathew, O.P., Fisher, J.T. and Sant'Ambrogio, F.B. Laryngeal receptors responding to transmural pressure, airflow and local muscle activity. *Respiration Physiology* 54:317-330, 1983.

Sauerland, E.K. and Harper, R.M. The human tongue during sleep: Electromyographic activity of the genioglossus muscle. *Experimental Neurology* 51:160-170, 1976.

Sauerland, E.K. and Mitchell, S.P. Electromyographic activity of intrinsic and extrinsic muscles of the human tongue. *Texas Reports on Biology and Medicine* 33(3):445-455, 1975.

Schwieler, G.H. Respiratory regulation during postnatal developmant in cats and rabbits and some of its morphological substrates. *Acta Physiologica Scandinavia* 72(Supp):1-123, 1968.

Shelton, R.L. and Bosma, J.R & J.F. Maintenance of the pharyngeal airway. *Journal of Applied Physiology* 17(2):209-214, 1962.

Sica, A.L., Cohen, M.I., Donnelly, D.F. and Zhang, H. Hypoglossal motoneuron responses to pulmonary and superior laryngeal afferent inputs. *Respiration Physiology* 56:339-357, 1984.

Sica, A.L., Steele, A.M., Gandhi, M.R. and Prasad, N. Factors affecting central inspiratory modulation of hypoglossal motoneuron activity in newborn pigs. *Journal of Developmental Physiology* 10:285-295, 1988.

Siesjö, B.K. and Nilsson, L. The influence of arterial hypoxemia upon labile phosphates and upon extracellular and intracellular lactate and pyruvate concentrations in the rat brain. *Journal of Clinical Laboratory Investigation* 27:83-96, 1971.

St.John, W.M. and Bledsoe, T.A. Comparison of respiratory-related trigeminal, hypoglossal and phrenic activities. *Respiration Physiology* 62:61-78, 1985.

St.John, W.M. and Bartlett, D.Jr. Comparison of phrenic montoneuron responses to hypercapnia and isocapnic hypoxia. *Journal of Applied Physiology* 46(6):1096-1102, 1979.

St.John, W.M., Knuth, K.V. and Rist, K.E. Dynamic changes of hypoglossal and phrenic activities by hypoxia and hypercapnia. *Respiration Physiology* 56:237-244, 1984.

St.John, W.M. Influence of reticular mechanisms upon hypoglossal, trigeminal and phrenic activities. *Respiration Physiology* 66:27-40, 1986.

Stark, A.R. and Thach, B.T. Recovery of airway patency after obstruction in normal infants. *American Review of Respiratory Disease* 123:691-693, 1981.

Strohl, K.P., Wolin, A.D., Van Lunteren, E. and Fouke, J.M. Assessment of muscle action on upper airway stablilty in anesthetized dogs. *Journal of Laboratory Clinical Medicine* 110:221-230, 1987.

Suguihara, C., Bancalari, E. and Hehre, D. Brain blood flow and ventilatory response to hypoxia in sedated newborn piglets. *Pediatric Research* 27(4):327-331, 1990.

Sumi, T. Functional differentiation of hypoglossal neurons in cats. *Japanese Journal of Physiology* 19:55-67, 1969.

Taasan, V.C., Block, A.J., Boysen, P.G. and Wynne, J.W. Alcohol increases sleep apnea and oxygen desaturation in asymptomatic men. *The American Journal of Medicine* 71:240-245, 1981.

Thach, B.T., Menon, A.P. and Schefft, G.L. Effects of negative upper airway pressure on pattern of breathing in sleeping infants. *Journal of Applied Physiology* 66(4):1599-1605, 1989.

Uemura-Sumi, M., Itoh, M. and Mizuno, N. The distribution of hypoglossal motoneurones in the dog, rabbit and rat. *Anatomy and Embryolgy* 177:389-394, 1988.

Ugolini, G., Küypers, H.G.J.M. and Simmons, A. Retrograde transneuronal transfer of Herpes simplex virus by type 1 (HSV 1) from motoneurones. *Brain Research* 422:242-256, 1987.

Ukabam, C.U., Knuth, S.L. and Bartlett, D.Jr. Phrenic and hypoglossal neural responses to cold airflow in the upper airway. *Respiration Physiology* 87:157-164, 1992.

Van Lunteren, E., Haxhiu, M.A., Mitra, J. and Cherniack, N.S. Effects of dopamine, isoproterenol, and lobeline on cranial and phrenic motoneurons. *Journal of Applied Physiology* 56(3):737-745, 1984a.

Van Lunteren, E., Van de Graaff, W.B., Parker, D.M., Mitra, J., Haxhiu, M.A., Strohl, K.P. and Cherniack, N.S. Nasal and laryngeal reflex responses to negative upper airway pressure. *Journal of Applied Physiology* 56(3):746-752, 1984c.

Van Lunteren, E. and Dick, T.E. Intrinsic properties of pharyngeal and diaphragmatic respiratory motoneurons and muscles. *Journal of Applied Physiology* 73(3):787-800, 1992.

Van Lunteren, E., Martin, R.J., Haxhiu, M.A. and Carlo, W.A. Diaphragm, genioglossus, and triangularis sterni responses to poikilocapnic hypoxia. *Journal of Applied Physiology* 67(6):2303-2310, 1989.

Van Lunteren, E. and Strohl, K.P. The Muscle of the Upper Airway. *Clinics in Chest Medicine* 7(2):171-188, 1986.

Van Lunteren, E., Strohl, K.P., Parker, D.M., Bruce, E.N., Van de Graaff, W.B. and Cherniack, N.S. Phasic volume-related feedback on upper airway muscle activity. *Journal of Applied Physiology* 56(3):730-736, 1984b.

Watchko, J.F., Klesh, K.W., O'Day, T.L., Weiss, M.G. and Guthrie, R.D. Genioglossal recruitment during acute hypoxia and hypercapnia in kittens. *Pediatric Pulmonology* 7:235-243, 1989.

Weigand, L., Zwillich, C.W. and White, D.P. Collapsibility of the human upper airway during normal sleep. *Journal of Applied Physiology* 66(4):1800-1808, 1989.

Weiner, D., Mitra, J., Salamone, J. and Cherniack, N.S. Effect of chemical stimuli on nerves supplying upper airway muscles. *Journal of Applied Physiology* 52:530-536, 1982.

Weiner, D., Mitra, J., Salamone, J., Nochomovitz, M. and Cherniack, N.S. Effect of chemical drive on the electrical activity and force of contraction of the tongue and chest wall muscles. *American Review of Respiratory Disease* 121(418):418-419, 1980.

Wilson, S.L., Thach, B.T., Brouillette, R.T. and Abu-Osba, Y.K. Upper airway patency in the human infant: influence of airway pressure and posture. *Journal of Applied Physiology* 48(3):500-504, 1980.

Withington-Wray, D.J., Mifflin, S.W. and Spyer, K.M. Intracellular analysis of respiratory-modulated hypoglossal motoneurons in the cat. *Neuroscience* 25(3):1041-1051, 1988.

Wood, G.A. and Harding, R. The effects of pentobarbitone diazepam and alcohol on oral breathing in neonatal and mature sheep. *Respiration Physiology* 75:89-104, 1989.

Wood, G.A. and Harding, R. The effects of pentobarbitone, diazepam and alcohol on oral breathing in neonatal and mature sheep. *Respiration Physiology* 75(1):89-104, 1989.

Yarom, Y., Sugimori, M. and Llinas, R. Ionic currents and firing patterns of mammalian vagal motoneurons in vitro. *Neuroscience.* 16(4):719-737, 1985.

Younes, M.K., Remmers, J.E. and Baker, J. Characteristics of inspiratory inhibition by phasic volume feedback in cats. *Journal of Applied Physiology* 45(1):80-86, 1978.

Zhang, S. and Mathew, O.P. Decrease in lung volume-related feedback enhances laryngeal reflexes to negative pressure. *Journal of Applied Physiology* 73(3):832-836, 1992.

APPENDICES

APPENDIX A.

Electrical stimulation in the rostral pons induces apnoea in kittens

Although the following study is only preliminary I have included it in the thesis because I feel that it is important when considering the mechanisms and targetting the area which may be affecting the activity of the hypogossal nerve during hypoxia in kittens.

Introduction

The ventilatory response to hypoxia in the neonate has been well characterised in both the human infant and a variety of newborn animals (for references see Rigatto, 1984, Henderson-Smart, 1984, Neubauer et al., 1990). During the first one to two minutes of hypoxia there is an increase in ventilation followed by a return after approximately five minutes to levels approaching baseline.

The underlying reasons for the respiratory depression during hypoxia are not clearly understood, but a number of mechanisms have been proposed including a reduction in pulmonary compliance (La Framboise et al., 1981 & 1983) and a reduction in metabolism (Grunstein et al., 1981). However more recently a number of studies have attributed the decrease in ventilation during hypoxia in neonates to a central mechanism (Blanco et al., 1984, Lawson & Long, 1983, Martin-Body & Johnston, 1988).

In fetal lambs, hypoxia reduces or abolishes movements of the lungs known as breathing movements (Boddy et al., 1974). Further studies have demonstrated that, although pre-collicular transection of the brainstem does not influence the inhibition

of breathing movements during hypoxia, following midcollicular brainstem transection the inhibition of breathing movements is absent (Dawes et al., 1983). Lesion studies suggested that the inhibition is mediated in the lateral pons slightly rostral to the trigeminal nucleus and at the level of the fifth nerve and middle cerebellar peduncle (Gluckman & Johnston, 1987, Johnston & Gluckman, 1989).

In the neonate it was originally believed that the fall in ventilation was due to a general depression of the respiratory centers (Cross et al., 1954). However, more recent studies suggest that, as with the depression of breathing movements in the fetus during hypoxia, the fall in ventilation in the neonate is due to a mechanism sited in or above the upper pons (Martin-Body & Johnston, 1988, Hanson & Williams, 1989). In these studies, the secondary fall in ventilation which occurs in neonatal rabbits (5-10 days old) and rats (4-7 days) during hypoxia (O_2 7% & 8%, respectively) was absent following decerebration at or near the midbrain/pontine junction (Martin-Body & Johnston, 1988, Hanson & Williams, 1989). In contrast pre-collicular decerebration did not prevent the secondary fall in ventilation (Hanson & Williams, 1989).

More recent studies have shown that electrical stimulation at a site in the rostral ventrolateral pons at a level just rostral to the trigeminal nucleus induces apnoea in the newborn lamb (Coles et al., 1989). Isocapnic hypoxia increased the activity of units recorded with extracellular microelectrodes within this area (Noble & Williams, 1989). however, these units were not affected by a transient intense carotid chemoreceptor stimulation (Noble et al., 1990). More recently the secondary fall in ventilation has been prevented by cooling the rostral pons at the level of the middle cerebellar peduncle (Moore et al., 1991).

Aim

The aim of this preliminary study was to establish whether stimulation of the area just rostral to the trigeminal nucleus at the level of the middle cerebellar peduncle also induces apnoea in kittens. This study extended the work of Coles et al. (1989) (for review see Noble 1991).

Methods

Three kittens were used in this study, each was approximately 2 months old and weighed between 800 and 950g. These studies were all performed at Reading University and the animals were brought into the animal house up to a week prior to the experiment.

Surgical preparation

Anaesthesia was induced and maintained with pentobarbitone sodium (30-35mg/kg,I.P, Sagatal). The depth of anaesthesia was assessed by the absence of flexor reflexes and by the degree of pupillary constriction. The tracheal area and inner side of a rear leg were shaved. The trachea was exposed and intubated below the larynx with a paediatric tracheal tube with an inner diameter of 2.5mm. The composition of gases in the delivery line was set by rotameters connected to oxygen and air cylinders. During the surgery and at intervals between recordings the animals were artifically ventilated and PaO_2 was maintained above 100mmHg and $PaCO_2$ between 35 and 45mmHg. A femoral artery was cannulated for the monitoring of blood pressure. The blood pressure transducer was calibrated prior to each experiment. Blood pressure was used to assess the level of anaesthesia. Blood samples (0.3ml) were taken for arterial gas analysis. The femoral vein was cannulated and further doses of anaesthetic were

administered intravenously. Both the femoral arterial and venous cannulae were filled with heparinised saline (10 units/ml). The rectal temperature was maintained at ca. 38°C using a homeothermic blanket control and heat lamps.

The animal was turned into the prone position and the head fixed into a stereotaxic frame. The medulla oblongata and pons were exposed by an occipital craniotomy and cerebellectomy.

At the end of the experiment the animals were killed with the administration of a large dose of pentobarbitone sodium (200mg,i.v). The brainstem was removed and fixed with 10% formalin (w/v) and stored in a fume cupboard. The area of stimulation was verified histologically in each case.

Experimental protocol

During recordings the animals were allowed to breath air spontaneously.

A concentric bipolar electrode (Rhodes Metal RNE 100) was used to stimulate (0.1ms pulses, 100 Hz for 10secs) in a series of electrode penetrations made in rows across the pons at and around the level of the trigeminal motor nucleus. This level was located by visual identification of the facial colliculi and middle cerebellar peduncle on the exposed floor of the fourth ventricle. In each experiment the electrode was initially lined up with the midline and then positioned with the use of the stereotaxic frame. During each penetration a stepping-motor microdrive (Significat, Digitimer) was used to move the electrode down through the tissue in 0.5mm steps to a final depth of 4.5. For each penetration, zero depth was taken at the floor of the ventricle as measured at the midline. The electrode penetrations were made in rows 1mm appart, each starting 1mm lateral from the midline. Each row consisted of upto 6 tracks. At

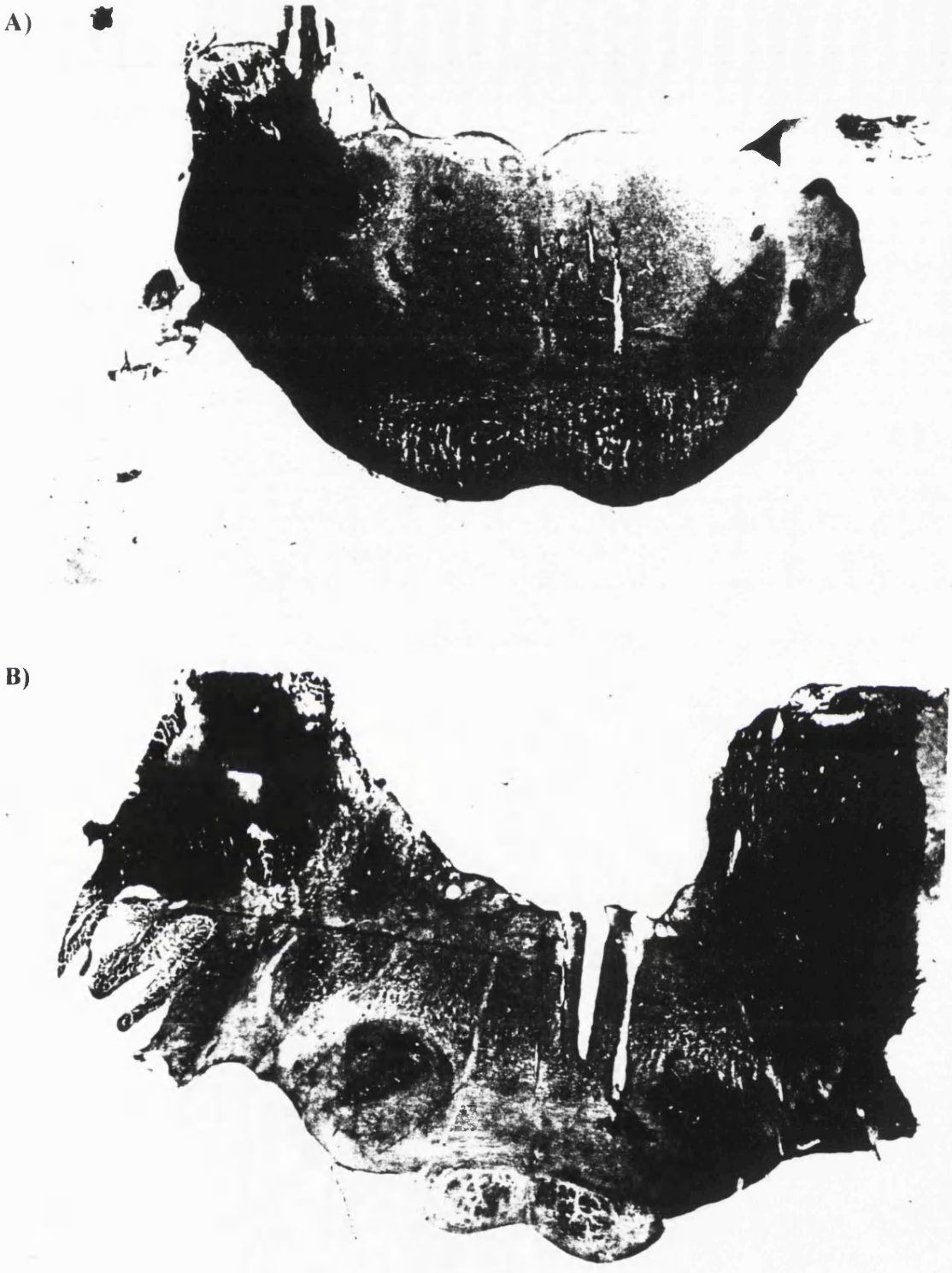
each step down through the tissue electrical stimulation (1-10 volts)was applied to determine whether such stimulation induced apnoea i.e an extended T_E for the duration of the stimulus. If such stimulation induced apnoea the apnoeic threshold was determined i.e the minimum strength of stimulus required to induce apnoea. Voltage was used to represent the stimulus strength because the relative apnoeic thresholds were required rather than the absolute stimulus strength and furthermore a constant current stimulator was not available at the time.

Histology

The brain was transected so that the area in which the tracks were made were included within the section. The section was washed in distilled water and then placed and frozen on a microtome. One hundred micrometer thick transverse sections of the pons were taken, removed with a paintbrush and placed on agar coated slides. The slides were then left to dry overnight.

Luxol fast blue and cresyl violet were used to stain the sections of the pons, making histological reconstructions easier. The slides were stained with Luxol fast blue overnight in an oven at 60°C. After rinsing in 95% alcohol and distilled water, the slides were dipped in lithium carbonate (0.05%) for a few seconds before being left to differentiate in 70% alcohol for 30 seconds or until a clear distinction is seen in the tissue. The slides were washed well in distilled water and then counterstained in 0.1% cresyl fast violet in 1% acetic acid for 10 minutes at 56°C. The slides were again rinsed in distilled water and then the counterstain differentiated in 95% alcohol. The slides were mounted in DPX in a fume cupboard.

The slices containing the tracks were identified (see photograph 1) and the area within the pons determined with the use of a stereotaxic cat atlas. The area was mapped out



Photograph 1. Showing two transverse sections of the pons from a neonatal kitten. The tracks made by the electrode through the tissue are clearly visible. The reconstructions from these slices are shown in figure 1.

using a camera lucida. No calculations were made for shrinkage.

Results

In two kittens the histological reconstructions verified that the electrical stimulations had been at locations in the pons at the level of the trigeminal nucleus (Photograph 1.). Electrical stimulation at certain loci within this area inhibited breathing for the 10 second duration. In the third kitten the tracks were located further rostral and at the level of the lateral lemnisci nucleus.

Figure 1 is a reconstruction from the transverse sections of the pons from one kitten. It shows the apnoeic threshold profile recorded at the level of (a) and 1mm caudal to (b) the trigeminal nucleus. The reconstruction indicates a region at the level of the trigeminal nucleus adjacent to the middle cerebellar peduncle which when stimulated induces apnoea. This region is contained mainly in the lateral pons but spreads medially a further 1 mm caudal. Furthermore, lower apnoeic thresholds were recorded at this more caudal level (2mm caudal to the cerebral aquaduct).

Figure 2 shows the reconstruction from transverse sections of the pons from the second kitten. The tracks were more rostral than those depicted in figure 2 and higher stimulus levels were required to induce apnoea. Figure 3 shows the results from the third kitten. Again the tracks are further rostral than those of the first kitten but in this case the area of stimulation was not at the level of the trigeminal nucleus. The lower apnoeic thresholds ($\leq$ 3 volts) were more medial than those recorded in the more rostral sections.

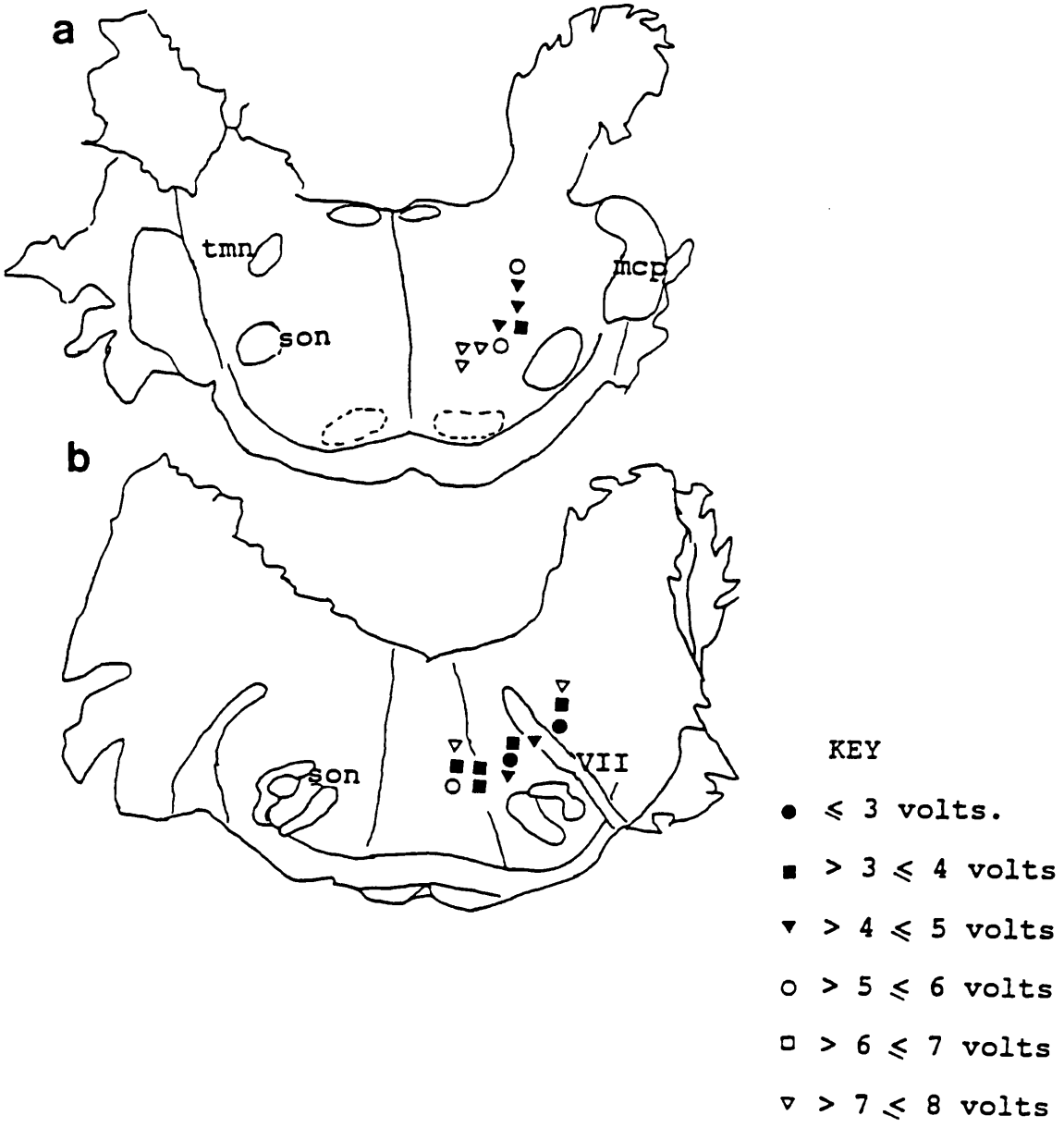


Figure 1. Reconstruction from transverse sections of the pons of a neonatal kitten (850g) to show the apnoeic threshold profile recorded at the level of a),and 1mm caudal to b), the trigeminal motor nucleus (tmn)(son=superior olivary nuclei, VII = facial nerve, mcp=middle cerebellar peduncle). The apnoeic thresholds determined at each stimulus location in a grid of electrode tracks are represented by symbols as indicated.

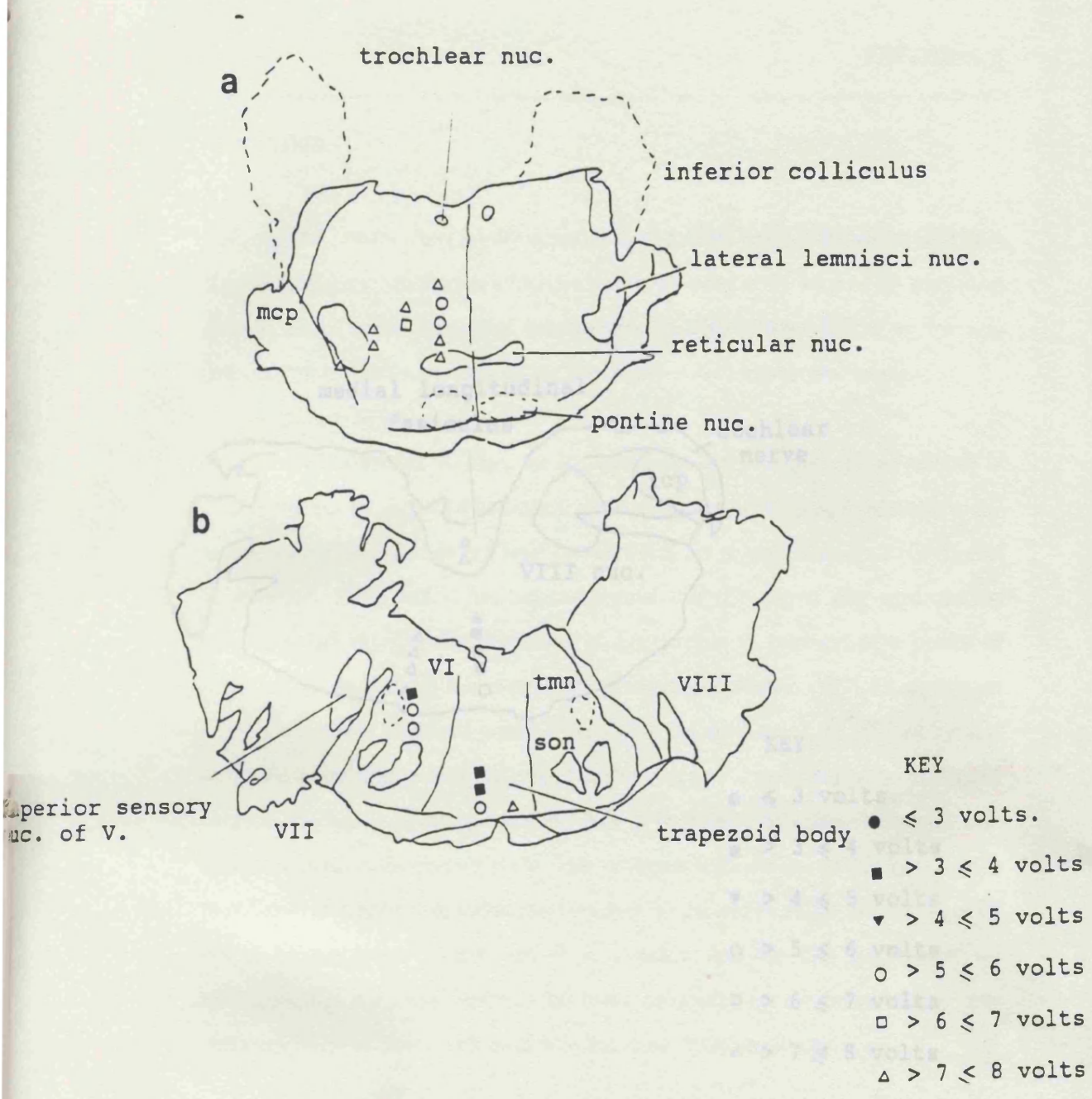


Figure 2. Reconstruction from transverse sections of the pons of a neonatal kitten (850g) to show the apnoeic threshold profile recorded 1mm (a) and 2mm caudal to b) the cerebral aqueduct (tmn= trigeminal motor nucleus, son=superior olivary nuclei, VII = facial nerve, VIII = vestibular nerve, VI= abducens nerve, mcp=middle cerebellar peduncle). The apnoeic thresholds determined at each stimulus location in a grid of electrode tracks are represented by symbols as indicated.

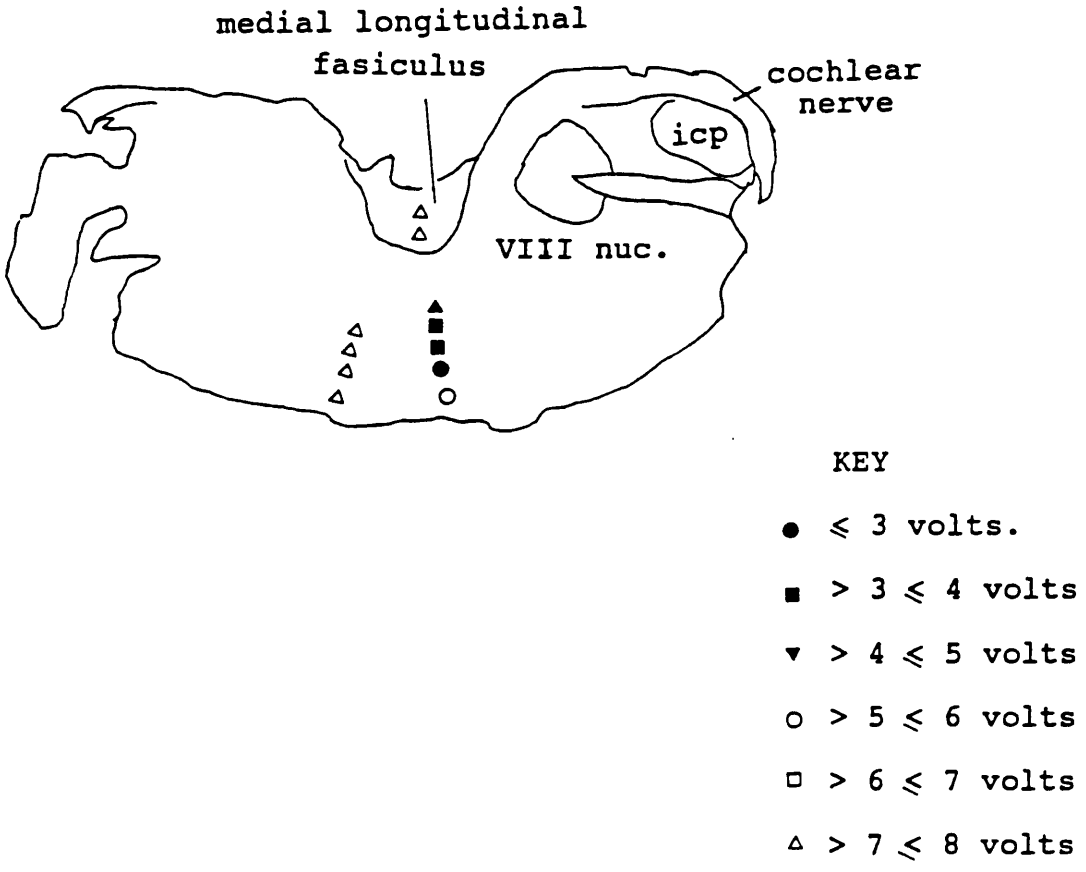


Figure 3. Reconstruction from transverse sections of the pons of a neonatal kitten (950g) to show the apnoeic threshold profile recorded at a level just caudal to the cerebral aqueduct (mcp = middle cerebellar peduncle). The apnoeic thresholds determined at each stimulus location in a grid of electrode tracks are represented by symbols as indicated.

Discussion

Although the present study is very preliminary and rather crude in its technique it does show that in the kitten there is a location within the pons at the level of the trigeminal nucleus which when stimulated induces apnoea. This is very similar to the area mapped out by Noble and colleagues (see Noble, 1991) in neonatal lambs.

As described in the introduction, the hypoxic suppresion of breathing movements in fetal sheep can be abolished by lesions in the ventrolateral brain stem adjacent to the middle cerebellar peduncle at a level just rostral to the trigeminal nucleus (Gluckman & Johnston, 1987) and it has been suggested that this region also mediates the secondary fall in ventilation observed in the neonate in response to a period of hypoxia (Martin-Body & Johnston, 1988., Hanson & Williams, 1989). In the present study the apnoeic threshold profiles show that the apnoea can be elicited by low threshold stimulation at a ventral site at the level of or 1 mm caudal to the trigeminal nucleus. The lesions of Gluckman and Johnston were more dorsolateral however than area stimulated in the present study. The variations between the studies may be due to differences in species or technique. However the present study did not extend as far lateral as the lesion studies and it is possible that the two suggestions are corresponding; the more ventromedial locus designated by the present study may represent tracts of fibres with origins in the more dorsolateral area.

Further studies are required to try and establish whether the inhibition seen upon stimulation originates at the level of the trigeminial or elsewhere in the brain stems, for example the parabrachial or Kölliker-Fuse nucleus. This issue may be resolved by the microinjection of amino acids such as glutamate which would only excite cell bodies but not axons. Although this study has not yet been carried out in the kitten and lamb, Farber (1990) demonstrated that apnoea could be evoked in the young

opposum after glutamate (1M) was injected into the ventrolateral pons.

References (not found in main reference list)

Boddy, K, Dawes, G.S., Fisher, R., Pinter, S. and Robinson, J.S. Foetal respiratory movements, electrocortical and cardiovascular responses to hypoxaemia and hypercapnia in sheep. *Journal of Physiology*. 253:599-618, 1974.

Cross, K.W., Hooper, J.M.D. and Lord, J.M. Anoxic depression of the medulla in the new-born infant. *Journal of Physiology*. 125: 628-640.

Dawes, G.S., Gardner,W.N., Johnston, B.M. and Walker D.W. Breathing in featal lambs: The effect of brain stem section. *Journal of Physiology*. 335: 535-553.

Farber, J.P. Effects on breathing of rostral pons glutamate injection during opossum development. *Journal of Applied Physiology*. 69(1) 189-195. 1990.

Gluckman, P.D. and Johnston, B.M. Lesions in the upper lateral pons abolish the hypoxic depression of breathing in unanaesthetized fetal lambs in utero. *Journal of Physiology*. 382: 373-383.1987.

Johnston, B.M. and Gluckman, P.D. Lateral pontine lesions affect central chemosensitivity in unanaesthetized fetal lambs. *Journal of Applied Physiology*. 67(3): 1113-1118. 1989.

Lawson, E.E. and Long, W.A. Central origin of biphasic breathing pattern during hypoxia in newborns. *Journal of Applied Physiology*. 55(2): 483-485, 1983.

[*From the Proceedings of the Physiological Society*, 28–29 *June* 1990
Journal of Physiology, **429**, 81*P*, 1990]

Transient, intense carotid chemoreceptor stimulation does not affect discharge of rostral pontine neurones found to be excited during hypoxia in anaesthetized newborn lambs

By Ray Noble, Bridget A. Williams, Mark A. Hanson and Julie A. Smith*.
Fetal and Neonatal Research Group, School of Animal and Microbial Sciences, University of Reading, Whiteknights, Reading RG6 2AJ

In the neonate, the ventilatory response to hypoxia is 'biphasic', with an initial increase followed by a return to, or to below, pre-hypoxic levels. Lesions and transection studies suggest that the fall in ventilation is mediated in the rostral pons (Martin-Body & Johnston, 1988). We have recently found an area at this level in which electrical stimulation inhibits breathing in newborn lambs (Coles *et al.* 1989) and from which we have made recordings of neurones which increase their discharge frequency during isocapnic hypoxia (Noble & Williams, 1989). In a preliminary study, we have now investigated whether peripheral chemoreceptors also excite such neurones.

Newborn lambs aged 1–2 days were anaesthetized with halothane (4% in O_2 for induction, 1–2·5% for maintenance) and artificially ventilated. The pons was exposed and tungsten microelectrodes were used to record the discharge of neurones in the area delineated in previous stimulation and recording experiments. As reported, neurones were found in this area which increased discharge markedly during isocapnic hypoxia ($P_{a,O2} = 38{\cdot}7 \pm 1{\cdot}9$; $P_{a,CO_2} = 36{\cdot}7 \pm 0{\cdot}7$; mean ± S.E.M.). In seven neurones from three lambs, we found no change in discharge when a bolus of 0·5–1·0 ml of CO_2-saturated saline was injected towards the carotid body via a catheter inserted into the ipsilateral lingual artery.

If these pontine neurones were driven in hypoxia by peripheral chemoreceptor excitation, we would have expected their discharge to be affected by the CO_2-saline injections, as this method produces an intense, transient excitation of the carotid chemoreceptors and changes ongoing respiration (Black & Torrance, 1971). We cannot, however, rule out the possibility that more prolonged chemoreceptor stimulation somehow modulates the activity of these neurones.

Supported by the Wellcome Trust and The Foundation for the study of Infant Deaths.

REFERENCES

Black, A. M. S. & Torrance, R. W. (1971). *Resp. Physiol.* **13**, 221–237.
Coles, S. K., Kumar, P. & Noble, R. (1989). *J. Physiol.* **405**, 66*P*.
Martin-Body, R. L. & Johnston, B. M. (1988). *Resp. Physiol.* **71**, 25–32.
Noble, R. & Williams, B. A. (1989). *J. Physiol.* **417**, 146*P*.

* Reading University Endowment Scholar.

The effect of mild hypoxia on discharge frequency of hypoglossal motoneurones in anaesthetized neonatal kittens

Julie A. Smith*, Fei Li and Ray Noble

Department of Obstetrics & Gynaecology, University College London, Gower Street, London WC1E 6BT

In adults, respiratory-related genioglossus muscle activity is augmented by hypercapnia and hypoxia (Önal *et al.* 1981) and plays an important role in maintaining upper airway patency (Brouillette & Thach, 1980). The proportion of kittens which show genioglossus recruitment increases with postnatal age. Futhermore, if recruitment occurs in the neonate it is not always sustained (Watchko *et al.* 1989). We have investigated the effect of hypoxia on the discharge frequency of hypoglossal motoneurones (HMN) in neonatal kittens.

Seventeen kittens (13–27 days, 279–650 g) were anaesthetized with α-chloralose (50 mg kg^{-1}, I.V.), after induction with ketamine (15–30 mg kg^{-1}, I.M.) and halothane (1–2 %), paralysed with gallamine triethiodide and artifically ventilated. The brainstem was exposed and glass microelectrodes (3 M KCl, 60–80 MΩ) were used to record HMNs identified by antidromic stimulation. $P_{ET.CO_2}$ and $F_{I.O_2}$ were continually monitored (Ohmeda 5250 RGM).

Stable recordings were made from 46 HMNs (7 intracellular). Isocapnic hypoxia ($F_{I.O_2}$ 0.14–0.18, $P_{ET.CO_2}$ 35–56 mmHg, 2–10 min) increased the discharge frequency ($P < 0.05$, Student's t test) of 25 HMNs recorded extracellularly. However, only fourteen of these sustained the increase throughout hypoxia whereas eleven displayed a transient increase (at least 60 s duration), in some cases falling to below the control level ($P < 0.05$). Eight HMNs decreased their discharge during the hypoxia ($P < 0.02$) and six units showed no change. Hypoxia increased the amplitude of the 'respiratory-related' EPSPs (average change = 14.48 mV) and/or decreased the resting membrane potential (61.62 ± 2.62 in normoxia) by 10.24 ± 1.98 mV (mean ± S.E.M.).

Our results suggest that both respiratory- and non-respiratory-related HMN activity may increase during hypoxia. However, the poor recruitment of upper airway activity during hypoxia in the neonate may reflect our finding that the majority of HMNs either failed to sustain an increased discharge frequency or were inhibited during hypoxia.

*J.A.S. is an MRC Scholar. This work was supported by the Wellcome Trust.

REFERENCES

Brouillette, R.T. & Thach, B.T. (1980). *J. Appl. Physiol.* **49 (5)**, 801–808.

Önal, E., Lopata, M. & O'Connor, T.D. (1981). *J. Appl. Physiol.* **50 (5)**, 1052–1055.

Watchko, J.F., Klesh, K.W., O'Day, T.L., Weiss, M.G. & Guthrie, R.D. (1989). *Pediatr. Pulmonol.* **7**, 235–243.

Effect of isocapnic hypoxia on membrane potentials of hypoglossal motoneurones recorded in anaesthetized neonatal kittens

Julie A. Smith*, Fei Li and Ray Noble

Department of Obstetrics & Gynaecology, University College London, Gower Street, London WC1E 6BT

It has recently been shown that hypoxia depolarizes hypoglossal motoneurones (HMNs) recorded in rat brain slices *in vitro* (Haddad & Donnelly, 1990). Mifflin (1992) has shown that transient activation of peripheral chemoreceptors in adult cats can produce a depolarization of HMNs independent of its effect on respiratory-related EPSPs. Both these studies suggest that one way or another hypoxia might have an effect on hypoglossal output independent of its effect on respiratory drive. We have now recorded the effect of acute isocapnic hypoxia on the membrane potentials of HMNs recorded in neonatal kittens.

Eight kittens (17–23 days, 300–527 g) were anaesthetized with α-chloralose (50 mg kg^{-1}, I.V.), after induction with ketamine (15–30 mg kg^{-1}, I.M.) and halothane (1–2 %), paralysed with gallamine triethiodide and artificially ventilated. Anaesthesia was assessed by pupillary constriction, blood pressure, and the absence of flexor reflexes and muscle tone without gallamine. The brainstem was exposed and glass microelectrodes (3 M KCl, 60–80 MΩ) were used to record HMNs identified by antidromic stimulation. $P_{ET.CO_2}$ and $F_{i.O_2}$ were continually monitored (Ohmeda 5250 RGM).

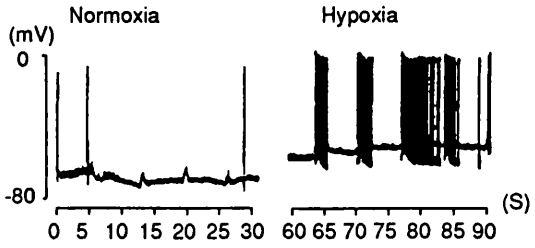


Fig. 1. Membrane potential of an HMN during normoxia ($F_{i.O_2}$ 0.21, $P_{a.O_2}$ 90 mmHg) and hypoxia ($F_{i.O_2}$ 0.15, $P_{a.O_2}$ 47 mmHg).

Stable intracellular recordings were made from fourteen HMNs. Resting membrane potentials in normoxia were 58.73 ± 3.11 mV (mean ± S.E.M.). During isocapnic hypoxia ($F_{i.O_2}$ 0.14–0.18, $P_{ET.CO_2}$ 29–48 mmHg) all HMNs, bar one, depolarized (mean depolarization ± S.E.M. = 12.95 ± 1.24 mV). An example is shown in Fig. 1.

These data support the hypothesis that hypoxia can excite HMNs independently of its effects through respiratory drive.

* J.A.S. is an MRC scholar. This work was supported by the Wellcome Trust.

REFERENCES

Haddad, G.G. & Donnelly, D.F. (1990). *J. Physiol.* **429**, 411–428.
Mifflin, S.W. (1990). *J. Appl. Physiol.* **69 (2)**, 700–709.