BRAINSTEM CONTROL OF RESPIRATION IN JAPANESE QUAIL

by

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ABSTRACT

The brainstem of Japanese Quail was studied systematically using small electrodes and small currents (10-40μA), and areas involved in the control of respiration were identified. Regions producing large and small increases in rate, inspiration and expiration were found. The latter two responses had differing post-stimulatory recovery patterns. The distribution of sites from which these four responses were elicited, was analysed three dimensionally; this indicated that they were anatomically separated in the brainstem.

Multiple unit recordings were made in the regions and peak neurone firing activity was found to occur at many different stages of the respiratory cycle. However, in some regions, there was a noticable predominance of activity just prior to the on-set of inspiration. It was suggested that this activity peak could be responsible for the initiation of inspiration. Three dimensional analysis indicated that early inspiratory activity sites were significantly separated from other areas.

During polypnoea brought on by heat stress, the electrical stimulatory threshold currents for both inspiratory and expiratory cramps were found to be on average 92.8% higher.

It was suggested that the increased ventilatory drive due to heat stressing, was antagonistic to any areas responsible for the respiratory arrest and the current thresholds rose as a consequence. Sites from which rate increase could be elicited at normal body temperatures produced slowing of respiration when the animal was heat stressed, threshold for the response increased by an average of 102%.

When carbon dioxide levels in the inspired air were elevated to 5%, the current thresholds for cramp regions increased, while those for the large rate sites decreased. At higher (10%) carbon dioxide levels, both cramp and large rate sites displayed a mixture of increased and decreased thresholds.

Basal respiratory properties were examined and unilateral (right nerve) vagotomy decreased frequency by 45.8% and increased depth by 13%.

A schematic model of the brainstem regions was proposed in the light of the responses seen. Input from chemoreceptors, stretch receptors and higher brain centres were included. Elements such as ramp generators, timers and reciprocal and recurrent inhibition were included in the model.

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Chapter 1

General Introduction

1.1 Previous Work

"Practically nothing is known about the location and function of the central respiratory control mechanism in birds" (Bouverot, 1978).

Respiratory periodicity was confirmed by transection to be generated in the Medulla oblongata in birds (von Saalfield, 1936). Further than this the only work on avian brainstem respiratory centres appears to be represented by two studies. An area eliciting a 7-20 fold acceleration of respiratory rate accompanied by a slight decrease in amplitude was located in the brainstem of the chicken (Richards, 1970a, 1971a). Richards used the word "brainstem" for all regions posterior to the cerebral hemispheres. Throughout this thesis, "brainstem" will mean Rhombencephalon. The stimulatory currents used were greater than those used in other brain stimulation studies, so responses due to current spread to other areas cannot be ruled out. The points identified were somewhat more anterior than would have been expected for final motor pathway areas and therefore may be concerned with polypnoea

for temperature control. Other work (Richards, 1968 and Ramirez and Bernstein, 1976) has indicated that the magnitudes of the respiratory rate increases elicited are similar to those obtained during heat stress.

The other previous study (Cohen and Schnall, 1970) was principally concerned with cardio-vascular control. However, some points eliciting respiratory arrest were identified in the pigeon brainstem together with others producing decreases in respiratory depth. It should be pointed out that these points were in the Rhombencephalon and are therefore likely to be directly concerned with respiration. Only eighteen birds were studied and the currents used were higher than in the present work.

Respiratory responses to stimulation of other, more anterior brain areas, have also been reported. For example Kotilainen and Putkonen (1972, 1974) found sites from which respiratory arrest was elicited, in areas of the forebrain around the Nucleus rotundus. Vedyaev (1963, 1964) elicited respiratory intensification and arrest from points at the Striatal and Thalamic levels. Richards (1970a, 1971a) identified midbrain regions from which he was able to produce polypnoea in the pigeon and chicken. Such responses are almost certainly mediated via respiratory centres in the brainstem, from higher "command centres".

In view of the fact that such little work has been conducted

in this field (Jukes, 1971), it seems presumptious to assume that bird respiration is controlled in the same way as the mammals (Jones, 1969). In fact, evolutionary, anatomical, physiological and environmental differences between the two classes, suggest that birds may not control their respiration in the same way as mammals.

1.2 Special Avian Demands

A partly aerial existance, coupled with the high energy requirements of flight (Tucker, 1968a, b, 1973, 1980), causes birds to require a very efficient oxygen extraction system. This is highlighted by the fact that they possess the most efficient respiratory system of all animals (King and Molony, 1971). It is reported (Tucker, 1968a, b) that a bird in flight can require up to 21 times as much oxygen than while at rest. Non-flying birds and birds whose flying capabilities are limited, have a less well developed respiratory system and if sustained flight activity is undertaken, an oxygen debt develops rapidly. This point illustrates that such an elaborate respiratory system is a direct reflection of the in-flight oxygen requirements.

Torre-Bueno (1977) carried out experimental analysis of in-flight respiration on starlings. The metabolic rate was measured by recording gas compositions and carbon dioxide production in the anterior and posterior air sacs during flight. It was found that the metabolic rate was 8.9 times

greater, and volume of gas moved by the system 8.2 times greater, in flight than at rest. However, most importantly, the proportion of oxygen extracted remained the same in flight as it was during rest. These facts demonstrate that the increased oxygen demand is met by moving more air, and not by an increased oxygen transport efficiency across the exchange surface. From this, it can be inferred that it is the peripheral and central chemoreceptors and their link with the brainstem control mechanisms, which cause the increase in gas movement to meet the in-flight oxygen requirements.

Besides the energy requirements of flight, birds exist at altitudes where similar size mammals would be in hypoxic collapse (Altland, 1961 and Colacino et al , 1977). So, not only do birds require more oxygen, but there is less available to them due to the rarefied atmosphere.

Many species of birds dive, and much work has been conducted on bradycardia and other respiratory reflexes, during and after diving (Orr and Watson, 1913; Feihl and Falkaur, 1963; Andersen and Lovo, 1964; Butler and Jones, 1968; Jones and Purves, 1970a and Jones, 1973) One important observation to come out of this work was that carbon dioxide acted as a respiratory supressor in birds, unlike in diving mammals where it stimulates respiration. However, it was postulated (Andersen and Lovo, 1964) that this did not happen when

partial pressures of carbon dioxide were as low as they naturally occured (Andersen, 1959). It was pointed out (Jones, 1976) that depression of medullary output, due to hypercapnea, was mediated not by peripheral, but by central brain chemoreceptors - another contrast with the mammalian system.

Vocalisation is an important part of the behaviour of most birds. Some of the demands made by it are reflected in the anatomy and control of the respiratory system. Rapid mini-breaths described by Calder (1970) involve very fast movements of air, which infer high frequency respiratory muscle oscillations.

Much work has been conducted into the central control of vocalisation and this has been the subject of several reviews (eg: Nottebohm, 1975; Brackenbury, 1980a and Seller, 1983). The Nucleus intercollicularis (ICo) has a central role in voice production, however its exact function has been questioned recently. It is known that the syrinx is innervated via pathways from the Nucleus medianus (Nottebohm et al , 1976). It has been suggested (Seller, 1981) that the ICo is a "command centre" innervating the syrinx via the Nucleus medianus and the brainstem respiratory centres via an as yet, unknown route. The syringeal muscles and the respiratory muscles would thus be coordinated in such a way as to produce what is recognised as vocalisation. This idea

is further supported by work (Manogue and Paton, 1982) showing gating in the hypoglossal nerve innervating the syrinx. More hypoglossal nerve fibres were found to fire during expiration than during inspiration. At the very least, this indicates the presence of a feedback mechanism from the respiratory centres to the hypoglossal motor nucleus.

Birds also put demands on their respiratory muscles to move air at high speeds during thermal regulation (Ramirez and Bernstein, 1976). Regions in the midbrain eliciting polypnoea responses have been identified (Richards, 1970a, 1971a) in the pigeon. The responses elicited consisted of a 7-20 fold acceleration of respiratory rate and a decrease in depth. Respiratory rate increases of this order of magnitude have only previously been observed naturally in birds subjected to severe heat stress (Richards, 1970b). It would therefore seem logical to conclude that the region of study (Richards, 1970a and 1971a) was concerned with thermal regulation, and respiratory rate was only seen to increase as a result of innervation of pathways to the brainstem respiratory centres. Equally, it would seen unlikely, especially in the light of the current study, that this area is analogous to the mammalian pneumotaxic centre as proposed by Richards (1971a). Indeed, it is more probable that the area is analogous to the proposed vocalisation command centre (Seller, 1981). If this is so, routes between it and

the brainstem respiratory centres are yet to be identified. Fig1 summarises the peripheral mechanisms which effect the central respiratory rhythm generator in birds.

1.3 Avian Respiratory Anatomy and Mechanics

The mammalian respiratory system is anatomically and functionally quite simple; air is sucked from the atmosphere to inflate two bags, where gases are exchanged, after which it is forced back into the atmosphere. In birds, the system is known to be a good deal more complex, since it is very well researched and understood. The degree of specialisation and complexity of the avian respiratory system, is a direct reflection of the demands placed upon it by the behaviour of the bird.

1.31 The Lung Air-sac System

The anatomy of the avian lung air-sac system has been studied in detail and descriptions of the system are available for many species, including the turkey (Rigdon et al , 1959 and Cover, 1953) and the Pekin duck (Rigdon, 1954). A comparison of the systems found in the duck, fowl and pigeon was presented by Akester (1960). Generalised accounts are also available (Schmidt-Nielson, 1972, 1975a, Gordon, 1972) and a diagram of the system is shown in Fig2.

The route of air through this complex system has been a controversial matter since early work on the subject by Soum

Fig 1 Schematic diagram of the peripheral factors influencing the brainstem respiratory regions. These are:- lung mechano-receptors, lung chemoreceptors, peripheral systemic chemo-receptors, central chemoreceptors, temperature receptors in the spinal cord and the thalamus and vocalisation controlling centres such as the Nucleus intercollicularis.

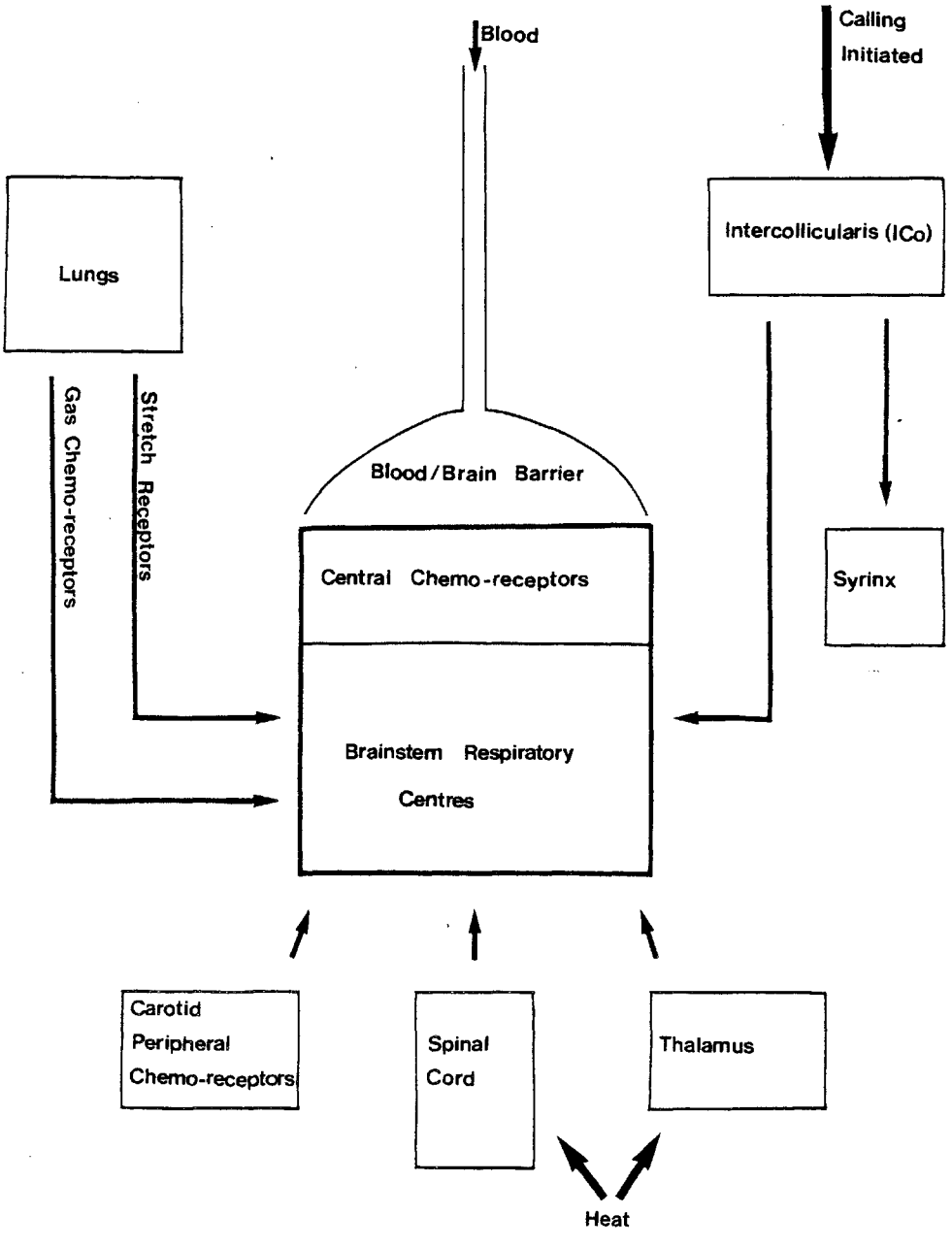
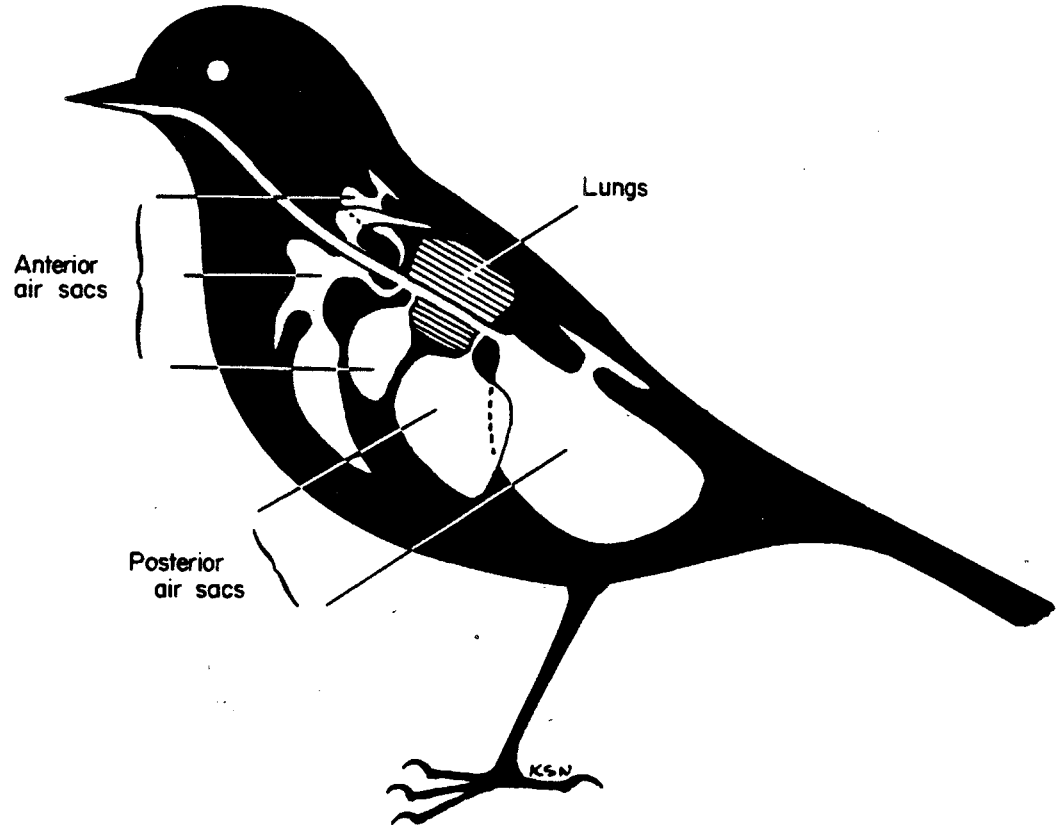

Blood
Calling Initiated
Lungs
Intercollicularis (ICo)
Gas Chemo-receptors
Stretch Receptors
Blood/Brain Barrier
Central Chemo-receptors
Syrinx
Brainstem Respiratory Centres
Carotid Peripheral Chemo-receptors
Spinal Cord
Thalamus
Heat

Fig 2 The respiratory system of birds contains two functional groups of air sacs - the anterior (cranial) and the posterior (caudal). Compared with mammals, the lungs are relatively small and inexpandable. (From Schmidt-Nielson, 1972).


Lungs
Anterior
air sacs
Posterior
air sacs
KSN

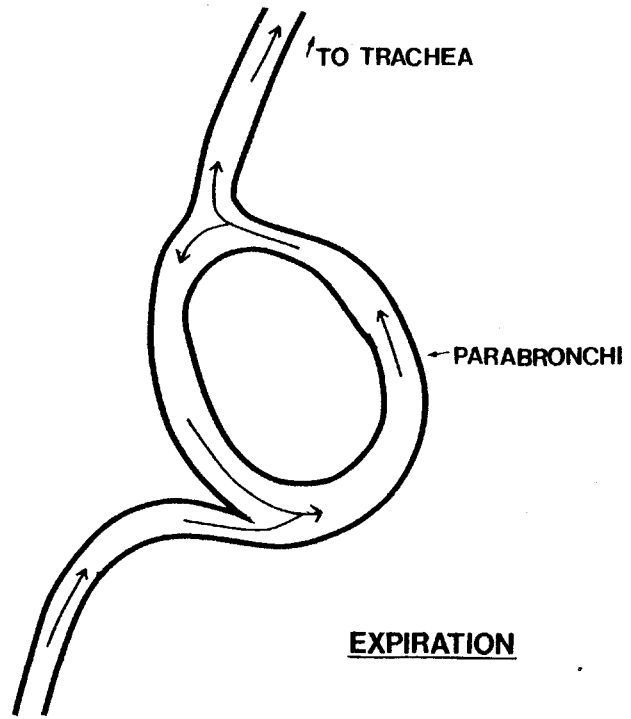
(1896). The work to date has been reviewed recently by Brackenbury (1980b). The idea of unidirectional gas flow over the parabronchi was first suggested by Brandes (1923, 1924) and Bethe (1925). They maintained that gas flowed in a "dorsobronchi to ventrobronchi" direction across the parabronchi during both inspiration and expiration. Later, with the use of glass models of the lung, (Dotterweich, 1936) it was proposed that valving of air was an aerodynamic process and that anatomical valves played no part in gas routing.

Hazelhoff (1943, 1951) conducted work using charcoal dust carried in air and formulated the well known "Hazelhoff Loop" (Fig3). He found that when he injected this smoke into the caudal air-sacs of dead animals (which mimicked expiration), the dorsobronchi became most laden with soot. When smoke was injected into the trachea (which mimicked inspiration), the dorsobronchi again bore soot deposits. Hazelhoff deduced from this that aerodynamic forces, generated in the mesobronchus, caused air to be directed into the dorsobronchi during both inspiration and expiration. He proposed a loop:-

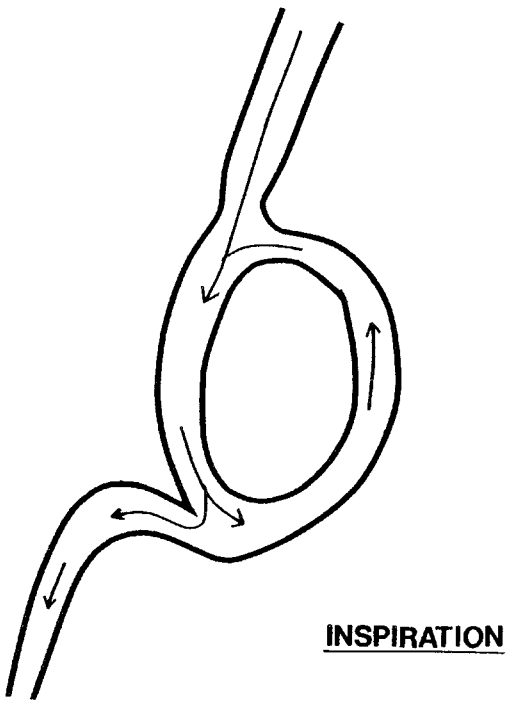
mesobronchus - dorsobronchi - parabronchi -
ventrobronchi - mesobronchus.

In other experiments, Hazelhoff used starch suspensions in a syringe attached to the trachea and was able to simulate

Fig 3 Hazelhoff proposed a unidirectional air route in the respiratory system of birds. During both inspiration and expiration, air is aerodynamically routed so that it always flows in the same direction across the exchange surface (parabronchi). (Modified from Hazelhoff, 1943).



EXPIRATION



INSPIRATION

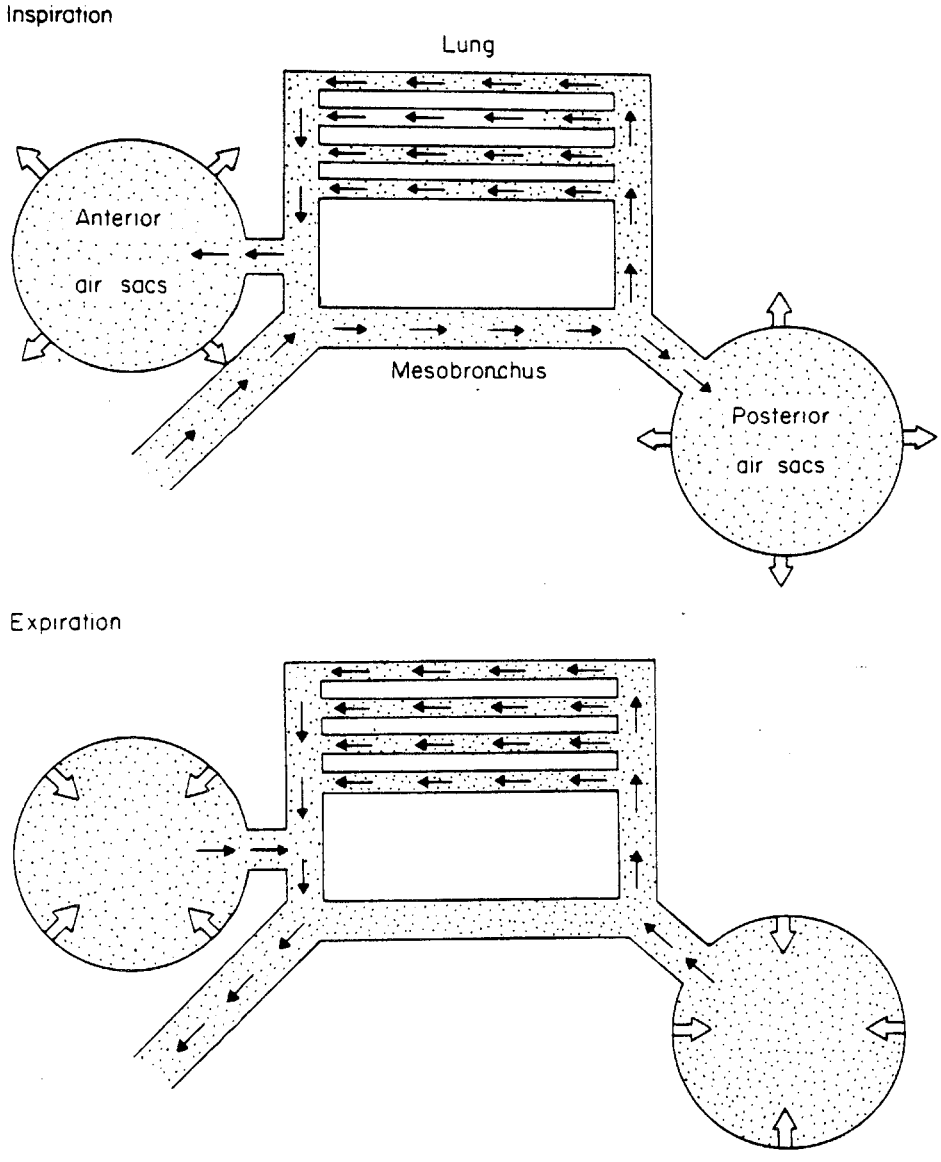
inspiration and expiration while examining the exact flow route of the starch.

Brackenbury (1971) confirmed that a unidirectional (dorsobronchi to ventrobronchi) gas flow over the parabronchi also took place in spontaneously breathing birds.

The gas compositions in the air-sacs have been examined (Shepard et al , 1959; Schmidt-Nielson et al , 1969; Jammes and Bouverot, 1975 and Torre-Bueno, 1977) and this has helped to highlight the importance of the air-sacs in the functioning of the loop (Fig4). It appears from this work that, during inspiration, the caudal air-sacs suck in a mixture of dead space gas and fresh air from the mesobronchus. At the same time, the cranial air-sacs draw used air out of the parabronchi and it is replaced with fresh air from the mesobronchus. When expiration occurs, the relatively fresh air mixture being stored in the caudal air-sacs is forced into the dorsobronchi and aerates the parabronchi before re-entering the mesobronchus. In the mesobronchus, it joins stale air from the collapsing cranial air sacs and is expelled.

It should be mentioned that some recent studies have questioned the functioning of the Hazelhoff loop. Bretz and Schmidt-Nielson (1971) found air to pass in both directions in the mesobronchus and the ventrobronchi. If the caudal

Fig 4 Diagram showing the use of air sacs in the mechanics of air flow in the avian respiratory system. The most essential feature is the unidirectional flow of air through the lung in the anterior direction during both inspiration and expiration. The air sacs fill with air during inspiration and this is pumped over the exchange surface during expiration so that a continuous current is maintained. (From Schmidt-Nielson, 1975b).


Inspiration
Lung
Anterior
air sacs
Mesobronchus
Posterior
air sacs
Expiration

air sacs were ruptured (Brackenbury, 1979) and a main air stream was applied from the trachea to the air-sacs, air was found to flow from the mesobronchus to the dorsobronchi. However, when the main stream was reversed, so that it flowed from the caudal air-sacs to the trachea, air was found to flow in the opposite direction, from the dorsobronchi to the mesobronchus. If the caudal air-sacs were then resealed, air flowed from the mesobronchus to the dorsobronchi regardless of the direction of the main stream. This study stressed the importance of the air sacs in the valving of air over the parabronchi.

1.32 Musclature

It is well known that in mammals, inflation and deflation of the lungs is performed, in the main, by the external and internal intercostal muscles acting with the diaphragm (Campbell et al , 1970). Since birds possess no diaphragm, the way in which gas is pumped in and out of the respiratory system will exhibit differences.

The pectoral muscles and the ventral and dorsal intercostal muscles were examined for activity during inspiration and expiration (Burkart and Bucher, 1961). This work demonstrated that there was activity in the muscles during expiration, especially towards the end of the cycle, as well as during inspiration. Expiration was thus demonstrated to be an active process.

De Wet et al (1967) went on to show exactly which muscles were resposible for inspiration and expiration. Their findings together with other work are presented in the review by King and Molony (1971).

Electromyography was conducted on the respiratory muscles of the fowl (Fedde et al , 1969) and ideas of how motor units were recruited were postulated. They found that 0-4% carbon dioxide in the ventilatory gas produced apnoea in the Transverse Abdominus muscle, during which electrical activity was present in both the muscle and its efferent nerve. 5-14% carbon dioxide caused phasic contraction of the muscle, an increase in electrical activity and selective recruitment of other motor units. The study thus proved that this muscle was directly concerned with respiration.

1.4 Mammalian Brainstem Respiratory Control

Mammalian brainstem respiratory control has been the subject of much research and discussion since the middle of the last century. As a consequence of this interest, the respiratory areas are now well defined in many species and ideas are developing as to how the centres operate on a neuronal basis. This work provided essential background for the present study and a brief review of the work is therefore relevant.

1.41 The Pontine Pneumotaxic Centre

Hering and Breuer (1868 and Breuer 1868; see Chapter 3) began work which was to show that respiratory rhythmicity was partly controlled by a feedback loop, via the vagus nerve, from mechanoreceptors in the lung. As the animal inhaled, an increasing inhibition of inspiration occurred and expiration was stimulated: the reverse was true during expiration. However, when the vagus link was cut, rhythmic respiration, although slightly modified, continued to take place. It was believed that a central rhythm generating mechanism, or pneumotaxic centre, was now setting the respiratory pace.

It is a well established fact (Liljestrand, 1958) that the cortex, cerebellum and the anterior part of the brainstem, are not necessary for normal respiratory rhythmicity. However, some higher centres, if not vital, do have some influence on the rate and depth of respiration (Allen, 1927). The pneumotaxic centre was, by ablation of the cortex, cerebellum and anterior brainstem, localised to the posterior brainstem.

By transecting the posterior brainstem, Lumsden (1923a, b) found that a border line of transection, beyond which respiratory rhythmicity was destroyed, existed near the anterior border of the Pons. Transection at this level caused inspiratory cramp (apneusis). After some time, the

animal was reported to start apneustic breathing (gasping with the inspiratory phase predominating). Lumsden (1923a) concluded from these results, that the upper Pons contained a pneumotaxic centre, and that this inhibited an apneustic centre, so allowing normal breathing to take place.

Transecting the brainstem in cats (Tang, 1953) was reported to eliminate normal respiratory periodicity. However, after vagotomy, it was found that the level of the transection which eliminated normal respiration had changed - periodicity was no longer under vagal influence and more of the brainstem was now required, since it alone was controlling rhythm generation. In order to eliminate peripheral feedback, animals were routinely vagotomised before ablation and transection studies.

The pneumotaxic centre has been localised further to the tegmentum of the upper few millimetres of the Pons. The area has also been found to be larger, and electrical stimulation of the cat brain (Ngai and Wang, 1957) has extended the region to parts of the dorsolateral Reticular Formation. This area includes the Locus Coeruleus (Russell, 1955), which was examined using electrolytic lesions (Johnson and Russell, 1952). Electrical stimulation of the Locus Coeruleus (Johnson and Russell, 1952) gave inconclusive results, producing both inspiratory and expiratory pauses in respiration. Ngai and Wang (1957)

obtained accelerations in respiratory rate whatever stimulus parameters they used. This contradicts work by Johnson and Russell (1952) who reported that only a low frequency stimulus elicited such responses. These diverse results have been attributed to current spread (Liljestrand, 1958). Wang et al (1957) suggested that the apneustic centre was responsible for rate setting and that its effect could be modified by input from the pulmonary stretch receptors and from the pneumotaxic centre.

Salmoiraghi and Burns (1960) recorded from the brainstem and obtained rhythmic output in the absence of vagal input, demonstrating the presence of a central rhythm generator. However, they found no evidence for respiratory pacemaker neurones. It is reasonable to assume from this work, that populations of neurones, and not single cells, are responsible for rhythmicity.

Pathways were located linking the pontine regions discussed with areas in the Medulla (Ngai and Wang, 1957).

1.42 Medullary Inspiratory and Expiratory Centres

When Lumsden (1923a) transected the brainstem just caudal to the Striae Acousticae, the apneustic respiration changed to gasping with neither respiratory phase predominating. He concluded that the Striae Acousticae contained an "apneustic" centre and that a gasping centre was present in

the rostral Medulla. He later identified an expiratory centre too (Lumsden, 1923b). The apneustic centre reported by Lumsden, appears to correspond with the inspiratory centre later identified by Pitts et al (1939a, b). When they electrically stimulated a small part of the Reticular Formation, respiration was arrested in the inspiratory position. This was termed the "inspiratory centre" and just rostral to it was located the "expiratory centre". This work confirmed earlier ideas about the location of the respiratory centres by Henderson and Sweet (1929). The fact that these results were obtained many times in the cat and other species such as sheep (Amoroso et al , 1951) and the albino rat (Ondina et al , 1960), and were invariable, continued to support the existence of the centres.

The dynamics and physical characteristics of the apnoeas and apneusis elicited, were examined in detail in the cat (Breckenridge and Hoff, 1950) and the dog (Hoff and Breckenridge, 1949). In both species apneusis was reported to be temporary, but this could have been due to system fatigue during stimulation. In the dog, apneusis was seen to be superimposed on normal respiratory periodicity - this fact may help in future evaluations of the functioning of the system.

In conclusion, it appears that the respiratory rhythm is maintained and modulated both by the pneumotaxic centre and

vagal afferents from lung mechanoreceptors. This rhythm generator exerts control on the medullary inspiratory and expiratory centres which cause the respiratory muscles to produce rhythmic oscillations.

1.43 Recent Advances

Recent studies into central respiratory mechanisms in mammals have tended to concentrate at a neuronal level, both anatomically and functionally.

Berger (1981) was able to divide the medullary neurones into groups according to their intrinsic and extrinsic properties. On examining their anatomy, two separately defined populations became apparent. These were represented by neurones in the ventro-lateral nucleus of the Tractus Solitarius (Madden and Remmers, 1982) in the dorsomedial Medulla (dorsal respiratory group) and by those in the Nucleus Ambiguus and Retroambigualis. Kalia (1981) used horseradish peroxidase (HRP) tracing techniques in order to relate anatomy with the function of neuronal populations. Extrinsic properties (Berger, 1981) revealed a variety of firing patterns which led to the production of a model including elements of reciprocal inhibition as well as recurrent inhibition and excitation.

Cohen (1981) proposed, like Berger, that reciprocal and recurrent inhibition could be responsible, wholly or in

part, for rhythm generation. Phase switching mechanisims were suggested for both inspiration to expiration and expiration to inspiration. Cohen (loc. cit.) suggested that the inspiration to expiration switch was activated by Beta inspiratory neurones which received information from the central rhythm generator as well as from the pulmonary stretch receptors. An "off switch" neurone population was brought to threshold by input from the Beta inspiratory neurones during inspiration. This "off switch" terminated inspiration and, probably by means of recurrent excitation, initiated expiration.

About 5% of neurones in the dorsal respiratory group (DRG) (Berger, 1981) have activity which predominates at the start of expiration. They start to discharge as phrenic activity falls and reach a peak in early expiration, after which their activity decays. If the time for expiration is lengthened, the rate of decay of discharge of the DRG neurones slows. It was postulated that the DRG neurones, when activated, inhibit the succeeding inspiration by preventing the "expiration to inspiration" switch from operating. If expiration is slow, the DRG's activity is prolonged and inspiration is thus delayed. Other groups of neurones, which may contribute to the "expiration to inspiration" switch, were described.

Feldman (1981) proposed models in which he suggested

inclusions of both "Threshold Phase Timer" and "Phase Timer" ramp generators. He proposed that phasic inspiratory output could be used in two ways to activate respiratory phase switches.

Fig5a shows a threshold phase termination ramp generator. The phasic inspiratory activity is fed to the inspiratory ramp. The ramp gradient depends on variables such as carbon dioxide tension and so therefore does the inspiratory period. Pulmonary stretch receptor input feeds the inspiratory threshold terminator and causes it to be lowered and thus threshold is reached faster. This causes the switch to expiration to follow sooner and consequently respiratory rate increases.

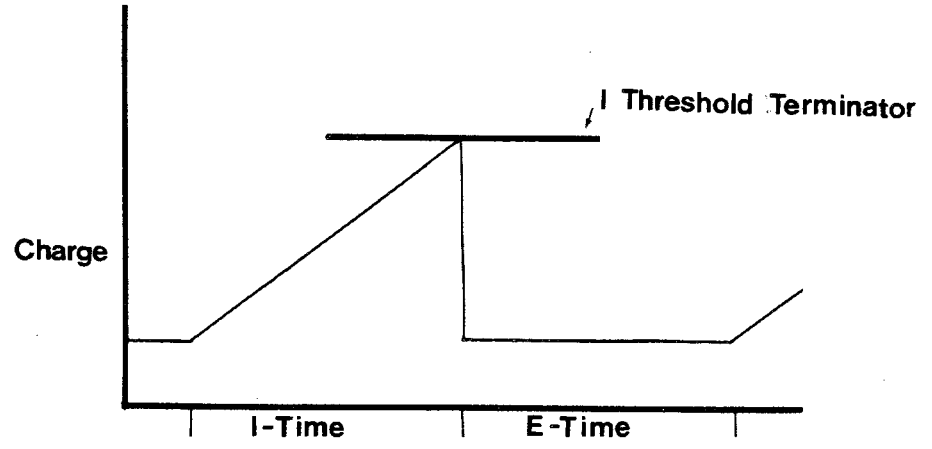
Fig5b shows a phase timer ramp generator. At the end of expiration, the inspiratory timer is released from inhibition and, at the same time, generation of the ramp starts. After a time, which is variable according to carbon dioxide tension and other variables, inspiration is turned off and expiration is excited irrespective of the position of the ramp.

Merrill (1981) collected information about all types of medullary neurones so far reported, from which it was possible to construct the plan shown in Fig6. His ideas did not support the hypothesis that stable respiratory oscillations can be provided by the medullary neurones

Fig 5 A. Diagram of a threshold phase termination ramp generator. During inspiration the ramp climbs towards a pre-set charge threshold. At this inspiratory threshold terminator, discharge occurs and inspiration is terminated. Termination of inspiration may be accompanied by excitation of expiration, or expiration may merely be released from inhibition.

B. Diagram of a phase termination ramp generator. During inspiration the ramp climbs, as in A, but discharge occurs at the end of inspiration irrespective of any threshold charge being reached. Expiration and inspiration are timed by independent timers in this model. As before, expiration is either excited or released from inhibition.

A


I Threshold Terminator
Charge
I-Time
E-Time

B

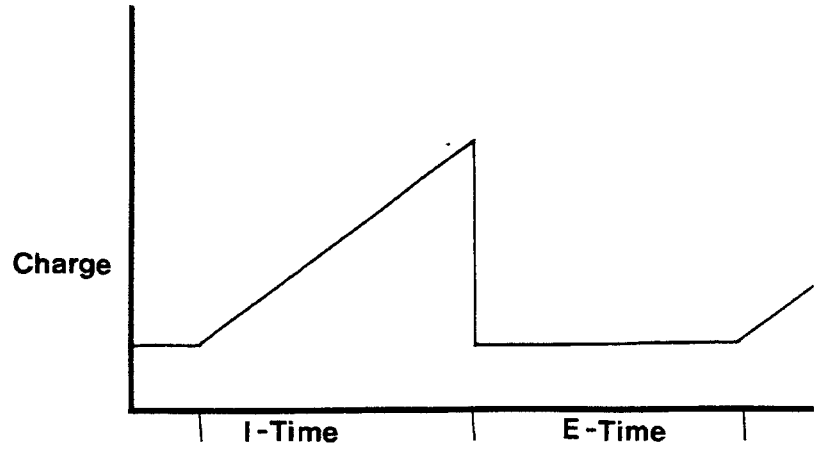
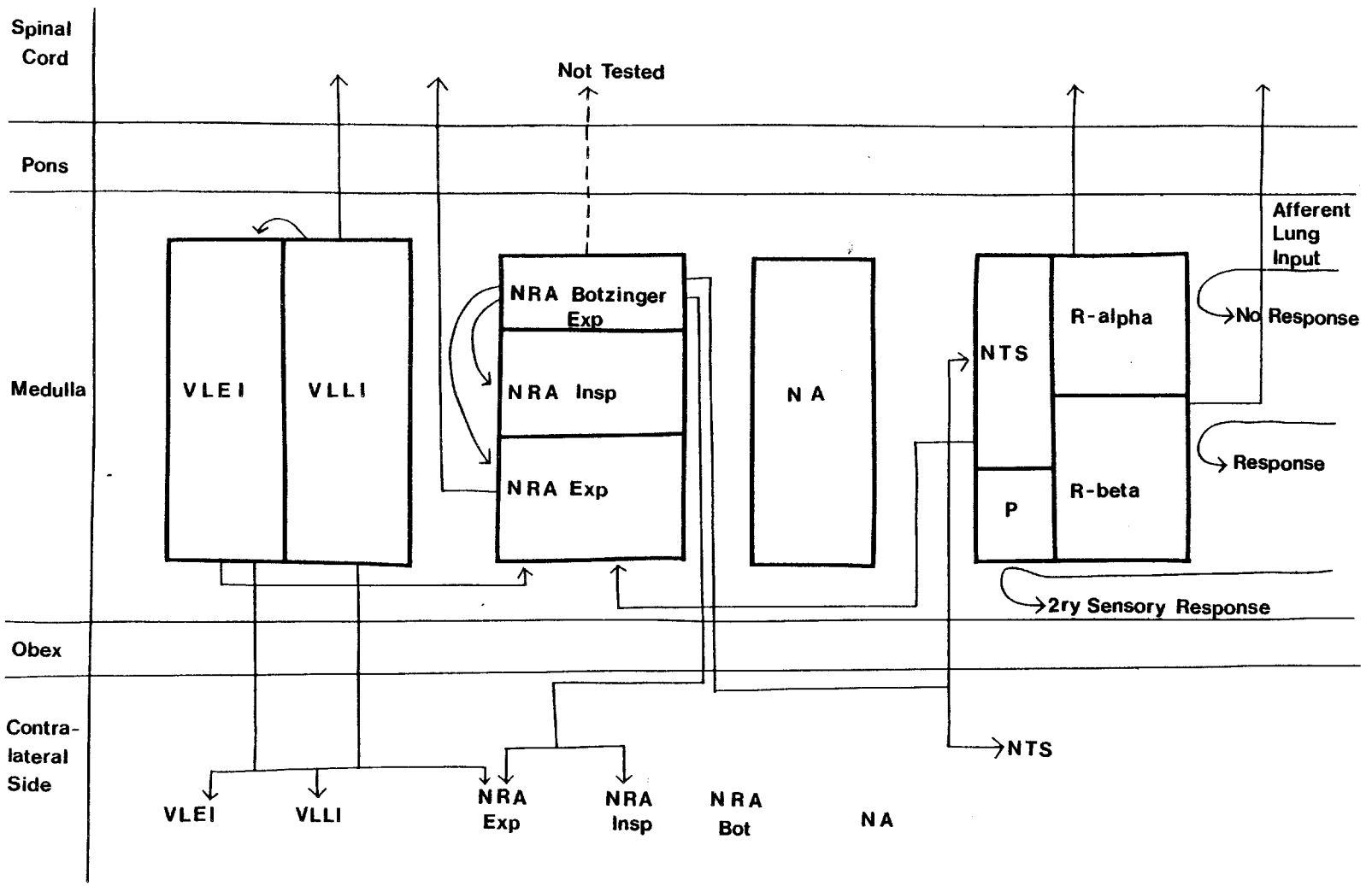

Charge
I-Time
E-Time

Fig 6 Diagram summarising the pathways between populations of respiratory neurones in the mammalian brainstem. (Based on Merrill, 1981). The dorsal group population contains four sub-populations of neurones:- the NTS, R-alpha, R-beta and "P" cells. It is this population which receives afferents from lung stretch receptors (Hering-Breuer reflex). Abbreviations: Bot Botzinger Complex; NA Nucleus ambiguus; NRA Nucleus retroambigualis; NTS Nucleus of the solitary tract; VLEI Ventrolateral early inspiratory; VLLI ventrolateral late inspiratory.


Spinal Cord
Pons
Medulla
Obex
Contra-lateral Side
Not Tested
VLEI
VLLI
NRA Botzinger Exp
NRA Insp
NRA Exp
NA
NTS
R-alpha
P
R-beta
Afferent Lung Input
No Response
Response
2ry Sensory Response
NTS
VLEI
VLLI
NRA Exp
NRA Insp
NRA Bot
NA

already known to exist. He proposed that to date, extracellular recording methods have failed to recognise important aspects of the medullary respiratory mechanism.

In spite of years of extensive work in the subject, neuronal interaction in mammalian brainstem respiratory control is still not wholly understood. Generation of the respiratory rhythm has recently been reviewed by Richter (1982).

Chapter 2

Experimental Methods

2.1 Animals

Japanese Quail (Coturnix c. japonica) of 2 - 8 months old were used in the study. Birds of both sexes were purchased from a breeding colony (Houghton Poultry Research Station) in batches of 40 at about six weeks old.

The quail were kept in wire mesh cages (60 X 45 X 35cm). They were divided by sex and not more than six birds were put into each cage. Food and water were available from arrival and all animals received the same diet. The fact that calorific value of food influences the body temperature (Robinson and Lee, 1947) was thus eliminated. The cages were kept in a thermostatically controlled room with the temperature maintained at 21^{o} C. The birds were not used for at least three days after arrival; by then their body temperature had stabilised and they had recovered from the shock of their journey. As far as possible, birds were not used until a body weight of 120g was attained.

Before each experiment, the animal was starved for 4 - 5 hours to help prevent choking while under anaesthesia.

2.2 Anaesthesia and preparation

As has been discussed (see Chapter 1) birds have a more complex respiratory system than mammals and this needs to be taken into consideration when selecting an anaesthetic. The presence of air sacs can lead to "pooling" of anaesthetic and consequently delayed recovery and responsiveness to changes of the anaesthetic mixture ratio. The unidirectional gas flow over the parabronchi (Hazelhoff, 1943, 1951) can lead to too much anaesthetic in the blood, since more diffusion across the exchange surface is permitted. This flow system also has an advantage, in that induction is faster.

Halothane (Fluothane; ICI Ltd) is a very successful avian anaesthetic (Marley and Payne, 1964) and was used during all operations. The anaesthetic technique was as described by Marley and Payne (1962, 1964). Since oxygen was used as the carrier gas the respiratory rate was slightly depressed due to hyperoxia (see Chapter 3). This had no detectable adverse effects on the experimental results and made changes in respiratory rate easier to monitor.

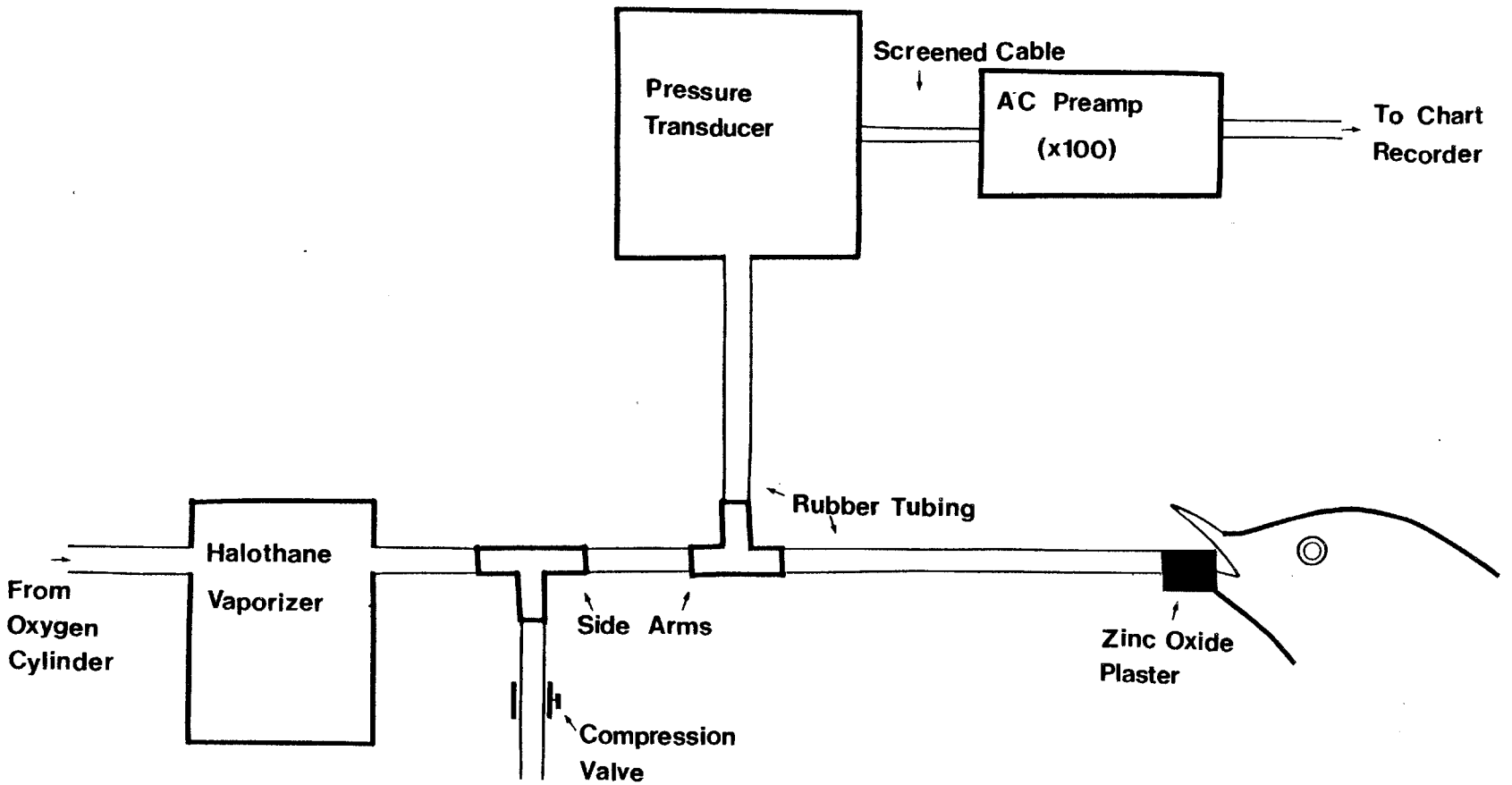
The oxygen was supplied from a 48cu.ft. cylinder (BOC), via a pressure valve and flow meter (BOC; 15-litres per min max) and rubber tubing to the vaporizer. The anaesthetic was fed into the carrier gas from a Goldman Halothane vaporizer (BOC) with a calibrated supply from zero to three percent of

Halothane. In order to prevent pressure from building up in the system, a variable leak was provided to allow excess gas to escape. This consisted of a right-angle branch junction to which was attached a small length of rubber tube with a compression valve for varying the flow rate of the dumped gas. A second right-angle branch was connected via rubber tubing to a pressure transducer for monitoring the respiratory cycle (see 2.3). The Halothane mixture was delivered to the quail via rubber tubing which was taped over the tongue and mandible with zinc oxide plaster (Fig7). Since the larynx of quail is mounted on the back of the tongue, the end of the pipe could be placed very close to the tracheal opening.

Induction was achieved with an oxygen flow rate of about 1 litre per minute, containing three percent of Halothane. After about two minutes surgical anaesthesia was reached and the Halothane level was reduced to 1.5-2.5 percent of the mixture, and maintained constant for the rest of the experiment. During experiments in which thermal polypnoea was induced, the Halothane level had to be further reduced to less than one percent in order to prevent over-anaesthesia and death.

Since anaesthesia had a cooling effect and this in turn affected respiration (see Chapter 6), temperature was continually monitored and regulated using a thermostatically

Fig 7 Schematic diagram showing the system used for administering Halothane anaesthesia and for monitoring the respiratory cycle. Pressure build up in the system was eliminated by means of a side arm dump with a variable compression valve. The output of the pressure transducer was amplified 100 times so that it was large enough to produce a reasonable trace on the chart recorder.


Pressure
Transducer
Screened Cable
A.C. Preamp
(x100)
To Chart
Recorder
Rubber Tubing
Halothane
Vaporizer
From
Oxygen
Cylinder
Side Arms
Zinc Oxide
Plaster
Compression
Valve

controlled animal electric blanket (Harvard). The blanket system consisted of a rectal probe for monitoring core temperature, a control box with a temperature meter, and a plastic covered low voltage electric blanket. Control body temperature of quail was measured as 40.45^{o} C (see Chapter 6) and this was maintained throughout all experiments except those in which the effects of temperature change were of interest.

Prior to both recording and stimulatory experiments the right vagus nerve was transected in order to give a slower and deeper respiration (see Chapter 3). The vagi in quail run adjacent to the jugular vein, so locating them presented little problem. Once exposed, the right vagus nerve was carefully separated from the jugular vein for about 1.5cm in the cervical region. It was then transected at about the level of the second cervical vertebra. A deeper respiration resulted in better operation of the transducer and a decrease in respiratory rate enabled the respiratory cycle to be followed more accurately.

2.3 Respiratory rate and depth monitoring

Pressure changes in the anaesthetic supply were used to monitor respiratory rate and depth. A branch pipe close to the animal, led via rubber tubing to a Mercury pressure transducer. The time separating two adjacent positive or negative pressure peaks represented the inter-breath

interval, from which the instantaneous respiratory rate could easily be calculated. Since the transducer monitored pressure, it was necessary to know if the size of its output bore any relation to respiratory depth.

Pressure (P) is proportional to the volume (V) of a gas passing a given point per second (t):-

P is proportional V/t

From this:-

V is proportional to Pt

where V is the volume of one breath and t, the time for which a pressure P is maintained (ie: the time used for inhalation and exhalation of one breath).

The volume of several breaths (nV) is therefore proportional to the average pressure (Pav), multiplied by the average time for which that pressure is sustained (tav) multiplied by the number of breaths (n):-

nV is proportional to Pav X tav X n

So the size of the transducer's output (magnitude of pressure) can be said to be proportional to volumes of breaths as long as the pressure continues to change (ie: the animal continues to breath). However, the pressures

registered by the transducer were only relative to each other in magnitude within the same experiment and real values of pressure and capacity were not measured. This was due to variables such as leakage from the bird's mouth, slightly differing oxygen pressures and leakage from the dump valve. Even so, real values of respiratory rate and indications of relative respiratory depth were available from the output of the transducer.

The transducer's output was amplified 100 times using an a.c. preamplifier (CFP 8121). This output was fed to both an instantaneous rate meter (Neurolog NL250; Digitimer) and one channel of a hot wire pen recorder (Lectromed MX 212). The output from the rate meter could be fed to a second chart recorder channel if required. A rapidly updated digital display of instantaneous rate was always available from the rate meter. A third pen on the chart recorder gave a time trace. A sample of the sort of trace produced is illustrated in Fig8.

2.4 Stereotactic electrode implantation

A Stoelting Stellar Stereotactic Instrument (No.51400M; Fig9), designed for small rodents, was used in the study. The stereotactic atlas prepared for quail by Bayle et al (1974) was referred to in conjunction with the Karten and Hodos pigeon atlas (1967) in order to ascertain rough coodinates for likely respiratory areas. Coordinates in the

Fig 8 (Plate 1) A typical chart record being produced. Traces (left to right):- time marked off in seconds, respiratory rate, respiratory cycle, marker showing the stimulatory period (see 2.5).

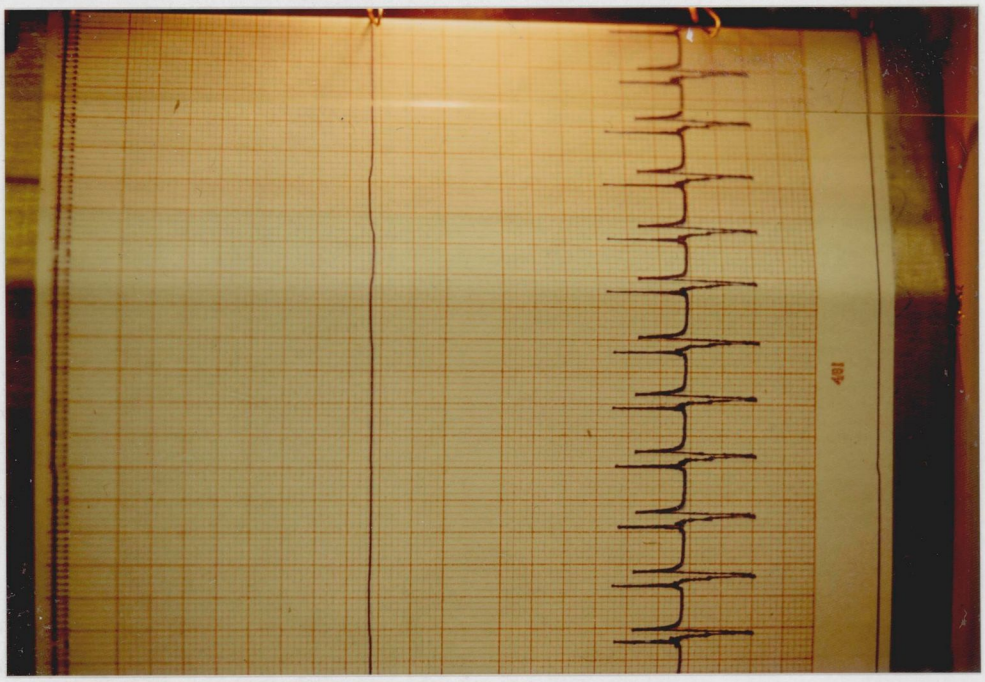
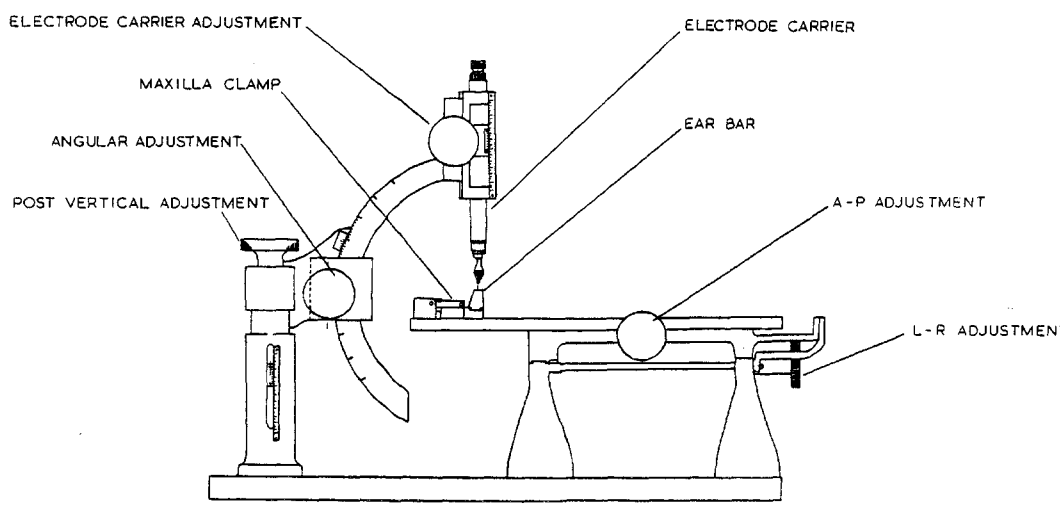


Fig 9 Side elevation of Stoelting Stellar Stereotactic Instrument (No. 51400M).


ELECTRODE CARRIER ADJUSTMENT
MAXILLA CLAMP
ANGULAR ADJUSTMENT
POST VERTICAL ADJUSTMENT
ELECTRODE CARRIER
EAR BAR
A-P ADJUSTMENT
L-R ADJUSTMENT

ranges of anterio-posterior 4 to 4.5; lateral 0.5 to 1.2 and height -1 to 3mm were used for most electrode implants.

The electrodes for all experiments were made from short lengths of 0.076mm diameter insulated platinum wire (Grade 4 PTM Trimmel; Johnson and Matthey Metals Ltd). One end of the wire was held over a lighted match to burn off 1-1.5cm of insulation. This end was then scraped clean with a scalpel and doubled back on itself to provide a double portion of exposed platinum for mounting in the chuck of the electrode carrier. For all stimulatory experiments the standard electrode carrier was used. However, due to voltage drop in the cable, a X1 head stage amplifier combined with electrode holder (Neurolog NL100; Digitimer) was used when recording from the brain (see 2.6). The other end of the electrode was cut off with scissors so that the base only was exposed (ie: a circle 0.0045mm2 in surface area). The electrode carrier or head stage was then added to the stereotactic instrument. By moving the controls, the electrode tip was centred above the small hole in the table between the ear bars, at the height of the centre of the ear bars. The electrode tip was at that stage positioned at height, anterio-posterior and lateral coordinates zero, with respect to the animal. Readings from the instrument scales of all three coordinates were noted as correction factors to be added or subtracted from the desired implant coordinates, to give real implant readings on the stereotactic instrument

scales. The vertical post assembly was then swung away together with the electrode carrier in order to facilitate operative procedures.

The quail was anaesthetised as previously described and placed on the stereotactic instrument table. The rate meter and chart recorder were switched on to provide a respiratory chart record and rate reading (see 2.3). The ear clips were inserted into the external auditory meati and agitated slightly until firmly seated. The head was centred so that both ear bar scales gave the same reading; this was normally between 9 and 10mm. The bird was then wrapped in the electric blanket and the temperature probe was inserted (see 2.2).

Feathers were cut away from the dorsal surface of the head and for about 1-2cm down the neck. An incision was made along the dorsal midline of the scalp from a point between the eyes extending for about 1.5cm down the neck. The periosteum and some neck musclature were scraped off using a periosteal elevator. Blood was wiped away with cotton wool and bleeding was quenched with a cautery (R.B. Model 708 FP74).

The two beak bars were positioned, one above and one below the maxilla and by adjusting their height, the dorsal surface of the exposed cranium was levelled. Once the cranium was as horizontal as possible, the two bars were

tightened against each other holding the maxilla firmly between them and preventing the head from rocking about the ear bars.

Using the lateral control on the stereotactic instrument, the electrode was placed over the midline of the cranium and a new lateral zero reading was noted, from which the real implant coordinate was calculated. The electrode carrier was swung back into position and the anterio-posterior and lateral controls were set to the calculated coordinates. Using a felt tip pen, a small dot was drawn on the cranium, marking the implant position. The electrode carrier was swung away again to facilitate drilling.

Using a dental drill, a small hole was bored in the cranium at the marked position. Great care was necessary to ensure that the drill was withdrawn before reaching the brain. Usually, one or more of the three dura were still intact and these had to be penetrated to allow passage of the electrode into the brainstem without buckling. The dura were broken by inserting a suture needle carefully into the hole. Very often, profuse bleeding through the electrode hole occurred, although this usually stopped after the electrode had been implanted for a few minutes. The area immediately surrounding the electrode hole was roughened using the dental drill. This procedure ensured good bonding if the electrode were later to be cemented to the skull.

The electrode carrier was swung back into place over the cranium and the electrode was lowered through the hole until the desired height coordinate was shown on the height scale of the stereotactic instrument (Fig10).

Sometimes, the electrode was cemented into position which allowed removal of the electrode carrier and ear bars. This facilitated easy access to the animal, as well as preventing possible movement of the electrode. A quick drying, autopolymerising dental cement (Simplex Rapid; Dental Fillings Ltd) was used for this procedure.

2.5 Stimulatory Procedure

The stimulator used (Hi-Med HG203S) was designed to deliver a constant preset current up to a resistance of 10 megohms by using an automatically varying voltage. The stimulator was in two parts; one supplying the negative pulses and the other the positive pulses. The output from these two parts were connected in parallel, so that when activated by the timer, an alternating square wave current was produced.

An elaborate timer (Hi-Med) was used to activate the stimulator and was optically isolated from it. Although all parameters on the timer were variable, they were maintained at prescribed values for all experiments. A cycle length of 130 seconds was set; this consisted of 10 seconds of stimulation followed by 120 seconds of rest. After

Fig 10 (Plate 2) The head is firmly clamped between the ear bars and the stimulatory electrode has been implanted into the brainstem and cemented in at the pre-set coordinates. The indifferent electrode is connected via a crocodile clip to the ear bar furthest from the camera. One of the beak bars has been removed to allow a better view of the head.

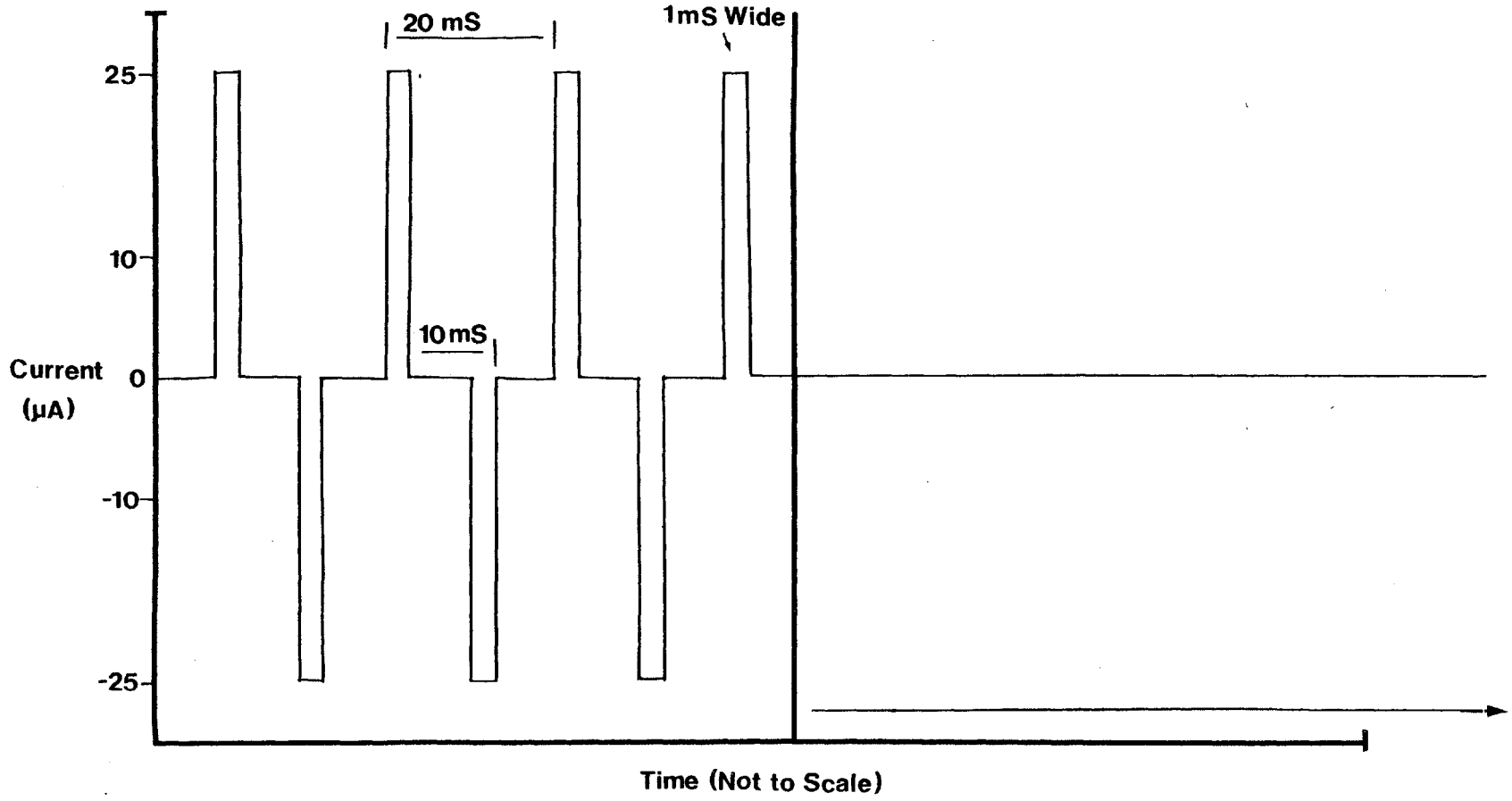


switching on, the cycle was repeated for a programmed number of times before stopping. The stimulator produced a 50Hz pulse train in which the pulses were 1 milli second in duration. Since an a.c. current was used, there was no net charge in the tissue. Positive and negative pulses were always kept equal in size and all currents quoted hereafter are peak to peak values (ie: a 50μA current would have been 25μA positive and 25μA negative). One cycle of such stimulation is shown graphically in Fig11.

As well as activating the stimulator during the stimulatory period, the timer also activated two relays. One relay produced a closed circuit for 250 milli-seconds and the other for 10 seconds (the stimulatory period). The 250 milli-second closed circuit reset a digital stop clock, so that at any stage during an experiment, it was possible to determine how far into the cycle the timer was and consequently when the next stimulatory period was due. The 10 second closed circuit operated a marker pen on the chart recorder during the stimulatory period. It was therefore possible to associate any response to the stimulus with the stimulatory period.

One lead from the stimulator was connected to the electrode via the electrode carrier and the other (indifferent electrode) was attached to one of the ear bars with a crocodile clip.

Fig 11 Graphical example of the electrical stimulus applied, here 50μA peak-to-peak at 50Hz. Pulses were of alternate polarity, 10ms apart, each 1ms long. Pulse trains continued for a maximum of 10 seconds and were applied at least 120 seconds apart. (The time axis is not to scale).


20 mS
1mS Wide
25
10
10mS
Current
(μA)
0
-10
-25
Time (Not to Scale)

When the electrode had been lowered to the desired height coordinate, the timer was reset and started. To begin with, the stimulator current was set to 50μA. Since the cycle always began with 10 seconds of stimulation, responses, if present, were immediate. Between periods of stimulation the electrode was lowered in steps of 0.2mm until a response was elicited. Once a response area was located, the electrode was moved in small steps to determine the position of the best response. When this had been found, the current was reduced in steps of 5μA in order to find the response threshold. For the purposes of finding the threshold, a response giving a change in respiratory rate or depth of equal to or greater than 10 percent was counted as significant. Any respiratory changes of less than 10 percent were disregarded.

After the threshold for the response sites had been determined, the electrode was cemented into position with dental cement (Simplex Rapid; Dental Fillings Ltd). The site was marked with a small lesion for histological identification, using a d.c. current of 1.5mA positive for 10 seconds followed by 10 seconds of negative current. Any change in the respiratory pattern following lesioning was duly noted. The birds were killed by decapitation at the end of the experiment.

Using the statistical package "MINITAB" (Pennsylvania State

University) on the College computer, graphs of respiratory rate and depth against time were plotted. Pooled results of each response type were also plotted to give average response characteristics. This made response categorising easier, as well as showing up more subtle differences between types of response.

2.6 Recording Procedure

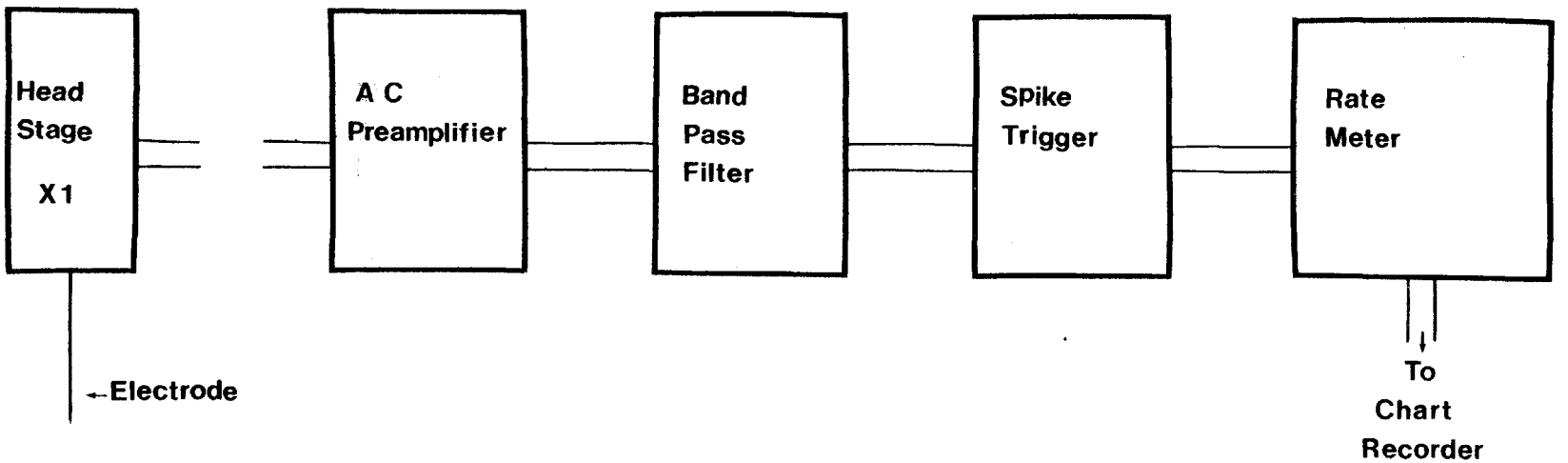
The recording experiments were carried out after the series of stimulatory implants, so there was some indication of coordinates from which good recordings were likely to be obtained. The electrodes were the same as those used in the stimulatory study and, due to their large size, it was estimated that firing patterns of up to 40 neurones were being simultaneously recorded.

The recording apparatus consisted of a Neurolog system (Digitimer), the modules of which and their layout are shown in Fig12. As mentioned previously (see 2.4) the electrode was mounted in a head stage unit (Neurolog NL100; Digitimer) which amplified the multiple unit activity by a factor of X1; this ensured that information was not lost through voltage drop in the cable.

Experiments were made inside a wire mesh Faraday cage which had a common earth line with all the electrical equipment. In this way it was ensured that the casing of each item of

Fig 12 The Neurolog system used consisted of a head stage with an amplification of X1. The output from this was fed to an a.c. preamplifier following which the spikes were filtered. The spike trigger converted spikes within a certain pre-set amplitude range into pulses of equal amplitude and duration. The rate meter counted these pulses and produced an analogue output which was fed to the chart recorder.

THE NEUROLOG SYSTEM USED



apparatus had only one route to earth. This prevented an "earth loop" from developing that could have caused a serious interferance problem. The quail was connected to earth via the ear bars which, as in the stimulatory work (see 2.5), acted as the indifferent electrode.

The output from the head stage unit was fed into an a.c. preamplifier (Neurolog NL104; Digitimer) which amplified the signal a further 2000 times. The signal was then filtered using a band pass filter module (Neurolog NL125; Digitimer) on which the upper filter limit was set to 1kHz and the lower to 100Hz. As well as this, a notch filter, which was factory set to 50Hz, was always switched on to eliminate any remaining line interferance. After filtering, the signal was passed through a spike trigger module (Neurolog NL200; Digitimer). This module took the form of a gated amplitude discriminator, producing separate outputs for spikes which: A. crossed an upper threshold; B. crossed a lower threshold and C. crossed a lower but not an upper threshold. It was the latter output with which we were most concerned. The upper threshold was set to 1.5 volts and the lower to 0.5 volts, so that the trigger responded only to spikes with an amplitude of less than 1.5 volts and greater than 0.5 volts. Each spike within this range triggered the module to produce a single pulse at its output. A pulse train resulted in which all pulses were of the same amplitude; this enabled analysis to be much simpler. The

pulse train from the spike trigger was fed into a rate meter (Neurolog NL255; Digitimer) and the rate of spiking was displayed digitally in pulses per second. The rate meter also produced an output voltage which varied in magnitude according to rate of pulsing and this was fed to the second chart recorder channel (respiratory cycling being on the other).

The electrode was moved up and down until a good recording was obtained. A meaningful recording was easily distinguishable by the output on the chart recorder trace which showed spiking rate increasing in phase with the respiratory cycle. Often small adjustments of the filter and spike trigger thresholds were necessary in order to increase the clarity of the result.

Once a useful recording was underway, the apparatus was left for about 5 minutes with the chart recorder running, in order to produce enough data for future analysis. After this period the electrode was cemented into the brain using dental cement (Simplex; Dental Fillings Ltd). When the cement was dry, the head stage amplifier was removed and the stimulatory apparatus was connected to the bird. Stimulation was carried out as earlier described (see 2.5).

The charts were analysed and graphs were plotted of respiratory cycling and spiking rate against time. In this way, any correlation between spiking and respiratory cycling

was clearly shown up.

2.7 Changing the Carrier Gas Mixture

The stimulatory procedure was carried out as previously described (see 2.5). When a low threshold response site was located the details were noted and the electrode was cemented into the brain. For these experiments, it was not normal procedure to vagotomise the birds.

Several cylinders of oxygen and carbon dioxide gas mixtures were obtained from BOC. These contained 0%, 5% and 10% of carbon dioxide. A second pressure valve and flow meter (BOC; 15 litres per minute max) was fitted to a cylinder containing the desired proportion of carbon dioxide. The gas was switched on and the flow control was carefully adjusted to give the same flow rate as that used with the pure oxygen (about 1 litre per minute). The feed tube to the vaporizer (see 2.2) was disconnected from the pure oxygen cylinder and connected to the flow valve on the mixture cylinder. The flow rate was then finely adjusted until the transducer was operating over the same pressure range as it was with the oxygen. This was a good indication that the pressure and consequently the flow rate of the second cylinder were the same as those of the first. With practice, the cylinder change-over could be accomplished in under 30 seconds and this afforded minimal disturbance to the preparation. As far as possible, less concentrated

carbon dioxide mixtures were used first, since this helped to eliminate the effects of "pooling" in the air sacs.

Once the new mixture was satisfactorily linked up, the bird was left for at least 10 minutes to stabilize. During this period, the chart recorder was left running so that any changes in the respiratory pattern could be identified. When stabilization had taken place, the animal was stimulated again and any changes in the response or its threshold were noted and added to the data store.

When all the mixtures required had been tested, the oxygen was reconnected. Usually, at least one hour was required for the respiratory rate and depth to return to their original basal levels. Once basal levels were restored, the animal was stimulated a third time and any changes in the threshold and response were noted.

2.8 Changing the Core Temperature

Prior to bringing the bird into the laboratory, the temperature of the cage in which it was living was noted. Once in the laboratory, the bird was left to equilibrate to the ambient temperature for about 4 hours. Temperatures of both the cage and the laboratory were, as far as possible, maintained at 21^{o} C.

In these experiments it was not normal procedure to vagotomise the animals. A mercury thermometer was inserted

into the bird's rectum and anchored with cotton. The core temperature was noted and it was always within 1^{o} C of the normal quail temperature of 40.45^{o} C (see Chapter 6). The animal was then wrapped in the electric blanket (see 2.2) which was switched on. The blanket monitoring probe was suspended in the laboratory atmosphere, so that the animal was warmed to increase core temperature.

To begin with, respiratory rate was noted after each 0.5^{o} C increase in core temperature, but from the onset of thermal polypnoea, readings were taken after every 0.1^{o} C increase. The animal was stimulated after each 1^{o} C increase in temperature and more frequently during and after the onset of thermal polypnoea. Any change in either the response elicited or its threshold was noted. When thermal polypnoea had reached a peak rate, the bird was stimulated once more before the electric blanket was switched off and removed. Further respiratory rate readings were taken during the cooling period and any changes in response characteristics were noted. Cooling was allowed to continue until the control body temperature was regained.

2.9 Histological Procedure

After decapitation, the eyes and lower jaw were removed from the head which was fixed by soaking in 1% formal acetic for at least a week. The brain was carefully removed from the skull, a procedure simplified because the formal acetic had

softened the skull bones. Then the brain was returned to formal acetic for at least two more days to ensure that fixing was complete.

The brain was freeze sectioned, which not only made the procedure faster and simpler, but also reduced the possibility that the brain might distort during sectioning. The anterior 8mm of the brain was sliced off leaving a flat surface on the posterior portion for mounting on the chuck of the microtome. The chuck was fastened over a jet of liquid carbon dioxide and a liberal amount of O.C.T. compound (Tissue-Tek; Lab Tek Products) was squirted onto the surface. The brain was then placed, cut surface first, into the globule of O.C.T. compound. The carbon dioxide jet was turned on until the preparation was frozen; this was indicated by the O.C.T. compound changing from translucent to opaque. The chuck was then mounted on the microtome in the cryostat (S.L.E.E. Ltd) and left for about 30 minutes to freeze thoroughly.

Sections were cut at 20um and mounted on slides which had previously been coated with a 1 percent gelatine solution to ensure that the sections would adhere. They were easily collected from the knife by placing the gelatine treated face of a slide over them and lifting them free.

The sections were fixed in formalin vapour for 24 hours at room temperature and stained using Luxol Fast Blue (LFB) for

myelin, with a counter stain of Cresyl Fast Violet (CFV) (Lewandowsky, 1904). The two stains were made up as follows:-

Luxol Fast Blue

0.5g of LFB powder (Raymond A. Lamb; London) and 5ml of 10 percent glacial acetic acid were added to 500ml of 95 percent alcohol. This was filtered through medium fast, qualitative filter paper. The resulting solution could be kept indefinitely and used several times over.

Cresyl Fast Violet

0.5g of CFV powder (Raymond A. Lamb; London) and 4 drops of glacial acetic acid were added to 100ml of distilled water. This was boiled and filtered through medium fast, qualitative filter paper. The resulting solution could be used several times over but had a limited storage life.

Staining Procedure

1) Dehydrate in 50 percent alcohol for 1 minute.
2) Dehydrate in 70 percent alcohol for 1 minute.
3) Dehydrate in absolute alcohol for 2 minutes.
4) Stain with Luxol Fast Blue solution for about 16 hours or over night at 70° C.
5) Wash in absolute alcohol for 1 minute.
6) Rinse in distilled water for 1 minute.

7) Differentiate in 0.05 percent lithium carbonate for 50 - 60 seconds (time varies according to LFB staining period).

8) Wash in 70 percent alcohol for 20 - 30 seconds.

9) Rinse in distilled water for 1 minute.

10) Repeat steps 7, 8 and 9 until clearly differentiated.

11) Stain with Cresyl Fast Violet for 6 - 7 minutes.

12) Rinse in distilled water for 1 minute.

13) Differentiate in absolute alcohol for 1 minute.

14) Repeat steps 12 and 13 until clearly differentiated.

15) Dehydrate in absolute alcohol for 1 minute.

16) Clear in xylene I for 1 minute.

17) Continue to clear in xylene II for at least 1 minute.

After staining, the sections were mounted in Canada balsam under glass cover slips. The slides were placed on a drying table at a temperature of 80^{o} C. They were usually dry enough to view under a microscope after 3 to 4 days.

A stereoscopic dissecting microscope was used for identifying the lesion sites (Fig13). When a lesion was clearly visible, the three coordinates of its position were estimated, allowing for shrinkage of about 15 percent, and noted. The site position was also marked on a transverse map of the medulla at anterio-posterior zero. The anterio-posterior coordinate was the most difficult to estimate. Tracts and nuclei were often present over a large range of coordinates but these, together with the shape of

Fig 13 (Plate 3) A brain section taken at anterio-posterior zero. Staining is with Luxol Fast Blue and Cresyl Fast Violet. An electrolytic lesion can be seen in the Medulla (left), marking the site of previous electrical stimulation or recording. Most of the tissue damage seen was caused by passage of the electrode; the lesion itself is at the base of the tract.



the ventricle, the number of folds in the cerebellum and the general shape of the brain provided reasonably accurate estimates. The lateral and height coordinates were much easier to estimate and consequently, probably proved to be more accurate. The numbers of points at each of the three coordinates were plotted to give distribution graphs of the response sites.

Chapter 3

General Results and Discussion

3.1 Hyperoxia and Anaesthesia

The use of 100% oxygen throughout all experiments as the carrier gas, may have been expected to cause the animal to exhibit the symptoms of hyperoxia. Jones and Purves (1970b) reported that, in ducks, only a few breaths of pure oxygen were required to decrease the respiratory rate by up to 17%. This decrease was abolished after denervation of the carotid bodies, which indicated that hyperoxia is monitored peripherally in ducks.

The first rate readings for each experiment were taken after connecting up the transducer and were usually of the order of 12 to 15 breaths per minute. After five minutes, these values had usually decreased to about 10 to 13 breaths per minute. This represented a decrease in rate of 13%.

Due to the nature of the apparatus, an effect as pronounced as that of Jones and Purves (1970b) could not be seen in Japanese Quail when carrying out the normal experimental procedure. This is because the supply of oxygen and anaesthetic was always linked to the bird up to two or three minutes before the transducer was adjusted to give a

satisfactory rate reading. Since so few breaths are required (Jones and Purves, 1970b), the bulk of the effect, if it exists, would have already occurred. However, during the first five minutes for which the transducer was in operation, a decrease in respiratory rate was noted. It would be incorrect to assume that this drop in respiratory rate was a direct reflection of the bird breathing 100% oxygen. The anaesthetic supply was linked up at the same time as the oxygen, and this itself may have a retarding effect on the rate of respiration (Marley and Payne, 1964).

Because all experiments were performed in the same manner, the degree of hyperoxia would have been similar in all the birds tested.

3.2 Basal Respiratory Pattern (Before Vagotomy)

Many physiological factors influence respiratory rate, such as oxygen and carbon dioxide levels in the blood which are affected by metabolic rate. When an animal is at rest, there is a minimum respiratory rate and depth which will maintain life - this is known as basal respiration. This state is most nearly approached naturally during sleep and artificially during anaesthesia.

3.21 Results

When the animal had reached surgical anaesthesia and equilibrated for about 5 minutes, a stable, even respiratory

pattern became evident on the chart recorder (Fig14).

The respiratory rate was measured instantaneously in numbers of full respiratory cycles (time between two inspiratory or expiratory peaks) in one minute. Once equilibrated, the respiratory rate settled down to an average of 12.2 (+/- 9.7%) breaths per minute (BPM).

The respiratory depth, could only be compared within one experiment (see Chapter 2) and once equilibrated, it remained at a constant level (+/- 4.2%).

A graph of two cycles of respiration against time, is shown in Fig15. From this it is clear that, like mammals (Ondina et al , 1964), there was a relatively long pause (in this case, 4 seconds) between the end of an expiration and the start of the next inspiration. Successive pairs of inspiratory or expiratory peaks were separated by a period termed the inter-breath interval (IBI), in this case six seconds. The quail from which this graph was drawn, was therefore breathing at a rate of 10BPM.

From rest to peak inspiration took 0.5 seconds, this was followed by a pause of a further 0.5 seconds. Expiration took place, taking 1 second to reach a peak; the transducer took a further 1 second to equilibrate. Quite often a "bounce" was seen in the inspiratory direction, following immediately after the pen returned to zero after expiration.

Fig 14 (Plate 4) A typical chart recording showing an even basal respiratory trace. Surgical anaesthesia has set in about 5 minutes prior to this.

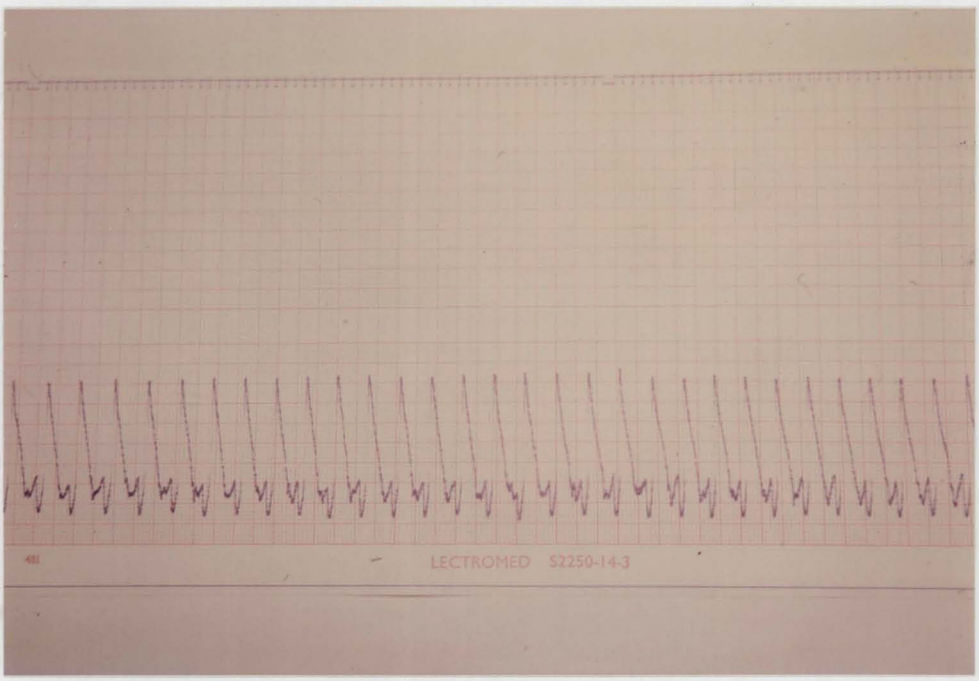
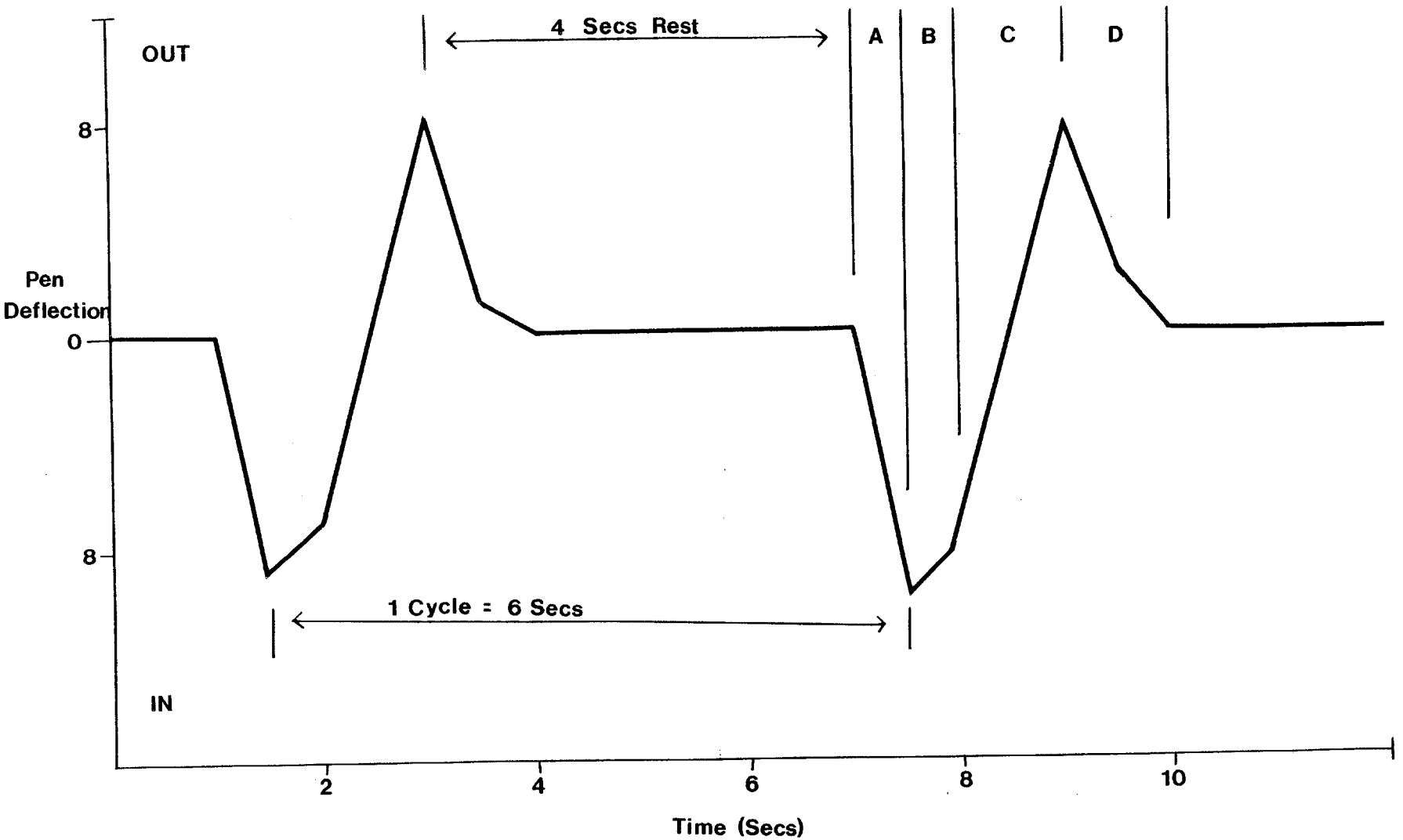

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Fig 15 A graphical representation of two cycles of basal respiration. One cycle (the distance between two inspiratory peaks) lasted six seconds. The time taken to inspire (A) was 0.5 seconds and inspiration was held (B) for a further 0.5 seconds. Active expiration (C) lasted for 1 second and it took another second for the recording equipment to return to basal levels (D). There was a rest period (between full expiration and the start of the following inspiration) lasting 4 seconds.


4 Secs Rest
A
B
C
D
OUT
8
Pen
Deflection
0
8
1 Cycle = 6 Secs
IN
2
4
6
8
10
Time (Secs)

This was due to an oscillation of the air in the tube causing a pressure change which was monitored by the transducer.

Active inspiration took half the time of active expiration and the system was in the expiratory position for 8 times as long as it was in full inhalation. From this data it can clearly be seen that there was plenty of scope for increasing respiratory exchange merely by increasing breathing rate.

3.22 Discussion

When analysing the basal respiration, it must not be forgotten that birds have a uni-directional air flow system over the parabronchi (Hazelhoff, 1943, 1951; Jones, 1979 and Brackenbury, 1971, 1980). By using the air sac system, the flow is organised so that fresh air passes over the exchange surface during both inspiration and expiration. Air was thus moving over the parabronchi during the inspiratory period (0.5 seconds) and the expiratory period (1 second). The system was full of fresh air for a further 0.5 seconds. So, of the 6 second cycle length, 2 seconds (33% of the time) could have been used for gas exchange.

By increasing the respiratory rate by 50%, to 15BPM, air would be pumped over the exchange surface for 30 seconds in every minute, (that is 50% of the time) provided that

inspiratory and expiratory periods remained the same. Further, by increasing the rate by 100% from basal, air would be pumped over the exchange surface for 40 seconds in every minute (66% of the time). Clearly, there is much to be gained by increasing respiratory rate, since all the time the respiratory muscles are pumping, air is passing over the exchange surface.

Increasing respiratory depth, keeping the rate constant, would result in more air passing the exchange surface in the same time. However, this would not necessarily give rise to increased exchange; for example the biochemistry of exchange could be a limiting factor and already be running at its maximum rate.

3.3 Vagotomy

3.31 Introduction

Hering and Breuer (1868 and Breuer, 1868) first found evidence for a vagus nerve mediated link between stretch receptors in the lung and the central respiratory control centres in the brainstem of mammals. This work led to what was later coined "The Hering-Breuer Reflex". As the animal breaths in, lung mechanoreceptors are stretched, and these excite a vagus mediated response resulting in an increasing inhibition of inspiration. This work was later to be confirmed and extended by other workers such as Lumsden

(1924a, b).

It was not until much later that the birds were examined to see if the Hering-Breuer reflex contributed to their respiration. Slowing of respiratory rate accompanied by a depth increase in response to bilateral vagotomy was first reported by Babak (1921). In 1940, Graham performed many experiments on the domestic fowl, originally conducted on mammals by Hering and Breuer. He too found that birds' respiration slowed when cervical vagotomy was performed and that fast inflation of the lungs led to expiration. His work also demonstrated that clamping the trachea after expiration, led to inspiration and vice versa. As well as these lung responses, he found that an expiratory air flow, independent from the lungs, inhibited inspiration. From this early work, it looked very likely that birds had, at least in part, a mammal-like Hering-Breuer reflex. Later work (Miller, 1977) showed that increasing pulmonary pressure in the chicken, caused a decrease in respiratory depth which was dependent on intact vagus nerves. A more pronounced effect was elicited by increasing air sac pressure, indicating the presence of mechanoreceptors there too.

Sinha (1958) worked on pigeons and found, if he vagotomised the birds or blocked the vagi by cooling, that rate and amplitude decreased. By stimulating afferent vagal fibres,

he could restore normal respiration, provided the stimulus parameters were appropriate.

Fedde et al (1961), contrary to earlier work (Graham, 1940), reported no Hering-Breuer reflex in fowl, while Richards (1968, 1969) elicited such responses in pigeons, ducks and quail. Richards (1968) found that, in all animals tested, unilateral vagotomy resulted in decreased respiratory rate and increased depth. However, he reported that the effect was enhanced by bilateral vagotomy only in duck and quail. The work also showed that, in ducks and quail, a vagal link was necessary to maintain thermal polypnoea (see Chapter 8), and that pigeons continued to pant after both vagi were cut. King et al (1968a), like Sinha (1958), were able to restore original respiratory rate and depth by stimulating the cervical afferent stumps of the vagi in fowl.

Although the avian lung was reported to be inexpandable (King et al , 1968b), it was shown (King et al , 1968c; Leitner, 1972 and Molony, 1972) by recording from the vagi, that the central nervous system was constantly being informed of the stage of the respiratory cycle. The vagal fibres were divided into discrete groups, according to when they fired during the respiratory cycle. In essence, it was found that 95 (78%) of the fibres studied fired predominantly during inspiration and only 27 (22%) during expiration.

Burger (1968) first suggested that, what up until now were thought to be stretch receptors, could be monitoring carbon dioxide in the avian lung. Even in mammals, the lung receptor discharge was reported to increase at the end of inspiration, when carbon dioxide is at a minimum (Mustafa and Purves, 1972). Later some vagal fibres were found to respond to mechanical lung stimulation (20%) but most (56%) to carbon dioxide levels in the lung (Molony, 1974).

Jones (1976) suggested an evolutionary divergence, where mammals had developed stretch receptors and birds carbon dioxide receptors. If the lung carbon dioxide receptors were excited maximally to low carbon dioxide levels (peak inspiration), and less to high carbon dioxide levels (late expiration), the output patterns seen in vagal recordings could be explained (Peterson and Fedde, 1968 and Fedde and Peterson, 1970). Other work (Kunz and Miller, 1974 and Kunz et al , 1973), noted that avian respiration appeared to "pace" itself with lung carbon dioxide tension.

3.32 Results

For the recording and stimulatory experiments, unilateral (right nerve) vagotomy was a part of the experimental procedure. Nevertheless, the effects of this were noted and are of some interest in their own right.

The right vagus nerve was transected in the cervical region,

of 83 Japanese Quail (in fact 123 birds were vagotomised but in 40, exact readings were not noted). Left nerve vagotomy, when performed, had an effect indistinguishable from right, and bilateral vagotomy caused too much slowing for our purposes; sometimes resulting in death (Davey and Seller, in prep). Readings of changes in respiratory rate and depth were taken after five minutes when respiration had stabilised.

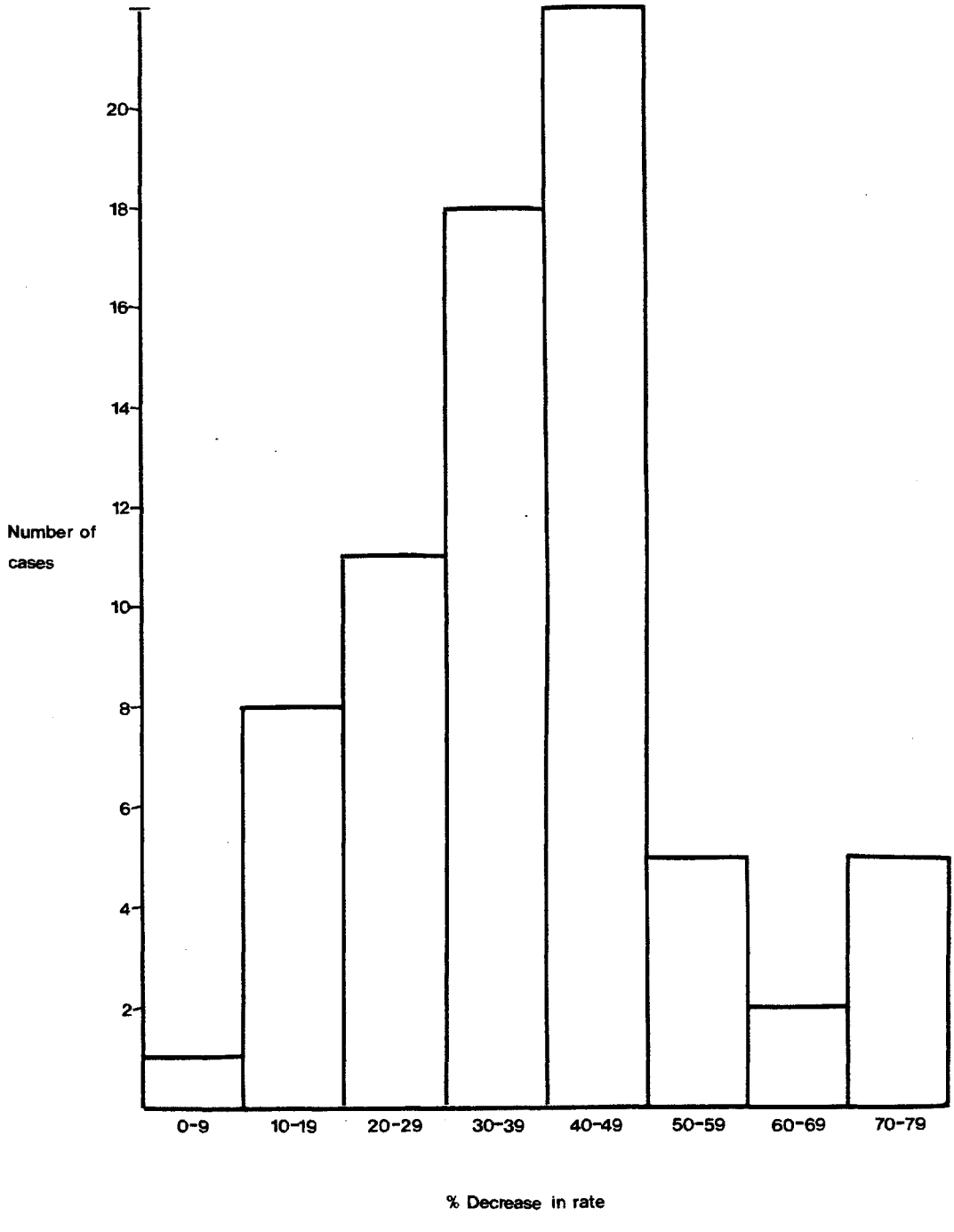
The respiratory rate decreased by an average of 45.8% (n=83, range 6.25-75%, $P<0.001$) from basal levels. The histogram in Fig16 shows the distribution of percent decrease in rate, in response to right nerve cervical vagotomy.

A change in respiratory depth took place in 77% (n=64) of the birds studied, but the degree of change was noted in only 24% (n=20). The average depth change was an increase of 13% (n=20, range 5-40%).

3.33 Discussion

These responses are to be expected in the light of the extensive work by Graham (1940), Miller (1977) and others. However, Fedde et al (1961) reported that there was no Hering-Breuer response in fowl. Their work involved experiments in which the lungs were artificially inflated: no reflex expiratory response was found. Further, the work did not include transection of either vagus nerve. These

Fig 16 Histogram showing the effects of unilateral (right nerve) vagotomy in quail. The operation caused an average slowing of rate of 38%. The histogram clearly shows that most birds fell into a range where the rate decreased by 30-50%.


Number of cases
20
18
16
14
12
10
8
6
4
2
0-9
10-19
20-29
30-39
40-49
50-59
60-69
70-79
% Decrease in rate

results have not been confirmed subsequently. Rather Miller (1977) did obtain reductions in respiratory amplitude after forced inflation of the lungs in fowl.

In conclusion it is probably most likely that although stretch receptors do play a part (Graham, 1940 and Miller, 1977), the main response is mediated by carbon dioxide cycling in the lung (Burger, 1968; Molony, 1974 and Jones, 1976).

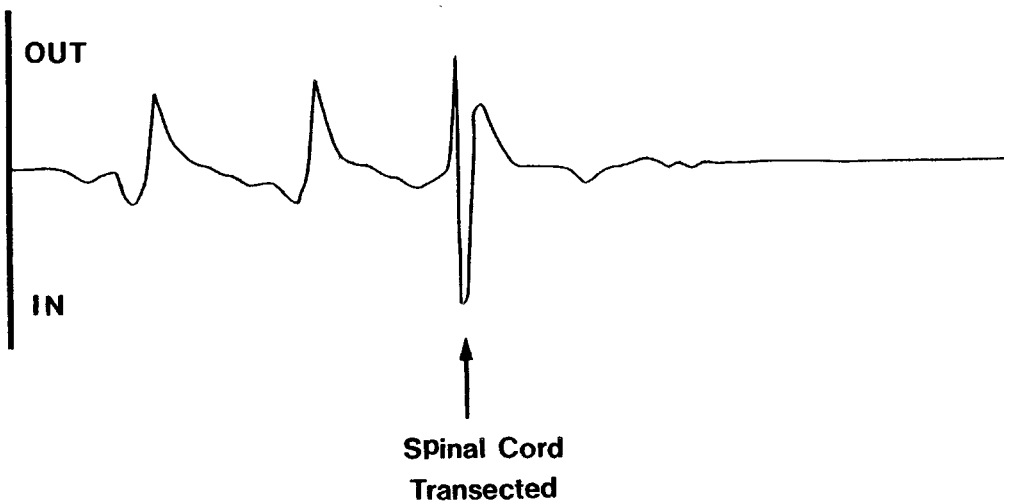
3.4 Transection of the Spinal Cord

The spinal cords of two birds were transected in the cervical region after bilateral vagotomy. Both birds immediately stopped breathing and died. A chart recorder trace of this response is shown in Fig17. These results confirm the assumption that respiratory motor drive is mediated via spinal routes.

3.5 Reactivity Time of the Transducing Equipment

During experiments in which brain recordings were made (see Chapter 5), a direct correlation in time between peak spiking rate and its position in the respiratory cycle was required. It was therefore necessary to make sure that there was no latency in the operation of the pressure transducing or pen recording equipment. It was arranged so that the marker pen would operate at exactly the same time as pressure was applied to the transducer. This was

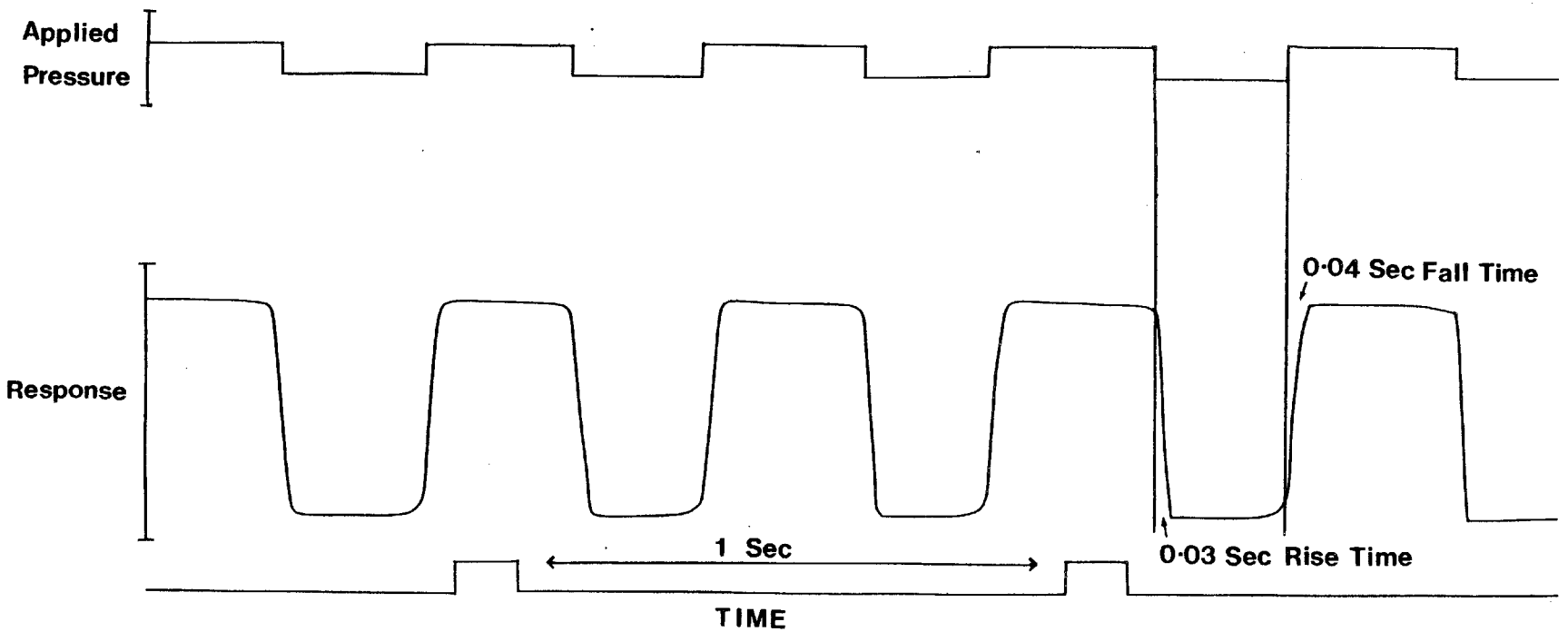
Fig 17 Respiratory trace showing the effects of spinal transection after bilateral vagotomy. Immediately following transection a few respiratory muscle spasms can be identified but respiration has stopped. As predicted, motor routes to the respiratory muscles are mediated spinally.


OUT
IN
SPinal Cord
Transected

achieved by taping the end of the tube (that was normally attached to the bird's mandible) over the marker pen button on the chart recorder. Consequently, when the button was depressed, the tube was constricted and a pressure consequently applied to the transducer. The result was recorded at 10mm/sec and is illustrated in Fig18.

It can be seen that there was a slight delay before the transducer reached peak output as well as for it to return to basal output when the pressure was released. These delays were very small (0.03 secs rise time and 0.04 secs fall time) and introduce no significant error into the results, particularly the multiple unit recordings undertaken (see Chapter 5).

Fig 18 Alternating pressure was applied to the transducer and the response was examined on the chart recorder. This experiment was designed to highlight any delay in the transducing system used. On application of pressure, the pen recorder took 0.03 seconds to reach a peak and on removal of the pressure basal levels were restored in 0.04 seconds. For the purposes of the experiments to be conducted, the transducer delay was minimal.


Applied
Pressure
Response
0·04 Sec Fall Time
1 Sec
0·03 Sec Rise Time
TIME

Chapter 4

Location of the respiratory regions in the Quail brainstem by electrical stimulation

4.1 Introduction

Much of the work elucidating the whereabouts of brainstem respiratory regions in mammals involved electrical stimulatory techniques (eg: Pitts *et al*, 1939b; Hoff and Breckenridge, 1949; Breckenridge and Hoff, 1950; Amoroso *et al*, 1951 and Ondina *et al*, 1960). This method was not often used in the evaluation of the mammalian pneumotaxic centre, although some studies employed it extensively (eg: Johnson and Russell, 1952 and Ngai and Wang, 1957).

As mentioned previously (see Chapter 1) two brief studies examining the avian brainstem for respiratory responses have been undertaken. Richards (1970a, 1971a) identified points in the fowl and pigeon brains, slightly anterior to the present work, around the Tractus vestibulo-mesencephalicus and the Nucleus mesencephalicus profundus in the Mesencephalon. These sites yielded 7-20 fold accelerations of respiratory rate on stimulation. He also reported other scattered points from which apnoea and apneusis were

elicited, but gave no details concerning their position in the brain or their response characteristics.

Cohen and Schnall (1970) located regions causing respiratory arrest (not specified whether inspiratory or expiratory), decreases in respiratory depth or increases in respiratory depth and rate in the pigeon. Details of these sites were not provided, probably because the study was essentially concerned with cardio-vascular responses. However, the points located were in the brainstem and provided some indication of the likely implant coordinates.

Even though almost nothing was known about the avian brainstem respiratory regions (Bouverot, 1978), it was suggested that they may lie in regions similar to those of mammals. There is no atlas of the quail brain covering regions this far posterior. Implant coordinates had therefore to be based on estimates formed using a quail brain atlas (Bayle et al , 1974) dealing with more anterior regions. Other works for the pigeon (Karten and Hodos, 1967) and the fowl (van Tienhoven, 1962) brains, as well as an extensive study on the avian brain in general (Pearson, 1972), were also referred to.

It was reported by Kappers et al (1936), that although a pontine region has been postulated in birds, no definitive pontine structure had been identified. Later, Brodal et al (1950) anatomically identified two nuclei near the ventral

surface of the Rhombencephalon, between the exit levels of the CN V and VIII. They believed these nuclei to be homologous to the mammalian Pons and called them the Nucleus pontis medialis and the Nucleus pontis lateralis. These nuclei were explored in detail during the study.

In the light of the limited work on birds and the extensive literature on mammals, it seemed reasonable to begin a study concerned with the identification of avian respiratory regions, using similar stimulatory techniques.

4.2 Responses Elicited

The brainstem was examined in 123 animals. Four main types of response were elicited on stimulation with 50Hz peak-to-peak currents of less than 50μA.

4.21 Rate Changes

Rate changes were obtained in 57 (46%) of the birds tested. The threshold currents required to elicit responses were all 50uA or less: most (79%) were 20-30μA. All thresholds quoted are those which elicited a 10% or greater change in respiratory rate.

Two different sorts of rate change were produced on brainstem stimulation. The first and more common, was a substantial rate increase averaging 140% (+/- 21%, n=42). As the stimulatory current was increased above threshold,

the response became more pronounced until a maximum rate was reached. All rate increases quoted refer to this maximum. Very often, the threshold current and the current required to elicit the peak rate, were very close; the difference often being less than 10μA. The rate returned to control levels within 30 seconds of the end of the 10 second stimulatory period. Sometimes a compensatory decrease in rate, averaging 10% (+/- 6%, n=11), was observed immediately following the stimulus and lasted up to 20 seconds (Fig19). There was always a change in respiratory amplitude accompanying the increase in rate. The amplitude decreased during stimulation, by an average of 30% (+/- 5%), and during recovery showed an average 60% (+/- 7%) compensatory increase. Basal amplitude was always restored within 30 seconds. Sometimes, the response consisted of a decreasing amplitude throughout the stimulatory period, however, this never led to respiratory cramp during stimulation. Occasionally a compensatory cessation of breathing was seen immediately after the stimulus, this never lasted more than 10 seconds, and there was no definite trend towards either inspiratory or expiratory cramp. An example of a rate increase response showing this phenomenon is given in Fig20. These responses and the sites in the brainstem from which they were produced, were termed "large rate".

With the help of recording work (discussed in the next chapter), a second less pronounced rate change area was

Fig 19 Graph showing the average (n=6) percent change from basal respiratory rate during large rate region stimulation. The bar marks the stimulatory period. During the stimulus, the rate increased by 140%. Basal rate was restored within 10-20 seconds, following a small compensatory rate decrease. (Cf small rate response).

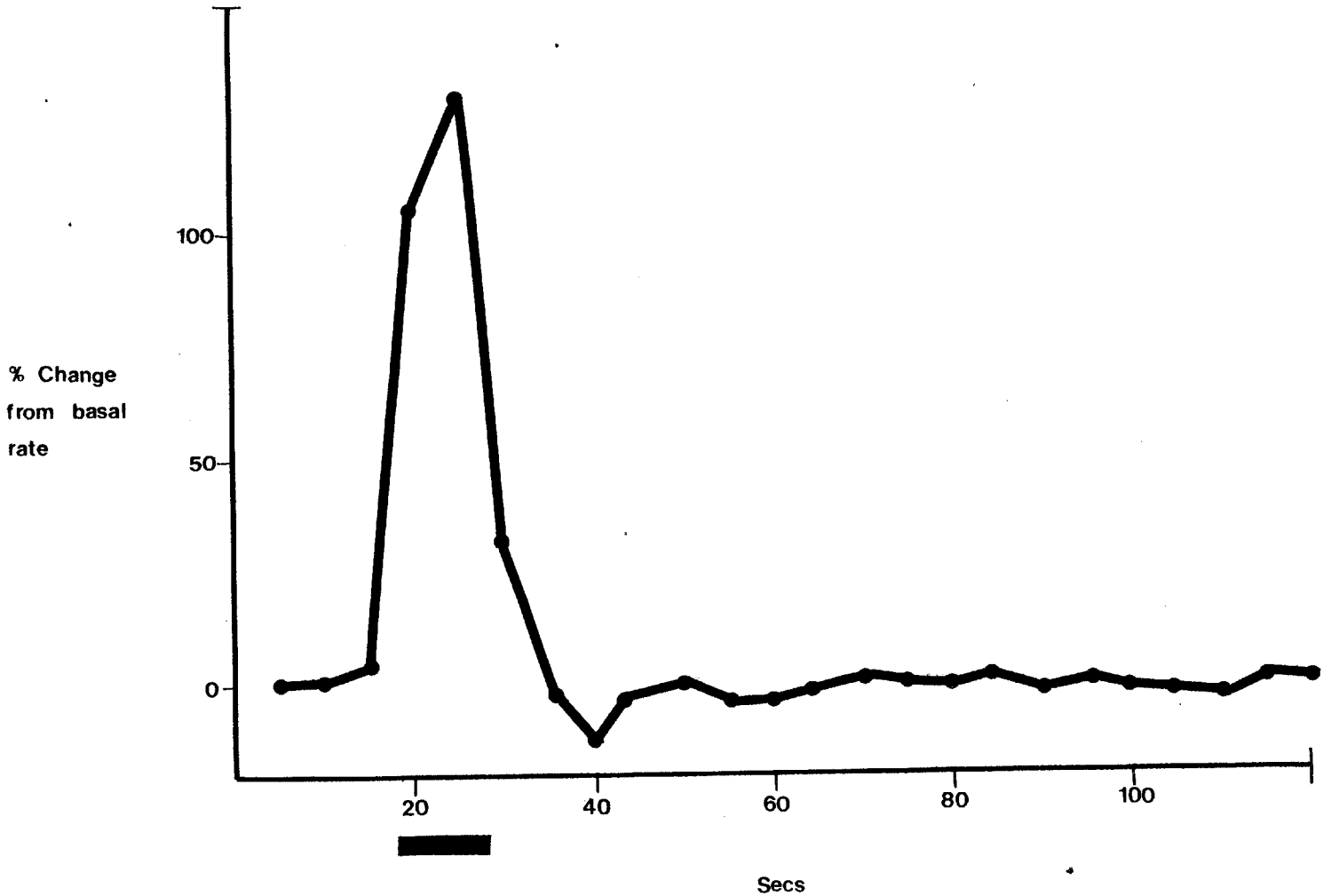
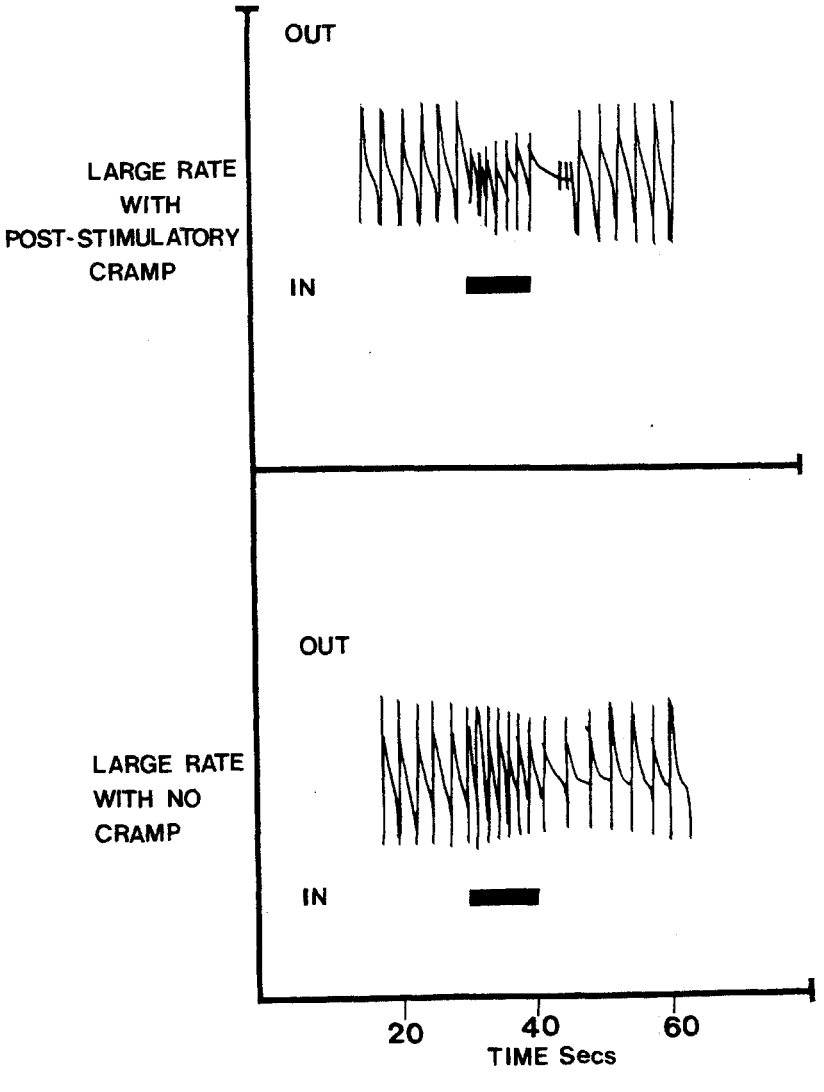

% Change
from basal
rate
100
50
0
20
40
60
80
100
Secs

Fig 20 (Top) Some large rate responses are followed by a compensatory cessation of breathing. This arrest usually lasts for 5-10 seconds after which breathing restarts at small amplitudes. Basal depth is recovered within about 5 seconds.

(Bottom) More usually, large rate responses have no compensatory cramp, although they may be followed by a slight slowing (see Fig 19).


OUT
LARGE RATE
WITH
POST-STIMULATORY
CRAMP
IN
OUT
LARGE RATE
WITH NO
CRAMP
IN
20
40
60
TIME Secs

identified on stimulating the brainstem. The respiratory rate increased by 35.5% (+/- 19%, n=15), and basal levels were restored immediately after the stimulatory period (Fig21). There was no detectable change in respiratory amplitude. Unlike the "large rate" response, the rate increase was all or nothing. The thresholds referred to for this response, thus refer to the lowest current at which it could be elicited. These responses and the sites from which they were produced, were termed "small rate".

4.22 Respiratory Cramps

Responses consisting of respiratory cramps in both expiration (apnoea) and inspiration (apneusis) were identified. The thresholds quoted for both responses refer to currents causing a 10% decrease in respiratory depth. However, in all (n=66) cases cramp was elicited at currents below 50μA: usually (n=53, 80%) 20-30μA. Very often, the threshold current and the current resulting in cramp, were within 10μA of each other.

Apneusic responses (n=32) and regions producing them were termed "inspiratory". Stimulation caused a cessation of breathing in the inspiratory position (Fig22). After stimulation, breathing restarted following a delay of up to five seconds and amplitude was gradually recovered thereafter. The amplitude rose to about 10% above basal levels before returning to control, about 30 seconds after

Fig 21 Graph showing the average (n=6) percent change from basal rate during small rate region stimulation. The bar marks the stimulatory period. During the stimulus, the rate increases by 20%. Basal respiratory rate is restored almost immediately with no compensatory slowing. (Cf large rate response).

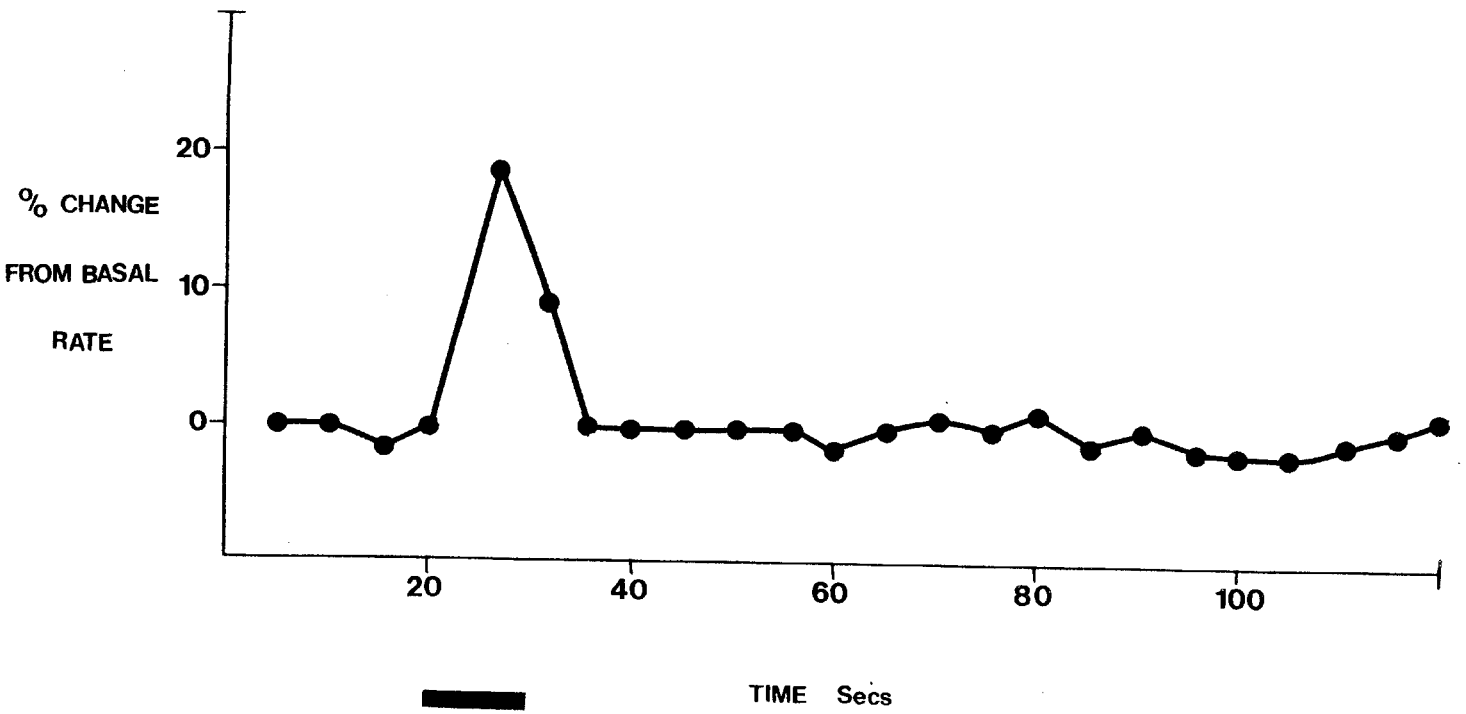
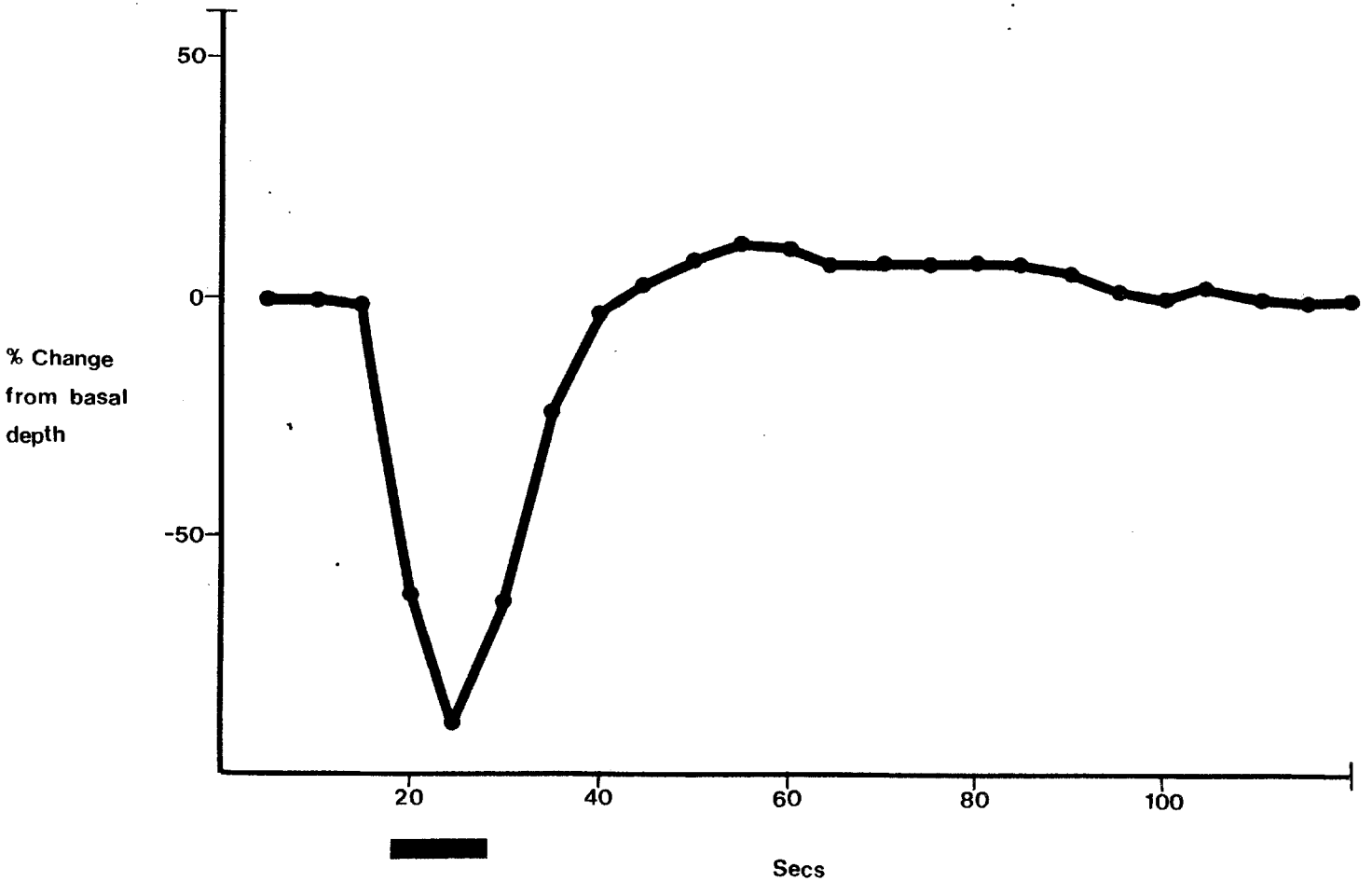

% CHANGE
FROM BASAL
RATE
20
10
0
20
40
60
80
100
TIME Secs

Fig 22 Graph showing the average (n=6) percent change from basal amplitude during inspiratory region stimulation. The bar marks the stimulatory period. During the stimulus the amplitude decreased by almost 100%. Following stimulation, respiration restarted after about 5 seconds and basal respiratory amplitude was slowly restored after a slight (10%) compensatory increase in depth. Basal depth was fully recovered within 25-30 seconds of the end of stimulation. (Cf expiratory response).


50
0
-50
% Change
from basal
depth
20
40
60
80
100
Secs

the end of stimulation. Respiratory rate remained constant while the animal continued to breath. Even during the apneusis, occasionally small respiratory movements of the animal at the basal frequency were seen but these rarely resulted in movement of air.

Apnoeic responses (n=34) and the regions producing them were termed "expiratory". They consisted of a cessation of breathing, during the stimulatory period, in the expiratory position (Fig23). After stimulation, breathing immediately restarted with amplitudes of up to twice the control levels being reached within 10 seconds. From this peak, amplitude gradually returned to control levels and basal depth was restored within 40 seconds of the end of stimulation. Like inspiratory responses, respiratory rate remained constant, at basal levels, and traces of it were seen superimposed over the apnoea.

4.3 Anatomical Separation of Different Response Sites

Each of the four responses described above was elicited from a region of the brainstem which was anatomically separated from areas producing other responses. A transverse map, at anterio-posterior zero, showing the points identified for all four response types is presented in Fig24.

The large rate sites clustered in two discrete areas. One of these was in the ventro-lateral part of the Nucleus

Fig 23 Graph showing the average (n=6) percent change in respiratory amplitude during expiratory region stimulation. The bar marks the stimulatory period, during which the amplitude decreased by 100%. Following the stimulus, breathing restarted immediately with depths 50% higher than basal levels. Basal depths were recovered 30-40 seconds after the end of stimulation. (Cf inspiratory response).

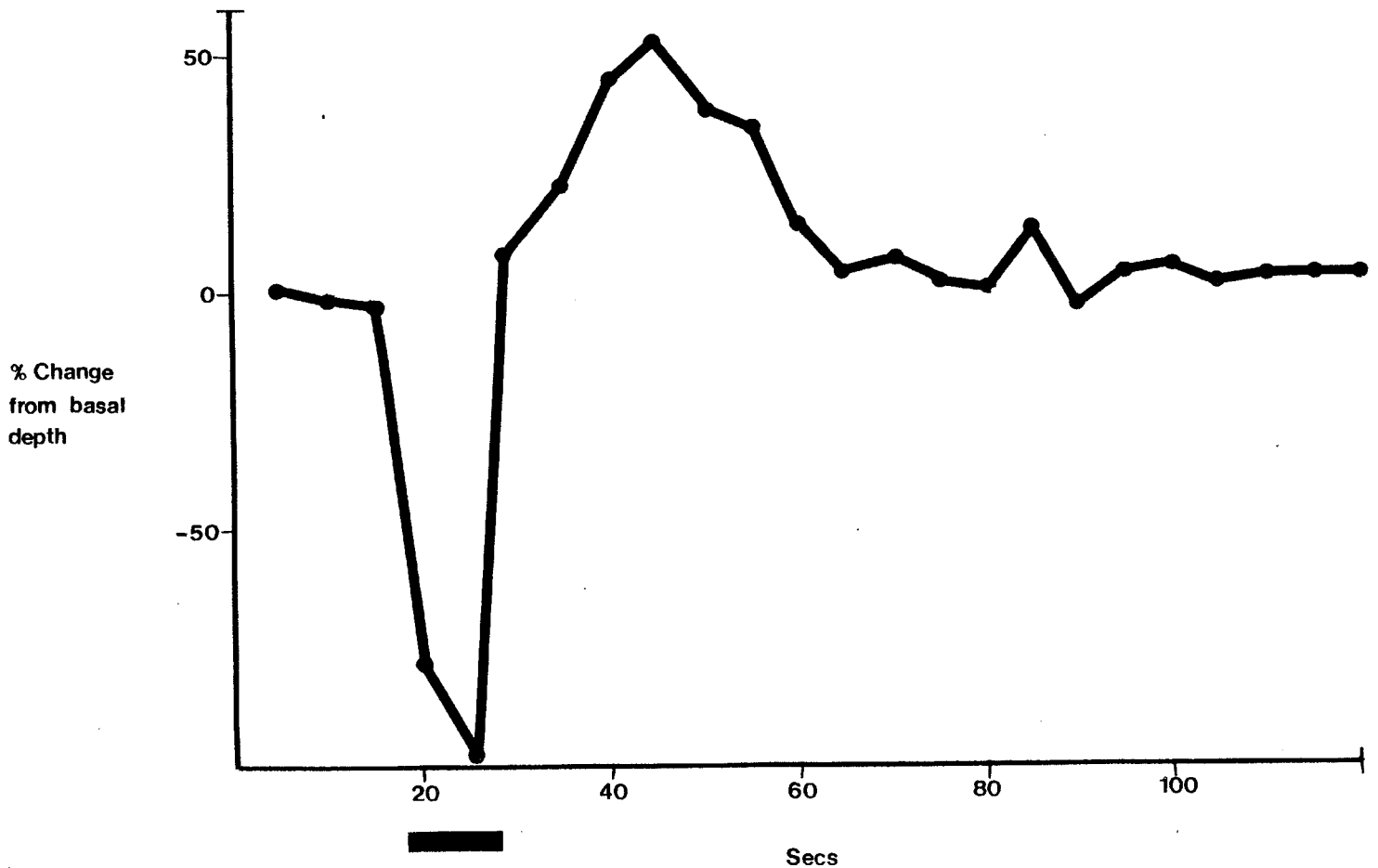
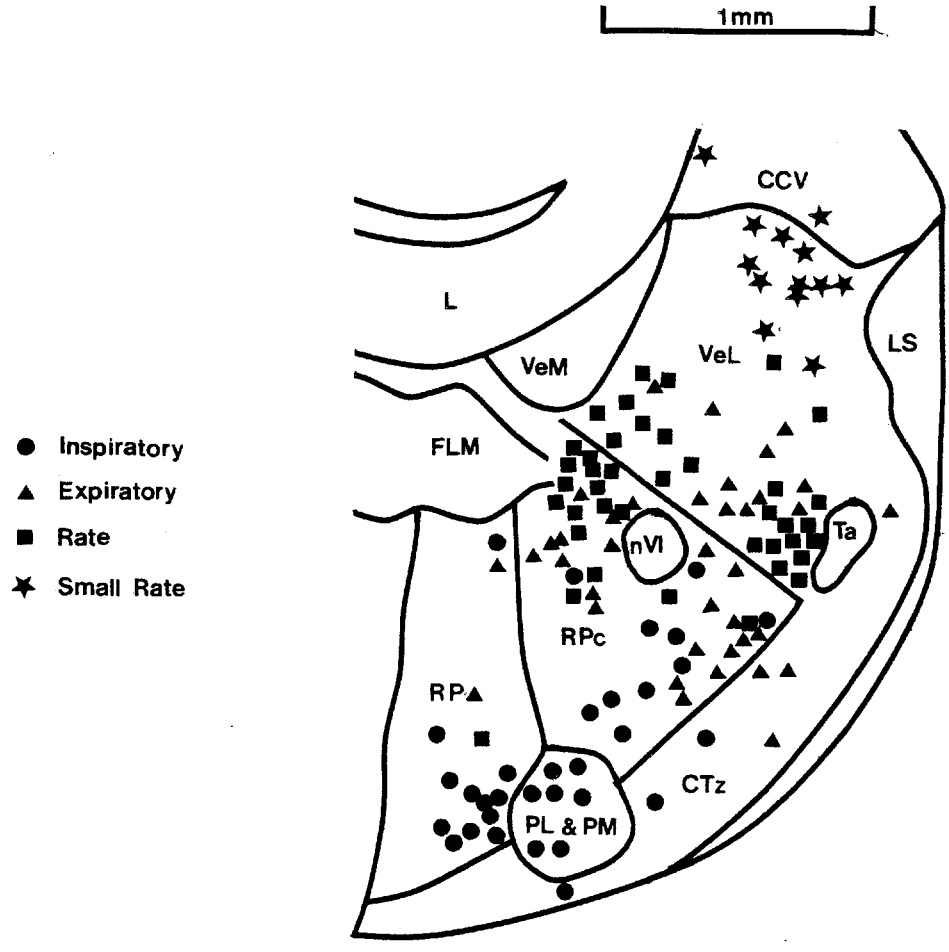

50
0
-50
% Change
from basal
depth
20
40
60
80
100
Secs

Fig 24 Map of one side of the Medulla oblongata showing sites where large rate, small rate, inspiratory and expiratory responses were obtained. The anterio-posterior axis is compressed so that all sites are plotted at A/P 1. Abbreviations:- CCV Commissura cerebellaris ventralis; CTz Corpus trapezoideum (Papez); FLM Fasciculus longitudinalis medialis; L Lingula; LS Limniscus spinalis; nVI Nervus abducens; PL Nucleus pontis lateralis; PM Nucleus pontis medialis; RP Nucleus reticularis pontis caudalis; RPc Nucleus reticularis parvocellularis; Ta Nucleus tangentialis (Cajal); VeL Nucleus vestibularis lateralis; VeM Nucleus vestibularis medialis.


1mm
CCV
L
VeM
VeL
LS
FLM
nVI
Ta
RPc
RPA
CTz
PL & PM
Inspiratory
Expiratory
Rate
Small Rate

vestibularis lateralis just medial to the Nucleus tangentialis. Some points were scattered locally around the main cluster in the dorsal region of the Nucleus reticularis parvocellularis. This was termed the "large rate lateral region" (LRLR). The other cluster area was more medial in the brainstem, in the dorsal part of the Nucleus reticularis parvocellularis, between the Fasciculus longitudinalis medialis and CN VI (abducens). There was local spread of positive sites into the Nucleus vestibularis lateralis. This was termed the "large rate medial region" (LRMR).

The small rate sites were situated much more dorsally in the brainstem, in the dorsal region of the Nucleus vestibularis lateralis, spreading into the Commissura cerebellaris lateralis.

The inspiratory sites appeared to cluster ventrally in and around the two pontine nuclei (Nucleus pontis lateralis and Nucleus pontis medialis). However, some were spread locally in the Nucleus reticularis pontis caudalis and, more laterally, in the Nucleus reticularis parvocellularis. In the latter, some sites, although few in number, were identified dorsally approaching the border of the Nucleus vestibularis lateralis.

In contrast, the expiratory sites were more widely scattered but in general, appeared to form a band which was sandwiched dorsally between the two large rate regions. The band

stretched from central regions of the Nucleus vestibularis lateralis to, at its ventral end, ventro-lateral regions of the Nucleus reticularis parvocellularis. A small cluster of points outside the band, was found medial to CN VI (abducens) extending ventro-medially, with one point in the Nucleus reticularis pontis caudalis.

Although there was some overlap between the regions, generally it is clear that each region can be isolated from its neighbours by a boundary line drawn around definite clusters of positive points.

4.4 Three-Dimensional Distribution and Statistics

From Fig24 it is evident that the distribution of points is only being represented in two dimensions - dorso-ventral and lateral.

4.41 Distribution Graphs

Each stereotactic coordinate was divided into sectors 0.25mm in width. The number of positive sites falling in each of these sectors was plotted on a distribution graph (Figs 25-27). The graphs include all points identified except the small rate sites, since these were not abundant enough to be included in statistical analyses. All positive points, were plotted along each dimension, together with graphs of large rate, expiratory and inspiratory points.

Fig 25 Graphs showing the numbers of positive sites identified by stimulation with respect to the anterio-posterior coordinate:- (top to bottom) all sites stimulated, expiratory sites, inspiratory sites, large rate sites.

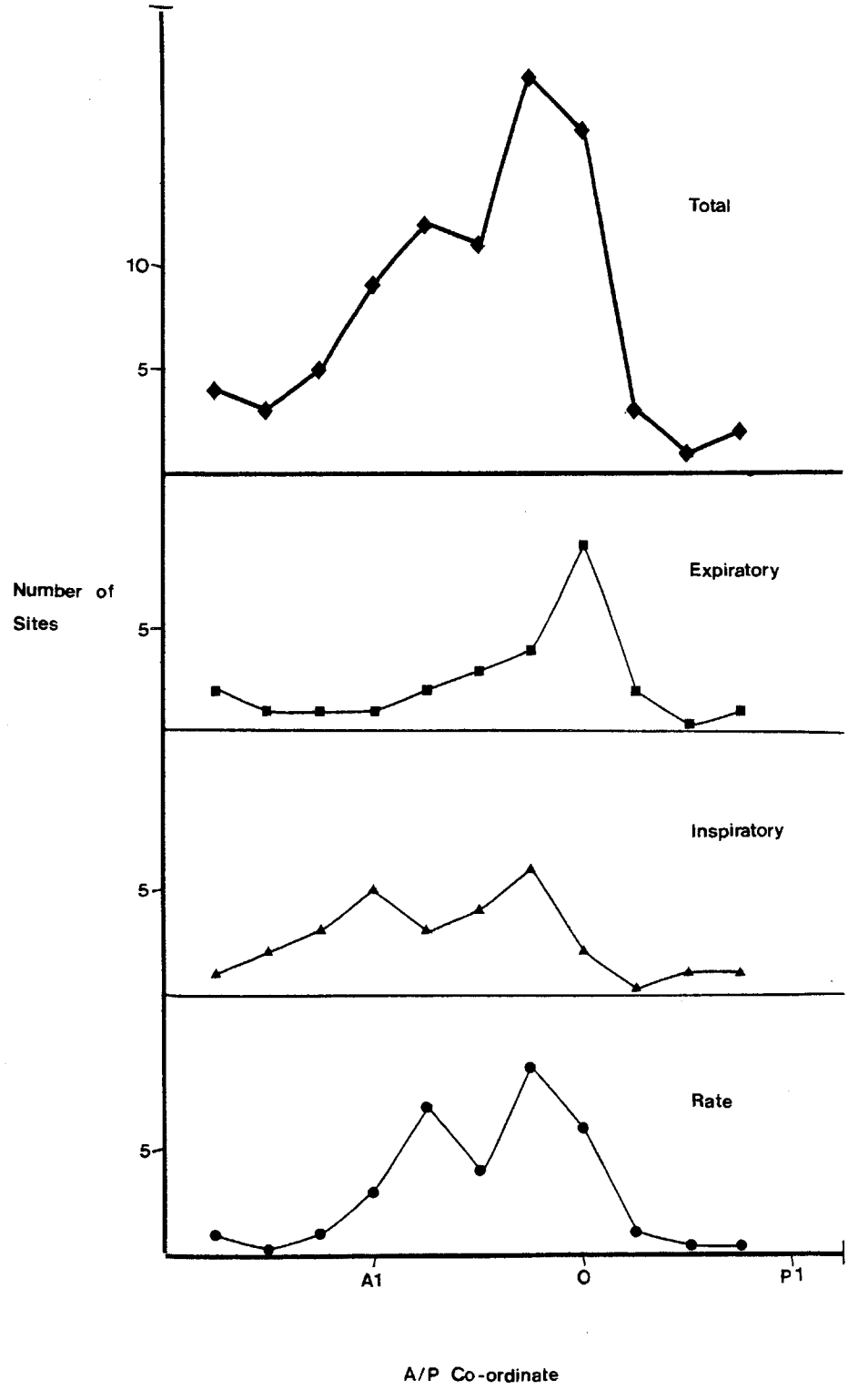

Total
10
5
Expiratory
5
Inspiratory
5
Rate
5
Number of Sites
A1
O
P1
A/P Co-ordinate

Fig 26 Graphs showing the numbers of positive sites identified by stimulation with respect to the lateral coordinate:- (top to bottom) all sites stimulated, expiratory sites, inspiratory sites, large rate sites.

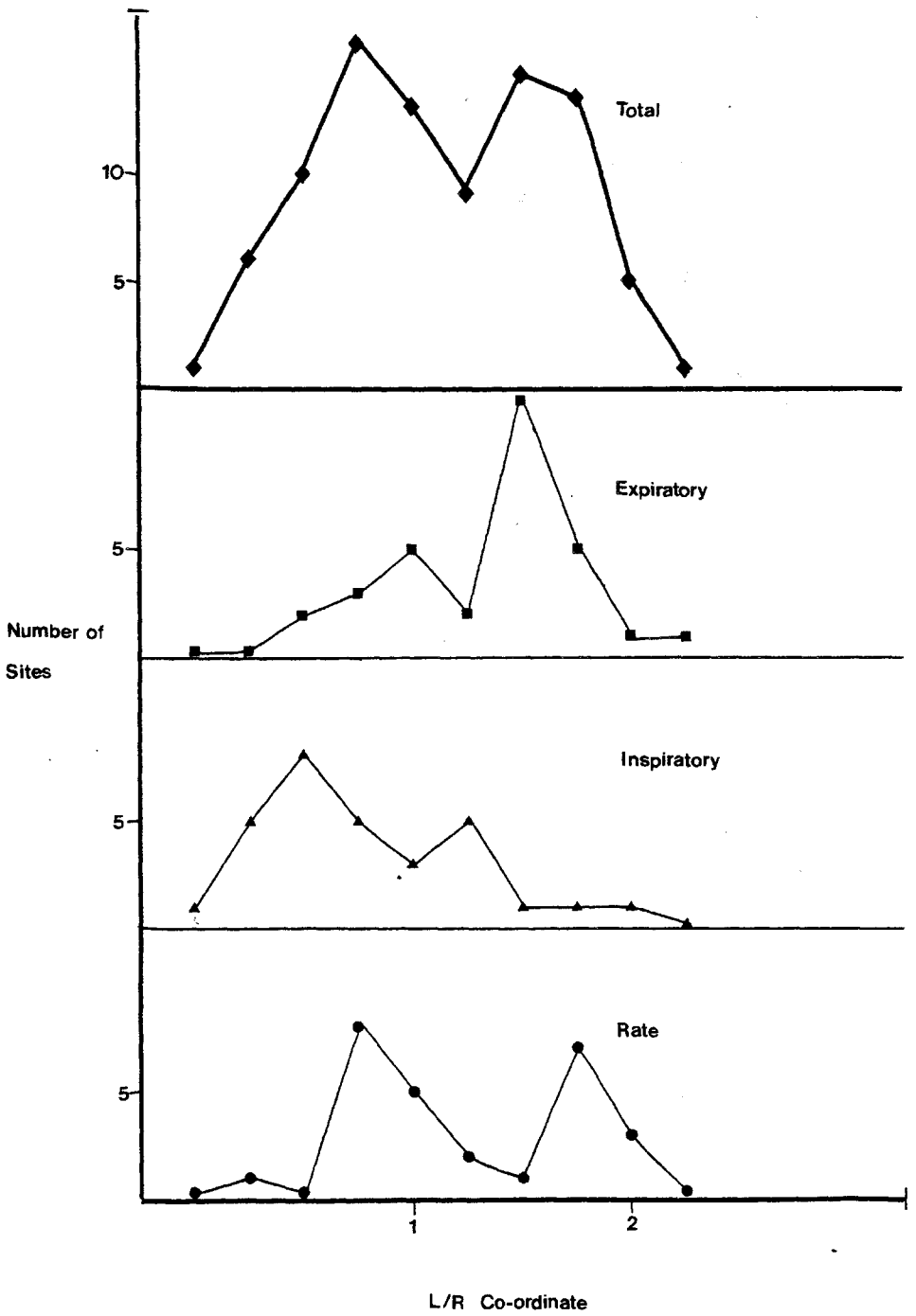
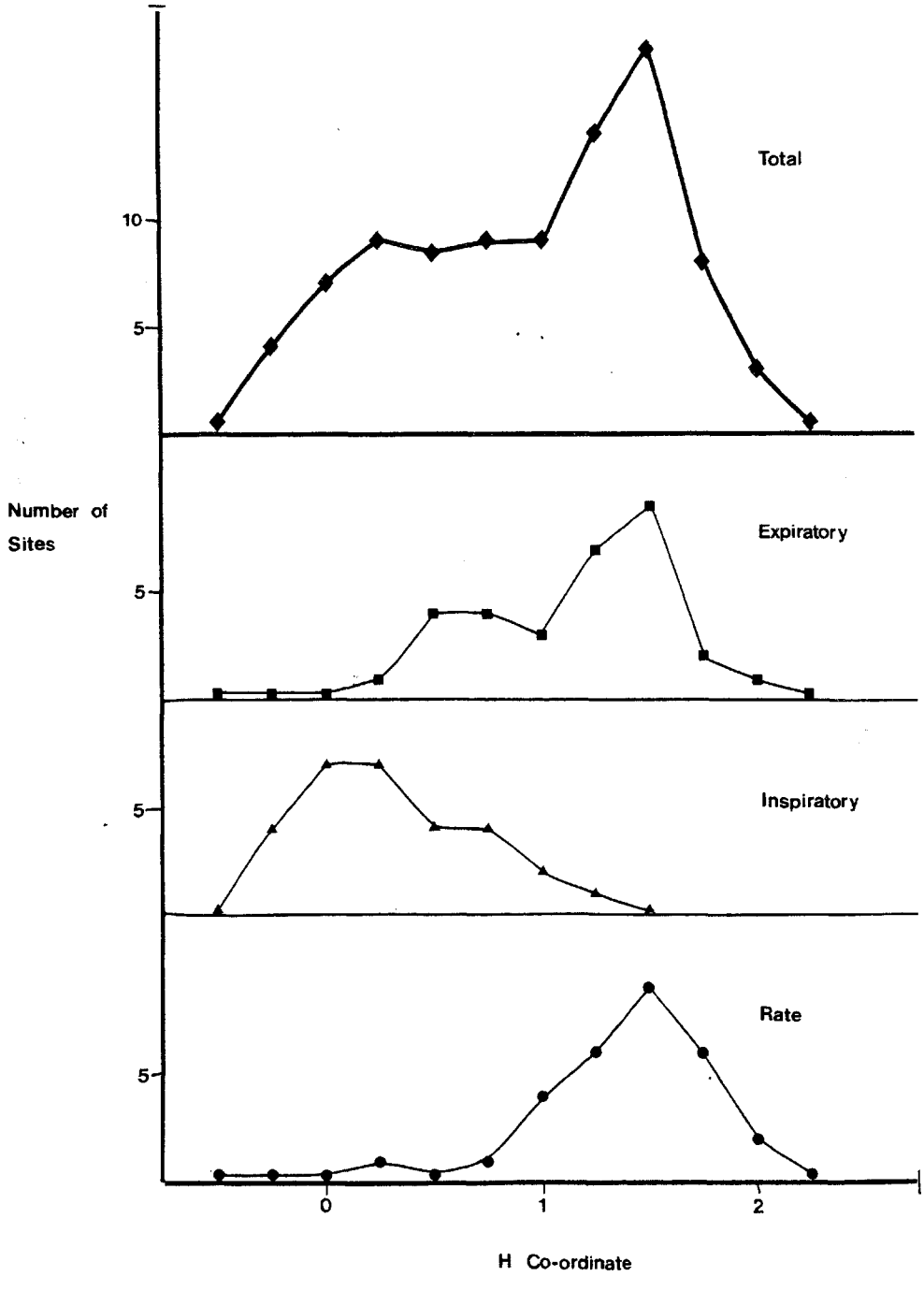

Total
10
5
Expiratory
5
Number of
Sites
Inspiratory
5
Rate
5
1
2
L/R Co-ordinate

Fig 27 Graphs showing the numbers of positive sites identified by stimulation with respect to the height coordinate:- (top to bottom) all sites stimulated, expiratory sites, inspiratory sites, large rate sites.


Total
10
5
Number of Sites
Expiratory
5
Inspiratory
5
Rate
5
0
1
2
H Co-ordinate

On the anterio-posterior coordinate graph (Fig25), the expiratory sites were more posterior than the inspiratory ones. By using a Mann-Whitney statistical test, it was confirmed that the inspiratory and expiratory regions were significantly separated ($P < 0.05$). The large rate sites fell between the inspiratory and expiratory sites in the anterio-posterior dimension. Although, on the graph, they look well displaced to the anterior of the expiratory sites, this was not found to be sufficient for statistical significance at the 5% level ($P > 0.1$). The large rate sites were only slightly displaced posteriorly to the inspiratory sites and again, this did not prove to be a statistically significant separation ($P > 0.1$).

In the lateral dimension (Fig26) is quite clear that the inspiratory sites are more medial to the expiratory sites and indeed this separation bore statistical significance ($P < 0.005$). The inspiratory sites were also significantly ($P < 0.005$) more medial than the large rate sites. Although, from the graph, it looks as though the peak of the expiratory sites, at about L 1.5, falls in the trough of the large rate sites, and that the large rate medial peak is in an area with few expiratory sites; this separation was not significant at the 5% level ($P > 0.05$).

In the dorso-ventral coordinate (Fig27) the separation was much clearer and each of the three regions was proven to be

significantly isolated from the others ($P < 0.05$). The large rate regions were most dorsal, with expiratory sites just below, and inspiratory sites placed very much more ventrally.

In conclusion, the large rate sites lay dorsally in two clumps, one medial and one more lateral and, anterio-posteriorly, they lay between the inspiratory and expiratory regions. Inspiratory sites lay ventrally and medially, being anterior to the large rate sites. Expiratory sites lay posterior and slightly ventral to the two large rate sites and were sandwiched between them in the lateral coordinate.

4.42 Discriminant Function Analysis

Any pair of response regions need not be separated in all three coordinates in order that they are significantly separated three-dimensionally in the brainstem. For example, although large rate and inspiratory sites were very significantly separated in the height coordinate, their separation anterio-posteriorly was not significant at the 5% level. This does not mean that the two regions are not separated overall since they could be one above the other at the same anterio-posterior coordinate. In order to look at the region separation using the three coordinates together, a discriminant function analysis test (Moroney, 1951) was applied to each pair of response types.

All three comparisons (inspiratory vs expiratory, inspiratory vs rate and expiratory vs rate) indicated significant separation at the 1% level. It is therefore certain that each response type was derived from a discrete region of the brainstem.

4.5 Discussion

4.51 Rhythm Generation

The work by Richards (1970a, 1971a) identified regions in the pigeon and fowl Mesencephalon which gave increases of respiratory rate on stimulation. The magnitudes of these rate increases were much greater than in the present study; Richards reported a 7-20 fold increase, while the present work found increases of about 1.5 times. Both the position of the sites in the brain and the magnitude of the response suggest that the regions identified in the Richards study were involved with polypnoea for thermal panting. If this hypothesis is correct, it is possible that these are higher control centres which mediate their effect via more caudal respiratory centres such as those forming the subject of the present study.

Richards (1970a, 1971a) suggested that the region he identified was analogous to the mammalian pneumotaxic centre because of its anatomical situation and because stimulation elicited a respiratory rate increase. This suggestion can

be questioned since stimulation of the mammalian pneumotaxic centre gave inconclusive results (Johnson and Russell, 1952 and Ngai and Wang, 1957) : these included respiratory accelerations as well as slowing by insertions of inspiratory and expiratory pauses. No rate slowing was reported by Richards and none was found in the present study. Further, the mammalian pneumotaxic centre was originally identified, not by electrical stimulation, but by transection and ablation studies (Lumsden, 1923a, b). From this, none of the three regions (Richards' region, large rate and small rate) is analogous to the mammalian pneumotaxic centre. Indeed, if central control of avian respiration is different from that in mammals, then there may be no exact pneumotaxic analogue in birds.

Following the stimulation of large rate sites, cessation of respiration was often seen and lasted up to 20 seconds (Fig20). This pattern could be due to one of the following reasons. Firstly, after breathing at more than twice the basal rate for 10 seconds, it is possible that the bird would be suffering from hyperoxia or hypocapnia (especially since it was breathing 100% oxygen). In order for the oxygen level to fall, or for the carbon dioxide level to rise, a compensatory depression or cessation of respiration would be expected. Indeed, it has been shown in the fowl, that a reduction of arterial carbon dioxide to levels of

3-4%, caused depression of respiration and complete cessation has been reported below these levels (Ray and Fedde, 1969). Secondly, subjecting a putative rhythm generator (see Chapter 8) to large stimulatory currents, abolished the basal respiratory rate and replaced it with a faster one. Following stimulation, the basal rhythm may take time to recover, perhaps due to system fatigue.

From the study, there were quite definitely two discrete responses involving increases of respiratory rate. However high the current used to stimulate the small rate regions, no larger increases in respiratory rate were produced. It is quite obvious from this, that the small and large rate regions are functionally, as well as anatomically separate. Further, stimulating the Vestibular nuclei in the cat (Bassal and Bianchi, 1982) also elicited a respiratory response; onset of inspiration was induced. This helps to support an anatomically separate response region in the Nucleus vestibularis lateralis.

4.52 Respiratory Cramps

The regions identified by Cohen and Schnall (1970) producing apnoea in the pigeon, appear to be anatomically equivalent to the expiratory regions found in the present study. Although they used the term "apnoea", they do not appear to differentiate between inspiratory and expiratory cramps and use it to refer to any respiratory arrest. Sites identified

by them producing decreases in respiratory amplitude, may also represent the expiratory regions of the present study being stimulated with insufficient current to elicit complete cramp.

The inspiratory sites appear to cluster around the two pontine nuclei proposed by Brodal et al (1950) to be homologous to the mammalian Pons. This respiratory connection supports the idea of a homology between the two regions, although the inspiratory responses produced, cannot be construed as originating from a mammalian type pneumotaxic centre.

Hoff and Breckenridge (1949) reported noticing traces of the basal respiratory rhythm superimposed on respiratory cramps in the dog. Similar traces of rhythm were noticed in the present work. It is probable that, for example during an inspiratory cramp, not all of the axons involved in inspiration are stimulated. Motor output and lower control centres may be made up of discrete regions controlling their own specific muscle blocks. If this is so, on stimulating an inspiratory region, not all of the muscle fibres concerned with that phase of respiration may contract. Those fibres which may not have contracted would continue to oscillate at the original basal respiratory rate, controlled by their own unaffected brain regions. If this occurred, it would be likely to manifest itself as the very small

respiratory movements sometimes reported during cramp. Further, often there is a range of stimulatory currents resulting in respiratory depth decreases, below the current at which cramp sets in. At these currents, it is possible that some muscle fibres are already in cramp. The others would be operating at the normal rhythm but would be hindered by those in cramp, resulting in a decreased respiratory depth.

At the end of three experiments, on lesioning dead animals' brainstems after stimulating inspiratory sites, muscle contractions causing inspiratory movements were seen. This indicates that some of the sites found are either motor centres, or intimately linked with motor centres.

The results showed radically differing recovery patterns after stimulation of inspiratory and expiratory sites. While after apneusis, restarting of respiration was delayed followed by a gradual recovery of depth; after apnoea, restarting was immediate at up to twice basal depths. Although the avian respiratory system operates by continuously passing air uni-directionally over the parabronchi, there are obviously net losses and gains of air to the system as a whole. During inspiration, the rib cage is lifted by the intercostal muscles (de Wet et al , 1967 and King and Molony, 1971). Since one third of the lungs is imbedded between the ribs (Scharnke, 1938) it is likely this

lifting causes them to expand slightly and thus take in more air. Further, although compression of the lungs is minimised during expiration (Fedde et al , 1964) by contraction of the costopulmonary muscles, it is not totally eliminated.

In conclusion, it would appear probable that during inspiration, more air is in contact with the exchange surface than during expiration. This being the case, during apnoea, due to less gas exchange being fa cilitated, an oxygen debt resulting in hypoxia could occur. It has been reported that hypoxia in ducks (Orr and Watson, 1913) and fowl (Richards and Sykes, 1967) leads to increased respiratory frequency and depth. This hypothesis provides one explanation for the greatly elevated respiration seen following stimulated apnoea.

Chapter 5

Multiple Unit Recording from the Respiratory Regions of the Quail Brainstem

5.1 Introduction

Mammalian respiratory centres were first looked at using ablation and transection techniques (Lumsden, 1923a, b). Later, other work using electrical stimulation

localised the respiratory regions more finely (eg: Pitts et al , 1939b and Ngai and Wang, 1957). Following this work, both Multiple Unit Recording (MUR) (Salmoiraghi and Burns, 1960) and Single Unit Recording (SUR) (eg: Howard and Tabatabai, 1975; Berger, 1981 and Feldman, 1981) techniques have added to information concerning the functioning of the rhythm generators themselves. This new information pool has led to the production of models of rhythm generators and phase switching mechanisms (Feldman, 1981; see Chapter 8).

As far as recording from the avian respiratory centres is concerned, no work appears to have been conducted. When the present series of experiments was started, most of the electrical stimulation work, discussed in the previous chapter, had been finished. Regions identified by their

responses to electrical stimulation were therefore looked at with great interest, since it was from these that good recordings were to be expected. However, in spite of this added information, brain atlases were still required to help calculate implant coordinates and to identify the response sites after lesioning and sectioning. Works on the anterior quail brain (Bayle et al , 1974), the pigeon brain (Karten and Hodos, 1967) and the fowl brain (van Tienhoven, 1962) as well as Pearson's book (1972) were used extensively.

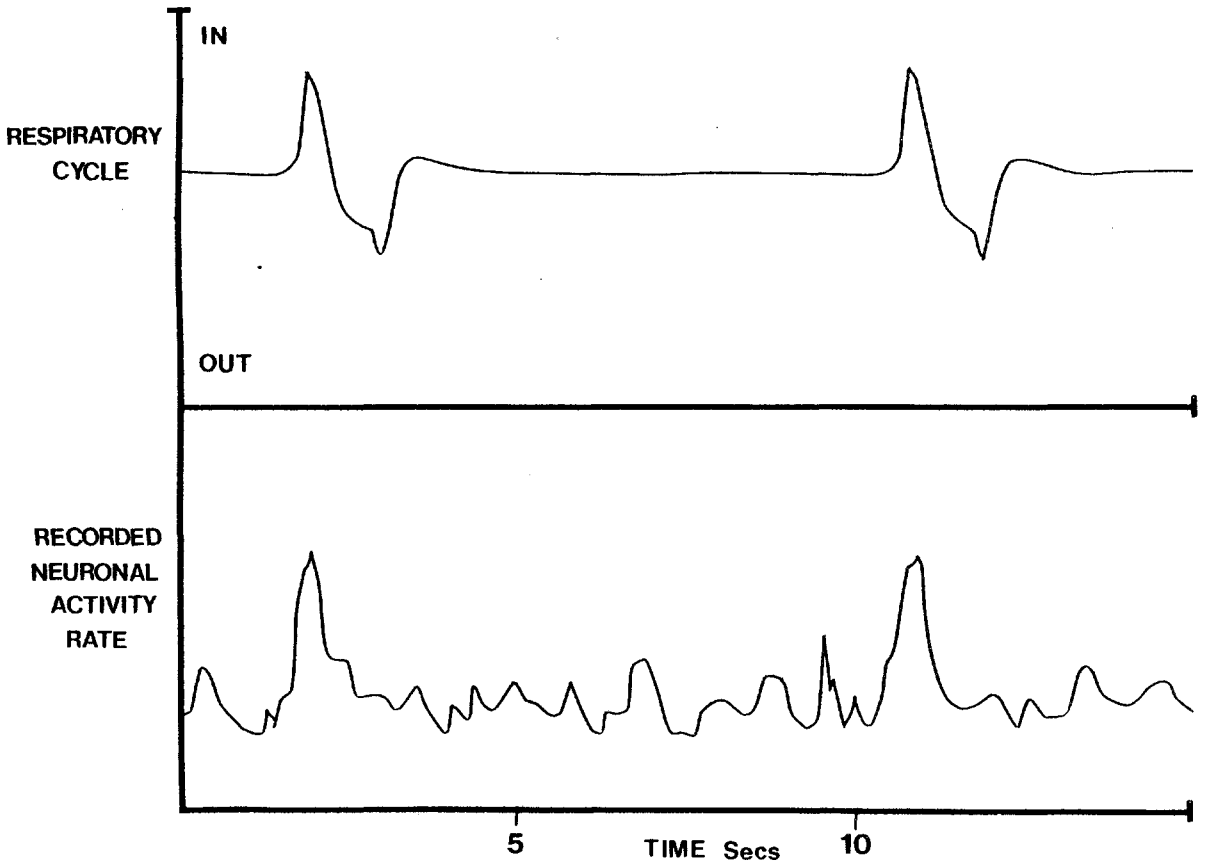
5.2 Recordings Obtained

5.21 General

The brainstem was explored for nervous activity associated with respiration in 70 birds. When a brainstem respiratory region was located, characteristic bursts of neuronal firing activity were seen. These bursts were in phase with respiration and occured at definable points during the cycle. At the peak of each burst, firing rates of up to 700 spikes per second were reached, even when the raw output was band-passed filtered and gated to remove interference. Such a burst is shown together with the associated respiratory cycle in Fig28.

In the case of the bird from which Fig28 was produced, firing rate began to increase just as inspiration started and it reached a maximum soon after the start of expiration.

Fig 28 Example of a multiple unit recording made from the brainstem respiratory regions. In the example the rate of spiking peaks during expiration and in thus a "rest of cycle" response.


IN
RESPIRATORY CYCLE
OUT
RECORDED NEURONAL ACTIVITY RATE
5
TIME Secs
10

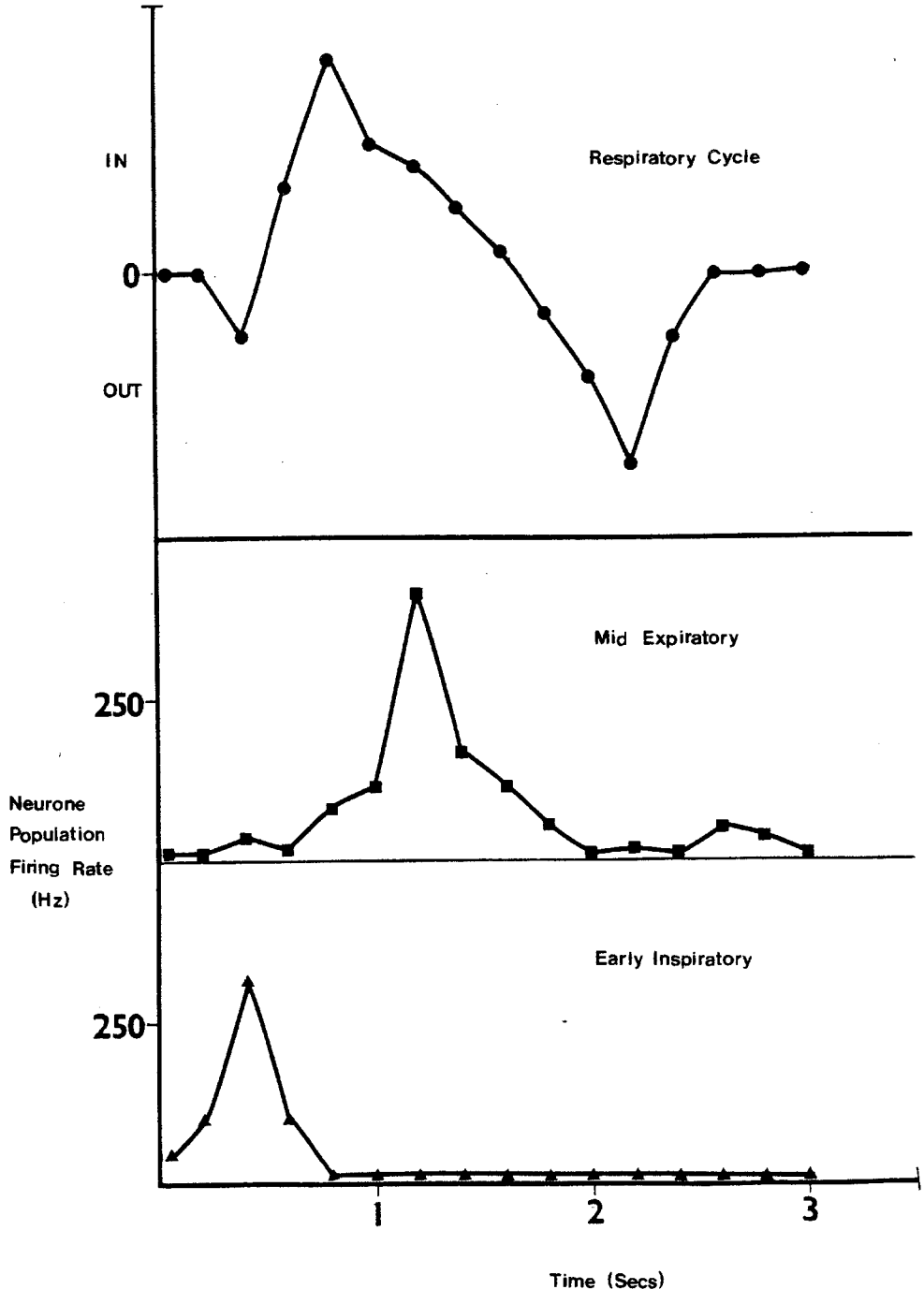
The return to basal levels was not complete until about 1.5 seconds after peak inspiration. The neurones recorded from here, are probably concerned with the onset of expiration.

5.22 Types of Response

Responses seemed to occur at many stages during respiration and it was difficult to put them into categories which occurred in a small number of discrete sub-divisions of the cycle. Broadly, responses could be divided into two groups - those associated with inspiration and those associated with the rest of the cycle. Of the 70 implants made, 26 (37%) had responses associated with the inspiratory phase of the respiratory cycle and 41 (59%) had responses associated with the rest of the cycle. 3 (4%) of the implants made gave variable activity peaks.

All 26 inspiration-associated responses peaked at either full inspiration or, more usually, sometime before this. These responses were termed "Early Inspiratory", while the others were termed "Rest of Cycle". Fig29 shows an average (n=6) early inspiratory response plotted together with a cycle of respiration and a rest of cycle response with its peak at mid-expiration. Firing rate begins to rise very early on in the cycle and the peak is reached just at the onset of inspiration. The decay of firing was just as rapid as its build up, returning to background levels by the time maximum inspiration was reached, 0.2 seconds after peaking.

Fig 29 Graphical representation of a rest of cycle (mid-expiratory) response (centre) and an early inspiratory response (bottom) plotted together with a cycle of respiration (top). The early inspiratory response is is very discrete and has virtually no background activity, whereas the rest of cycle response has a longer duration and a high level of background firing.


IN
0
OUT
Respiratory Cycle
Mid Expiratory
250
Neurone
Population
Firing Rate
(Hz)
Early Inspiratory
250
1
2
3
Time (Secs)

One of the features of the mid-expiratory response (Fig29), which it shared with other rest of cycle responses, was a long post-peak decay time. Peak firing of the rest of cycle response was reached about 0.6 seconds after the rise started and the decay took a further 0.8 seconds. In all, the rest of cycle burst took 1 second (250%) longer than the inspiratory burst. Figs 30-31 show actual responses obtained on chart records displaying the early inspiratory and rest of cycle responses. It was not possible to average the rest of cycle responses from several birds because the peaks occured at differing times during the respiratory cycle.

5.3 Anatomical Separation of Positive Sites

For the purpose of looking at anatomical distributions of positive sites, they were again divided into a group associated with the rest of the cycle and one associated with the onset of inspiration. Fig32 shows the distribution of these two response groups on a transverse brainstem section cut at anterio-posterior zero.

The early inspiratory sites appear to cluster in one main region, with two smaller clusters lying peripherally to this. The main cluster is in the ventro-lateral part of the Nucleus vestibularis lateralis just medial to the Nucleus tangentialis. A few points are situated dorsally to the main cluster but are placed along the same lateral

Fig 30 Example of an early inspiratory response. The neurone spiking rate peaks are very clearly distinguishable and occur about 0.3 seconds before maximum inspiration occurs.

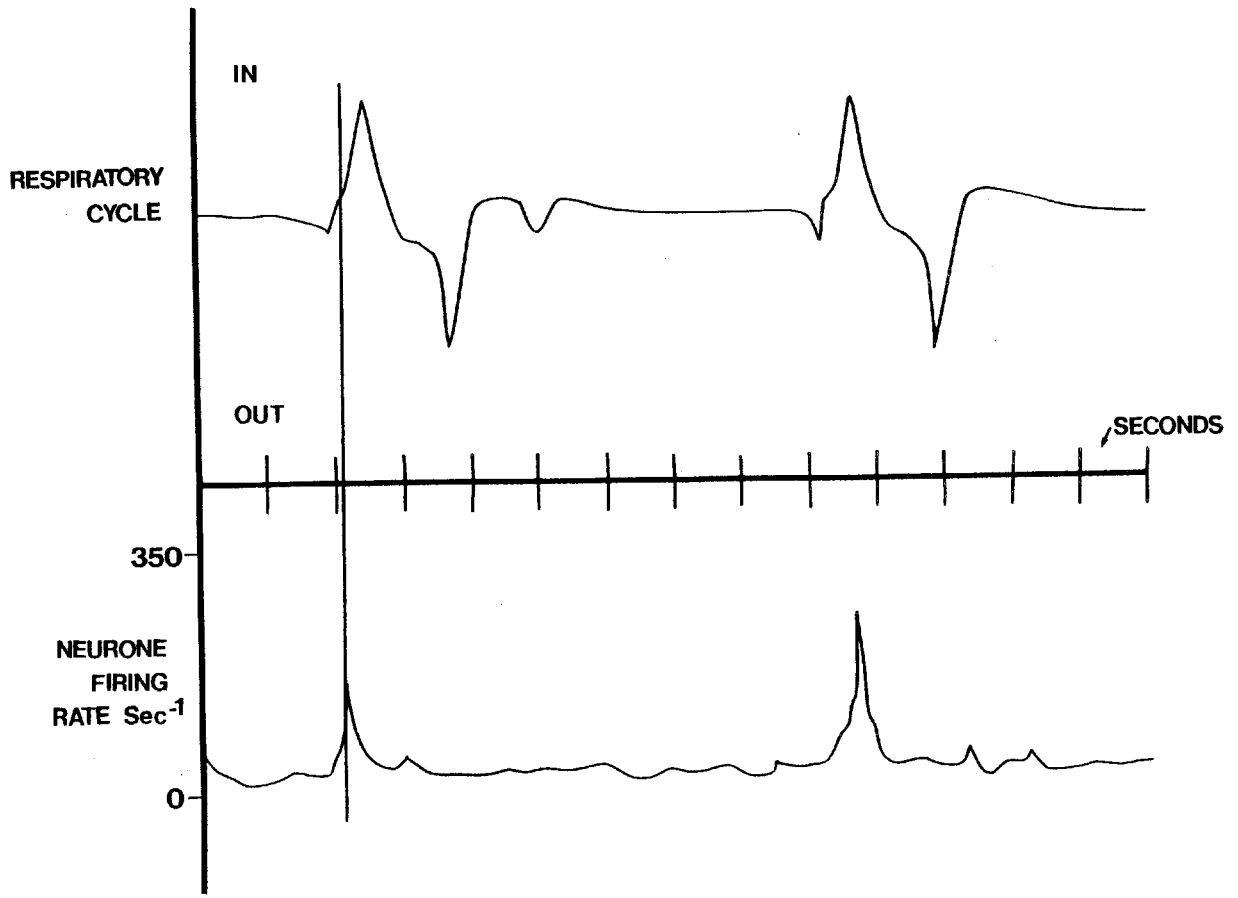

IN
RESPIRATORY
CYCLE
OUT
SECONDS
350
NEURONE
FIRING
RATE Sec-1
0

Fig 31 Example of a rest of cycle response. The neurone spiking rate peaks are less discrete than those of the early inspiratory regions. However, in this example, the peak activity clearly occurs just after the start of expiration.

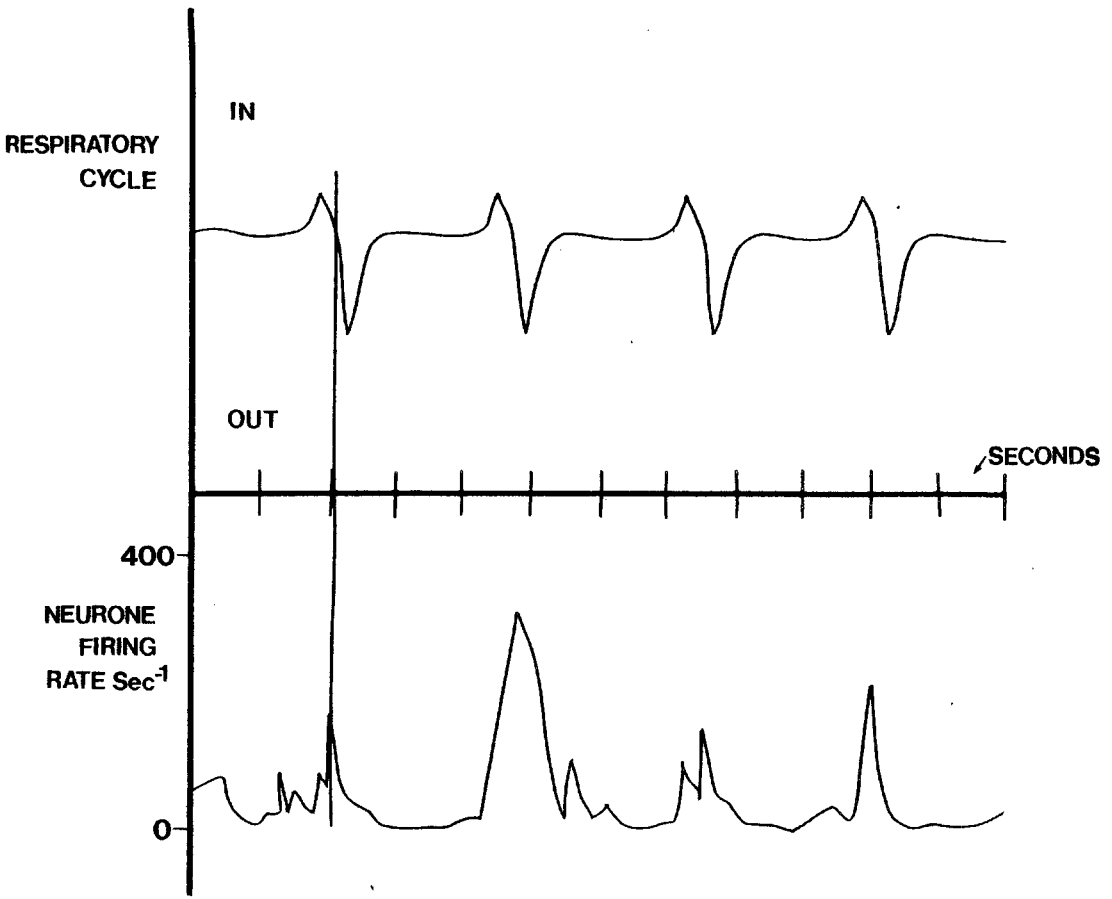
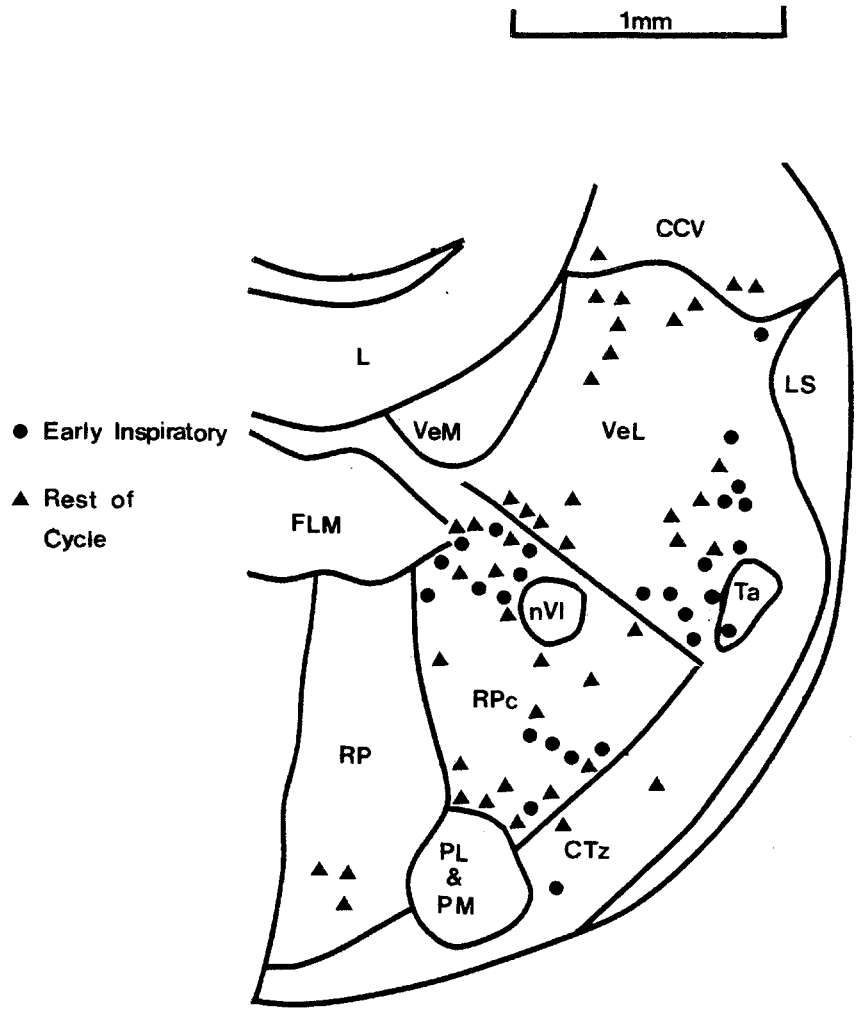

IN
RESPIRATORY
CYCLE
OUT
SECONDS
400
NEURONE
FIRING
RATE Sec-1
0

Fig 32 Map of one side of the Medulla oblongata showing sites where early inspiratory and rest of cycle recordings were made. The anterio-posterior coordinate is compressed so that all sites are plotted at A/P 1.

Abbreviations:- CCV Commissura cerebellaris ventralis; CTz Corpus trapezoideum (Papez); FLM Fasciculus longitudinalis medialis; L Lingula; LS Limniscus spinalis; nVI Nervus abducens; PL Nucleus pontis lateralis; PM Nucleus pontis medialis; RP Nucleus reticularis pontis caudalis; RPc Nucleus reticularis parvocellularis; Ta Nucleus tangentialis (Cajal); VeL Nucleus vestibularis lateralis; VeM Nucleus vestibularis medialis.


1mm
● Early Inspiratory
▲ Rest of Cycle
CCV
L
LS
VeM
VeL
FLM
nVI
Ta
RPc
RP
PL & PM
CTz

coordinate. The first of the smaller clusters, containing only six points, was located medially in the dorsal section of the Nucleus reticularis parvocellularis between the Fasciculus longitudinalis medialis and, more laterally, CN IV (abducens). The second small cluster contains only five points, and these are more widely spread than in the other regions. However, in gross terms, it can be said to lie in the ventral part of the Nucleus reticularis parvocellularis approaching the dorsal borders of the Nucleus pontis medialis and the Nucleus pontis lateralis. One point is more ventral and is lateral to the pontine nuclei, in the Corpus trapezoideum.

Sites associated with the rest of the cycle, being in a much broader category, are more widely spread than the early inspiratory sites. Notwithstanding this, they divide broadly into three clusters. The first is isolated from any early inspiratory sites and occurs in the dorsal region of the Nucleus vestibularis lateralis spreading into the Commissura cerebellaris ventralis. The second cluster is positioned in the dorsal part of the Nucleus reticularis parvocellularis and lies between the Fasciculus longitudinalis medialis and CN IV (abducens). There is a local spread both dorsally into the Nucleus vestibularis lateralis and more laterally into the Nucleus reticularis parvocellularis. The final rest of cycle region lies in the ventral part of the Nucleus reticularis parvocellularis and

surrounds the dorsal borders of the two pontine nuclei. A few sites were seen in the Nucleus reticularis pontis caudalis (n=3), the Corpus trapezoideum (n=1) and mixed with the early inspiratory response region in the ventro-lateral part of the Nucleus vestibularis lateralis (n=5).

The regions were not as clearly separated as those identified from the electrical stimulation work. Reasons for this more interdigitated and mixed layout of sites will be discussed later.

5.4 Three-Dimensional Distribution and Statistics

As with the electrical stimulation work, the preliminary distribution map (Fig32) only shows the position of the sites in two dimensions. A three-dimensional study was undertaken to include the anterio-posterior coordinate.

5.41 Distribution Graphs

Each coordinate was divided into sectors 0.25mm in width. The number of points falling within each of these sectors was plotted on a distribution graph (Fig 33-35). All points located, together with rest of cycle and early inspiratory responses were plotted along each dimension.

From the graph (Fig33) showing the distribution of points along the anterio-posterior coordinate, it is clear that the early inspiratory sites are more posterior than those

Fig 33 Graphs showing the numbers of positive sites identified by recording with respect to the anterio-posterior coordinate:- (top to bottom) all sites recorded from; rest of cycle sites; early inspiratory sites.

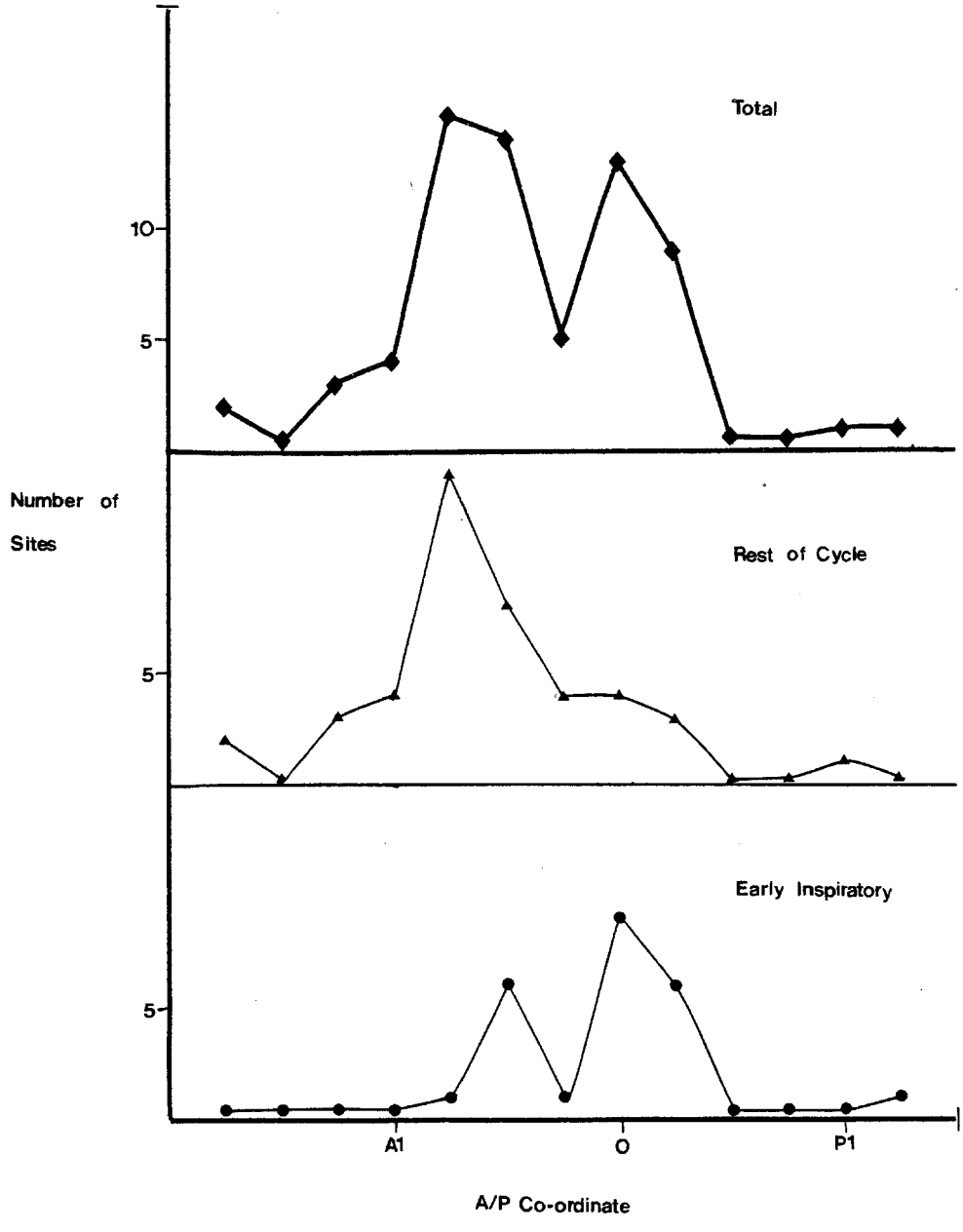

Total
10
5
Number of Sites
Rest of Cycle
5
Early Inspiratory
5
A1
O
P1
A/P Co-ordinate

Fig 34 Graphs showing the numbers of positive sites identified by recording with respect to the lateral coordinate:- (top to bottom) all sites recorded from, rest of cycle sites, early inspiratory sites.

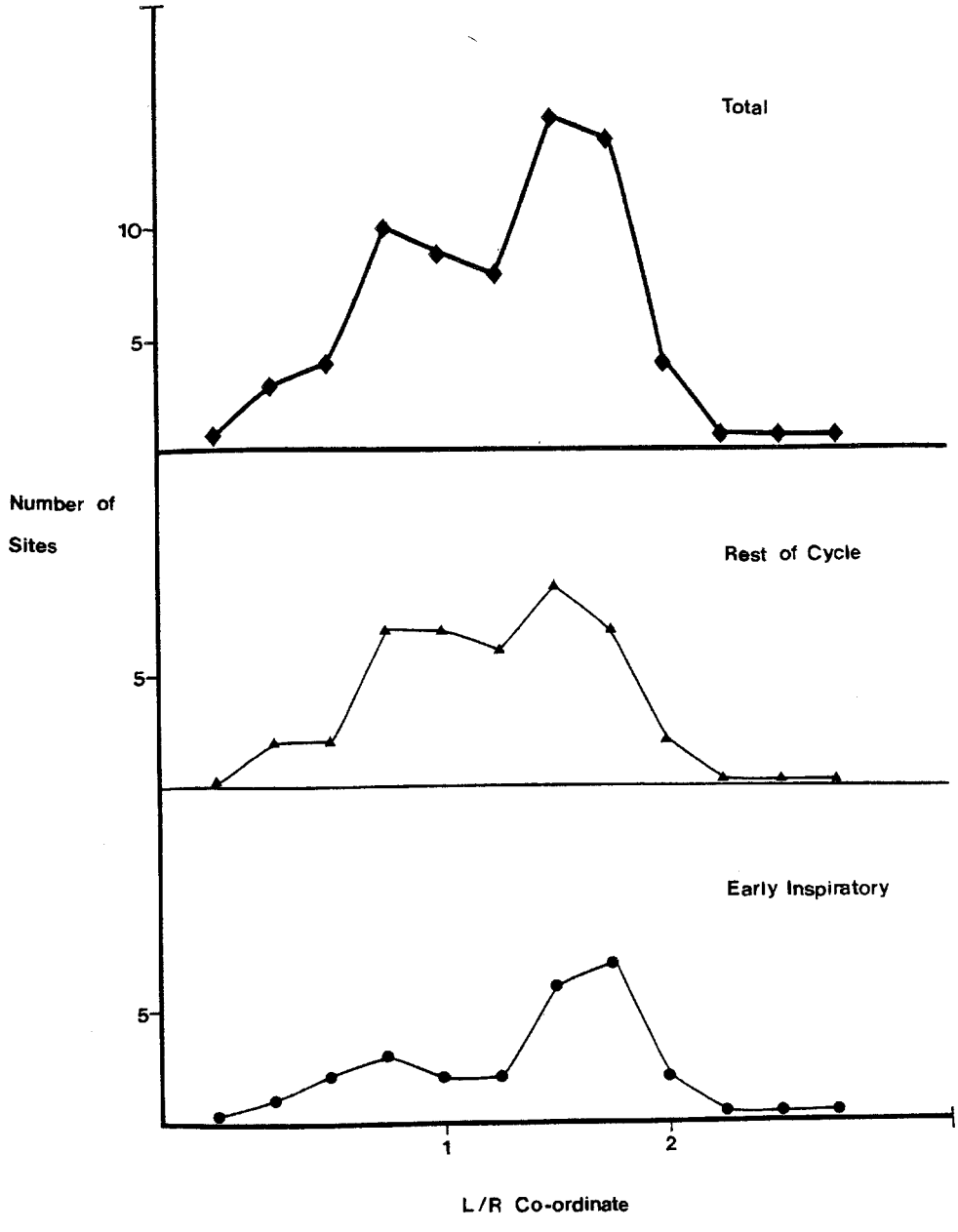
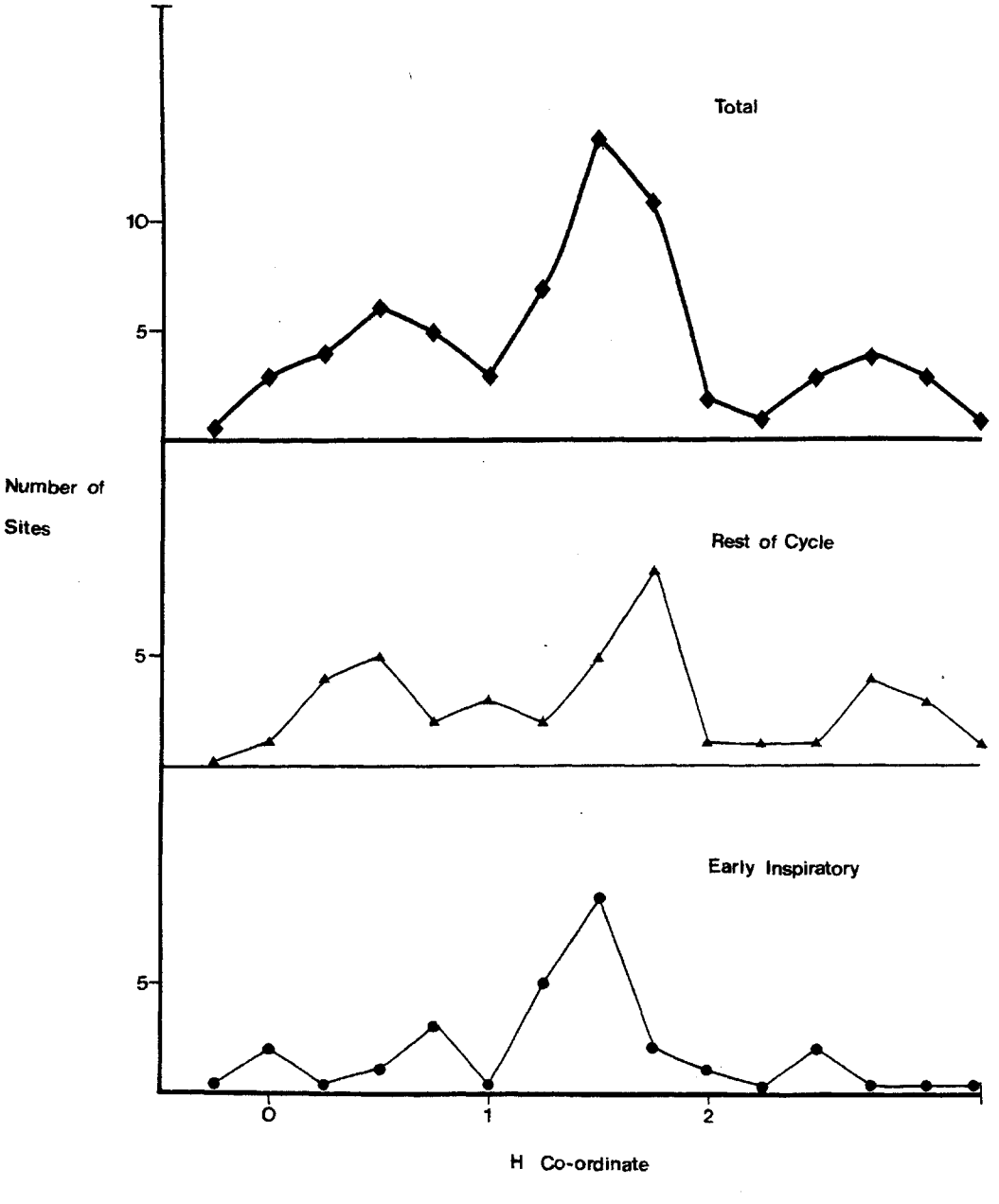

Total
10
5
Number of
Sites
Rest of Cycle
5
Early Inspiratory
5
1
2
L/R Co-ordinate

Fig 35 Graphs showing the numbers of positive sites identified by recording with respect to the height coordinate:- (top to bottom) all sites recorded from, rest of cycle sites, early inspiratory sites.


Total
10
5
Number of
Sites
Rest of Cycle
5
Early Inspiratory
5
0
1
2
H Co-ordinate

associated with the rest of the cycle. The peak number of sites associated with the rest of the cycle is at about anterior 0.75, while the early inspiratory sites form two peaks at anterior 0.5 and anterio-posterior zero. From this it can be calculated that a typical rest of cycle site falls over 0.5mm more anterior than an early inspiratory site. Mann-Whitney statistical analysis demonstrated that the two response types were significantly separated ($P < 0.005$, $n=67$) in the anterio-posterior coordinate.

In the lateral coordinate (Fig34), the points associated with the rest of the cycle are quite evenly distributed along the axis between L 0.75 and L 2.0. In contrast, the early inspiratory points are restricted to coordinates L 1.5 to L 2.0 - a much narrower range. However, over the entire early inspiratory distribution range, rest of cycle sites are heavily represented. It is for this reason that the site types were not found to have statistically significant separation.

In the height coordinate (Fig35), the two response types have their distribution peaks close together, in a range H 1.25-1.75. However, the early inspiratory peak is 0.25mm more ventral than the rest of cycle peak. There was no statistically significant separation of the two response groups in the height coordinate.

In conclusion, the early inspiratory sites occur more

posteriorly, slightly more ventrally and, on average, more laterally than the rest of cycle sites.

5.42 Discriminant Function Analysis

The Mann-Whitney tests carried out only indicated separation or not in one dimension of the brainstem. In order for one region to be separated from another adjacent region it need not be significantly separated in all three dimensions. For example, the early inspiratory regions were found to be very significantly separated from regions giving responses during the rest of the cycle in the anterio-posterior coordinate. However, in the other two coordinates, separation was not statistically significant. In reality it is possible that the two regions could be at the same height and lateral coordinates in the brainstem while being one posterior to the other. If this were the case the regions would be separated from each other.

A discriminant function analysis (Moroney, 1951) was carried out on the two regions and they were confirmed to be significantly separated three-dimensionally in the brainstem at the 1% level. It can be stated with some degree of certainty that the early inspiratory recordings were made in discrete regions of the brainstem isolated from other areas.

5.5 Discussion

5.51 Multiple Unit Recordings

Although multiple unit recordings have been useful in furthering the elucidation of the avian respiratory regions as a whole, they leave the properties of individual neurones relatively unknown. The size of the recording electrode used was a circle 0.076mm in diameter (ie: 0.0045 square mm) and it was estimated that firing patterns of around 30-40 neurones were likely to contribute to the activity recorded. The firing pattern seen at the apparatus output was the summation of the activity of all the individual neurones in contact with the electrode tip. It should be remembered that whether they were inhibitory or excitatory neurones, their effects would still summate to produce the recorded output. This effect alone could cause considerable confusion.

If for example, a motor output region for inspiration was being examined; on inspiration the activity of excitatory neurones would cause an increase in overall firing rate, and on expiration inhibitory neurone activity would do the same. The output would therefore be one of steady and continual firing. In reality this would rarely occur because it is unlikely that the recording electrode would be picking up equal numbers of inhibitory and excitatory neurones. If for example, thirty inhibitory and ten excitatory neurones were

being recorded from, there would be a "background activity" of 10 neurones, which would appear as constant firing. However, when the inhibitory neurones were firing, it would appear to the experimenter that twenty neurones were spontaneously firing, and a burst would be seen. Further, during expiration, the inspiration-associated neurones would be inhibited resulting in suppressed background activity. This would lead to an apparently enhanced burst frequency during inspiration. Perhaps the recently perfected technique for recording from single central neurones in freely moving quail (Foster et al , 1982) will prove to be useful in future work.

Besides this problem, there were very likely other non-respiration-associated neurones present which could also have had influence on the response.

5.52 Categorising Responses

Early inspiratory responses and those associated with the rest of the cycle were only arbitrarily chosen groupings to help make identification easier. Because many neurones were being recorded from simultaneously, in theory an infinite number of response types would be possible. Mammalian work has shown that individual neurones fire at many varying different points during the respiratory cycle (Berger, 1981 and Merrill, 1981). In the case of the present work the same appears to be true, because the positions of the burst

peaks in the cycle varied very slightly between implants. This implies that summation of neurones firing out of phase with one another was occuring. Indeed this is probably why the rest of cycle bursts lasted longer than the early inspiratory bursts. The methods used could not distinguish between bursts occuring a few milliseconds apart in the cycle. For early inspiratory responses it was possible, because of their similarity, to obtain average response characteristics from several birds (Fig29).

5.53 Response Types

In spite of the difficulties encountered when using multiple unit recording techniques, definite neurone types were both identified and inferred from the results.

The early inspiratory response was very well defined and occurred in bursts with short durations. This indicates that the response is dominated by a small number of neurone types firing together in the cycle. The burst peak occurred in a similar place in each bird examined and Fig29 shows that the response was little affected by averaging activity from several birds (n=6). It is not known whether the response seen is predominantly excitatory or inhibitory; in fact it is unlikely that all three early inspiratory regions are the same in this respect. It is interesting to note that single unit recordings made in the mammalian brainstem have demonstrated that inspiratory phase neurones

predominate (Richter, 1982). This further supports the idea that the early inspiratory response category is a functional one.

Since this response occurred at the beginning of inspiration, before any substantial respiratory muscle movement, it is probably partly responsible for triggering inspiration (details of triggering and phase-switching mechanisms are discussed in Chapter 8). The response is unlikely to be connected with the termination of expiration due to the large pause between expiration and the following inspiration (see Chapter 3). Expiration had always finished long before the early inspiratory burst was seen.

The early inspiratory burst may also be responsible for starting an inspiration timer in order to prevent overlapping with expiration. A similar timing mechanism, triggered at the onset of inspiration, may also be responsible for timing the whole cycle. The amount of gas exchanged in the respiratory system of a bird depends on the volume of air pumped over the parabronchi. This in turn depends on how many cycles of respiration are achieved in unit time and on their depth - which both depend on the cycle length. Therefore, a whole cycle timer could ultimately be responsible for gas concentrations in the blood (see Chapter 7). In conclusion, the early inspiratory burst could operate a trigger setting off inspiration or a

timer for timing either just the inspiratory phase or the whole respiratory cycle.

Although the rest of cycle responses were less well defined, they did demonstrate that a variety of neurone types fired during expiration. Bursts occurred throughout the expiratory phase and they had long durations; this demonstrates the presence of neurones firing at all stages during expiration. No neurone type could be said, from the work so far, to predominate in the rest of cycle regions. Even though any particular burst had a peak representing its position in the cycle, other neurones firing before and after it contributed to the build up and decay of that peak. This evidence serves to demonstrate further that the firing positions of neurones in the expiratory phase of respiration are by no means predictable or invariable.

Since expiration was followed by a long pause before inspiration, it is improbable that the rest of cycle responses are concerned with the onset of inspiration. In all cases the burst had occurred before the end of expiration. Like the early inspiratory response, these bursts could could be responsible for terminating expiration or for starting a timer to time either the expiration-inspiration pause, or the whole cycle. As with the early inspiratory responses, such timers would be intimately concerned with the control of gas levels in the

blood (see Chapter 7).

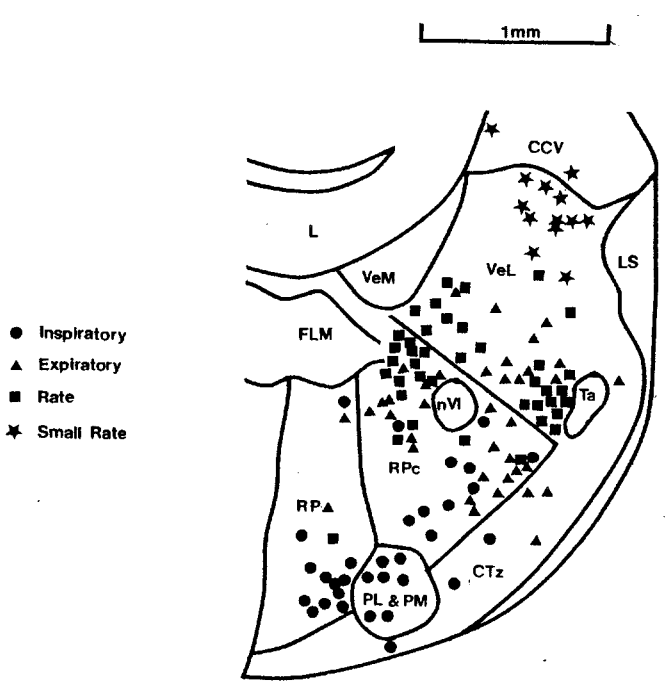
5.54 Response Site Distribution

In spite of the problems arising with the techniques and the difficulties in categorising the responses, there is a remarkably clear distribution of positive sites. The early inspiratory responses were particularly well separated which suggests that "early-inspiratory" was a functional response category. In turn, this supports the evidence that most of the early inspiratory neurones belong to the same neurone group.

There is (Fig36) a remarkable similarity between the distributions of positive sites identified from this study and from the stimulatory work. Further, regions devoid of positive sites in the electrical stimulation work, also show an absence of sites in the recording study. All sites recorded from were subsequently stimulated and all elicited positive responses of the types described in the previous chapter. This confirms that the two studies involved identical neurone groups. The early inspiratory responses predominated in the LRLR (large rate lateral region) identified from stimulation work. Some early inspiratory responses are also seen in the LRMR (large rate medial region) and a few in the inspiratory region. The rest of cycle sites, occupied the slow rate, inspiratory, and expiratory regions as well as some of the LRMR. Table 1

Fig 36 Maps of one side of the Medulla oblongata showing sites where:- (left) large rate, small rate, inspiratory and expiratory responses were obtained; and (right) early inspiratory and rest of cycle recordings were made. The anterio-posterior coordinates are compressed so that all sites shown are plotted at A/P 1.

Abbreviations:- CCV Commissura cerebellaris ventralis; CTz Corpus trapezoideum (Papez); FLM Fasciculus longitudinalis medialis; L Lingula; LS Limniscus spinalis; nVI Nervus abducens; PL Nucleus pontis lateralis; PM Nucleus pontis medialis; RP Nucleus reticularis pontis caudalis; RPc Nucleus reticularis parvocellularis; Ta Nucleus tangentialis (Cajal); VeL Nucleus vestibularis lateralis; VeM Nucleus vestibularis medialis.


1mm
Inspiratory
Expiratory
Rate
Small Rate
CCV
L
VeM
VeL
LS
FLM
nVI
Ta
RPc
RPA
PL & PM
CTz

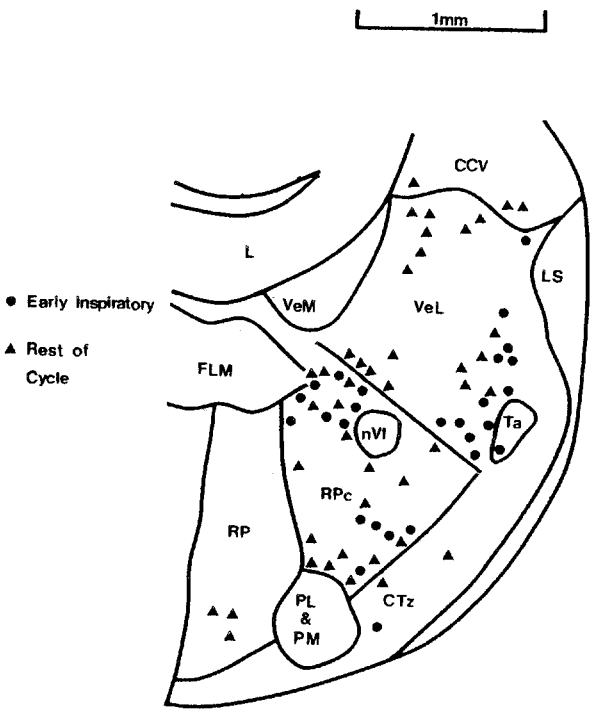

1mm
Early Inspiratory
Rest of Cycle
CCV
L
VeM
VeL
LS
FLM
nVI
Ta
RPc
RP
PL & PM
CTz

Table 1 Summary of results from stimulation and recording work.

Summary Table of Responses Elicited and Recordings Made from the Avian Brainstem Respiratory Regions

Region	Response to Stimulation	Multiple Unit Activity
CCV	Small Rate Increase	RoC
Ventral VeL Medial to Ta	Large Rate Increase (LRLR)	Mainly EI
Dorsal RPc Medial to nVI	Large Rate Increase (LRMR)	RoC and EI
Lateral RPc and Ventral VeL	Expiratory Cramp	Mainly RoC
PL and PM	Inspiratory Cramp	RoC and EI

Abbreviations:- EI Early Inspiratory; LRLR Large Rate Lateral Region; LRMR Large Rate Medial Region; RoC Rest of Cycle.

CCV Commissura cerebellaris ventralis; nVI Nervus abducens; PL Nucleus pontis lateralis; PM Nucleus pontis medialis; RPc Nucleus reticularis parvocellularis; Ta Nucleus tangentialis; VeL Nucleus vestibularis lateralis.

shows the responses to electrical stimulation and those obtained by MUR techniques for each region.

Chapter 6

Electrical Stimulation of the Respiratory Regions in the Quail Brainstem During Thermal Polypnoea

6.1 Introduction

Birds are homeotherms and as such need to maintain their body temperature within a narrow range that is typical for each species under all environmental conditions. If either the environment or physical activity causes the body temperature to move outside that range, behavioural and physiological processes act to return the temperature to acceptable levels. Heat stress can be caused by either high environmental temperature or, more usually, by excessive physical activity such as flight. One of the methods employed by birds to cool themselves is polypnoea; the respiratory system oscillates at up to 20 times the basal frequency in order to remove the excess heat with the expired air.

6.11 Temperature Monitoring

Changes in core temperature are monitored both peripherally and centrally in birds. Kitchell et al (1959) recorded changes in activity from the glossopharyngeal nerve according to tongue temperature. The activity diminished or

was inhibited on warming the tongue and increased up to 15 fold on cooling by 9^{o} C. More recently (Necker, 1972) it was demonstrated that neurones in the beak of the pigeon were also temperature sensitive. Electrodes were placed in the Trigeminal ganglion and although most of the neurones identified were cold sensitive, a few warmth sensitive ones were also reported.

Central temperature sensitive neurones have been indirectly demonstrated by heating local areas of the central nervous system and watching for a particular effector mechanism (Richards, 1975). Rautenberg identified central receptor mechanisms in the spinal cord (Rautenberg, 1967, 1969) and also in the hypothalamus (Rautenberg et al , 1972) of the pigeon. The brainstem was less sensitive than the spinal cord; no amount of brainstem cooling elicited shivering and heating rarely led to polypnoea. Further, panting, once elicited, was abolished more frequently on cooling the spinal cord than on cooling the hypothalamus. In conclusion, panting and shivering seem to be activated from spinal receptors with the hypothalamus playing only a mediating role.

Contrasting with this work, Richards (1970b, 1971b) concluded that the hypothalamus in chickens played a more important role in temperature regulation. Panting could not be elicited without a rise in brain temperature. However,

even temperature increases as great as 3.5° C produced no response unless accompanied by a corresponding increase in colon temperature. It was suggested that this effect may have been due to inhibition from spinal receptors. Nevertheless the results do illustrate that thermal reception may differ between species. This work cannot be compared directly with that of Rautenberg who used selective localised heating, rather than Richards' method of using infra-red irradiation to heat the head as a whole. More recently though, two types of temperature reception mechanisms have been substantiated and it appears that in pigeons the anterior spinal cord is more important as a receptor than the hypothalamus (Rosner, 1978). In contrast, it has been shown that at least some thermoregulatory responses are controlled by the hypothalamus in chickens as well as in some other birds. Simon et al (1976) demonstrated that heating the hypothalamus of the Adelie penguin resulted in a reduction of heat production and peripheral vasodilation causing a fall in core temperature. Seller and Stephenson (1983) produced a review of thermoregulation in birds.

6.12 Integration and Control

Richards (1971a) identified regions in the pigeon and fowl brains from which he elicited polypnoea during electrical stimulation. The frequencies of these polypnoeas were

similar to those elicited in heat-stressed animals; an increase in respiratory rate of up to 20 fold. It therefore seems reasonable to hypothesise that the region identified was concerned with the type of polypnoea brought on during periods of excessive heat-stress. Richards (1971a) suggested the region might be equivalent to the mammalian pneumotaxic centre. However, in the light of the present work (see Chapter 4) this seems unlikely due to the anterior position of the region in the Mesencephalon and its response characteristics. In the fowl the region is situated in the Nucleus mesencephalicus profundus at an anterio-posterior level of about A1.5-4. The region engulfs the Tractus vestibulo-mesencephalicus and the Nucleus ruber.

Richards (1971a) transected the brain at various levels and tested the fowl for the ability to thermal pant. He found that with a transection just caudal to the hypothalamus, the bird could still pant but the threshold temperature for this was raised 2-3^{o} C above normal. However, if the transection was just caudal to the region from which stimulation elicited polypnoea, then no amount of heat-stress produced panting. It is clear then, that although Richards' polypnoea region is required for thermal panting, the hypothalamus, although probably involved, is not essential. This further supports the idea that Richards' region is intimately involved with thermal panting. It is possible that the region has an integrative function and exerts its

effect on the more posterior brainstem respiratory regions identified in the present study.

If the above hypothesis has any substance, during heat-stress the posterior brainstem regions may be expected to receive information from the more anterior thermal regulation integration centres. If, during heat-stress, an increased respiratory frequency is a result of increased neuronal excitation of the brainstem respiratory regions, then the response thresholds can be expected to alter. For example, a region from which rate increases were elicited at basal temperature, would be expected to produce similar increases at lower current thresholds when under heat-stress. The reverse would be true for apnoea or apneusis; an increased respiratory rate due to heat-stress infers an increased neuronal drive. Consequently a higher current would be expected to be required to cause respiratory arrest during heat-stress, than while at basal temperatures. The purpose of this part of the study was to compare responses to electrical stimulation of brainstem respiratory regions and their threshold currents, at basal temperatures and during heat-stress.

6.2 Results

18 birds were heat-stressed externally, by wrapping them in a thermostatically controlled electric blanket (see Chapter 2), until thermal panting began. Each bird was electrically

stimulated in the brainstem respiratory regions before, during and after stressing. Normal body temperature in the quail, immediately after induction of anaesthesia, was found to average 40.45° C (n=18, range 38-41.8).

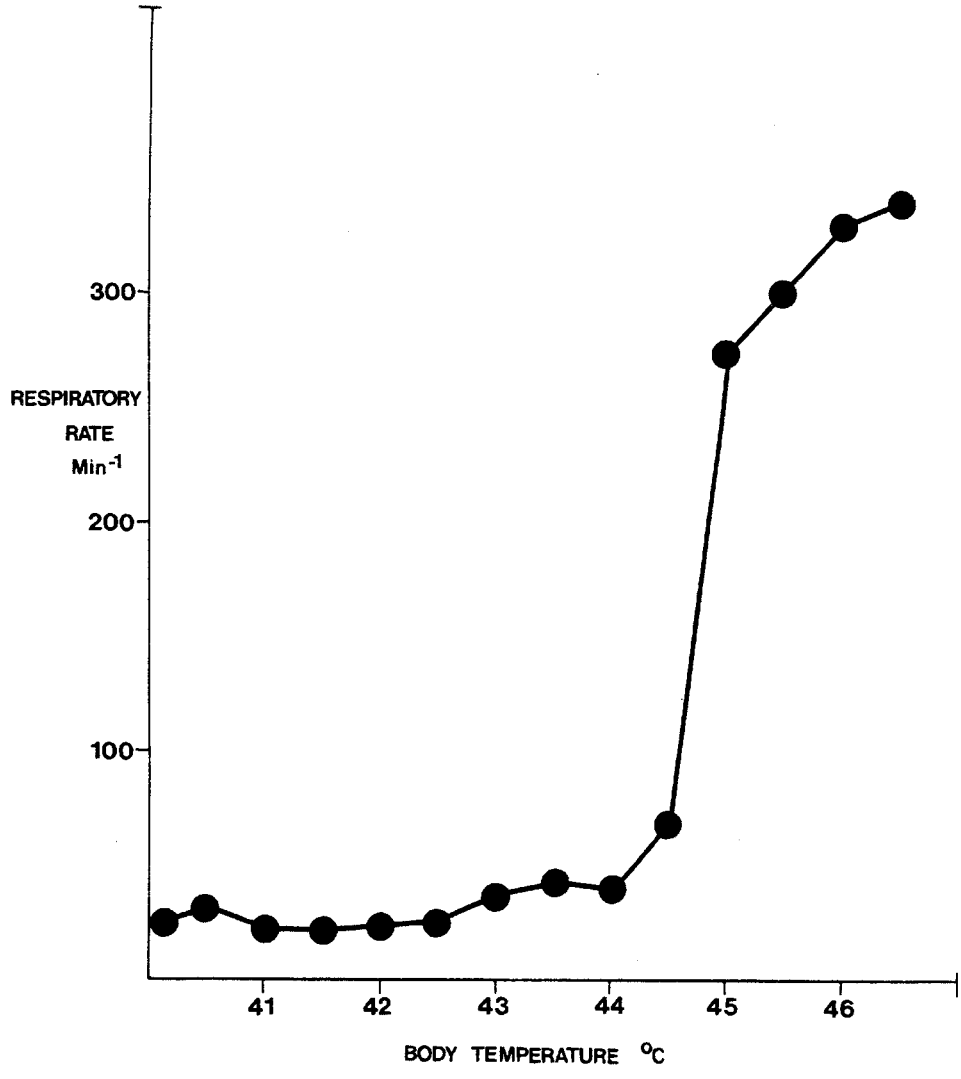
6.21 Effect of Heat Stressing on Respiratory Frequency and Amplitude.

As the environmental temperature was increased, respiratory rate remained constant. Thermal panting set in at an average temperature of 45.1° C (n=18) but at about 0.5° C below this temperature the respiratory frequency increased gradually until it was about 50% above basal levels. At the same time, respiratory depth also began to rise in most birds, but the increase was very small.

Thermal polypnoea began very suddenly and reached a peak within 0.2° C of onset (Fig37). The onset temperatures ranged from 42.7-46.8° C with an average of 45.1° C (n=18). Just before full onset of polypnoea, the animal breathed at accelerated rates (average 984%, n=18) for short periods interspersed with periods of basal respiration. During full polypnoea, respiratory rate increased by an average of 1648% (n=18, range 1300-3400%) and the amplitude by an average of 149% (n=18, range 134-400%). Net ventilation, taken to be rate multiplied by depth, rose by 4251% during polypnoea.

On cooling, thermal panting ceased, again very abruptly, at

Fig 37 Graph showing on-set of thermal polypnoea as body temperature was artificially increased. Data from 18 birds.


300
200
100
RESPIRATORY
RATE
Min-1
41
42
43
44
45
46
BODY TEMPERATURE °C

temperatures ranging from 40.2-45.3° C (average 42.9, n=9). Respiratory frequency remained at levels averaging 130% (n=9) above basal for up to 30 minutes. An average response to post polypnoea cooling (n=9) is shown in Fig38 and Table 2 summarises all the results from this study. The table below summarises the data in this section.

Mean body temperature - 40.45° C

Mean polypnoea onset temperature - 45.1° C

Mean temperature at which
polypnoea ceased on cooling - 42.9° C

6.22 Stimulation of Cramp sites During Thermal Polypnoea

Respiratory regions eliciting cramps were stimulated at normal body temperature in 8 birds. Both apnoeic (expiratory, n=3) and apneusic (inspiratory, n=5) sites were stimulated and the responses were as described earlier (see Chapter 4).

When thermal polypnoea had been produced by heat-stressing, the same sites were stimulated again and the responses and thresholds were compared with those noted at basal temperature. In all birds tested, the response remained unchanged during polypnoea. However, the same was not true for the threshold current required to elicit the cramp. In

Fig 38 Graph showing the return to basal respiration on lowering the body temperature after a period of thermal polypnoea. Basal rate is gradually recovered and no threshold temperature for a sudden change is apparent. Data from 9 birds.

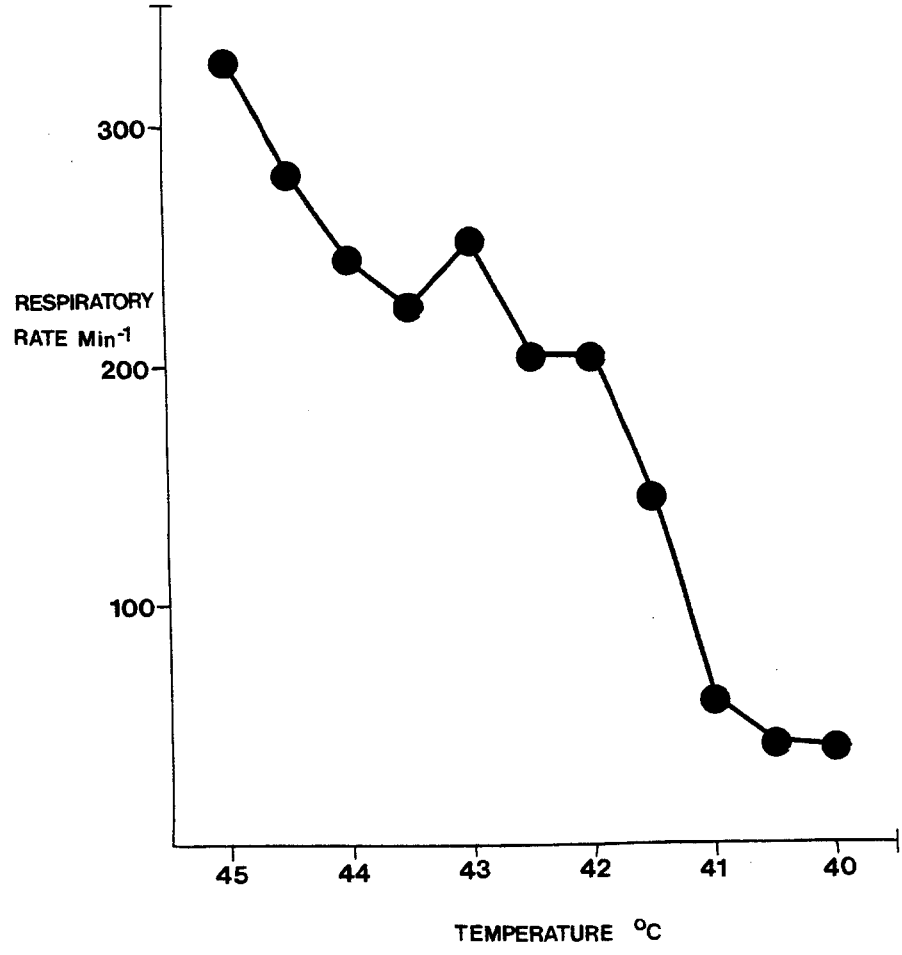

300
200
100
RESPIRATORY
RATE Min-1
45
44
43
42
41
40
TEMPERATURE °C

Table 2 Summary of changes caused to respiratory depth, rate and stimulatory responses and thresholds, before, during and after thermal polypnoea.

Summary Table of Changes in Respiration and in Response Thresholds During Thermal Panting

a. At Normal Body Temperature

Respiratory Depth	4.35 (n=18)
Respiratory Rate	15.42 BPM (n=18)
Ventilation	67.1
Cramp Threshold	17.5 µA (n=8)
Rate Threshold	17.5 µA (n=10)

b. During Thermal Panting

Respiratory Depth	10.83 (n=18, +149%)*
Respiratory Rate	269.6 BPM (n=18, +1648%)*
Ventilation	2919.8 (+4251%)^{o}
Cramp Threshold	33.75 µA (n=8, +92.8%)&
Rate Threshold	35.5 µA (n=10, +102%)*

c. After Cooling to Normal Body Temperature

Respiratory Depth	4.58 (n=9, +5.5%)
Respiratory Rate	35.5 BPM (n=9, +130%)&
Ventilation	162.6 (+141%)^{o}
Cramp Threshold	18 µA (n=2, +2.8%)
Rate Threshold	26.4 µA (n=7, +51%)^{x}

Level of Significance

* P less than 0.1%
& P less than 1.0%
x P less than 5.0%
o not tested

all cases the threshold rose, in one case by 150% and the average increase was 92.8% (n=8). The average increase for the inspiratory threshold was 90% (n=5) and 100% for the expiratory sites (n=3). The rise in threshold current occured very abruptly, just as the polypnoea began. Fig39 shows the threshold changes involved.

On cooling, the basal thresholds for respiratory cramp were usually recovered. In two cases (apneusic) however, the threshold after cooling remained slightly elevated even though basal depth and rate were fully recovered. (See Table 2)

6.23 Stimulation of Large Rate Sites During Thermal Polypnoea

Large rate sites were electrically stimulated in 10 birds at normal body temperature and the responses produced were as described earlier (see Chapter 4). After polypnoea due to heat-stressing had set in, the same sites were stimulated again and the responses and their thresholds were compared with those recorded at basal temperatures. Both the response and its threshold were found to have changes in heat-stressed birds exhibiting polypnoea. The response threshold increased by an average of 102% (n=10, range 50-400%). A diagram showing these increases in threshold is shown in Fig40.

Fig 39 Threshold currents required to elicit respiratory arrests (apneusis and apnoea) at normal body temperatures and during periods of thermal polypnoea at artificially elevated body temperatures.

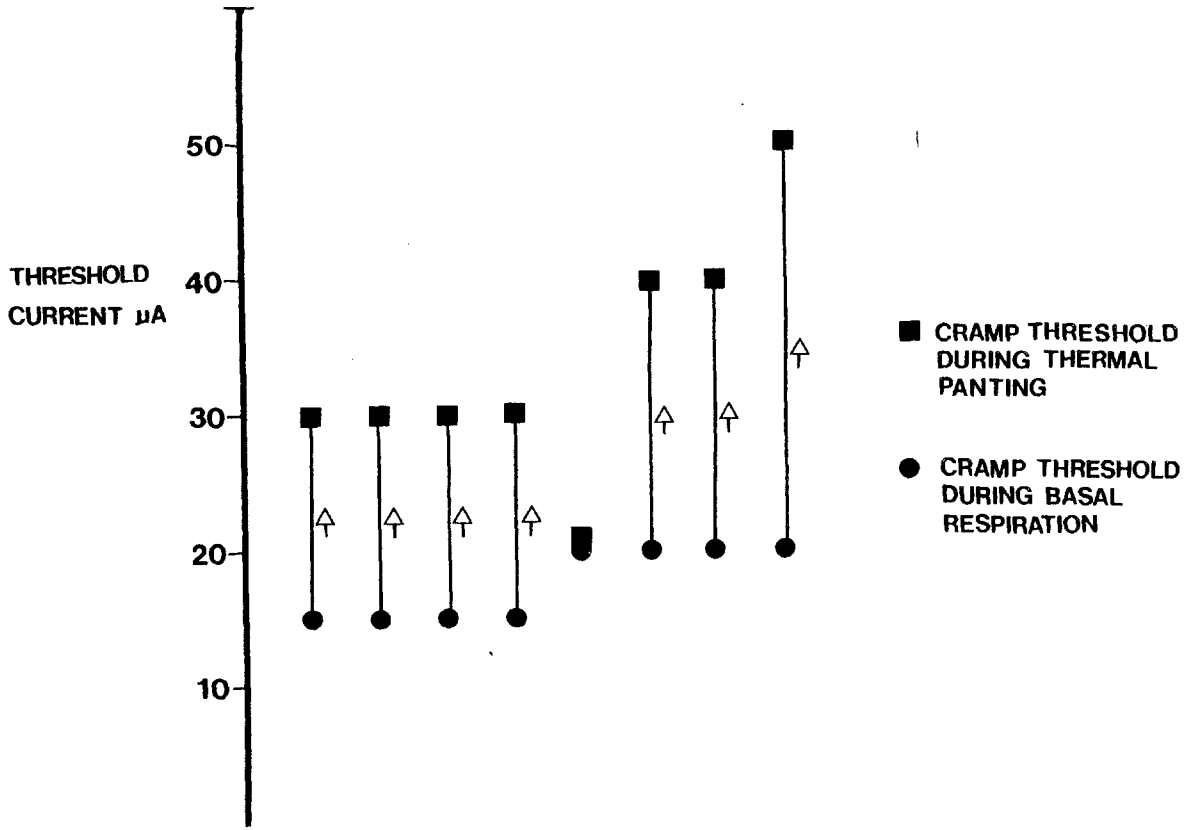
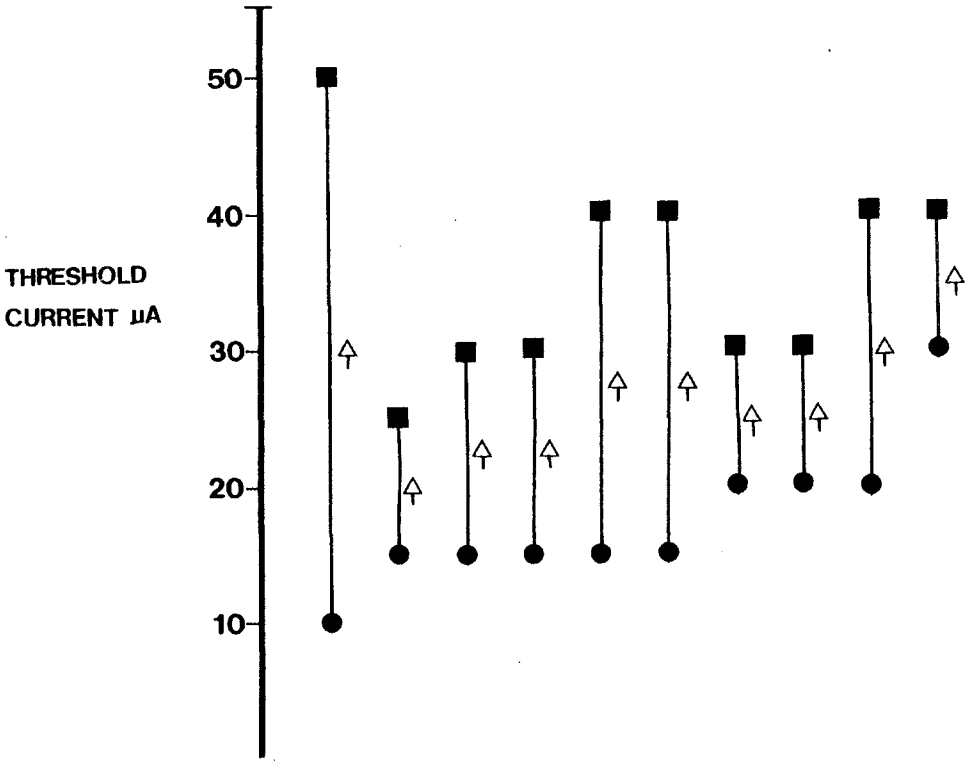

THRESHOLD
CURRENT µA
50
40
30
20
10
CRAMP THRESHOLD DURING THERMAL PANTING
CRAMP THRESHOLD DURING BASAL RESPIRATION

Fig 40 Threshold currents required to elicit a response from large rate sites at normal body temperatures and during periods of thermal polypnoea at artificially elevated body temperatures.


THRESHOLD
CURRENT µA
50
40
30
20
10
LARGE RATE THRESHOLD DURING THERMAL PANTING
LARGE RATE THRESHOLD DURING BASAL RESPIRATION

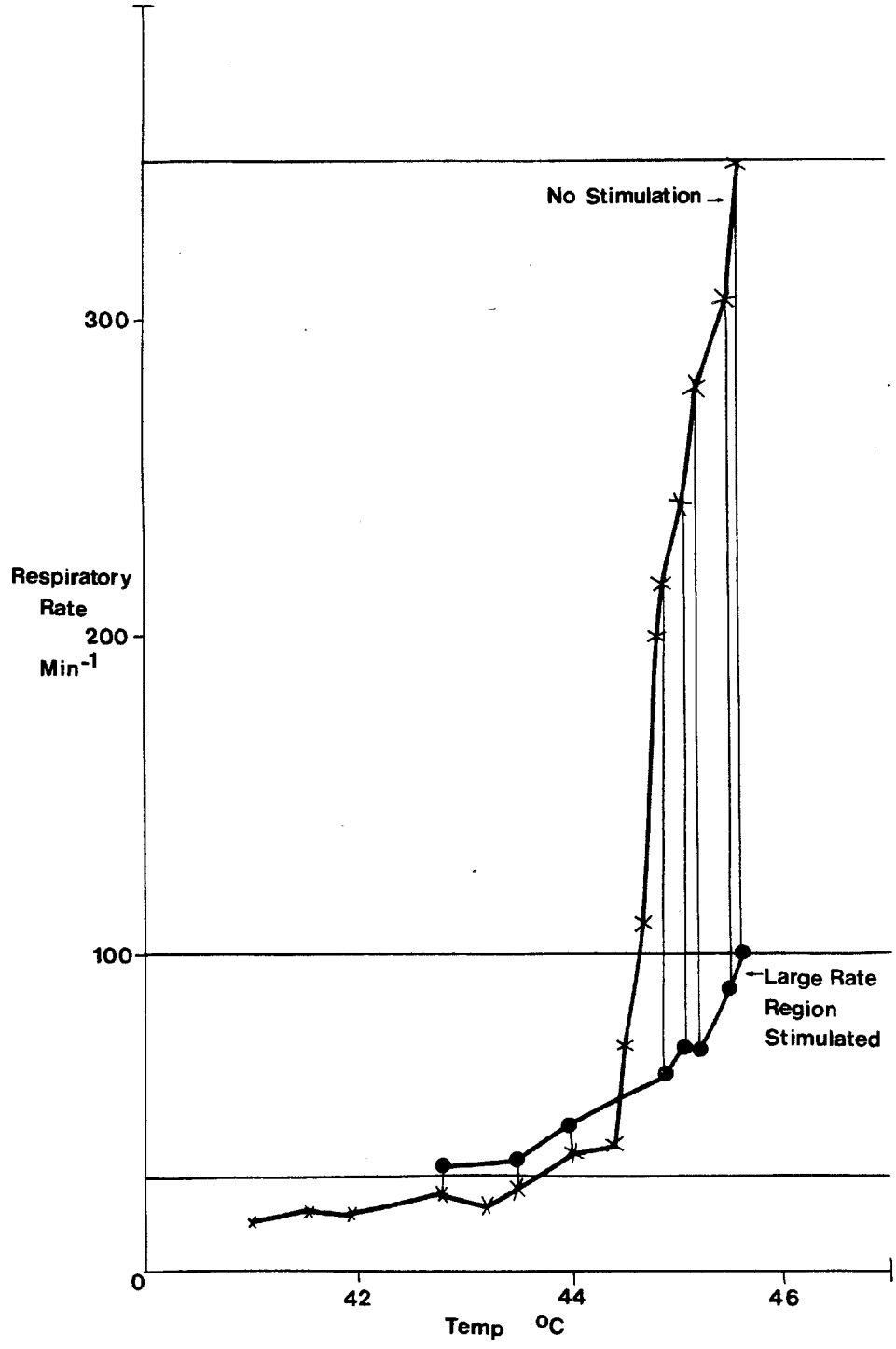
The response itself was the reverse of that elicited at basal temperatures. Instead of a respiratory rate increase, stimulation now slowed or abolished thermal polypnoea, reducing the respiratory rate by up to 84% (average 64.7%, n=10, range 10-84%). Fig41 shows the nature of the response both at normal temperatures and during heat-stress polypnoea. It is clear from the figure that large rate site stimulation always brings the respiratory frequency into a certain narrow range. In fact, over the whole temperature range used, the respiratory rate range is reduced by 72.2% (n=10, range 12-94%) during stimulation.

When the rate and amplitude of breaths had returned to basal levels after cooling, the site was stimulated a third time. The response had now reverted to the large rate type, exhibiting rate increases of up to 140%. The threshold however, remained an average of 51% (n=7, range 25-100%) above original basal levels. (See Table 2)

6.24 Statistical Analysis

The Student's T-test was carried out on the data to establish whether the changes of amplitude, rate and response thresholds during polypnoea were significant. Also, the significance of these factors following cooling was examined. Changes of amplitude, rate, cramp threshold and large rate threshold during polypnoea were all significant at the 1% level. After cooling, the rate

Fig 41 Graphs of respiratory rate against body temperature showing the changing effect of stimulating in a large rate site. In the unstimulated animal normal thermal polypnoea developed. Stimulation of the large rate site elevated respiratory rate at body temperatures without polypnoea, but markedly decreased them during thermal panting.


No Stimulation
300
Respiratory
Rate
200
Min-1
100
Large Rate
Region
Stimulated
0
42
44
46
Temp °C

($P<0.01$) and the large rate threshold ($P<0.05$) were still significantly elevated; in spite of this, rate, amplitude, cramp threshold and large rate threshold had all significantly changed ($P<0.05$) towards basal levels.

6.3 Discussion

6.31 Effects of Heat Stress on Respiratory Depth and Frequency

Subjecting cats to heat-stress while recording phrenic nerve activity (Chapot, 1967) resulted in increased respiratory frequency, while the depth of breathing remained unchanged. Mather *et al* (1980) stressed 7 fowl until they began to exhibit polypnoea of about 140 breaths per minute. They reported that respiratory depth decreased in all cases during polypnoea. Since this was a subjective observation, no exact figures for the decreases were given. Calder and Schmidt Nielson (1968) reported similar results in eight other species. However, these responses contrast with the present work in which respiratory depth increased by an average of 149%. Although Mather *et al* (1980) reported eventual respiratory depth increases (above 43.5^{o} C) these were accompanied by decreases in frequency. The present study demonstrated that all birds abruptly began a polypnoea of increased rate and depth; this was unchanging until death occured at the upper lethal temperature. The shallow breaths reported by Mather *et al* are possibly a result of

the chicken having a larger respiratory system with a lower resonant frequency than the quail. In view of its smaller respiratory system, with a higher resonant frequency, the quail is able to oscillate its system at higher speeds more efficiently. From this, it follows that less energy would be required for it to breath at large depths during polypnoea. However, by cooling with rapid small breaths, the chicken is able to delay the onset of alkalosis and reduce its extent. The quail on the other hand, would succumb to this much more rapidly since more air is being moved per breath across the exchange surfaces. Extending this arguement, the quail in the present study was breathing 100% oxygen, which suggests that the onset of alkalosis would be even more rapid. In spite of these facts, there was no outward evidence for alkalosis, and the quail continued to breath at high rates and depths for up to 7 minutes, after which cooling was permitted. If alkalosis had been in evidence, the polypnoea may have been expected to slow or decrease in depth (Mather et al , 1980).

Mather et al (1980) proposed that alkalosis (hypocapnia) led to further increases in rate and decreases in respiratory depth. In the present study both rate and depth remained constant during polypnoea. This indicated that there was no apparent feedback from alkalosis-monitoring chemoreceptors.

Various ways in which alkalosis during thermal panting could

be avoided have been proposed. Bouverot et al (1974) suggested that alkalosis could be minimised or avoided by dead space ventilation. Ramirez and Bernstein (1976) described a compound biphasic ventilation in pigeons. The fast component proved to have a tidal volume 0.24 times that of the trachea, thus avoiding any contact with the exchange surfaces. The slow componant was similar to that seen during normal respiration. Bech et al (1979) noted, in the greater flamingo, a combination of the above mechanisms. The bird was reported to pant at tidal volumes equivalent to the dead space and this was interrupted by brief regular sequences of deep breaths; probably allowing gaseous exchange. None of the hypothesised methods appears to be responsible for the apparent absence of alkalosis in quail.

Perhaps the quail is able to inhibit its chemoreceptors during thermal panting, so that any alkalosis would not interfere with cooling. Such a system would indicate a hierarchy of afferent respiration-influencing input in which thermal stress was dominant to hypocapnic alkalosis. During activity such as flight, cooling may be more important to the animal than monitoring blood gas levels.

6.32 Effect of Thermal Stress on Electrical Stimulation of Cramp Regions

In order for the animal to breath faster, rate controlling regions of the brainstem must be setting a faster rhythm.

If the rhythm is controlled by ramp generators (see Chapter 8) this means that either the input to these is increased or their threshold is decreased. If the input is increased, the threshold charge would be reached sooner. Since discharge would follow immediately, the time between successive discharges would decrease. Assuming that ramp generators, at least in part, set the frequency, more input would result in faster respiration. Coupled with the increased rate, was an increase (149%) in respiratory depth. This phenomenon probably resulted from an increased input causing increased motor output. Possibly, if the resonant frequency of the system were reached, the depth would have increased without an increase in muscle activity.

When the inspiratory or expiratory regions were stimulated, the threshold for cramp was found to rise by up to 150% during polypnoea. Presumably, in addition to the arrest, the stimulus had to counteract the effect of any afferent activity - in this case from temperature receptors. Input from hypothalamic and spinal thermal receptors, as mentioned, probably resulted in increased motor drive (greater respiratory depth) and increased charge/discharge of ramp generators (greater respiratory frequency). Any inhibitory or phase reversal responses elicited during basal respiration, would now be acting directly against both increased depth and frequency. It is thus, only to be expected that more current would be required to overcome the

effects of thermal stress, which were antagonistic to the response.

6.33 Effect of Thermal Stress on Electrical Stimulation of Large Rate Areas

From the principles outlined above, a region eliciting an increase in respiratory frequency on stimulation, would be aided rather than antagonised during heat-stress. It is possible that the rate increase during thermal panting occured due to lowering the ramp generator threshold or increasing the input current resulting in a faster charge time and a higher respiratory frequency. Assuming that the large rate increase was due to stimulation of the ramp generator, then during thermal polypnoea, a response would be expected at a lower threshold. This was not observed. Instead, stimulation elicited decreases in respiratory rate of up to 84%, very often eliminating polypnoea totally. It is not easy, at this stage, to explain this result. In terms of ramp generators, either the input is inhibited, or the threshold raised.

The result however, does lend support to the idea that the large rate region has functions similar to the pneumotaxic centre of mammals. Richards (1971a) obtained no respiratory slowing from his suggested pneumotaxic centre in birds. Although this was not proof that it had no pneumotaxic function, it added no support to the argu ment. The large

rate region has produced both slowings and accelerations of respiratory rate on stimulation. However, the slowings occurred at vastly accelerated rates due to thermal stress and the accelerations at slightly decreased rates due to hyperoxia (see Chapter 3). The large rate region appears to be maintaining a "status quo" by accelerating respiration at rates below this "status quo" level and decreasing it at higher rates. In fact, the respiratory rate range was decreased by 72% during stimulation (ranging from hyperoxia to thermal polypnoea) from non-stimulated levels. Viewed in this way the large rate regions appear, at least partly, to perform the functions of the mammalian pneumotaxic centre. These regions probably set a basal rhythm which is modified by input from chemoreceptors, thermal receptors etc. However, it seems that the balance between the basal rhythm and the externally modified rhythm is a fine one. The afferent activity has to be large enough to override the internal rhythm set by the region. It is probable that the region's output is variable in order to enable a hierarchy of external influencing factors to act on respiration. When the region is electrically stimulated, the afferent rhythm effecting mechanisms are overriden and the basal rhythm is re-established.

In conclusion, in the light of the work performed with heat-stressed quail, it is apparent that the large rate region has at least some features of the mammalian

pneumotaxic centre.

Chapter 7

Electrical Stimulation of the Respiratory Regions in the Quail Brainstem During Periods of Elevated Environmental Carbon Dioxide

7.1 General Introduction

Air passes unidirectionally over the parabronchi in birds (see Chapter 1) where it gives up oxygen and takes on carbon dioxide. Factors such as body temperature and activity level cause the metabolic rate to vary, these in turn result in changes in the demand for oxygen and production of carbon dioxide. During heavy exercise large amounts of oxygen are required while similar quantities of waste carbon dioxide are produced. In order to keep up with the demands, as far as possible, the rate at which air passes over the parabronchi must be increased during such periods. In order to do this effectively, the bird is able to monitor gas concentrations in the lung air and lung and arterial blood. If the carbon dioxide level rises, the chemoreceptors trigger the brainstem respiratory centres (see Chapters 4,5 and 8) to increase ventilatory drive in order to return the carbon dioxide to basal levels.

7.2 Central and Peripheral Chemoreceptors

There is plentiful evidence for the existence of peripheral chemoreceptors in birds, but support for the existence of central chemoreceptors is based largely on inference (Jukes, 1971). Peripheral chemoreceptors have been studied extensively in birds and fall essentially into two categories; systemic (those associated with major arteries) and pulmonary (those in the lung blood system or those directly sensitive to inspired gas).

7.21 Systemic Receptors

Carotid and aortic bodies as well as nerve endings in the pulmonary arteries were identified in the chicken (Fedde, 1970). Although he presented no evidence concerning the function of these bodies, he assumed them to be responsible for monitoring levels of oxygen and carbon dioxide in the blood. Later that year experiments were carried out in which respiratory gas concentrations were changed before and after denervation of the carotid bodies in ducks (Jones and Purves, 1970). Before denervation, administering 100% oxygen resulted in an 11-17% decrease in ventilation. Reducing the oxygen to levels of less than half the control, resulted in an average ventilatory increase from basal levels of 190%; this increase was developed further by increasing the level of carbon dioxide present. With oxygen concentrations at control levels, raising arterial carbon

dioxide to 45mm Hg resulted in a doubling of ventilation. After carotid body denervation all of these responses were modified. The ventilatory fall resulting from breathing 100% oxygen was abolished in all ducks tested and the increased ventilation caused by reduced oxygen levels was abolished in 66% of the birds. The increased ventilation resulting from raising the arterial carbon dioxide was unaffected, although the rate of increase in response to 4% carbon dioxide was markedly reduced. This work indicated that the carotid bodies were intimately involved with monitoring blood gas levels in order to mediate changes in the respiratory pattern. However, it would appear that they respond to hypocapnia and hypoxia to a greater extent than hypercapnia.

Bouverot and Leitner (1972) found that in fowl, 12% of the vagal fibres were responsive to arterial oxygen and carbon dioxide levels; they called these "arterial chemoreceptor fibres". They reported that these fibres exhibited permanent activity under control conditions. However, during transient hyperoxia the fibres showed a transient decrease in activity which accompanied the decrease in respiration. An increase in firing rate occured in response to transient hypoxic or hypercapnic conditions. Bouverot (1977) went on to cut the vagi in Pekin ducks and reported that the responses were abolished.

Estavillo and Youther (1977) stimulated the middle cardiac nerve (MCN) in the chicken at varying intrapulmonary and arterial carbon dioxide levels. They found that at all levels of carbon dioxide, stimulation of the MCN produced a reduction in respiratory movements. However, it was pointed out that this response was maximal at low carbon dioxide levels. Receptors with axons in the MCN therefore were thought to be important in avian respiratory control.

7.22 Intrapulmonary and Respiratory Tract Receptors

It was reported (Hiestand and Randall, 1941) that in some species of birds inhalation of carbon dioxide caused respiratory stimulation, while in others it caused cramp. The inhibitory responses to carbon dioxide were said to be due to reflex stimulation of receptors in the naso-pharynx of the upper respiratory tract. No evidence for such receptors was found in rabbits. Much later Fedde (1970) confirmed the existence of these receptors and stated that they had been functionally shown to alter respiratory movements in ducks. Bamford and Jones (1974) proposed that the response of these receptors was mediated via the glossopharyngeal nerve while Leitner et al (1974) proposed the hypoglossal nerve.

Molony (1971) described carbon dioxide sensitive receptors in the lower respiratory tract of the chicken which mediated their responses vagally. He proposed that it was the

elimination of the action of these receptors which was mainly responsible for the slow deep breathing produced after vagotomy (see Chapter 3).

The carbon dioxide receptors in the lung are probably more important to respiratory homeostasis than those in the respiratory tract; certainly much more work has been diverted towards their elucidation. Hiestand and Randall (1941) placed a cannula into the lungs of a duck through which carbon dioxide was pumped. They reported a marked stimulation of respiration which gave the first indication that this type of receptor existed in the lung. Fedde et al (1963) found that bilateral vagotomy in the thoracic region, leaving the nerve supply to the carotid bodies intact, resulted in similar respiratory changes to those following cervical vagotomy. The presence of additional peripheral receptors was thus indicated. Peterson and Fedde (1968) demonstrated the presence of receptors sensitive to the removal of carbon dioxide from the lung. Apnoea was induced in the chicken 0.64 seconds after rapid lowering of the respired carbon dioxide concentration. Afferent impulses from these receptors too, were reported to be mediated via the vagi.

Small blood vessels in the wall of the primary bronchus were seen to be accompanied by nerve bundles (Cook and King, 1969). A sensory function was speculated for these nerve

cells. Work the following year (Fedde, 1970 and Peterson and Fedde, 1971) confirmed the existence of these receptors and that their effects were mediated via afferent fibres in the vagi. Their firing rate increased as carbon dioxide concentration decreased. At carbon dioxide concentrations of 7-8% most of the fibres had stopped firing although some continued to show activity up to levels of 14%. It was suggested that these responses could be responsible for informing the central nervous system about the metabolism of the peripheral tissues. They may therefore, be involved with responses to hypercapnia resulting from exercise.

Abrupt elimination of carbon dioxide caused transient burst activity in some afferent vagal fibres which rapidly adapted to constant discharge (Fedde and Peterson, 1970). Neither hypoxia (5% oxygen) or hyperoxia (80% oxygen) had any effect on the discharge but 10% carbon dioxide silenced the receptors. As expected, the increased firing resulting from the abrupt removal of carbon dioxide, resulted in inhibition of breathing (Peterson and Fedde, 1971). This response was slowed but not abolished by bilateral vagotomy (Eaton *et al*, 1971), which indicated the presence of other peripheral receptors (see 7.21).

Burger *et al* (1974) reported that the cardiac sympathetic nerves, as well as the vagi, were involved in mediating the intrapulmonary receptor response. They also pointed out

that the receptors were sensitive to carbon dioxide in the lung blood as well as lung air. Their location was confined to the parabronchi and it was suggested that they monitored ventilation (lung air) as well as venous and mixed venous carbon dioxide levels.

Banzett and Burger (1977) recorded the discharge from individual intrapulmonary neurones in the left Nodose ganglion of the fowl. When carbon dioxide levels in the pulmonary artery increased, the discharge frequency of all 21 receptors studied decreased. The response was similar to increased carbon dioxide levels in the airway gas. Because the receptors inhibit respiration, it was suggested that they might:-

1. serve to adjust minute ventilation according to the rate of metabolic carbon dioxide production

or

2. regulate individual breath size.

As already discussed (see Chapter 3) it has been suggested that the receptors may be involved with pacing respiration with carbon dioxide levels in the lung gas. Maximal excitation of the receptors and thus inhibition of inspiration, would occur towards the end of inspiration (high oxygen and low carbon dioxide levels in the lung air) and minimal excitation and thus release of inhibition, during expiration (low oxygen and high carbon dioxide in the

lung air).

7.3 Respiratory Responses to Changing Arterial and Pulmonary Carbon Dioxide Levels

The carbon dioxide-sensitive receptors of the naso-pharynx (see 7.22) were by-passed (Johnston and Jukes, 1967). Any peripherally mediated responses were therefore due to intrapulmonary and arterial chemoreceptors only. As inspired carbon dioxide was increased from zero to 6.5%, pulmonary ventilation increased exponentially. Curves of similar shapes have been obtained from several mammals and it is for this reason that a common control mechanism was hypothesised.

During hypoxia in the fowl, respiratory rate increased to begin with but later fell off to below basal levels (Richards and Sykes, 1967). When hypoxia was gradually induced in the chicken (Butler, 1967), pigeon and duck (Butler, 1970) respiratory rate increased slightly during the early stages. Subsequently, contradicting Richards and Sykes, the rate was reported to increase still further. After vagotomy, the increase was abolished, indicating (see 7.2) that the vagus is essential for the rise in respiratory frequency.

During hypercapnic conditions, the respiratory rate was found to decrease while the amplitude increased (Richards

and Sykes, 1967). Ray and Fedde (1969) looked more closely at the response in chickens. Respiratory rate and depth were found to remain at basal values when carbon dioxide levels were below 4%. At higher concentrations (4-20%) the amplitude was reported to increase and the frequency drop while resistance to air flow in the respiratory tract decreased. Osborne and Mitchell (1977) concluded that while arterial gas levels remained normal, the intrapulmonary chemoreceptors mediated the ventilatory reaction in response to inhaled carbon dioxide. Further, changes in parabronchial ventilation were thought to be responsible for maintaining arterial carbon dioxide levels constant.

The responses to changing both intrapulmonary and arterial carbon dioxide levels were consolidated by Osborne et al (1977). Increasing intrapulmonary carbon dioxide (from 0-35 Torr) while maintaining arterial carbon dioxide levels constant, at 29 Torr, resulted in increased amplitude and decreased frequency. Increasing arterial carbon dioxide levels (from 19-61 Torr) while maintaining intrapulmonary carbon dioxide levels constant also increased depth and decreased frequency. In both cases any further increases in carbon dioxide concentrations had only minimal effects. Reviews of peripheral avian respiratory control have been published (Jukes, 1971 and Jones, 1976).

7.4 Results

14 birds were subjected to 5% carbon dioxide in oxygen and 19 birds to 10%. The effects of these elevated carbon dioxide levels on respiratory rate and depth were looked at in all birds. Individuals from each group were electrically stimulated in the brainstem respiratory regions (see Chapter 4) and any changes in the responses or the current thresholds required to induce them were recorded.

7.41 Effect of Elevated Carbon Dioxide Levels in the Inspired Gas on Respiratory Frequency and Amplitude

Generally, increasing the carbon dioxide level in the inspired gas caused a decrease in respiratory depth and an increase in amplitude. The effect was more pronounced using 10% carbon dioxide than with 5%.

With carbon dioxide at the 5% level in the inspired oxygen the respiratory rate dropped by an average of 17.9 (n=14) and the amplitude rose by an average of 37.9% (n=14). With 10% carbon dioxide the rate dropped by an average of 31.4% (n=19) and the amplitude rose by 98.2% (n=19). When breathing 10% carbon dioxide, the rate was on average 16.5% lower and the amplitude 43.7% higher than with 5% carbon dioxide. Total ventilation was taken to be represented by respiratory depth multiplied by respiratory rate. Average change in total ventilation was calculated and with 5%

carbon dioxide the total ventilation rose by an average of 13.3% while 10% carbon dioxide caused a rise of 36%. (See Table 3)

On some occasions (n=3) while using the 10% gas mixture, the amplitude fell to a very low level along with the rate. In such cases the animal was usually dead within five minutes.

30 minutes after reconnection of the 100% oxygen, the basal amplitudes and frequencies were noted again. In the 5% carbon dioxide experiments the amplitude returned to a level that was on average, 11.8% (n=5) lower and the frequency 33% (n=5) lower; while following 10% carbon dioxide the amplitude was 51% (n=14) above basal and the frequency 1.3% (n=14) depressed.

7.42 Stimulation of Cramp Sites During Periods of Elevated Carbon Dioxide Levels in the Inspired Gas

Respiratory regions eliciting cramps were stimulated with no carbon dioxide (normal experimental conditions) in the inspired gas of 17 birds. Both apnoeic (expiratory, n=9) and apneusic (inspiratory, n=8) sites were stimulated and the responses were as described earlier (see Chapter 4).

The carbon dioxide levels were elevated to 5% in some birds (n=7) and 10% in others (n=10) and the sites were stimulated again. The responses and their thresholds were compared with those noted when carbon dioxide levels were zero. In

Table 3 Summary of changes caused to respiratory depth, rate and stimulatory responses and thresholds, before, during and after periods of elevated carbon dioxide in the inspired air.

Summary Table of Changes in Respiration and in Response Thresholds During Periods of Elevated Carbon Dioxide

a. 0% Carbon Dioxide (Basal)

Respiratory Depth	5.01 (n=33)	
Respiratory Rate	19.35 BPM (n=33)	
Ventilation	96.94	
Cramp Threshold	35.7 µA (n=7)	25.8 µA (n=10)
Rate Threshold	16.43 µA (n=7)	17.5 µA (n=9)

b. At Elevated Carbon Dioxide Levels

	5% CO_2	10% CO_2
Respiratory Depth	6.91 (n=14, +37.9%)&	9.93 (n=19, +98.2%)*
Respiratory Rate	15.98 (n=14, -17.9%)	13.27 (n=19, -31.4%)*
Ventilation	109.8 (+13.3%)^{o}	131.8 (+36%)^{o}
Cramp Threshold(s)	51.42 (n=7, +44%)*	39.9 (n=3, +55%)^{o}
	None	14.3 (n=7, -44.6%)^{o}
Rate Threshold(s)	None	29.25 (n=4, +67%)^{o}
	12.7 (n=7, -22.6%)	11.4 (n=5, -35%)^{o}

c. 30 Minutes After Reconnecting 0% Carbon Dioxide

Respiratory Depth	4.42 (n=5, -11.8%)	7.6 (n=14, +51%)^{x}
Respiratory Rate	12.92 (n=5, -33%)^{x}	19.1 (n=14, -1.3%)
Ventilation	57.1 (-41%)^{o}	145.2 (+49.8%)^{o}
Cramp Threshold(s)	Basal	29.2 (n=3, +13.3%)^{o}
	None	Basal
Rate Threshold(s)	None	20 (n=3, +14.3%)^{o}
	15.2 (n=5, -7%)^{o}	12 (n=4, -31.4%)^{o}

Level of Significance

* P less than 5% & P less than 10% x P less than 20%

o not tested (sample too small)

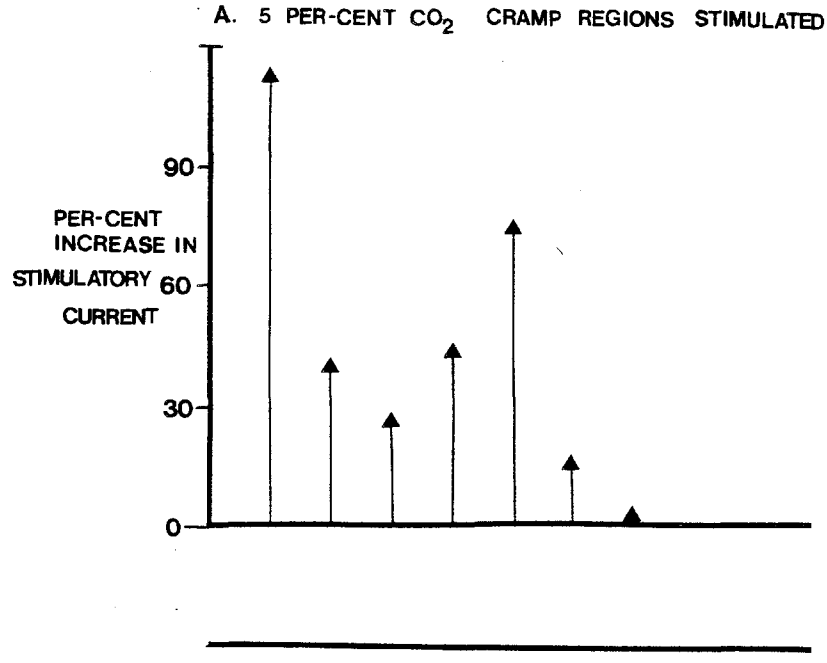
all birds tested the response remained unchanged during the periods of elevated carbon dioxide. The same was not true for the threshold current required to elicit the cramp. With 5% carbon dioxide all the thresholds rose; the average increase was 44% (n=7). The average increase for an inspiratory threshold was 37.2% (n=5) and for an expiratory one 61.1% (n=2). Fig42a shows the threshold changes involved.

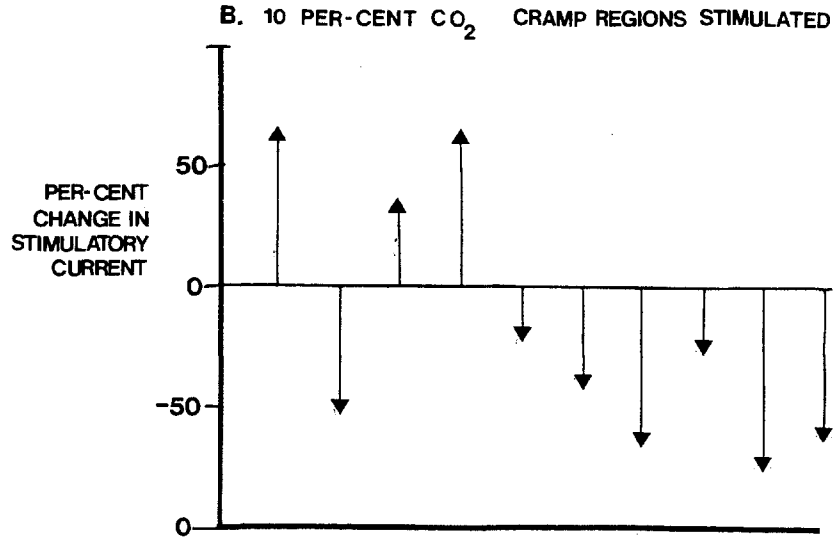
With 10% carbon dioxide the threshold showed both increases (n=3) and decreases (n=7). All the falls in threshold occured in birds whose respiration had become weak due to the high carbon dioxide levels. On average, the amplitude of respiration of such birds decreased by 9% (n=7) while those showing increases in cramp threshold exhibited increases in amplitude of 193% (n=3). Of those whose threshold rose, the average increase was 39.9% (n=3). The average increase for the inspiratory threshold was 42.9% (n=1) and for the expiratory threshold 37.6% (n=2). Of those whose threshold fell, the average decrease was 44.6% (n=7). The average decrease for the inspiratory threshold sites was 47.7% (n=2) and for the expiratory threshold 43.4% (n=5). Fig42b shows the threshold changes involved. (See Table 3)

30 minutes after reconnecting the 100% oxygen, thresholds for cramps were noted again: basal values were restored in

Fig 42 A. Percent increases in threshold currents required to elicit respiratory arrests (apneusis or apnoea) when carbon dioxide levels in the inspired gas are raised from zero to 5%.

B. Percent changes of threshold currents required to elicit respiratory arrests (apneusis or apnoea) when carbon dioxide levels in the inspired gas are raised from zero to 10%.


A. 5 PER-CENT CO_2 CRAMP REGIONS STIMULATED
PER-CENT INCREASE IN STIMULATORY CURRENT
90
60
30
0


B. 10 PER-CENT CO_2 CRAMP REGIONS STIMULATED
PER-CENT CHANGE IN STIMULATORY CURRENT
50
0
-50
0

those birds which were subjected to 5% carbon dioxide. Basal thresholds were also regained in birds which exhibited decreased thresholds when subjected to 10% carbon dioxide, those which exhibited increases, had thresholds remaining 13.3% (n=3) above basal.

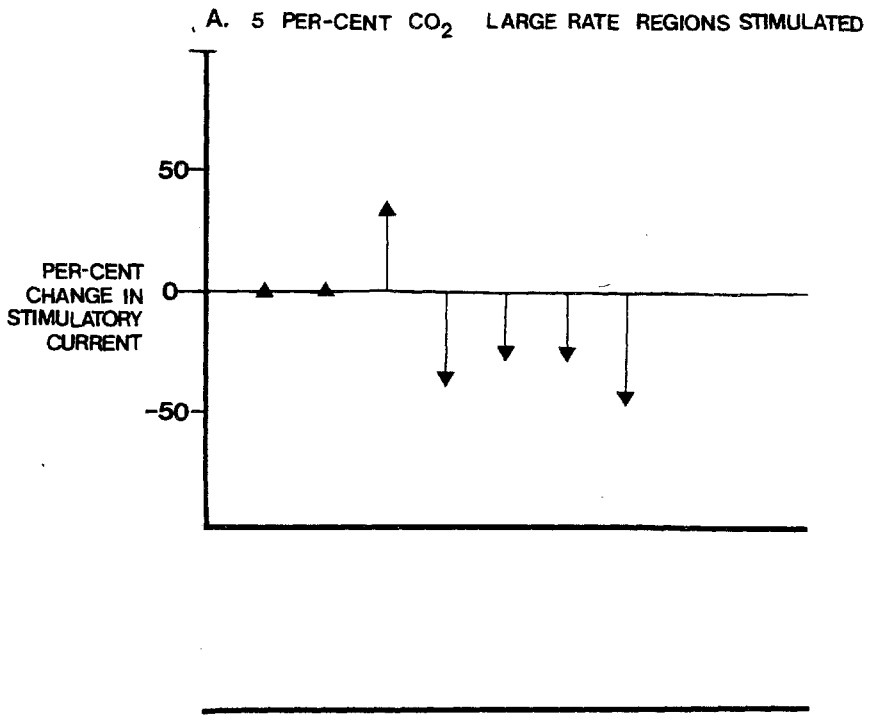
7.43 Stimulation of Large Rate Sites During Periods of Elevated Carbon Dioxide in the Inspired Gas.

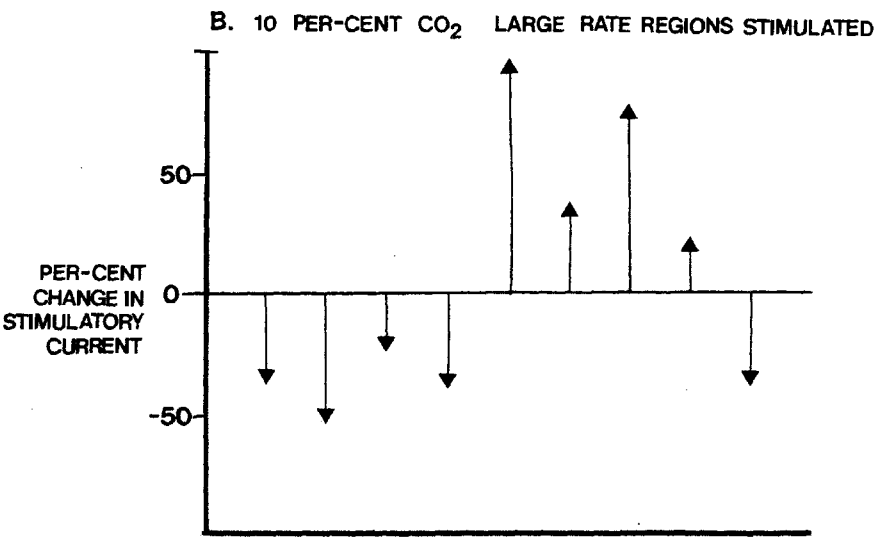
Large rate sites were electrically stimulated in 16 birds with no carbon dioxide (normal experimental conditions) in the inspired gas; the responses produced were as described earlier (see Chapter 4). The carbon dioxide levels were elevated to 5% in some birds (n=7) and to 10% in others (n=9) and the same sites were stimulated again. The responses and their thresholds were compared with those noted when the carbon dioxide levels were zero. In all birds tested the response remained the same during periods of elevated carbon dioxide concentrations. However, in most cases (n=14) the threshold required to elicit the large rate increase varied. With 5% carbon dioxide, the threshold changed very little, but in general it showed an average decrease of 22.6% (n=7). Fig 43a shows the threshold changes involved.

With 10% carbon dioxide, the threshold showed both decreases (n=5) and increases (n=4). When the threshold fell, the decrease averaged 35% (n=5) and in those birds showing an

Fig 43 A. Percent changes of threshold currents required to produce a large rate response when carbon dioxide levels in the inspired gas are raised from zero to 5%.

B. Percent changes of threshold currents required to produce a large rate response when carbon dioxide levels in the inspired gas are raised from zero to 10%.


A. 5 PER-CENT CO_2 LARGE RATE REGIONS STIMULATED
50
PER-CENT CHANGE IN STIMULATORY CURRENT
0
-50


B. 10 PER-CENT CO_2 LARGE RATE REGIONS STIMULATED
50
PER-CENT CHANGE IN STIMULATORY CURRENT
0
-50

increase, the average was 67% (n=4). Generally, the birds showing decreased thresholds were those whose respiratory amplitude had greatly increased with the introduction of carbon dioxide. On average, such birds showed an increased depth of 242% (n=5) while those whose response threshold increased, showed increased depth averaging only 34.7% (n=4). Fig43b shows the threshold changes involved. (See Table 3)

30 minutes after reconnection of the 100% oxygen, the thresholds were noted again: those birds subjected to 5% carbon dioxide had thresholds 7% (n=5) lower than basal. Of the birds subjected to 10% carbon dioxide, those which showed increased thresholds remained 14.3% elevated, while those showing decreases were 31.4% (n=4) below basal levels.

7.44 Statistical Analysis

The Student's T-test was carried out on those sets of data containing a large enough sample (See Table 3). Both amplitude and rate significantly changed ($P<0.05$) when the bird was subjected to 10% carbon dioxide; the response thresholds were not tested due to the small number of observations. Amplitude significantly changed ($P<0.01$) when the bird was subjected to 5% carbon dioxide as did the response threshold for cramp. However, no significant change was found for rate or the response threshold for large rate. 30 minutes after reconnection of the oxygen,

rate (following 5% carbon dioxide) and amplitude (following 10% carbon dioxide) were still changed ($P<0.2$) from basal. Response thresholds on recovery were not tested due to their small sample size.

7.5 Discussion

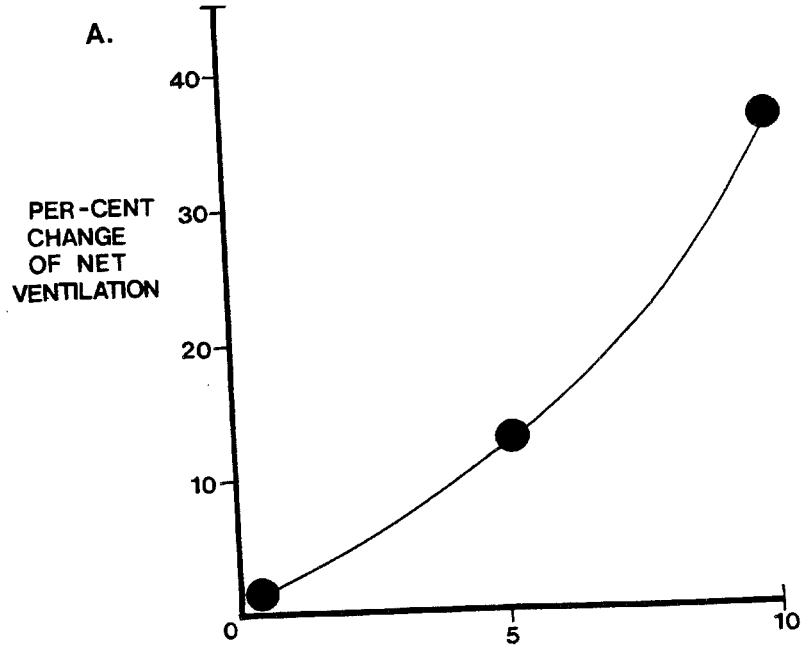
7.51 Effects of Elevated Carbon Dioxide Levels in the Inspired Gas on Respiratory Depth and Frequency

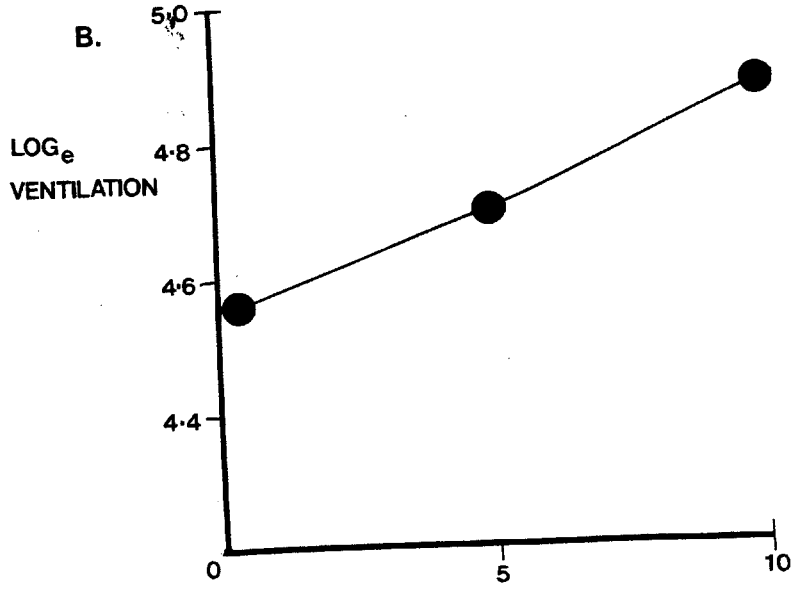
Richards and Sykes (1969) reported that hypercapnia (elevated blood carbon dioxide) in the fowl resulted in increased amplitude and decreased respiratory rate. This is consistent with the results obtained in the present study. Ray and Fedde (1969) noted that rate and depth remained at basal levels until carbon dioxide levels rose above 4%. In the present study, with carbon dioxide at the 5% level, amplitude rose by 37.9% and rate fell by 17.9%. It is unlikely that these responses would be eliminated by reducing the carbon dioxide level to 4%. This indicates that the quail is probably more sensitive to carbon dioxide than the fowl.

Johnston and Jukes (1967) found that total ventilation rose exponentially with carbon dioxide concentration at levels within 0-6.5%. Fig44a shows the same curve plotted with the three values used in quail; the relationship also appears to be exponential. This is confirmed when Log.e of the

Fig 44 A. Graph of ventilation plotted against carbon dioxide concentration in the inspired gas. The graph appears to bear an exponential relationship. Data taken from 14 birds.

B. Graph of Log.e ventilation plotted against carbon dioxide concentration in the inspired gas. Further weight is given to a genuine exponential relationship by the straight line produced. Data taken from 14 birds.


A.
40
30
20
10
PER-CENT CHANGE OF NET VENTILATION
0
5
10


B.
5·0
4·8
4·6
4·4
LOGe VENTILATION
0
5
10

increase in total ventilation is plotted against the carbon dioxide level (Fig44b) resulting in an almost straight line. A possible error caused by using a pressure transducer to monitor depth might cause some of the amplitudes recorded at the lower frequencies to be too low. If such an error existed, it would have resulted in the upper part of the curve having ventilation values too low. Consequently any correction for this error would improve the characteristics of the curve.

The results are again supported by Osborne et al (1977), who increased pulmonary and arterial carbon dioxide levels independently from one another. In both cases they reported increased depth and decreased respiratory rate.

Those birds showing smaller increases in depth and whose response thresholds changed in an unexpected direction while breathing 10% carbon dioxide, had probably been weakened by the unnaturally high level of carbon dioxide in the blood. Indeed, some birds (n=3) died before reconnection of the oxygen had taken place.

After reconnection of the 100% oxygen, restoration of basal amplitudes and depths was not complete after 30 minutes. Following subjection to 10% carbon dioxide the amplitude remained elevated by over 50% and the frequency slightly depressed - this resulted in an increase of total ventilation and helped to compensate for the hypercapnia.

After subjection to 5% carbon dioxide, both rate and depth were depressed which resulted in a decrease of net ventilation. This probably occurred following over-compensation for the hypercapnia resulting in hyperoxia.

In conclusion it seems likely, due to similarities in the responses, that quail monitor elevated carbon dioxide in a similar way to other birds.

7.52 Effect of Elevated Carbon Dioxide Levels in the Inspired Gas on Electrical Stimulation of Cramp Regions

Under hypercapnic conditions an increase in firing rate of arterial chemoreceptors has been reported (Bouverot and Leitner, 1972). This activity could result in increasing phase switching thresholds of ramp generators (see Chapter 8). This would lead to a longer time between consecutive phase switches and consequently a slowing of respiratory rate. Alternatively, the spike generator could be inhibited, making the ramp shallower and increasing the cycle time. Because of the increased cycle time, there is more time available for each inspiration or expiration; if the phases persist for longer, with all other factors remaining the same, an increase in amplitude is likely to result.

It is postulated (see Chapters 4,8) that the cramp responses

operate by rapidly charging and discharging the ramp generators. Under conditions where the switching threshold is raised, a larger current would be required to achieve the same rapid charge/discharge frequency which results in cramp; this is because the ramp generator now holds more charge before each discharge. This phenomenon may not completely explain the elevated response current threshold observed and the afferent activity from the chemoreceptors probably acts in other ways to increase respiratory drive. At whatever stage in the system this occurs, a higher stimulatory current might be required in order to counteract it.

The birds in which the threshold decreased were approaching hypercapnic collapse and consequently their respiratory drive was greatly reduced. This, in turn, caused a reduction of the cramp thresholds from basal - some birds (n=2) did not recover after stimulated cramps.

30 minutes after reconnection of the 100% oxygen, the basal thresholds of the birds subjected to 5% carbon dioxide were recovered as were those showing decreased thresholds in response to 10% carbon dioxide. Those showing increased thresholds in response to 10% carbon dioxide still had elevations of up to 33%. This suggests that the threshold for the response was directly related to the blood gas concentrations; the amplitude remained elevated and the

frequency slightly depressed, indicating that there was a pool of carbon dioxide remaining. Respiratory drive is increased (elevating the cramp thresholds) in order to clear this pool.

7.53 Effect of Elevated Carbon Dioxide Levels in the Inspired Gas on Electrical Stimulation of Large Rate Regions

With increased respiratory drive, any factor such as electrical stimulation would be expected to cause rate increases at lower thresholds. However, when subjected to high levels of carbon dioxide, the increase in respiratory drive is represented by increased depth; in fact respiratory rate decreases. Under such circumstances, it is difficult to suggest why the reported changes in the response threshold for large rate regions should occur.

Perhaps at lower respiratory frequencies, elements such as ramp generators and spike generators (see Chapter 8) become more sensitive in order to exert more accurate control over the rate. The ramp generators' ramps may not be linear, they could, for example, charge up in a reverse exponential manner. If this were the case, at the top of the ramp a small movement of the switching threshold would cause a large change in rate, while near the bottom even a large change in the switching threshold level would have a relatively small effect on the rate. This would give extra sensitivity to the system over the range of respiratory

rates used most frequently under normal conditions. When the switching threshold is raised (eg during periods of elevated carbon dioxide levels) a small change in current (at the top of the ramp) would result in a large change in rate. A significant (10%) change in rate would thus result at a lower current than when the switching threshold was lower.

Alternatively, the same principle could apply with the spike generator; there could be a range of spike frequencies which is more sensitively controlled. In this range either stimulation or inhibition of spikes would thus require less stimulatory current. Perhaps under conditions of elevated carbon dioxide, this range is intruded upon.

The increases in threshold occurred when the bird was suffering from hypercapnic collapse. During such periods, the respiratory drive would be reduced and a greater current would be expected to be required to increase the respiratory rate. In some cases (n=2) no current, however high (up to 100uA) could achieve this.

30 minutes after reconnection of the 100% oxygen, birds subjected to both 5% and 10% carbon dioxide still showed decreased response thresholds. As previously mentioned, the net ventilation was increased in response to increased drive which must have resulted from some remaining carbon dioxide pooled in the blood. As explained, increased respiratory

drive implies decreased large rate response threshold.

In conclusion, with the use of the proposed model (see Chapter 8) ideas can be put forward concerning the reasons for the stimulatory thresholds changing with elevated carbon dioxide levels. More work needs to be done to substantiate these reasons and as a result add to the model.

Chapter 8

Proposed Schematic Model and General Discussion

8.1 The Reasons for a Model

In recent years, schematic models have been developed in many biological areas to help consolidate and explain the meaning of pools of experimental data. As more results become available, the models are refined so that they are always both representative and supportive of all experimental evidence obtained.

As well as helping with an understanding of how individual neurone populations might be linked, such models help direct the course of future experimental work and suggest its likely outcome. If the predicted results do not concur with the results obtained, then the model must be modified appropriately. Alterations must be made so that the model fits the new evidence without losing any representation it had of previous data.

In the early stages of any model there are inevitable gaps resulting from lack of experimental results. The nature of these gaps helps the experimenter design further experiments in order to provide the missing material. In theory, a stage should eventually be reached where there are no gaps

and no experimental evidence contradicts the model's predictions. However, because of the variability of biological systems in general this stage is rarely attained.

A model of the brainstem regions responsible for controlling avian respiration was established at an early stage in this study. As well as elements based on areas within the brainstem, the model took account of all likely inputs and the effects they might exert on the respiratory rhythm. Many of the elements used in the model have been used previously in other models; particularly those proposed for mammalian respiration. The model outlined in this chapter, is likely to be a simplification of the true situation, especially on the single neurone interaction level and eventually the picture will probably look a great deal more complex.

8.2 Elements Used

Each response region was presumed to contain an element of the system responsible for the final respiratory rhythm. The element selected for a particular region was based on the available experimental evidence known about that region.

8.21 Threshold Phase Termination Ramp Generator

The threshold phase termination ramp generator was proposed as a likely basis for the generation of mammalian respiratory rhythmicity (Cohen and Feldman, 1977 and

Feldman, 1981). Fig45 shows the functioning of this as an oscillator when used for timing the inspiratory period and activating the inspiration-expiration phase switch. During expiration and the ensuing inspiration, the charge in the generator builds up along the ramp AB. When point B is reached, a time when the charge is maximum under prevailing conditions, the generator completely discharges along BG. As the discharge occurs, a switch is activated resulting in a change from inspiration to expiration. This switch possibly functions similarly to the proposed "off switch" neurone population proposed by Cohen (1981; see Chapter 1). After discharge, the generator begins to recharge up the gradient GH during the next expiration and inspiration.

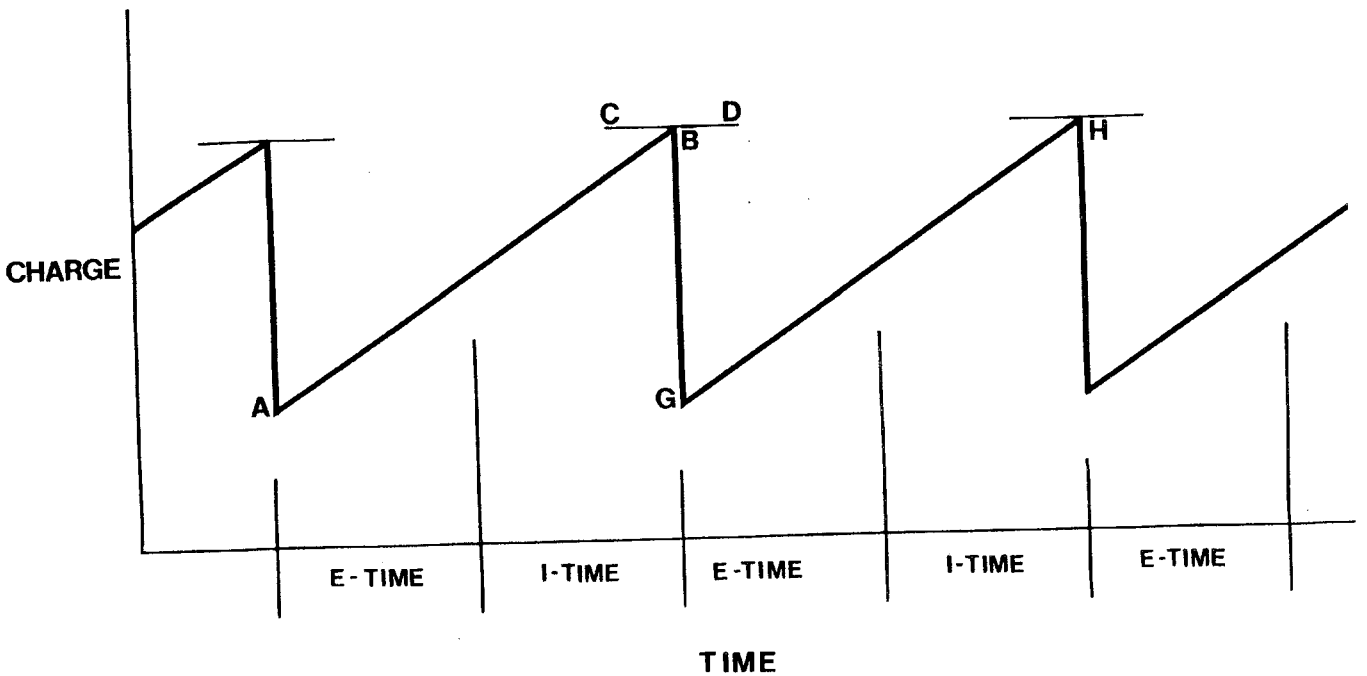
The time before the switch is activated, and consequently the inspiratory period, can be varied in two ways. Firstly, the threshold CD can be lowered or raised; in this way, with the gradient AB constant, the switch point B will be reached either earlier or later than usual. Secondly, the gradient AB can be made steeper or shallower, also altering the time at which the switch point B is reached. The factor controlling the gradient of AB is the rate of input of charge from other centres.

In the proposed model two such threshold phase termination ramp generators exist: one, as described, to operate the inspiration-expiration switch and one for the

Fig 45 Example of how a threshold phase termination ramp generator is used for timing the inspiratory period and activating the inspiration to expiration phase switch.

During expiration and the following inspiration, the charge in the generator builds up along the ramp A-B. When B is reached, the charge is maximum under prevailing conditions and discharge occurs along B-G. As this discharge occurs the inspiration to expiration switch is activated. A new ramp is built up along G-H during expiration and the following inspiration.

E-time = Expiratory period
I-time = Inspiratory period


CHARGE
A
B
C
D
G
H
E - TIME
I-TIME
E-TIME
I-TIME
E-TIME
TIME

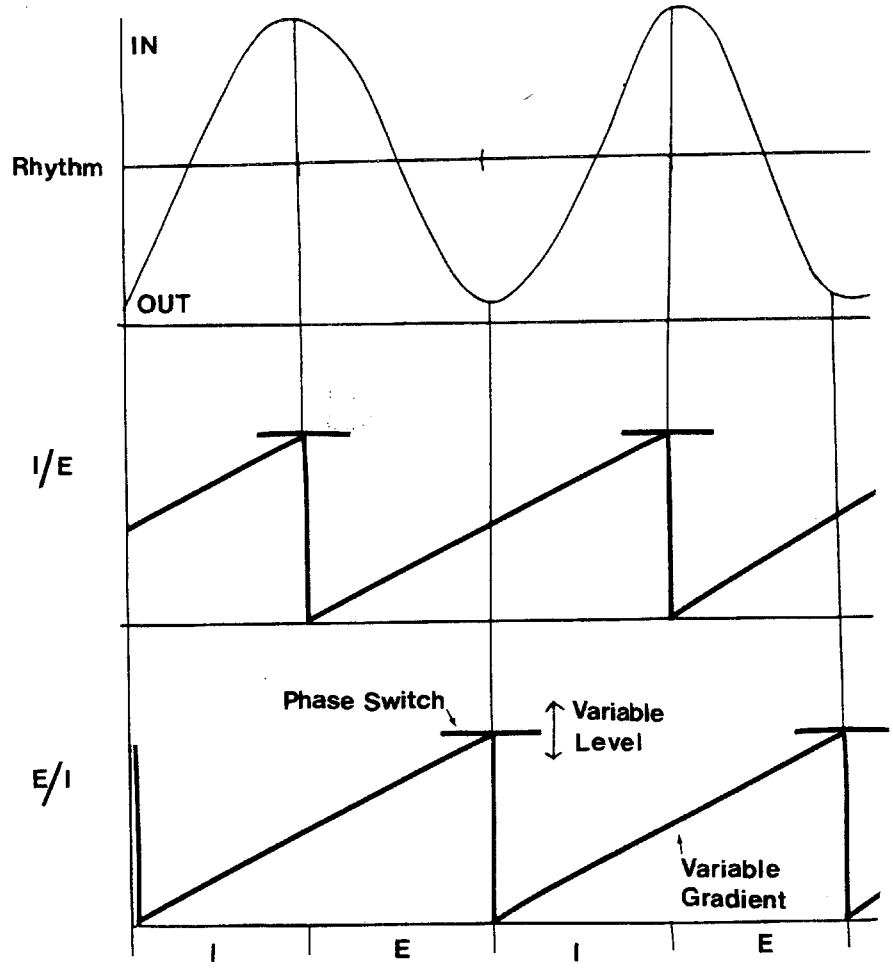
expiration-inspiration switch. If these two generators were to work together but 180 degrees out of phase, a rhythm similar to the one shown in Fig46 would be produced.

8.22 Spike Generator

In order for the ramp generators to reach threshold, they must be charged up along the gradient shown. If, as previously suggested, the gradients can change, then the rate of charge production must also be able to change. It is proposed that the model element responsible for producing this change is a "spike generator". This generator produces a continuous train of spikes which feeds the ramp generators (see 8.21) and charges them up to their threshold point. The spiking can continue at the same rate due to recurrent excitation (Cohen, 1981), whereby one action potential causes another to follow very quickly afterwards in the same neurone. Although basally, a constant rate of spike production is proposed, it is suggested that this rate can be modified by output from centres controlling factors such as vocalisation and temperature regulation. Such modifications would result in changes of the ramp generator's gradient, altering the time at which threshold is reached, which in turn would influence the respiratory frequency. Since there are two ramp generators, this system would allow the inspiration and expiration times to be modified independently.

Fig 46 Example showing how two phase termination ramp generators 180° out of phase with each other can create an oscillation - the basis of the respiratory rhythm. When the inspiration to expiration generator reaches threshold, maximum inspiration is reached; likewise maximum expiration is reached when the expiration to inspiration switching threshold is reached.

I/E = Inspiration to expiration
E/I = Expiration to inspiration


IN
Rhythm
OUT
I/E
E/I
Phase Switch
Variable
Level
Variable
Gradient
I
E
I
E

8.23 Modifier

Afferent inputs from vocalisation centres, pulmonary stretch receptors, thermal regulatory centres and central and peripheral chemoreceptors all have effects on the observed respiratory rhythmicity. It is suggested that the inputs from these regions feed into a "modifier" model element. The inputs are all correlated and given priorities over one another before action is taken in the form of outputs to ramp generator threshold changing mechanisms or spike generators. In this way an input from, for example, the thermal regulatory centres, requiring thermal polypnoea, might be given priority over one from a vocalisation centre. As a result the bird pants rather than vocalises. Under some circumstances, the cortex centres may be able to override this priority selection mechanism. As well as selecting a priority, the size of the respiratory change can be determined. Thermal panting requires a very large change in rate whereas hypoxia requires a relatively small one.

As discussed previously, the rate can be altered by adjusting the switching thresholds or by altering the gradient of the ramps in either of the ramp generators. It is proposed that the modifier exerts its effect by one or both of these mechanisms.

8.24 Motor Output and Mutual Inhibition

It is proposed that at least two populations of neurones (one for inspiratory and one for expiratory muscles) are involved in producing motor output to the respiratory muscles. In fact, the evidence suggests (see Chapter 4) that there are several groups involved for each respiratory phase.

The neurones are triggered off by receiving input from the phase switches (inspiration-expiration and expiration-inspiration) of the ramp generators. The activity of the motor neurones is possibly maintained by the use of recurrent excitation (Cohen, 1981). The inspiratory motor neurones are kept silent during expiration by mutual inhibition, whereby the expiratory motor neurones directly inhibit the inspiratory ones; and vice versa during inspiration.

Mutual inhibition is very likely to be used in many other parts of the system; whenever neurones related to expiration fire it is very likely that equivalent inspiratory populations are inhibited.

8.3 Linking the Elements

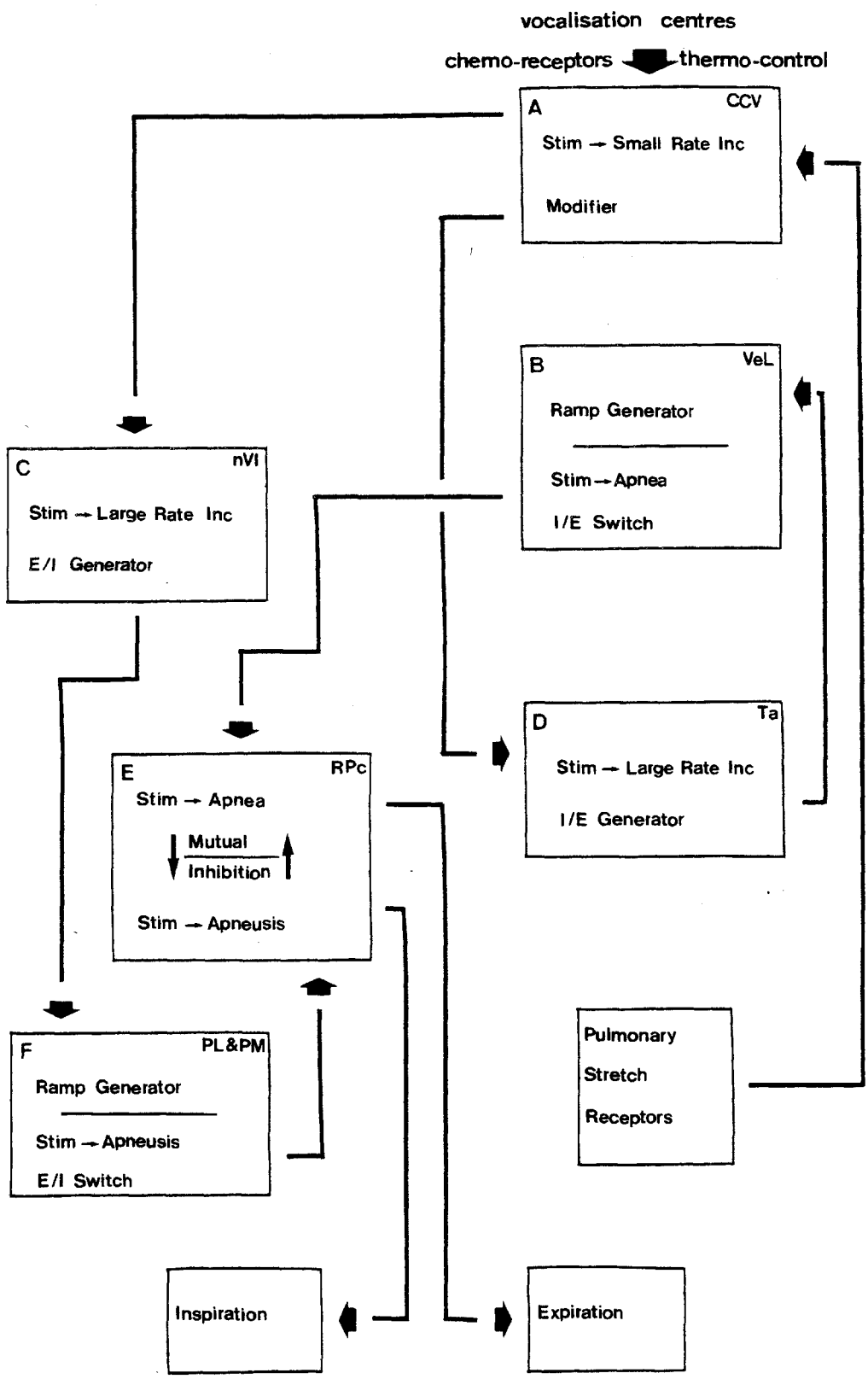
The elements outlined (8.2) are linked together in the proposed model (Fig47). The respiratory cycle continues (steps 1-4 operating inspiration-expiration and steps 5-8

Fig 47 Schematic model illustrating how ramp generators and spike generators might be linked to produce rhythmic respiration. For details see text.

Elements:- A Modifier
B Inspiration to expiration ramp generator
C Spike generator charging F
D Spike generator charging B
E Inspiration and expiration motor nuclei
F Expiration to inspiration ramp generator
Pulmonary stretch receptors
Inspiratory and expiratory muscles

Position in brainstem of elements and abbreviations:-

A CCV Commissura cerebellaris ventralis
B VeL Nucleus vestibularis lateralis
C nVI Nervus abducens
D Ta Nucleus tangentialis
E RPc Nucleus reticularis parvocellularis
F PL and PM Nucleus pontis lateralis and Nucleus pontis medialis.


vocalisation centres
chemo-receptors
thermo-control
A
CCV
Stim → Small Rate Inc
Modifier
B
VeL
Ramp Generator
Stim → Apnea
I/E Switch
C
nVI
Stim → Large Rate Inc
E/I Generator
D
Ta
Stim → Large Rate Inc
I/E Generator
E
RPc
Stim → Apnea
Mutual Inhibition
Stim → Apneusis
F
PL&PM
Ramp Generator
Stim → Apneusis
E/I Switch
Pulmonary Stretch Receptors
Inspiration
Expiration

operating expiration-inspiration) as follows:-

1. As expiration begins the spike generator (D) starts to charge up the ramp generator (B).
2. The ramp reaches threshold at the end of the following inspiration and discharges, activating the inspiration-expiration switch.
3. The expiratory motor neurones (E) are triggered and expiration occurs while inspiration is inhibited.
4. The procedure repeats from 1.
5. As inspiration begins, the spike generator (C) charges up the ramp generator (F).
6. The ramp generator reaches threshold at the end of the following expiration and discharges, activating the expiration-inspiration switch.
7. The inspiratory motor neurones are triggered and inspiration occurs while expiration is inhibited.
8. The procedure repeats from 5.
9. Throughout this time, the modifier (A) receives and consolidates afferent information and in response modifies the output of the spike generators (D and C).

In this way expiration and inspiration continue to occur out of phase with one another.

The model is somewhat simplified since mutual inhibition may well occur in other places, for example between the phase switches. Also, it illustrates only one side of the

brainstem and it should be remembered that any output is complemented by activity and appropriate cross-correlation from the contra-lateral side.

8.4 Experimental Evidence for the Model

The elements of the model (Fig47) are laid out so that they are loosely representative of anatomical regions of the brainstem from which responses were obtained. The response regions (see Chapters 4 and 5) were not quite as discrete as they appear in the model, but for ease of reference and terminology, each element has been allotted a nucleus which was well within each response area. For example, although expiratory sites covered a very much larger brain area, for the purposes of this model, they have been said to lie in the Nucleus vestibularis lateralis. Each element's box shows the response elicited on stimulation; this should help if any doubt is cast over identification.

8.41 Electrical Stimulation of Elements

Elements C and D represent the two brainstem regions from which large increases in rate were obtained during electrical stimulation. On the model, it is proposed that these regions contain spike generators which feed the expiration-inspiration and inspiration-expiration ramp generators 180 degrees out of phase (see Fig46). If either of these spike generator elements were to be stimulated, it

would be reasonable to expect that its output would be modified. Since this spiking activity contributes to the gradient of the ramp generator, if it were to increase then the switching threshold would be reached sooner. This means that the phase switch would be activated sooner and consequently the respiratory frequency would be expected to increase. As has been seen (see Chapter 4) this is the case. During large rate site stimulation, only one spike generator would be producing extra spikes - the other would be producing a spike train at the basal rate. Due to this, although one phase switching frequency would be increased, the other would be expected to remain at basal levels. In reality, if this type of system is involved, there is likely to be interaction between the two spike generators.

Accompanying the rate increase was a decrease in amplitude. This can be explained by an early phase switch cutting short the attainment of a full excursion in either the inspiratory or expiratory direction.

Elements B and F represent the inspiration-expiration and expiration-inspiration ramp generators and phase switches. If either of these regions were to be stimulated, the stimulatory current would contribute to increasing the gradient of ramp. The threshold would be reached and the generator discharged almost immediately; there are no interneurones acting as limiting factors as with the large

rate response. Assuming that the currents used are much higher than those naturally occuring, then the frequency of charge and discharge of the ramp generator would be large. The non-stimulated ramp generator would be functioning normally in an attempt to maintain the basal rhythm. However, the stimulated ramp generator would be producing a high rate of charge/discharge which in turn would be rapidly activating the associated phase switch. At such a high frequency, one phase of respiration would hardly have begun when termination and switching to the other phase would occur. Due to this, it would be unlikely that any physical respiratory movements would be seen - an observer would see the animal in apparent cramp; either expiratory or inspiratory. This is the response observed when the hypothesised ramp generator elements were stimulated.

Element E represents the nucleus of neurones responsible for motor output to the respiratory muscles. On stimulation of this element, the associated respiratory muscles would be expected to contract and remain contracted for the duration of the stimulus. Muscles associated with the opposite respiratory phase would be inhibited by mutual inhibition. It is unlikely that all the muscles concerned with one phase of respiration would be stimulated to contract together. Since higher rhythm-generating elements would be unaffected by the stimulus, muscles not in cramp might be expected to oscillate at basal frequencies. The observed result often

bore traces of the basal respiratory rhythm superimposed on the cramp (see Chapter 4).

Element A represents the region responsible for collecting and assimilating inputs from chemoreceptors, thermal receptors, stretch receptors, etc and modifying its output in response to them (the modifier). Its effect is exerted by feeding the spike generators causing them to produce spike trains at modified rates. However, since yet a further set of neurones is now involved, the response to stimulation is likely to be less pronounced than when stimulating the spike generators direct. As predicted, stimulation results in small increases in respiratory frequency (small rate response). By the same principle, it follows that if the input to this element were to be reduced, then respiratory rate would slow, due to the spike generators producing spikes at a slower rate. When the vagi were cut, reducing input to the modifier, the rate did slow. This reduction of frequency was accompanied by an increase in amplitude. A lower spike frequency from the spike generators resulted in shallower ramp gradients and consequently a larger gap between consecutive phase switches. As a result, since each phase persisted for a longer time, greater respiratory depths in either direction could be attained.

For both electrical stimulation of the respiratory regions

and vagotomy, the proposed model fits all the experimental data.

8.42 Recording from the Elements

The results from the multiple unit recording experiments (see Chapter 5) fitted the model with little need for modification. Generally, widely varying peak activity positions were found in each of the regions reported in the model. However, there was a predominance of early inspiratory activity seen in elements B,C and/or D. Element C forms the spike generator responsible for activating the expiration-inspiration phase switch. As such, its activity continues from early inspiration through to the end of expiration, where the expiration-inspiration phase switch is activated. It is likely, that as the ramp generator threshold is approached, there is increasing inhibition on the spike generator. In contrast, in the early stages of the charge build up, the spike generator may be expected to be least inhibited. The release of previous inhibition may be quite rapid, so that to the observer an apparent burst of activity may be seen. This explanation may require future modifications; with elements such as mutual inhibition, recurrent inhibition and recurrent excitation, which are not illustrated in the model, the picture is likely to become very much more complex. Further, as explained (see Chapter 5) many neurones are being simultaneously recorded from and

their effects summated and averaged.

8.43 Stimulatory Threshold and Response Changes Associated with Thermal Polypnoea and Elevated Carbon Dioxide Levels

The increased stimulatory threshold during polypnoea when stimulating centre B or E, is more difficult to explain in terms of the model. Perhaps, as well as increasing the gradient of the ramp (or decreasing the phase switching threshold), other changes take place to allow the rapid breathing seen during polypnoea to take place. It is possible that the regions in the midbrain identified by Richards (1970a, 1971a) might themselves become important as rhythm generators. If this were the case, stimulating ramp generators in the brainstem would have a reduced effect, or the same effect at a higher threshold. Perhaps the midbrain regions do not mediate their effect wholly through routes already identified or speculated in the model.

The change of a rate increase response (large rate) to a rate decrease response (abolition of polypnoea) on stimulation of the spike generators is simpler to explain. Stimulating the spike generator element could result in either more or less spikes. It is likely, based on inference from mammal pneumotaxic function, that the spike generator strives to produce activity at a constant rate. However, as suggested this effect can be overridden by afferent activity from, for example, chemoreceptors.

Stimulating this element possibly returns the spiking rate to its natural level. During basal conditions, chemoreceptors sensitive to hyperoxia were exerting their effect on the system because pure oxygen was being used as the anaesthetic carrier gas (see Chapter 3). This had a slowing effect on the respiratory frequency, possibly by reducing spike generator output. The large rate increase seen during such conditions may have been due to the spike generators increasing their activity to the basal level. In the same way elevated respiratory rate due to thermal stressing would cause the spike generators to produce spikes at a frequency above normal. Stimulation of the spike generators could return a natural spike frequency resulting in reversion to the basal respiratory spiking level. The natural spiking level could be set by means of delay circuits which are reduced or increased in number so as to modify the spiking rate according to peripheral input.

During hypercapnic conditions brought on by elevated carbon dioxide concentrations in the inspired gas, the stimulatory threshold generally rose for cramp responses and fell for large rate increase. These changes in threshold could merely be a reflection of the level of drive through the system. If, during periods of stress, drive is strengthened by bringing in duplicate interneurones, then inhibition of that drive by external stimulation would require greater current, while increasing it to produce an accelerated rate

would require less current.

The respiratory response to elevated carbon dioxide can be explained to some extent, in terms of the present model. When carbon dioxide levels rise, there is indication that systemic carbon dioxide sensitive chemoreceptors show increased activity (Bouverot and Leitner, 1972) while pulmonary receptors show a decrease in activity (Fedde, 1970; Fedde and Peterson, 1970 and Peterson and Fedde, 1971; see Chapter 7). The influence of these receptors would result in a changed input to the modifier (A); this input may be a summation of all chemoreceptor responses, or it may be from selected chemoreceptors only - for example, just pulmonary ones. It is suggested that the chemoreceptor input to the modifier ultimately results in a reduction of spike production rate in the two spike generators C and D. This would cause the gradients of the ramps in the two ramp generators to become shallower and take longer to reach threshold. Consequently an increased delay between consecutive phase switchings would result, which in turn would produce a slower respiratory frequency. The rise amplitude seen to be associated with this decreased frequency, can also be explained. Since the phase switchings are further apart, each set of muscles (inspiratory or expiratory) has a longer time in which to contract before phase is switched. The extra contraction time would permit a greater amplitude of contraction in both

phases of respiration. This effect was what was observed (see Chapter 7).

8.5 Conclusion

In conclusion, the experimental data to date fits the proposed model although gaps still exist in the available data. Further work at all levels is required before a full understanding of the proposed model elements can be reached.

Employing the technique of single unit recording for looking very much more closely at the regions identified, may help with the understanding of how the rhythm is set centrally. Further work examining the way in which peripheral factors and higher brain centres influence the rhythm, would be an invaluable contribution to the understanding of central control mechanisms in general. The motor aspect of respiratory control appears not to have been studied in detail in any species. It is probable that different muscle blocks concerned with respiration have their control exerted from different motor nuclei in the brainstem. This suggestion gains some weight when the avian respiratory system is implicated. The bellow-like way in which air is routed around the system and the absense of a diaphragm (see Chapter 1) directly implies that a wider variety of muscles must be involved than in the mammalian situation. Coupled with this, the timing of contraction of the various muscle groups must require additional central control mechanisms

and discrete motor nuclei. Preliminary observational evidence has illustrated that in addition to the major inspiratory and expiratory contractions, there are other smaller respiratory movements that occur throughout the cycle. It is likely that these minor movements are concerned with routing the air and emptying and filling the air sacs. Brainstem stimulation of identified areas coupled with muscle recordings would help with the understanding of both respiratory systems and control systems in general.

Chapter 9

Summary

1. Methods are described for recording the respiratory pattern of anaesthetised Japanese Quail. An account is given of the electrode implantation technique used together with both brain stimulation and recording methods. A description is given of how basal respiratory rate can be varied by subjecting the bird to heat stress, and elevated carbon dioxide concentrations in the inspired gas. Lesioning, sectioning and staining techniques carried out on the brainstem are explained.

2. Average basal respiratory rates and depths for the animals under Halothane anaesthesia are given and the distribution in time of the respiratory cycle is examined in detail.

3. The effect of unilateral (right nerve) vagotomy on respiratory rate and depth were measured; on average it resulted in a 45.8% decrease in rate and a 13% increase in amplitude.

4. The entire brainstem was surveyed using electrical stimulatory techniques and four types of respiratory

response were identified:- Inspiratory Cramp, Expiratory Cramp, Large Rate Increase (140%) and Small Rate Increase (20%). The Inspiratory and Expiratory Cramp responses had contrasting post-stimulatory recovery patterns.

5. All positive sites were marked on a two dimensional map of the brainstem. Distribution graphs for the three main response types were plotted on graphs against each of the three dimensions and Mann-Whitney analysis tests were conducted between each pair of site types; significant separations of the regions were established. Three dimensional statistical analysis were carried out and confirmed the separation.

6. Multiple unit recordings were made in the response sites previously identified; peak spiking rate was examined with respect to the respiratory cycle. Neurone populations were found with activity peaks at all stages of the respiratory cycle but some showed a predominance of activity prior to the on-set of inspiration, these were termed Early Inspiratory Regions. Recording sites were classified as either Early Inspiratory or Rest of Cycle, since the others were difficult to separate.

7. All sites recorded from were classified and marked on a two dimensional map of the brainstem. Distribution graphs were plotted and Mann-Whitney analysis tests were carried out for both recording types in each dimension; significant

separation of the sites was established. Three dimensional statistical analysis was carried out and confirmed the separation.

8. Body temperature was artificially increased to initiate thermal polypnoea; average respiratory rates and depths for the panting were recorded. During polypnoea, currents required to induce Inspiratory or Expiratory Cramps were on average 92.8% higher than at normal body temperature. Basal respiratory rates, depths and threshold currents were recovered on cooling.

9. Stimulation of large rate sites during thermal panting caused a reduction in respiratory rate of up to 84% (usually abolition of panting, n=8) with an increase in threshold of 102%. Having elicited both rate increases and decreases from the same sites, a pneumotaxic function was proposed.

10. Inspired carbon dioxide levels were raised from zero to 5% and 10% and the threshold currents for induction of cramps and the Large Rate response were examined. With 5% carbon dioxide the thresholds for the cramp responses increased by 44% while those for the large rate decreased by 22.6%. At carbon dioxide levels of 10%, both cramp and large rate sites displayed a mixture of increased and decreased thresholds. It was suggested that 5% carbon dioxide resulted in increased respiratory drive, which directly resulted in the threshold changes. However, 10%

carbon dioxide sometimes resulted in increased ventilation but also often resulted in the bird collapsing from hypercapnia. It was under the latter circumstances that respiratory drive fell and the thresholds for the responses changed in an unpredicted way.

11. A schematic model was proposed where each element represented a response region in the brainstem. The model fits all the data so far and helps to link the various experiments together. Gaps in the knowledge show up in the model and this helps to design further experiments as well as predict the results of current ones.

12. Little work has been previously conducted on Central Avian Respiratory Control and the study provides some insight into the workings of the system as well as forming the groundwork for future research.

Glossary of Common Names Used in the Text

Albino Rat	*Rattus norvegicus*
Adelie Penguin	*Pygoscelis adelinae*
Canary	*Serinus canaria*
Domestic Cat	*Felis catus*
Domestic Dog	*Canis familiaris*
Domestic Duck	*Cairina moschata*
Domestic Fowl	*Gallus domesticus*
Flamingo	*Phoenicopterus ruber*
House Sparrow	*Passer domesticus*
Japanese Quail	*Coturnix coturnix japonica*
Ostritch	*Struthio camelus*
Pigeon	*Columba palumbus*
Pekin Duck	*Anas platyrhynchos*
Rabbit	*Oryctolagus cuniculus*
Sheep	*Ovis aries*
Starling	*Sturnus vulgaris*
Turkey	*Meleagris gallopavo*

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