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CHARACTERIZATION OF VAGAL RESPIRATORY REFLEXES IN LAMBS

by

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The undersigned certify that they have read, and recommed to the Faculty of Graduate Studies for acceptance, a thesis entitled, " Characterization of Vagal Respiratory Reflexes in Lambs " submitted by Erika M. Ebly in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

The objectives of this study were to independently characterize the peripheral and central components of the Breuer-Hering inspiratory-inhibitory, expiratory-prolonging reflex in unanesthetized lambs.

Fetal and newborn lambs were chronically instrumented with stimulating and recording nerve cuff electrodes on the right cervical vagus nerve and diaphragm EMG electrodes. The peripheral component of the Breuer-Hering reflex was characterized using whole nerve recording of respiratory modulated vagal afferent activity from slowly adapting pulmonary stretch receptors. The central component of the reflex was characterized using reproducible, preferential electrical stimulation of the vagus nerve.

The results of these studies have demonstrated that respiratory modulated vagal afferents were active during fetal breathing as early as 130 days gestation. The magnitude of this activity was considerably smaller than in the newborn lambs, in whom respiratory modulated vagal afferent activity increased with increasing age. Graded electrical stimulation of the vagus nerve during inspiration or expiration elicited distinct within-phase and post-stimulus respiratory effects that were dependent on stimulus intensity, however similarly aged newborn lambs could respond differently to the same inspiratory vagal stimulus. The different responses to the same stimulus were correlated with differences in breathing pattern. There were no developmental changes in the central components of the vagally-mediated, inspiratory-inhibitory, expiratory-prolonging reflexes.

Taken together these results suggest that the inspiratory-inhibitory, expiratory-prolonging Breuer-Hering reflex increases in strength with age and that this increase is due primarily to an increase in afferent activity.

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INTRODUCTION

There is a significant gap in our understanding of the ontogeny of the respiratory system in the perinatal period. Early in gestation, fetuses make intermittent breathing movements in utero though pulmonary gas exchange does not occur. The transition from the fetal dependence on placental gas exchange to the air breathing of the neonate is complex, and involves maturation not only of the lungs and the respiratory musculature but also of the appropriate neural mechanisms that permit continuous air breathing after birth. Because of the complexities involved in the changeover from the liquid filled to the air filled lung, and the mechanical disadvantages inherent in the neonatal respiratory system (Mortola, 1987), the control of breathing in the newborn can be a difficult task and thus breathing may be unstable.

Indeed, respiratory control in the early neonate may be so complex that it is not always successful. Newborns, particularly the premature, frequently exhibit respiratory irregularities in the form of periodic breathing or apnea which, in the extreme case, can lead to death. For some of these irregularities an age dependence, increasing to a peak at two or three months after birth and then declining, has been reported, and it has been hypothesized that the mechanisms responsible must therefore contain a developmental factor (Johnson et al., 1983).

Shannon (1979) suggested that immature respiratory reflexes may underlie the respiratory irregularities observed in the neonate. Specific knowledge of the ontogeny of neural mechanisms involved in respiratory reflexes may provide important information on developmental changes in the neural control of breathing thus offering potential explanations as to why some newborns exhibit respiratory irregularities.

This project focuses on the development of vagal afferent activity and its role in respiratory reflexes in the perinatal period. The following section presents background material on breathing in general, the control of inspiration and expiration, vagal influences on breathing and the role of the vagus in breathing in the perinatal period.

BACKGROUND

Breathing, a critical physiological act which normally occurs with such remarkable regularity that it escapes notice, is accomplished by a multi-component respiratory system with several feedback loops. A respiratory controller, consisting of neural networks that reside in the brainstem, generates a rhythm that is transmitted to motor neurons whose axons innervate respiratory muscles. Contraction of the respiratory muscles creates pressure differences that move air in and out of the lungs resulting in pulmonary ventilation which eliminates $C0_2$ and delivers 0_2 to pulmonary capillaries.

Mechanical changes in the respiratory system associated with inspiration and expiration affect several types of receptors. Central and peripheral

chemoreceptors, as well as mechanoreceptors in the intra and extra-pulmonary airways, ribs, and respiratory muscles, are components of feedback loops that modulate normal breathing. These feedback loops allow the central nervous system (CNS) to match alveolar ventilation to metabolic rate.

Initially, inspiration and expiration were measured using plethysmographs and defined by the movement of air in and out of the lungs. Electromyographic (EMG) recordings from the diaphragm, showed that the muscle, unlike the temporal profile of air flow, had an augmenting pattern of discharge during inspiration. Consequently, the electrophysiological definition of inspiration is based on the output of the phrenic nerve. Inspiration is defined as the period from the onset to the peak of phrenic activity; expiration is defined as the period from the peak of phrenic activity to the onset of the next phrenic burst.

Subsequent studies, combining diaphragm EMG recordings with measurements of flow, defined a two phase expiratory cycle. During stage I, the first portion of the expiratory phase, lung volume declines slowly due to a variable amount of inspiratory motor activity. This post inspiratory activity (PIA) counteracts elastic recoil and retards the rate of exhalation (Remmers & Bartlett, 1977). The second portion of the expiratory phase, stage II, is usually passive except under conditions of strong ventilatory drive (von Euler, 1986).

Intra and extracellular recordings from neurons in the brainstem have identified cells with specific temporal discharge properties at particular points in the respiratory cycle relative to the activity of the phrenic nerve. There are inspiratory and expiratory neurons with augmenting, constant and decrementing

discharge patterns as well as neurons whose activity spans both phases (e.g.,Long & Duffin, 1986; Ezure,1990). The augmenting discharge of inspiratory medullary neurons is transmitted to the phrenic motor neuron pool in the spinal cord resulting in the augmenting discharge pattern of the phrenic nerve. Decrementing expiratory neurons, active during the first phase of expiration, inhibit inspiratory neurons. Residual (post) inspiratory activity is completely inhibited by the activity of augmenting expiratory neurons in the second phase of expiration. Expiratory activity is maintained until it is inhibited by a type of inspiratory neuron with a decrementing pattern of activity (Ezure, 1990).

While the coordinated activity of these and other respiratory neurons in the presence of tonic inputs offers a prospective model for the generation of respiratory rhythm (Ezure, 1990), in the whole animal, control of inspiration and expiration is subject to a variety of modulating factors.

CONTROL OF INSPIRATION

The termination of inspiration, or phase transition from inspiration to expiration occurs even in the absence of sensory feedback. However, normally inspiratory duration can be modified by many sensory inputs.

Several feedback loops can modify the duration of inspiration. For example, a rise in body temperature causes a decrease in inspiratory duration (Bradley et al., 1974b). An increase in inspired CO_2 also decreases inspiratory time but this effect may be secondary to the increase in tidal volume and may

only occur during anaesthesia (Bradley et al., 1974a; cf. Gautier, 1976; Clark and von Euler, 1972). Inspiratory duration is also shortened during exercise when breathing frequency increases (Agostoni & D'Angelo, 1976).

Electrical stimulation in the nucleus parabrachialis medialis and adjacent areas (NPBM complex) prematurely terminates inspiration (Cohen, 1971). Lesions in the same areas result in a prolongation of inspiratory duration in anaesthetized and conscious cats (Gautier & Bertrand, 1975). Activation of intercostal muscle spindle afferents also causes shortening of inspiration (Remmers and Martilla, 1975). Vagally mediated volume feedback from stretch receptors can evoke respiratory reflexes that either shorten (e.g., Gautier et al., 1981) or augment inspiration (e.g., Davies & Roumy, 1986). Regardless of the stimulus, the threshold for termination of inspiration decreases as the inspiratory cycle progresses (Younes & Remmers, 1981).

Spontaneous changes in inspiratory duration (Ti) occur in animals (Remmers et al., 1976) and humans (Newsom-Davis and Stagg, 1975). This normal variability in inspiratory duration is increased when the chemical drive for breathing is low (Bradley et al., 1974). During sleep, inspiratory duration in cats lengthens during slow wave sleep and shortens during rapid eye movement sleep (Remmers et al., 1976).

Typically, inspiratory duration ranges from 50 to 70% of expiratory duration (St.John and Knuth, 1981; Cohen, 1979). Inspiratory duration may also be affected by the duration of the preceding expiratory phase. Some investigators have described a weak positive relationship between inspiratory duration and the preceding expiratory duration (Benchetrit & Bertrand, 1978).

However, other reports suggest that expiratory duration can be altered without affecting the duration of the following inspiration (Cohen, 1979; Martin, 1978; von Euler, 1977).

Like duration, the temporal profile of inspiratory activity (ie., the slope of augmenting diaphragmatic inspiratory activity) can also be modified by a number of factors. Hypoxia (Gautier,1976), exercise (Agostoni & D'Angelo, 1976), hypercapnia and increased body temperature (von Euler & Trippenbach, 1976) increase the rate of rise of the inspiratory ramp while anaesthesia and narcotics decrease the slope of the inspiratory ramp (Younes & Youseff, 1978). Vagal reflexes can also alter the rate of rise of inspiratory output (e.g.,Cherniak et al., 1981; Bruce et al.,1982).

CONTROL OF EXPIRATION

Under normal conditions the duration of expiration (Te) can be prolonged or shortened by several factors. In this thesis, unless indicated otherwise, expiration will refer to the period from the peak of inspiratory diaphragmatic EMG activity to the onset of the next inspiratory burst. Stimuli administered during expiration can terminate expiration and initiate inspiration. St. John & Bartlett (1979) have shown that stimulation of the carotid body terminates expiration prematurely. Hypercapnia and hypoxia both decrease spontaneous expiratory duration (Gautier,1976). Other stimuli can prolong expiration. Vagally-mediated reflexes (Karczewski et al., 1980, Iscoe & Vanner, 1980) and electrical stimulation of the NPBM-Kolliker Fuse area of the pons (Cohen,

1971) can lengthen or shorten expiratory duration, depending on the intensity or location of the stimulus. Hyperthermia increases the effectiveness of a vagally mediated lung volume stimulus in prolonging expiratory duration (von Euler & Trippenbach, 1976).

The extent to which a stimulus prolongs expiration depends on blood gases (Younes et al., 1974). Similar to inspiration, the threshold for the termination of expiration decreases during the expiratory phase (Younes and Remmers, 1981). Stimuli administered during the last 30% of the expiratory phase are ineffective (Knox, 1973).

Expiratory duration can be dependent on the previous inspiratory duration. This linkage occurs with spontaneous variation in Ti (Newsom-Davis & Stagg, 1976) and is maintained for example, when Ti is altered by body temperature (von Euler & Trippenbach,1976).

VAGAL INFLUENCES ON BREATHING

In the control of breathing, how and when phase transitions occur are important. Many studies have investigated the significance of the vagus nerve in the control of breathing because vagal afferents play a role in phase transitions.

As early as 1908, Scott found that the duration of inspiration increased after bilateral vagotomy in dogs suggesting that the vagus was important in the termination of inspiration. Subsequent studies confirmed that vagotomy decreased respiratory frequency increasing both inspiratory and expiratory time

and that the amplitude of inspiratory efforts increased (e.g Widdicombe, 1964). In conscious dogs, vagotomy also increases the variability in resting tidal volume and breathing frequency (Kelsen et al., 1982) implying that the stability of normal breathing depends on the vagus.

Vagotomy abolishes or markedly reduces the effect of $C0_2$ in increasing breathing frequency (Lahiri et al., 1975); this CO_2 effect can be restored with electrical stimulation of the severed vagus nerve (Marsland, 1975). In conscious vagotomized dogs however, vagotomy changes minute volume of ventilation, respiratory frequency and tidal volume without changing resting arterial CO_2 tension (Phillipson et al., 1970; Widdicombe & Winning, 1974). Vagotomy has also been noted to decrease rather than abolish the occurrence of spontaneous augmented breaths (Knowlton & Larrabee, 1946 cf. Cherniak et al., 1981). Vagotomy does not change the increase in breathing frequency associated with exercise (Grunstein et al., 1973).

The effect of vagotomy on ventilation is most likely due to the removal of respiratory vagal afferent impulses since topical anaesthetization of the airways results in similar changes in breathing pattern (Jain et al., 1975). Several vagal respiratory afferent fibres have been described. Because the methodology employed by my experiments does not bear on unmyelinated fibres, only myelinated afferent fibres will be discussed here. These fibres carry afferent information from two kinds of pulmonary receptors, slowly adapting stretch receptors and irritant or rapidly adapting stretch receptors. The characteristics of these receptors will be described along with their reflex effects and their suggested role in respiration.

Afferents from Stretch Receptors

Pulmonary stretch or slowly adapting stretch receptors (SAR) are mechanoreceptors which are located in the trachea and bronchi (e.g.,Bartlett et al.,1976a; Sant'Ambrogio 1982) and are arranged in series with the smooth muscle of the airways (Widdicombe, 1954b, Bartlett et al., 1976b). These receptors are stimulated by distension of the lungs and airways; lung inflation increases the discharge of these receptors and recruits previously inactive receptors. There is evidence that SAR activity corresponds more closely to transpulmonary pressure than to volume. Contraction of airway smooth muscle also results in an increase in receptor discharge (Bartlett et al, 1976a). Slowly adapting stretch receptors may also have a limited chemoreceptive function. Carbon dioxide inhibits the activity of SAR; this may occur as a result of a change in the pH of the receptors' environment (Bartlett et al.,1976b).

The discharge pattern of slowly adapting pulmonary stretch receptors (SAR) was first described by Adrian (1933) based on single fibre recordings from large, myelinated vagal afferents. The activity of these fibres increased during inspiration and decreased during expiration, and showed a slowly adapting discharge in response to sustained lung inflation. A large percentage of SAR are also tonically active (Bartlett et al., 1976a).

Afferent fibres from SAR are among the fastest conducting fibres in the vagus nerve (Paintal, 1953). Primary afferent fibres from SAR terminate in the medulla in the region of the nucleus of the tractus solitarius (Berger & Averill,

1983).

Afferents from SAR mediate the inspiratory inhibitory and expiratory prolonging components of the Breuer-Hering inflation reflex, a volume feedback loop that helps maintain normal breathing. Volume feedback from the lungs shortens inspiratory duration; as tidal volume increases, the duration of inspiration decreases. Larger volumes elicit greater amounts of SAR afferent traffic which shut inspiration off earlier (Grunstein et al., 1973). The amount of inhibitory input necessary to terminate inspiration declines with inspiratory time (Younes & Polacheck, 1981).

The reflex effects of SAR have been tested in several ways. The classic test of the Breuer-Hering inflation reflex is to inflate the lung at end inspiration and measure the duration of the ensuing apnea and compare it to the normal duration of expiration. This method tests the expiratory prolonging component of the reflex. Younes et al., (1974) have criticized the use of lung inflation, showing that while the onset of the apnea is clearly related to SAR activity, the termination of the apnea (and hence the duration of expiration) is a function both of increasing chemoreceptor drive and of waning vagal inspiratory inhibition. The duration of an inflation-induced apnea may also depend on the level of anesthesia (Phillipson et al., 1971) and body temperature (von Euler & Trippenbach, 1976).

Another method of demonstrating the effects of stretch receptor activity is to occlude the airway at the end of expiration. When the airway is occluded, the next inspiratory effort occurs without any change in volume, hence stretch receptors are not activated. The occluded inspiratory time (without SAR) is

then compared to inspiratory time pre-occlusion (with SAR). This method tests the inspiratory inhibitory component of the Breuer-Hering reflex. Airway occlusion yields results that are similar to vagotomy in some animals but not in others. In anaesthetized cats, the duration of the occluded inspiration is identical to the duration of inspiration after vagotomy (Grunstein et al.,1973). In conscious dogs however, the duration of inspiration after occlusion is less than the duration of inspiration post vagotomy (Phillipson, 1974). Gautier et al., (1981) found that the ratio of occluded inspiratory duration to control inspiratory duration is greater with increased lung volume.

Electrical stimulation of the vagus nerve has been used to assess the effects of stimulating SAR; these fibres have a low threshold to stimulation because they are large, rapidly conducting myelinated fibres. Although other fibres are stimulated as well, stimulation of the vagus nerve at relatively low intensities produces inspiratory-inhibitory, expiratory-prolonging reflexes, suggesting that SAR mediated effects dominate the response. Classically, electrical stimulation of the vagus nerve throughout inspiration shortens inspiration compared to unstimulated breaths while stimulation throughout expiration (like lung inflation) prolongs that phase compared to unstimulated breaths (e.g. Iscoe & Vanner, 1980). Electrical stimulation of large myelinated vagal fibres, presumably from SAR has revealed that several reflex effects are possible. These effects may depend on the intensity of the stimulus as well as the timing of the stimulus relative to the respiratory cycle.

Initially it was thought that the vagal inspiratory inhibiting reflex did not exhibit an effect until shortly before the peak of inspiration, but more recent

evidence suggests that SAR mediated inspiratory inhibition occurs during the progress of inspiration in an additive, graded and reversible fashion and is followed by an irreversible cessation of inspiration (Younes et al., 1978).

Importantly, the magnitude of inspiratory shortening or expiratory lengthening in response to SAR stimulation changes if the timing of the stimulus is altered. If SAR are not stimulated until late in inspiration their inhibitory effect is decreased compared to a stimulus given throughout inspiration (e.g., Feldman & Gautier, 1976). A brief stimulus administered early in inspiration can sometimes produce an early, transient inhibition which causes the inspiratory ramp to follow a lower time course for the remainder of inspiration (Younes et al.,1978). As a result, inspiratory time is actually increased. This occurs with a subthreshold stimulus because a higher level of SAR input is needed to terminate inspiration early in the inspiratory phase. These findings suggest that SAR input undergoes complex central processing.

Vagal stimulation can also affect the slope of the inspiratory ramp. Some investigators have reported inspiratory facilitation in response to an SAR stimulus early in inspiration (Bruce et al.,1982) while another group (Karczewski et al., 1980) has described inspiratory facilitation just prior to inspiratory off-switching. Some discrepancies between different studies might be explained by the fact that few investigators, when reporting the effects of vagal stimulation, record a vagal compound action potential at their chosen intensity of stimulation. Given the large overlap in conduction velocities reported for fibres from SAR and fibres from irritant receptors (Paintal, 1973), it is difficult to determine, in the aforementioned studies, whether similar

degrees of SAR stimulation occurred.

Like lung inflation at end inspiration, electrical stimulation of SAR during expiration prolongs expiration. Whereas the last 30% of expiration is resistant to expiratory prolonging stimuli (Knox, 1973), until that point there is a declining threshold for the amount of vagal information necessary to prolong the breath (Younes and Polacheck, 1985). The magnitude of the expiratory prolonging response is decreased by anaesthesia and increased by hypercapnia (Bradley, 1977). Gautier et al., (1981) found that in cats there was a significant correlation with prolongation of expiratory duration via lung inflation and control expiratory duration.

In addition to the direct effects of an inspiratory or expiratory vagal stimulus there are post stimulus effects as well. The effects of an inspiratory inhibitory vagal stimulus persist longer than the stimulus itself (D'Angelo,1977; Zuperku et al.,1982). Younes & Polacheck (1985) have described post stimulus effects for two respiratory phases following an inspiratory stimulus. A vagal inspiratory inhibitory stimulus, even though it is removed during the subsequent expiratory phase, shortens that phase compared to control values. Even a subthreshold inspiratory stimulus produces a shortening of the following expiratory phase (Younes & Polacheck, 1985), however a larger stimulus will have a greater post inspiratory effect (Clark & von Euler, 1972; Grunstein et al, 1973).

The linkage between inspiratory and expiratory duration is unaffected by a change in body temperature (von Euler et al., 1976) or an increase in CO_2 either at rest or during exercise (Agostoni & D'Angelo,1976; Clark and von

Euler, 1972). Although von Euler (1976) has reported a linear relationship between stimulated inspiratory duration and the following expiratory duration, Younes and Polacheck (1985) report expiratory shortening even after a subthreshold inspiratory stimulus which prolongs inspiration. This means that the post stimulus effect is less closely linked to inspiratory duration than previously thought. After an inspiratory stimulus and a shorter subsequent expiratory phase, the next inspiratory effort is longer in duration.

The post stimulus effects of an expiratory stimulus were noted by Breuer and Hering (see Hering & Breuer, 1868) who found that after a lung inflation that reflexly prolongs expiration, the next inspiration was shorter in duration compared to control. Similar results have been found using vagal stimulation during expiration (e.g., Iscoe & Vanner, 1980). Feldman & Gautier (1976) found that the effect of prolonging expiration on the following inspiratory duration is much smaller than the effect of shortening inspiration on the following expiratory duration. However, Martin (1978) found that increasing functional residual capacity significantly increased expiratory time with only a variable effect on inspiratory time.

Sustained stimulation of the vagus has different effects. In anaesthetized adult mammals, sustained elevations of lung volume or continuous vagal stimulation have little or no effect beyond the first breath (e.g., D'Angelo & Agostoni, 1975). This adaptation to a sustained stimulus is not well understood and conflicts with results obtained in sedated, newborn lambs (Johnson, 1979). These lambs showed an initial decrease in breathing frequency followed by a gradual increase to a level higher than control values.

Afferents from Irritant Receptors

Irritant (IR) or rapidly adapting receptors are found in the airway mucosa. They respond rapidly and adapt quickly to vigorous lung inflations and deflations, chemical (histamine, gases) and physical (fine particles) stimuli, with an irregular pattern of discharge (Fillenz & Widdicombe, 1971; Sellick & Widdicombe, 1970) thus distinguishing them from SAR. Irritant receptors are located in airways greater than 0.3 mm in diameter (dogs, Mortola et al., 1975) and are less numerous than SAR. In cats, SAR outnumber IR by a factor of ten (Widdicombe, 1954a); in rabbits there are four times as many SAR as IR (Roumy and Leitner, 1980).

Impulses from irritant receptors are carried by myelinated fibres running in the vagus nerve. Although the mean conduction velocity reported is somewhat lower for irritants (23.3 m/s) than for SAR (32.3 m/s) (dogs - Sampson & Vidruk, 1975) the considerable overlap in their conduction velocities (cats - Paintal, 1973) has made it difficult to distinguish between the two fibre types based on this property alone. Afferents from IR terminate in the medial and comissural subnuclei of the NTS (Kubin and Davies, 1988).

Irritant receptors are thought to be responsible for augmented breaths (sighs) and rapid, shallow breathing (e.g.,Davies & Roumy,1986) and have been implicated in coughs (Widdicombe, 1964) and the transient tachypnea of the newborn infant (Avery et al, 1966; Mortola, 1987). Stimulation of presumed irritant fibres, through rapid inflation or deflation of the lungs results in

augmented inspiratory efforts or shortened expiratory phases, respectively (e.g. Cherniak et al., 1981).

Augmented breaths have a characteristic biphasic inspiratory ramp. The first portion of the augmented breath is similar to the ramp in control breaths whereas the second component of the augmented breath has a steeper rate of rise (Cherniak et al, 1981).

There is some evidence from studies using rapid, lung inflations to provoke augmented breaths, that the irritant mediated inspiratory response exhibits a refractory period, and hence can be difficult to evoke repeatedly (Reynolds, 1962; Glogowska et al., 1972 , Davies et al, 1978, Cherniak et al, 1981). There are also reports of post-stimulus effects due to irritant fibre stimulation. There is decrease in the amplitude and the duration of the inspiratory effort following an augmented breath and a brief overall decrease in breath duration (Glogowska et al., 1972; Cherniak et al., 1981). In addition, the rate of rise of inspiratory flow in post sigh breaths has been reported to be greater than in breaths occurring before the sigh (Cherniak et al., 1981). Only one group describes the expiratory phase after an augmented inspiratory effort; these workers found the expiratory phase to be lengthened (Comroe, 1974)

Although there are several reports of the effects of electrical stimulation of SAR fibres, electrical stimulation of IR fibres is less frequently reported. Electrical stimulation of IR fibres during expiration results in expiratory shortening (Karczewski et al, 1980) but electrical stimulation of irritant receptors during inspiration has not yet been recorded.

Whereas SAR have a breath by breath regulatory influence on respiratory timing, the role of IR in normal breathing is not as clear. The reflex effects of irritant receptors appear to be pro-inspiratory and defensive in nature. Irritant receptors react in response to noxious agents by increasing their frequency of discharge. Augmented breaths open atelectatic alveoli, and release pulmonary surfactant (Nicholas et al., 1982) producing a perceptible reduction in airway resistance and often an increase in lung compliance. Short expiratory phases could have two functions: 1) to increase the frequency of breathing and promote inspiration and 2) a shortened expiratory time may be an energy efficient means of maintaining lung volume (Mortola, 1987).

VAGAL ROLE IN THE PERINATAL PERIOD

Perhaps the first person to recognize the role of the vagus nerve in neonatal respiration was LeGallois. This nineteenth century investigator found that the *primum mobile* of respiration, in unanesthetized young rabbits , resided in a particular small portion of the medulla and was dependent on the presence of the vagi.

Since that time, several investigators have used vagotomy as a method of assessing the role of the vagus nerve in the control of breathing in the newborn. In 1930, Coombs and Pike reported that anaesthetized puppies and kittens, while initially exhibiting the slowing of respiratory rate observed in the adult mammal (Scott, 1908), died within several hours of bilateral vagotomy. In a similar study, Schweiler (1968) found the effects of vagotomy on breathing

frequency were stronger in the youngest kittens studied. In these newborn kittens, ventilation decreased after vagotomy while $PaCO_2$ increased and pH decreased. Older kittens showed only a transient decrease in ventilation with $PaCO_2$ and pH remaining constant throughout the experiment. More recently, Fedorko et al., (1988) have reported the effects of bilateral vagotomy on ventilation in unanesthetized newborn rats. These investigators found that vagotomy in newborns substantially decreased ventilation and increased tidal volume and inspiratory and expiratory time whereas in adult rats, although inspiratory and expiratory duration and tidal volume increased, ventilation was not compromised. Similar findings in newborns have been reported in lambs (Marsland, 1975), which are more mature at birth than kittens, puppies or rat pups. These data suggest that the vagus is important for breathing in the newborn.

Vagal reflexes in the newborn

Though the Breuer-Hering reflex has been postulated to be more important in the newborn than in the adult (Cross et al., 1960; Bodegard et al., 1969; Olinsky et al., 1969) developmental aspects of this vagal reflex remain unclear. Maloney et al., (1975), in chronically instrumented sheep fetuses, used lung inflation in excess of the volumes that would be normally inhaled in utero in an attempt to evoke the B-H inflation reflex. Increasing the volume of the lungs for ten minutes decreased the diaphragm EMG activity by roughly fifty percent. If this effect was indeed SAR mediated it suggests that

SAR in the fetus have an extended response to a sustained stimulus which is not apparent in the adult. However, this maneuver may have resulted in chest distortion and as a result actually revealed the presence of an inspiratory inhibitory intercostal reflex (Knill & Bryan, 1976).

In human infants, the presence of the B-H reflex is well established. Both the inspiratory inhibitory and the expiratory prolonging components of the reflex have been observed and numerous attempts have been made to characterize their development. The results of these studies conflict.

Premature infants have been shown to have similar (Fox et al, 1988; Thach et al, 1975), greater (Kirkpatrick et al, 1976; Olinsky et al, 1974) or lesser (Gerhardt & Bancalari, 1981) inspiratory prolongation via airway occlusion than full-term infants. Using the lung inflation technique Cross et al., (1960) found less expiratory prolongation in newborns than in older infants while Bodegard et al.,(1969) saw a decrease in expiratory prolongation postnatally. Martin et al., (1978) did not find an age dependent difference in reflex expiratory prolongation.

Similar controversies are found in studies on the neonates of other species. In response to lung inflation newborn animals have been shown to have a similar (kittens- Duron & Marlot, 1976) greater (rats- Smejkal et al., 1985) or lesser (rats,rabbits -Gautier & Mortola, 1981) expiratory prolonging BH reflexes than their adult counterparts. Smejkal et al., (1985) found that 8 day old rats had a weaker response than both the newborn and the adult.

Vagal reflexes from irritant receptors might be abnormal in premature infants (Ariagno,1979; Fleming et al.,1978). Fleming et al.,(1978) have

suggested that irritant receptors are immature in infants less than 35 weeks gestation (irritant reflex is absent), however Greenough and Morly (1984), who used positive pressure inflation, artificial ventilation in preterm infants, found that augmented breaths could not be evoked in infants, regardless of gestational age, after the first five days of life. Thach and Taeusch (1976) have also reported that the frequency of sighs decreases with increasing postnatal age.

Some studies report that the irritant receptor mediated deflation (expiratory-shortening) reflex is absent in newborn animals (rabbits - Schweiler, 1968; kittens- Marlot & Duron, 1979) while others report the presence of this reflex (opossums-Farber, 1975; kittens-Trippenbach, 1985; rabbits-Trippenbach, 1986).

Despite these conflicts, it is frequently assumed that vagal reflexes undergo developmental changes and thus may play a more important role in the control of breathing in the newborn than in the adult.

Vagal afferent activity in the newborn

There is no information on the spontaneous activity of vagal afferents from SAR in the unanesthetized fetus or newborn animal. Although it has been known for some time that the fetus makes breathing movements in utero it is not known whether this activity, which is unrelated to gas exchange, activates pulmonary stretch receptors thus providing the central nervous system with respiratory modulated sensory feedback from the fetal lung via the vagus.

In anaesthetized, exteriorized fetal lambs SAR activity has been recorded from a multi-fibre vagal preparation (Ponte & Purves, 1973). The activity consisted of a regular discharge which varied with intra tracheal pressure and increases with spontaneous respiratory movements.

Evidence describing SAR activity in the newborn also comes from studies of anaesthetized animals and with one exception suggests that afferent activity from SAR in newborn animals is lower than in adult animals. Lower discharge rates of SAR in newborns have been reported in kittens (Schweiler, 1968; Marlot & Duron, 1979), puppies (Fisher & Sant'Ambrogio, 1982) rabbits (Schweiler, 1968) and opossums (Farber et al.,1984). In the newborn lamb, which is more precocial than the kitten, puppie, rabbit or opossum, the firing pattern of SAR afferents is reported to be similar to that of the adult sheep (Ponte & Purves, 1973).

Some studies reported little or no tonic activity of SAR at end expiration in the newborn (Schweiler, 1968; Marlot and Duron, 1979). Ponte & Purves (1973) reported that the discharge of SAR in newborn lambs at end expiration was more variable than in the adult sheep. Harding (1980), also studying newborn lambs, found that though the frequency of SAR discharge was related to end-expiratory pressure, discharges still occurred in some units at subatmospheric pressures. In young opossums static SAR discharge at low transpulmonary pressure differed little from that of older age groups (Farber et al., 1984).

Previous studies of SAR activity were performed in anaesthetized, ventilated and sometimes vagotomized animals. It is known, for example, that

anaesthesia and mechanical ventilation can sensitize SAR above spontaneously occurring levels (Rehder et al., 1973; Sellick and Widdicombe, 1970) and that vagotomy, by destroying the parasympathetic innervation of smooth muscle, can result in an underestimation of SAR activity (Richardson et al., 1984; Mitchell et al., 1985).

Much less is known about irritant receptor (IR) activity in the newborn. In newborn opossums and dogs, IR constitute a smaller portion of the receptor population than in adult animals (Farber et al., 1984; Fisher et al., 1982). Fisher and Sant'Ambrogio (1982) found little irritant receptor activity in single fibre vagal recordings from puppies (Fisher & Sant'Ambrogio, 1982). Despite the apparent paucity of IR activity in the newborn, augmented breaths (sighs), presumably mediated by IR, are a common feature of breathing in the newborn and may be extremely important in combatting atelectasis.

RATIONALE

In order to understand the complexities of respiratory control in the neonate, it is essential to know how the neurophysiological variables involved operate at an early age.

Maturational changes in afferent activity from SAR might be expected since developmental changes in the mechanics of the respiratory system during the neonatal period (Mortola, 1987) may increase the amount of tension on the airways and hence affect the response of receptors located in the airways (Farber, 1982).

There is also evidence that the cellular properties of brainstem neurons undergo developmental changes (Haddad, 1987), hence central elements processing afferent information may have different properties in the newborn than in the adult. Lucier et al., (1979) found that respiratory modulation in brainstem neurons of the kitten could be suppressed by superior laryngeal nerve stimulation suggesting a central susceptibility to input from the periphery.

Our understanding of neonatal respiratory control would be enhanced by additional information on vagal influences on breathing early in development. It is important to describe, in the unanesthetized animal, the spontaneous activity of respiratory modulated vagal afferents from SAR (feedback to the respiratory controller) and the central response to different amounts of simulated vagal afferent input (gain of the respiratory controller). If both the feedback and the gain of the respiratory controller are found to undergo simultaneous developmental changes, then the complexity of the respiratory controller's task of providing continuous, effective respiratory control in the newborn will be better understood.

To test the hypothesis that respiratory control in the neonate involves immature SAR feedback or an inability to process SAR feedback in an appropriate manner, this research addressed the following questions:

1. Under normal conditions, do respiratory modulated vagal afferents provide sensory feedback from the lungs to the central nervous system during periods of fetal breathing in utero?

2. How does respiratory modulated vagal activity in the fetus compare to that of the newborn?

3. Does the amount of vagal afferent activity change significantly after birth?

4. What are the effects of vagal stimulation in the newborn? Can SAR effects be identified ? Can the stimulus intensity corresponding to SAR fibre activation be given reproducibly?

5. Do newborn lambs of the same postnatal age respond similarly to SAR stimulation?

6. Does the response to SAR stimulation change with age?

These questions were pursued in unanesthetized, state-monitored, chronically instrumented fetal sheep and newborn lambs of known gestational and postnatal ages.

METHODS

Sixteen lambs between 3 and 28 days were studied. Lambs were orphaned twenty-four hours after birth and bottle fed lamb's milk replacer(Land of Lakes). Fresh warm milk was delivered in 2 litre bottles at 8 am, 12 pm, 6 pm and 10 pm. Lambs generally gained 1 lb per day, except for the day of surgery.

Five fetuses were studied in utero at 130 days gestation. The ewes were kept in metabolic cages, were cared for by vivarium staff and always had access to hay and water. Sheep were Dorset, Suffolk or Dorset-Suffolk breed.

SURGICAL PREPARATION

Pregnant ewes (125-127 days gestation) were fasted for 12 hours prior to surgery. Ewes were given an intravenous induction dose of pentothal (25 mg/ml/kg), shaved, and intubated with a No.10 cuffed endotracheal tube. Anaesthesia was maintained by ventilation with 1.5% halothane in oxygen. End tidal CO_2 levels were monitored and maintained near 5%.

The fetus was partially exteriorized from the uterus via a left flank incision and implanted with electrodes and then returned to the uterus. Prior to closing the uterus antibiotics were given directly into the uterus. Post surgical analgesia was not given to the ewes because it would have compromised the cardiovascular function of the fetus. Ewes were able to stand

within two hours after surgery and would typically begin eating immediately. Temperature was monitored and antibiotics (Pen-Di-Strep 9,000 IU/kg; Gentocin 160 mg) were administered intra muscularly for 7 days after surgery.

Lambs were fasted for roughly 30 minutes prior to surgery. Anesthesia was induced with 5% halothane delivered in a mask. Lambs were intubated with a No. 5 cuffed endotracheal tube and anesthesia was maintained by ventilation with 1% Halothane in nitrous oxide. Lambs were ventilated at roughly 15 ml/kg and end tidal CO_2 was kept near 5%. After recovering from anesthesia, lambs were given ample warm milk and kept warm with a heating lamp for twelve hours after surgery. At the end of these twelve hours, a large portion of which the lambs spent asleep, newborn lambs were feeding vigorously and appeared completely recovered from the surgery. An antibiotic (Pen-Di-Strep) was administered for 7 days beginning on the day of surgery. Rectal temperature was monitored to insure that lambs were not developing infections.

CHRONICALLY IMPLANTED DEVICES

Fetal and newborn lambs of known gestational and postnatal age were chronically instrumented with electrodes for monitoring and evoking vagal activity and recording physiological/behavioral state.

Nerve Cuff Electrodes

Nerve cuff electrodes were implanted on the right cervical vagus nerve

in order to 1) record vagal afferent activity primarily arising from SAR (Ebly, 1986) and 2) to electrically stimulate the vagus nerve to elicit respiratory reflexes.

A tripolar nerve cuff recording electrode (Figure 1) consisted of three multi-stranded stainless steel electrodes (COONER 631, 40 gauge teflon insulated wire) symmetrically sewn (Figure 1b) inside a sleeve of silicone tubing (SILMED 2.5mm ID x 4.5mm OD). The silicone tubing had a longitudinal slit, which opened the cuff for nerve placement when the sutures, which were glued into position around the cuff at three different points, were pulled from either side (see Figure 1a). The sutures were then lightly tied over the flap which insulated the longitudinal slit. All bare wire on the external surface of the cuff was covered with insulative silicon sealant (General Electric). The tubing diameter was 1.5 times the diameter of the nerve as to allow for swelling of the nerve after surgery and for growth of connective tissue (Hoffer & Loeb, 1980).

Two nerve cuff recording electrodes were implanted on the right cervical vagus. One cuff was positioned close to the larynx; this cuff was referred to as the proximal cuff. The second cuff, was placed at a maximal distance caudal to the first cuff; this cuff was be referred to as the distal cuff. The distance between the two cuffs was measured and added to the appropriate interelectrode spacing for a measurement of conduction distance. Although the distal cuff sometimes contained additional closely spaced leads for stimulation, either cuff could function as a stimulating electrode. Some of the proximal recording cuffs had another set of electrodes sewn on the outside

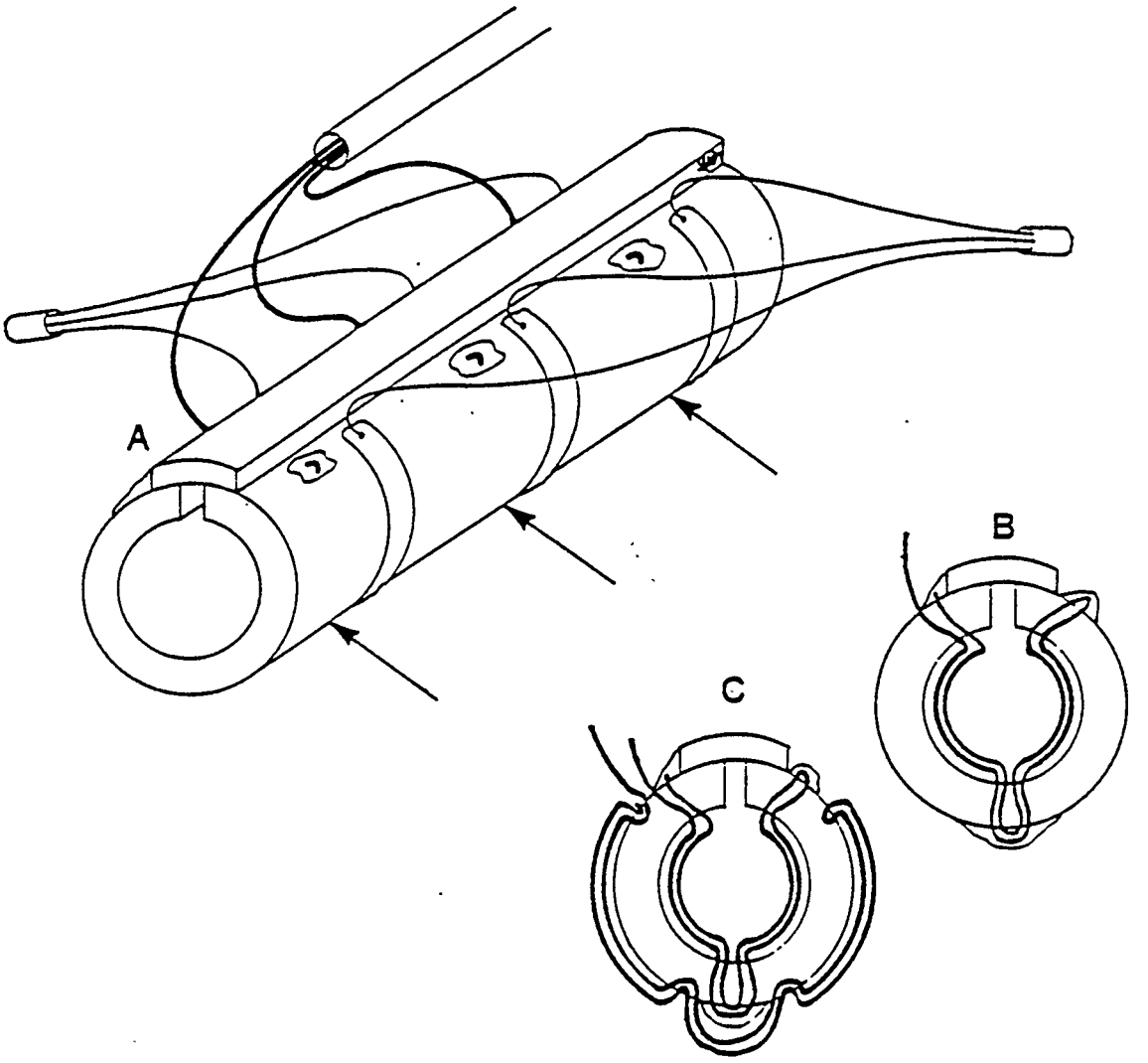

Figure 1. Vagal recording cuff electrode. The arrows in A point to the position of the electrodes within the cuff. A cross-sectional view is shown in B. External electrodes are shown in C.

of the silicone tubing (Figure 1c). The purpose of these external electrodes was to monitor ongoing activity outside the recording cuff electrode.

The signals from the nerve cuff electrodes were typically amplified by 1,000,000 via a NEOMEDIX amplifier. The filter settings on these amplifiers usually provided a bandpass between 50-7500 Hz. Signals were further filtered using KEMO 4 pole, 24 dB/octave filters at a bandpass of 800 - 8000 Hz.

There were several sources of noise in the signal: thermal noise, amplifier noise, EMG activity and EKG contaminants. Thermal noise, which is proportional to the square root of the source impedance (Stein et al, 1975), was minimized by keeping the impedance of the recording electrodes low. Typically the impedance of the nerve cuff electrodes was between 1 and 1.5 K. Amplifier noise was reduced by choosing an amplifier with exceptional signal to noise properties-- one that used a low input impedance bipolar transistor instead of a high input impedance field effect transistor (FET). The FET type headstages yield minimum noise but use high source impedances. With a bipolar headstage there is minimum noise and low (<10K) source impedance (Franz, 1982). The tripolar configuration of the nerve recording electrodes reject EMG better than a bipolar recording electrode configuration (Stein et al., 1975). Filtering (800 Hz) eliminated any remaining EMG as well as lower frequency EKG contributions.

The vagal nerve cuff electrodes were also used to electrically stimulate the vagus nerve. There were two types of stimulation, stimulation to monitor the recording environment and stimulation to elicit respiratory reflexes. Vagal stimulation evoked compound action potentials (CAPs) which reflected the

algebraic sum of the action potentials from the fibres being stimulated at a particular intensity.

Compound action potentials were elicited using bipolar, monophasic constant current stimulation at 0.1 msec duration at the stimulating cuff. Stimulus artefact, resulting from current spread between stimulating and recording leads, was minimized by the choice of stimulation configuration. A tripolar stimulus configuration minimized current spread by eliminating the gradient for current to flow outside of the cuff (Mortimer, 1981). Threshold for the CAP was defined as the minimum amount of current which could evoke(after 8-25 averages) a potential twice the size of the background noise. Compound action potentials were recorded at intensities corresponding to 1-5 times threshold in increments of 0.25 x threshold.

Compound action potentials (CAP) are potentially a powerful ally in chronic nerve recordings because they reflect the total amount of nerve activity and thus can provide a measurement of the integrity of neural recordings, as well as give information on the size of individual action potentials. The compound action potentials in these studies were used to 1) provide a measure of the sensitivity or efficiency of the neural recordings and 2) provide an index of the fibers being recruited.

Impedance measurements were made routinely as another measure to help verify the integrity of the nerve cuff recording electrodes (Stein et al., 1978). It is possible to generalize Ohm's law substituting "impedance" for "resistance" (Horowitz & Hill, 1980). Therefore a decrease in impedance would result in a decrease in voltage recorded. Impedance was measured using a

device meter modeled after a BAK Electronics impedance meter (model IMP-1). This device measured impedance at a frequency of one kilohertz and injected minimal current (50 nanoamps peak to peak) into the animal. Impedance values were recorded and used to help interpret measurements of neural amplitude as well as to identify electrodes which had broken or become unsoldered within the animal.

Diaphragm EMG electrodes

Diaphragm EMG electrodes provided information on central respiratory output. The diaphragm EMG electrode consisted of the tips of two seven millimeter dental broaches (KERR Barbed Broaches xx-fine) which were soldered to two lengths of COONER 631 wire. The solder joint was imbedded in a drop of FIVE MINUTE epoxy. The diaphragm was accessed via a 3 cm transabdominal incision and electrodes were implanted in muscle fibres just lateral to the sternocostal margin. A ground or reference wire (description follows) was inserted through the same incision.

The raw EMG signal from the diaphragm was usually amplified by 5000 on a NEOMEDIX amplifier. The signal was low pass filtered at 5000 Hz; the high pass filter setting varied depending on the amount of EKG contamination, but was typically set at 75 Hz.

To facilitate measurement of inspiratory and expiratory time as well as magnitude of inspiratory effort, the raw signal was full wave rectified and low pass filtered at 5 Hz. In the remaining text, this type of processing is indicated by the abbreviation RLPF.

Ground Wire

A nine cm loop of deinsulated wire (Cooner 631) secured by a small piece of silicone tubing (0.5 mm ID x 0.9 mm OD) was implanted in the peritoneal cavity to serve as a ground or reference for the amplifiers and as a current return.

State Electrodes

Electrocorticogram (ECoG), Electrooculogram (EOG) and nuchal EMG electrodes were used to assess the behavioral state of the animal.

ECoG

Electrocorticogram electrodes (SEMCOS Associates, Melbourne, Australia) consisted of two ball tipped gold leads, each of which was encased in a nylon self tapping screw. Two holes were drilled in the skull 1 cm distal to the parietal suture on either side of midline. The screws were then turned into these holes for placement of the ECoG electrodes. The signal from the ECoG electrodes was typically amplified 2000 times and bandpass filtered from .5 - 50 Hz.

Nuchal

The nuchal EMG electrodes consisted of two strands of individually knotted Cooner wire with a two millimeter deinsulated section just proximal to the knot. The nuchal electrodes were inserted in the deep muscle of the neck

about 3 inches caudal to the ears. The nuchal EMG was typically amplified by 2000 and bandpass filtered from 50 - 5K on a NEOMEDIX amplifier.

EOG

The EOG electrodes were identical to the nuchal electrodes described above. The electrodes were sewn on the inner and outer canthus of the right eye. The signal from the EOG electrodes was amplified by 5000 and bandpass filtered 3-50 Hz on a NEOMEDIX amplifier.

Connector and Cables

The wires corresponding to the implanted devices were left in a large loop for strain relief. In the fetuses all leads emerged through the maternal flank at which point they were soldered to a connector. In lambs the leads were routed subcutaneously and emerged at an incision on the lamb's back, where they were mated to the same type of connector.

The "backpack" connector (see Loeb & Gans, 1986) which served to relay the signals from the animals to the recording equipment consisted of two parts; a printed circuit board to which leads emerging from the animal were soldered and a 40-pin ribbon cable connector (3M).

EXPERIMENTAL CONDITIONS

Pregnant ewes were brought into the recording room at least one hour before an experiment and given free access to hay and water. Recordings

from fetal sheep were made in the late afternoon and evening. Data were collected during episodes of fetal breathing and during episodes of nonbreathing for comparison. Breathing was associated with active or rapid eye movement (REM) sleep. This state was identified by the presence of fast wave low voltage ECoG activity, rapid eye movements and the absence of activity on the nuchal EMG electrodes. When there were no breathing movements (quiet sleep) low frequency, high voltage activity on the ECOG and tonic activity occurred on the nuchal EMG.

After recovering from surgery newborn lambs were brought into the recording room. Newborn lambs were bottle fed until satiated before an experiment began. Awake lambs had an ECoG that was characterized by a high frequency, low voltage pattern, tonic activity of nuchal EMG and occasional eye movements. The lamb was placed either in a cardboard box with clean wood shavings or was held on my lap. Experiments were interrupted when the lamb became restless or hungry. Lambs were fed on demand with fresh, warm, milk replacer. When the lambs were well fed and comfortable they would lie quietly and for the recording session. Because body movements often produced massive movement artefact, a quiet, calm animal was essential for neural recordings.

EXPERIMENTAL PROTOCOL

Before recording commenced, impedance measurements were made to verify the condition of the recording electrodes. The amplitude of all the

signals was examined on a digital oscilloscope before they were input to a Neuro-corder digitizing unit for storage on VCR tape during recording. The latency and amplitude of the compound actions potentials at various stimulus intensities were assessed on line using a digital oscilloscope with averaging capabilities. During the course of the experiments all signals were visually monitored on an eight channel electrostatic chart recorder as they were being stored.

Data were digitally acquired using ASYST data acquisition software with Data Translation (DT 2801, DT 2821) 12 bit a/d boards on IBM AT compatible machines. Raw neural signals were sampled at a minimum of 15 KHz, processed neural and EMG signals were typically sampled at a minimum of 250 Hz. The sampling rate of the neural signals determined the sampling rate(s) for the other signals. Computationally intensive operations were performed on 20 Mhz 386 machines.

SPECIFIC EXPERIMENTAL

Ontogeny of Respiratory Modulated Vagal Afferents

The goal of these experiments was to discover whether respiratory modulated vagal afferents from SAR were active in utero, to compare the activity to the newborn lambs and to look for changes in respiratory modulated vagal afferents with age.

Raw vagal neurograms were recorded along with diaphragm EMG and

the signal from the state electrodes from fetuses during periods of breathing and non-breathing, and from quiet, awake newborn lambs. Raw vagal signals were only recorded when there was no activity on the external electrodes of the recording cuffs, thus decreasing the likelihood that phasic EMG activity from neighboring muscles would contaminate the neural recordings.

The raw neural signals were rectified and low pass filtered (RLPF). If there was no obvious modulated activity on the RLPF neurogram, then the RLPF neurogram was ensemble averaged using a manually placed marker at either the beginning of inspiration or the peak of inspiration, depending on which portion of the breath was of greater interest and required more accuracy, to synchronize the averages with respect to time. The origins of the modulated activity were identified using the cross correlation technique.

Respiratory Effects of Vagal Stimulation

Electrical stimulation of the vagus is a method of investigating the central mechanisms involved in respiratory rhythm generation. The advantage of electrical stimulation is that the stimulus to the central nervous system is very reproducible if CAPs are monitored. The disadvantages of electrical stimulation are that nerve damage can occur and that afferent fibres from SAR are activated synchronously as opposed to the naturally occurring asynchronous discharge from SAR. Synchronous activation is a much stronger input. Trenchard (1977) was able to reproduce the breathing pattern prevagotomy by electrically stimulating the cut ends of the vagus nerve. This

means that the effects of synchronous activation of vagal fibres were comparable to the effects produced by the naturally occurring asynchronous discharge from SAR. Though it can be argued that lung volume is a more natural stimulus, the central effects of electrical stimulation of the vagus resemble those of lung inflation (Euler & Trippenbach, 1976; Knox, 1979; Feldman & Gautier, 1976). A further advantage of mimicking lung inflation with electrical stimulation is that the animal can continue breathing during the mimicked maneuver thus minimizing changes in blood gases (Trenchard, 1977).

The goal of these experiments was to investigate whether the central component of the BH reflex undergoes maturational changes. Vagal stimulation during inspiration and expiration was used to examine the respiratory effects of stimulating the vagus at different intensities. The stimulus consisted of a train of monophasic, 0.1 msec pulses and ranged in intensity from 1-5 times threshold for the CAP. Stimulation occurred in increments of .25 times threshold in order to reveal subtle effects on respiratory pattern.

The purpose of stimulating at a variety of intensities was to identify intensities or fibre groups that had effects attributable to SAR -- inspiratory shortening in response to an inspiratory stimulus and expiratory lengthening in response to an expiratory stimulus. By using a range of stimulus intensities during each experiment, this intensity could be identified, allowing the assessment of the effects of stimulation on different days and between animals. The compound action potential was monitored in order to associate the effects of stimulation with the recruitment of particular fibre groups.

The procedure for stimulation was as follows. The rectified, low pass filtered diaphragm EMG was used to generate a +5 volt gate to enable the stimulator. For an inspiratory stimulus the gate turned on at the beginning of inspiration and turned off at the peak of inspiration. After a minimum of three breaths of similar shape and duration had occurred, the stimulator was turned on. The next gate delivered the stimulus train. For expiration the gate functioned similarly except that the gate turned on at peak inspiration and turned off at the beginning of the next inspiration. With very few exceptions stimuli were delivered for the duration of inspiration or the duration of expiration. Inspiratory and expiratory stimuli were repeated at least ten times for each stimulus intensity. Approximately 500 stimuli were delivered to the nerve during the course of an experiment. Because stable breathing (a minimum of two very similar breaths) was required before a stimulus was given, and matched frequencies of breathing over all stimulus intensities were preferred, a recording session would typically last a minimum of six hours.

DATA ANALYSIS

Data analysis was performed off line using custom programs with the ASYST 2.01 software package on IBM AT compatible, 20 MHz 386 machines and SUN spark stations. Much of the analysis used an Asyst cursor program. This program displayed consecutive segments of data and allowed manual placement of markers within the data. These markers were placed to indicate, with respect to the RLPF diaphragm EMG signal, the onset of inspiration, the

peak of inspiration and the end of expiration (or onset of the next inspiration). Marker positions were stored for use in cross correlation analysis and ensemble averaging.

Cross Correlation Analysis

Cross correlation analysis was used to establish the origin, afferent or efferent, of the vagal neurogram. In the simplest terms cross correlation can be explained as follows: an afferent action potential arrives at the distal recording cuff before arriving at the proximal cuff; an efferent impulse arrives at the proximal cuff before the distal cuff. Cross correlation uses these differences in timing and direction to determine the relative contributions of afferents and efferents to the neural signal.

The end product of the cross correlation analysis is a plot called a cross correlogram. The cross correlogram shows peaks at positive and negative time delays. The positive time delays correspond to the time delay between the cuffs for afferent components while the negative time delays correspond to efferent components. Since the distance between the cuffs at implant is known, conduction velocity (distance/time) for an afferent or efferent component can be computed.

In these experiments cross correlation analysis was computed two ways. The first type of cross correlation was performed on an entire segment of data. The second type of cross correlation was done as a function of time within segments of data. Specifically, cross correlation was performed as a function of the breathing cycle using the markers from the ASYST cursor program.

Each breath was divided in ten segments and cross correlation was performed within each of the ten segments. The end result was ten cross correlograms, each the average cross correlogram for a particular portion of the breath. While this technique could indicate the activity of fibres within the cycle it had the disadvantage of having total sampling size reduced by the number of segments the breaths were divided into. Hence, the signal to noise resolution from these type of cross correlogram was not as good.

Ensemble Averaging

In order to enhance the modulations in the raw vagal neurogram and make quantitative measurements, the RLPF vagal neurogram was averaged using peak inspiration markers (from the RLPF diaphragm EMG) to synchronize the averages with respect to time. Ensemble averages were performed using an Asyst averaging program. The averaged, modulated neural signal was low pass filtered and the amplitude of modulation was measured from peak to peak.

Compound Action Potentials

Vagal compound actions potentials were averaged using an ASYST averaging program to improve the signal to noise. The CAP is a reflection of the number of nerve fibres, their conduction velocity and the sensitivity of the recording electrodes. Changes in these variables on different recording days

and between animals were examined by comparing the area of the CAP. Compound action potential area was measured at the stimulus intensity where there was no further increase in the amplitude of the myelinated components of the CAP.

The CAP consisted of several components which represented discrete groupings of myelinated axonal conduction velocities. In order to quantify the contribution of different fibre groups during electrical stimulation on a particular recording day, the peak to peak amplitude of each component of the CAP was measured using the difference between the negative peak and the preceding positive peak at each stimulus intensity. Amplitude was expressed as a per cent of the maximum amplitude observed for each component.

Effect of Vagal Stimulation

The effects of vagal stimulation were evaluated over a range of stimulus intensities by comparing the features of stimulated phases with previous unstimulated breaths for a particular stimulus intensity. The effects were calculated by dividing the measured variable during electrical stimulation by the average of that variable during the two preceding unstimulated breaths. The normalized variable (percent of control values) was then plotted as a function of stimulus intensity. Variables measured were inspiratory duration, amplitude of the inspiratory burst, and expiratory duration. Post-stimulus variables were also compared to the average of the same variable pre-stimulus. For example, expiratory duration after an inspiratory stimulus was expressed as a percent

of the average duration of the two expiratory phases preceding the stimulus and then plotted as a function of stimulus intensity.

This analysis was performed by using cursors to mark the beginning, peak and end of inspiration and which breath was stimulated for a set of data at one stimulus intensity. Custom programs within ASYST then averaged these data and calculated the following variables as per cent of control values: for an inspiratory stimulus, mean inspiratory duration, mean amplitude of the inspiratory burst, mean post-stimulus expiratory duration and mean post-stimulus inspiratory duration. For an expiratory stimulus, the program calculated mean expiratory duration, mean post-stimulus inspiratory duration and mean post stimulus expiratory duration as a per cent of control values for each variable. The analysis was repeated for each stimulus intensity and the results were plotted as a function of stimulus intensity. Thus the stimulus intensity, relative to threshold for the vagal CAP, required for the inspiratory- shortening, expiratory-prolonging reflex could be identified. By reproducibly administering an inspiratory shortening, expiratory prolonging stimulus major changes in the output of the respiratory system on different postnatal days could be attributed to developmental changes in the central component of the reflex.

Statistical Analysis

One and two-way analysis of variance with repeated measures and missing values was performed using STATA. Linear and quadratic regression analysis was performed using GRTOOL software. Significance was .05 or better.

RESULTS

Development may alter 1) the activity of respiratory modulated vagal afferents or 2) the central processing of these vagal afferents.

Part one of this section examines respiratory modulated vagal afferent activity in the fetal and newborn lamb. First, the properties of the vagal neurogram in a typical newborn lamb are described. Second, the presence and identification of vagal afferents in the fetus in utero are detailed. Third, vagal afferent activity in the fetus is compared to that in the newborn. Finally, the development of vagal afferent activity after birth is described.

Part two of the results section examines the central processing of vagal afferents. First, the effects of vagal stimulation on the respiratory cycle will be characterized. Second, the effects of stimulation will be described in newborn lambs at different ages, using both intra and inter-animal comparisons.

VAGAL ACTIVITY IN FETAL AND NEWBORN LAMBS

Typical Neonatal Data

Figure 2 shows a typical example of a vagal neurogram recorded from an awake newborn lamb. The traces in Figure 2 from the top down are, the raw vagal neurogram, the same raw neurogram rectified and low pass filtered (RLPF) and the raw diaphragm EMG. The raw neural signal, though noisy, contained both a phasic and tonic component. The phasic component was

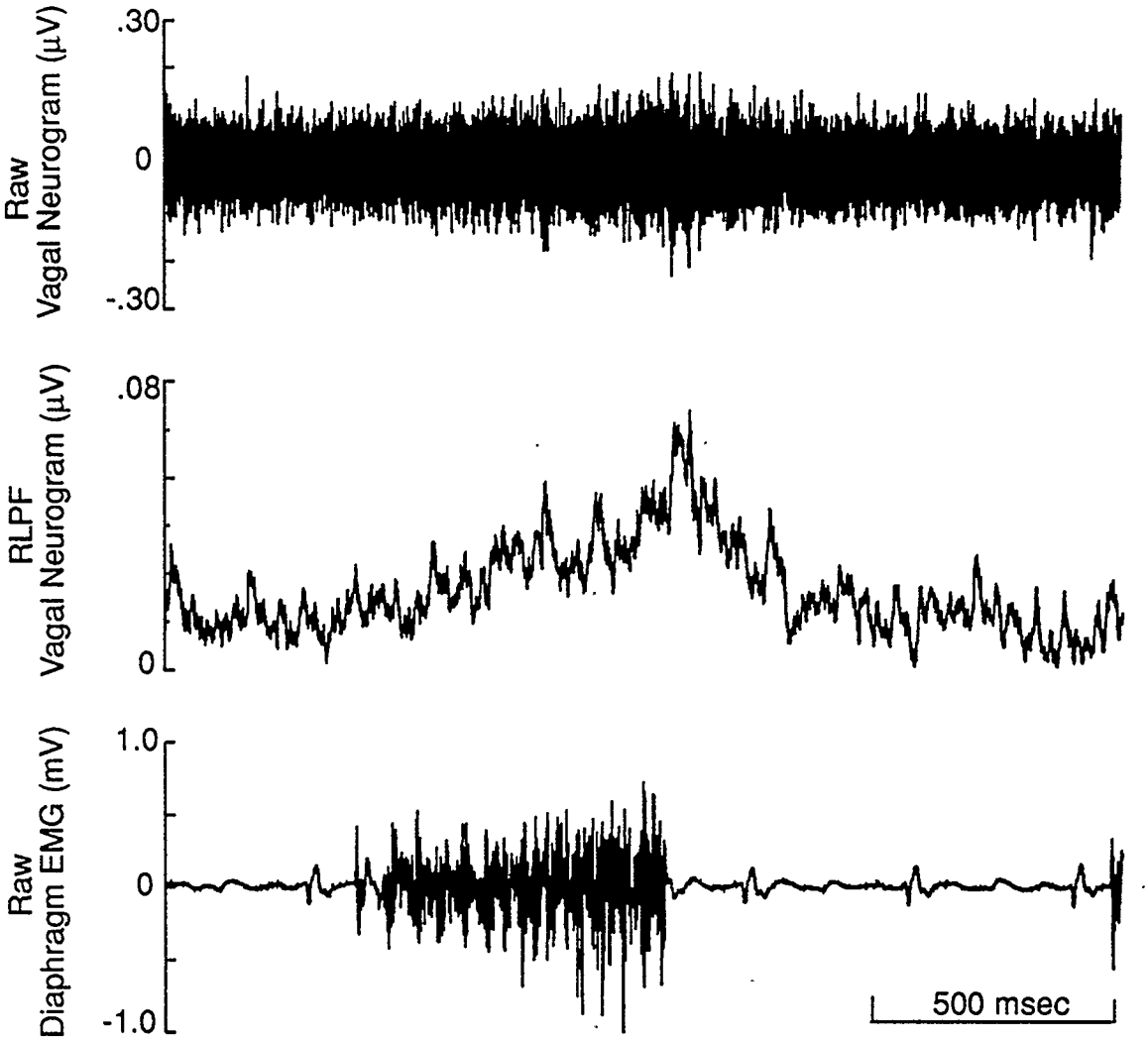

Figure 2. Vagal activity in the newborn lamb. The raw vagal neurogram (top trace) was slightly modulated during breathing; in some lambs modulation in the raw neuorgram was only apparent when the signal was played over an audio amplifier. The modulation in the raw vagal signal was accentuated when the signal was full wave rectified and low pass filtered at 10 Hz (RLPF). The RLPF signal showed clear respiratory modulation that reached a peak shortly after peak inspiration in the diaphragm EMG signal. All newborn lambs showed respiratory modulation in the RLPF vagal signal.

modulated with respiration, increasing with inspiration and decreasing with expiration. This modulation was enhanced when the signal was rectified and low pass filtered. In addition to the respiratory modulated phasic component of the signal, there was also a tonic component which, in the raw signal, was indistinguishable from noise. This tonic component was reflected by the difference between zero and the baseline voltage level of the RLPF vagal neurogram.

Because even the RLPF vagal neurogram was noisy, it was difficult to make quantitative measurements of the amount of respiratory modulated activity. In order to measure the amplitude of the modulated neural signal, the RLPF vagal neurogram was ensemble averaged using the peak of diaphragm EMG activity (transition between inspiration and expiration) as a synchronization point with respect to time. Ensemble averaging improved the signal to noise of the RLPF vagal neurogram. The ensemble average in Figure 3 revealed that the onset of the diaphragm EMG preceded the onset of modulation in the neural signal, and that peak diaphragmatic activity occurred 80 msec earlier than the peak of neural activity. The peak to peak amplitude of the modulated signal was approximately .08 microvolts.

Since the frequency bandwidth (800-8 Khz) of the respiratory modulated signal suggested a neural origin, it was necessary to further identify whether the signal resulted from efferent (motor) or afferent (sensory) vagal activity. Cross correlation is a technique which uses the differences in timing between the neurogram recorded at the proximal and distal recording cuffs to determine the relative contribution of afferent and efferent fibres to the signal. If the

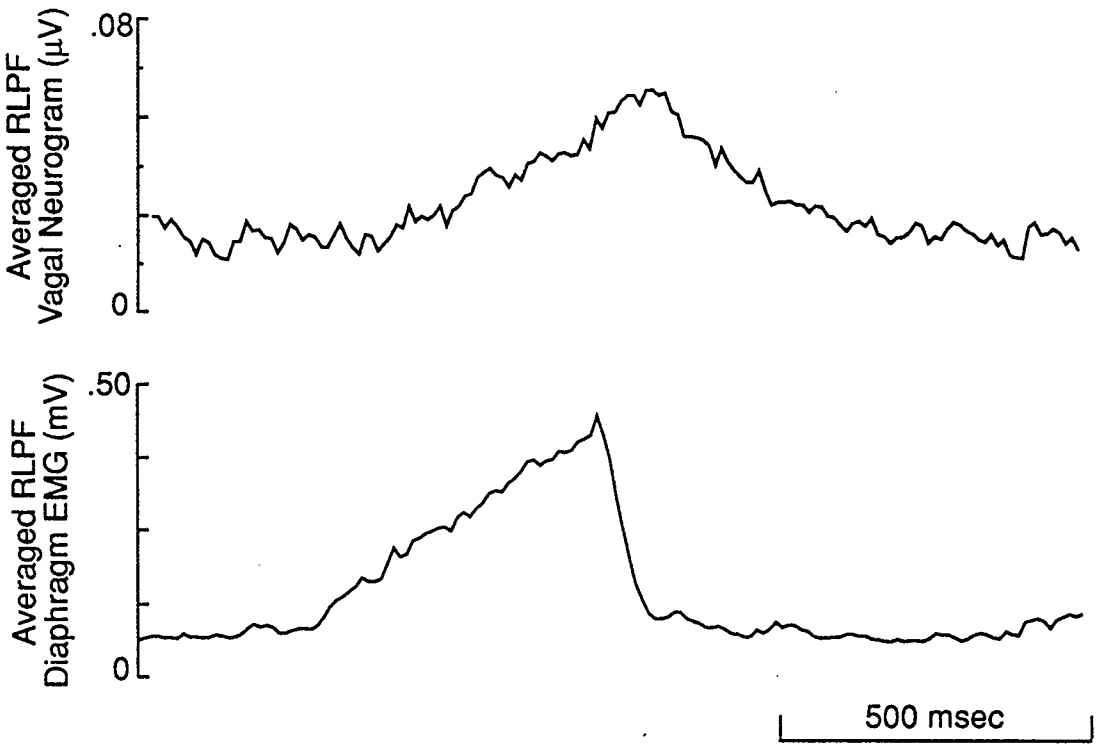

Figure 3. Averaged vagal activity in the newborn lamb. In the top panel the raw neurogram was filtered 800 Hz - 8 KHz and averaged in time using the peak of the diaphragm EMG as a synchronization point. The peak of the neural average occurred approximately 80 msec after the peak of the diaphragm EMG average (lower pane). The average was based on fifty sweeps.

conduction distance between the electrodes is known, the results of cross correlation analysis can also determine the conduction velocity of those fibres contributing most to the signal. Figure 4 shows an example of a cross correlogram from a 7 day old unanesthetized lamb for a period of quiet wakefulness. The large pentaphasic peak at +1.49 msec represents the activity of afferent fibres. Given a known conduction distance of 46 mm the latency of this peak corresponded to that of afferent fibres with a mean conduction velocity of 30.9 m/sec. Hence, the most active fibres in the neural signal were of afferent origin. The conduction velocity of the afferent peak of the cross correlogram was nearly identical to the conduction velocity of the second component of the electrically evoked vagal compound action potential (left corner inset Figure 4).

Cross correlation was also computed as a function of the respiratory cycle. Breaths were marked at the beginning of inspiration, peak inspiration and at end expiration. Each breath was divided into ten bins, five for inspiration and five for expiration. Data from each bin was processed via cross correlation. Hence, each bin yielded the average cross-correlogram for a particular portion of the breathing cycle. Thus it was possible to examine the amplitude of the afferent peak of the cross correlogram at different points in the breathing cycle. The afferent peak of the cross correlogram was modulated with breathing, increasing during inspiration and decreasing during expiration. The difference between the amount of afferent activity at peak inspiration and end expiration is illustrated by the cross correlogram in Figure 5.

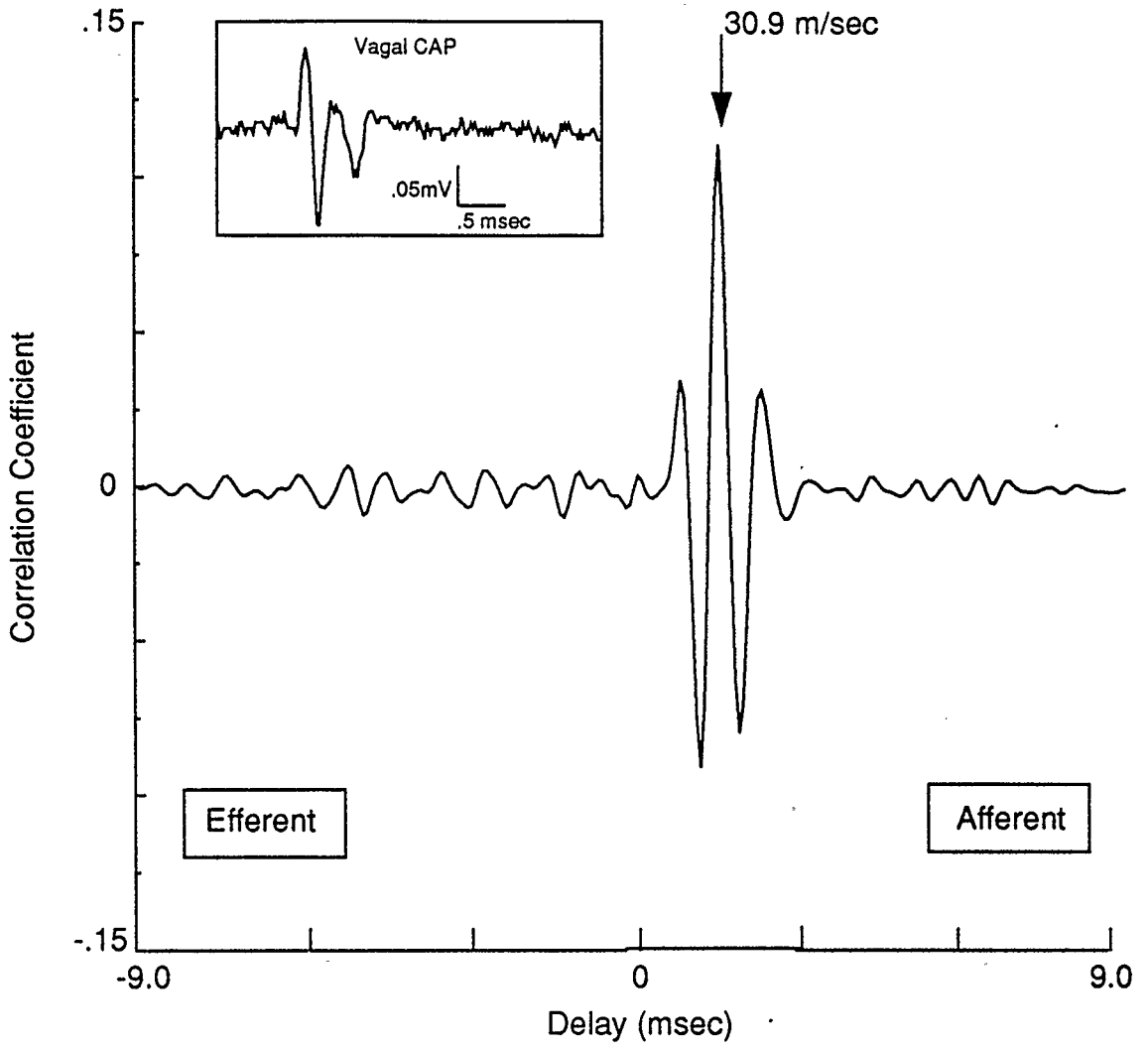

Figure 4. Cross correlation analysis in the newborn lamb. Cross correlation analysis between the signals from the two vagal recording cuff produced an afferent peak at1.49 msec. This afferent peak represented the most active vagal fibres. The conduction distance between the two electrodes was 46mm. The inset in the upper left hand corner shows that the second component of the electrically evoked vagal CAP had a latency of 1.7 msec; the conduction distance for the CAP was 52.75 mm. The conduction velocity of second negative component of the CAP had the same conduction velocity (31 m/sec) as the afferent peak of the cross correlogram. Thus the second component of the vagal CAP probably represented the most active afferent vagal fibres.

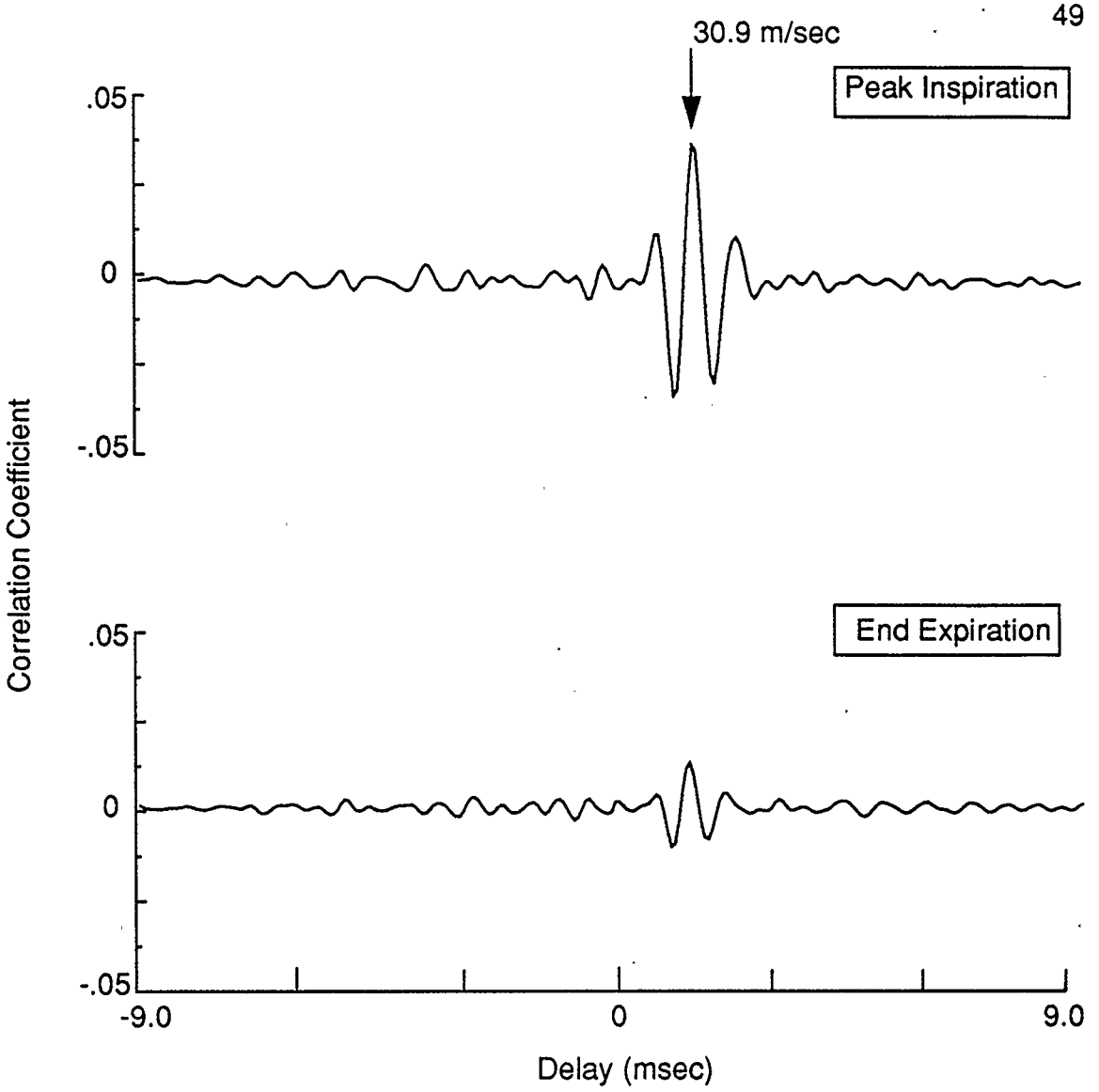

Figure 5. Cross correlation during inspiration and expiration. In the newborn lamb, the afferent peak of the cross correlogram was modulated with breathing and was larger at peak inspiration than at end expiration. The conduction velocity of the afferent peak did not change.

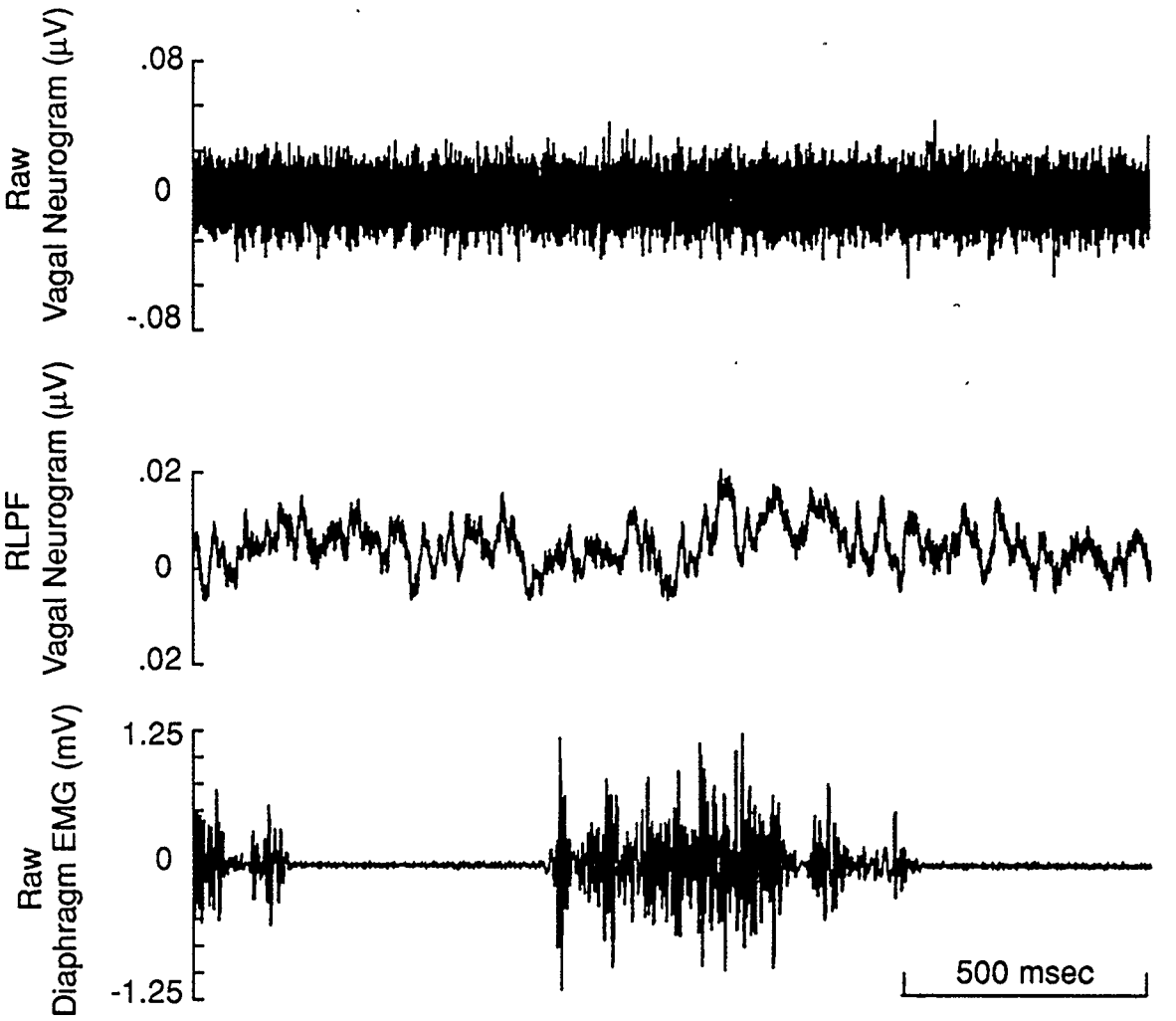

Figure 6. Vagal Activity in the fetal lamb. Although modulated activity was not obvious in the raw vagal neurogram, the RLPF vagal neurogram showed a hint of respiratory modulation. Data are from Fetus D at 130 days gestation.

From the cross correlation data it was possible to conclude that the vagal neurogram was afferent in origin, and that the modulation was due to the activity of afferents that were respiratory modulated. Based on previous work and the relative contributions of various vagal fibres to the modulated vagal neurogram, the evidence that this activity originates from slowly adapting pulmonary stretch receptors (SAR), though indirect, is strong (Ebly,1986).

Fetal Data

Figure 6 shows the raw and processed vagal neurogram and diaphragm EMG from Fetus D at 130 days gestation. Both the raw neural signal (top trace) and the rectified and low-pass filtered (RLPF) neurogram did not appear to contain any respiratory periodicity. Using peak inspiratory activity of RLPF diaphragm EMG as a synchronization point, the RLPF vagal neurogram was ensemble averaged to improve the signal to noise ratio. The top trace of Figure 7 shows that the ensemble averaged fetal neurogram was modulated with respect to the ensemble averaged diaphragm EMG signal (bottom trace). The amplitude of the modulation was approximately .02 microvolts. The peak of the RLPF neural signal lagged the peak of the RLPF diaphragm EMG by 150 msec. In three of the five fetuses (Fetuses A, B, and D) ensemble averaging the RLPF neural signal revealed clear respiratory modulation. In the other two fetuses (C and E) respiratory modulation was not clear. The maximum amount of possible modulated activity in these lambs was judged by the noise level in the RLPF averaged signal.

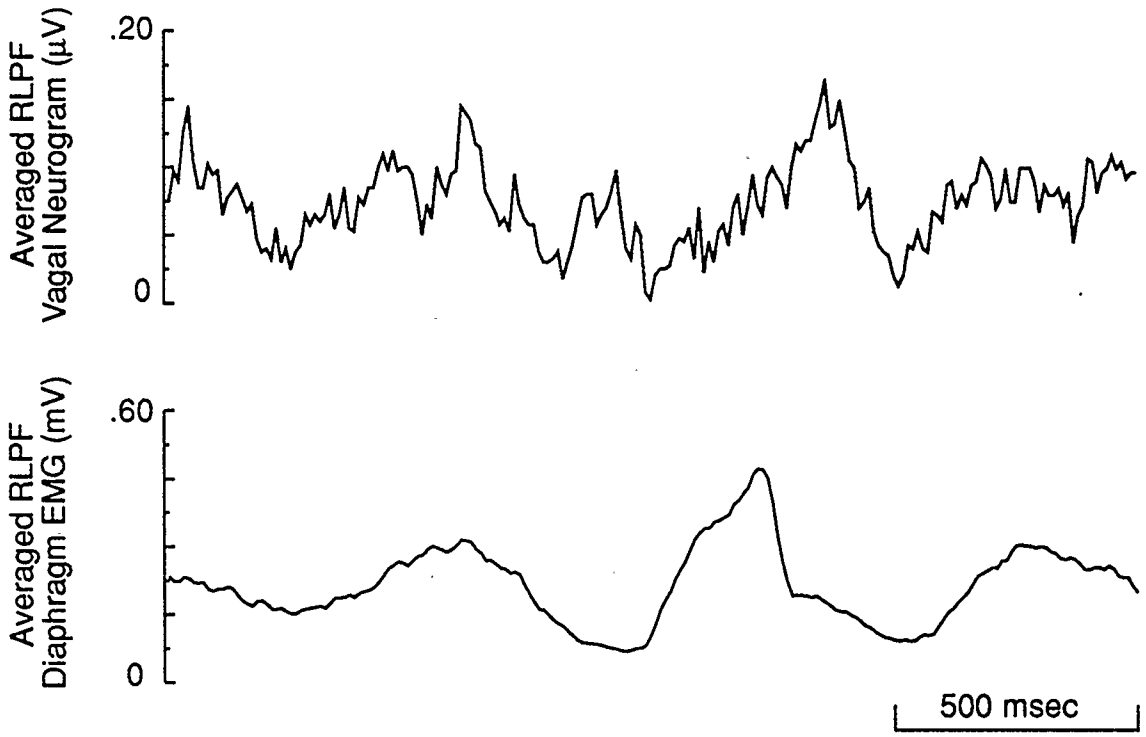

Figure 7. Averaged vagal activity in the fetal lamb. In the top panel the RLPF vagal neurogram was averaged in time using the peak of the diaphragm EMG as a synchronization point. This processing revealed respiratory modulated activity. The peak of the neural averaged occurred 130 msec after the peak of diaphragm EMG activity. Data are from Fetus D and consisted of 80 sweeps.

Because the vagal signals were quite small it was more likely that EMG activity, which is large, could significantly influence the neural recordings. In all five fetuses the same neural signals were processed (50 -350 Hz) to enhance low frequency components which were likely to arise from EMG pickup. Ensemble averaging revealed no low frequency modulated activity in four fetuses. An example of data from Fetus B is shown in panel A of Figure 8. In one fetus (Fetus D) a respiratory modulated low frequency component was present. Importantly, the low frequency modulation (middle trace, panel B. Figure 8) had a different time course and thus could not account for the high frequency respiratory modulation (top trace, panel B, Figure 8).

Cross-correlation was computed between the two recording electrodes to determine the relative contributions of afferent and efferent fibres to the neural signal recorded from the fetus. The cross-correlogram for one fetus during an epoch of fetal breathing is shown in Figure 9. The central peak in the cross correlogram reflects activity which occurred simultaneously in both recording cuffs. Possible sources of this activity include electronic noise and EKG. The right side of the cross-correlogram, represented the contributions of afferent fibres to the neural signal. A small peak occurred at a delay of 2.4 msec, which corresponded to a conduction velocity of 25.8 m/sec. Because the peak was small relative to what had been seen in the newborn it was necessary to discount the possibility that this peak occurred randomly and was due to noise. In all fetuses, multiple cross correlations were performed on different segments of data. Each cross correlogram revealed a very similar afferent peak. A histogram of the latency of the afferent peak for one fetus is

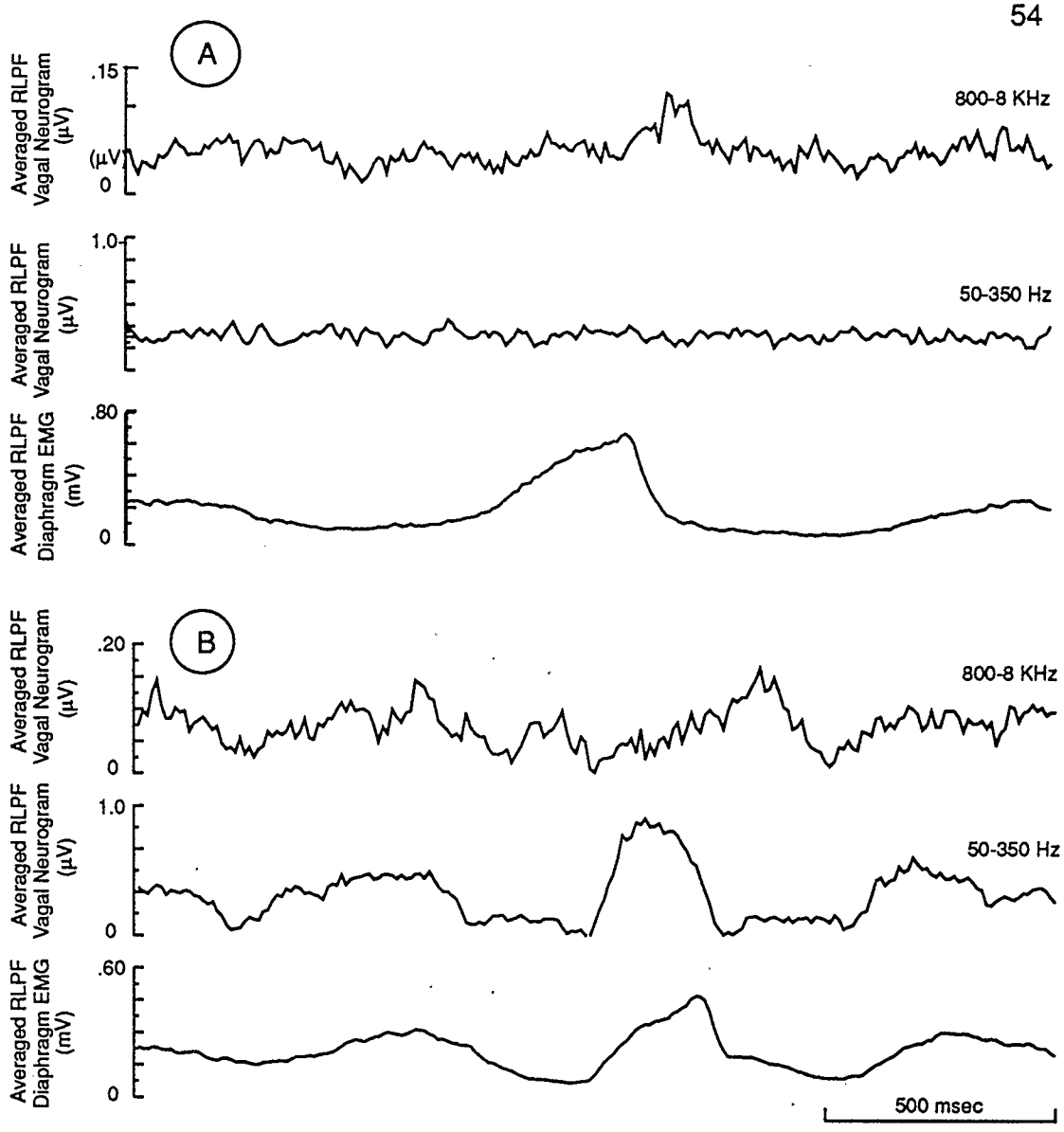

Figure 8. Low frequency respiratory modulation.. In panel A (Fetus A) the raw vagal neurogram was filtered 800 Hz - 8 KHz and averaged in time using the peak of the diaphragm EMG as a synchronization point. This processing revealed respiratory modulated activity. Data which were filtered 50-350 Hz showed no respiratory modulation. In panel B (Fetus D), the same data from Figure 7 were filtered 50 Hz- 350 Hz before RLFP and averaging. Processing this way revealed a signal whose peak modulated activity preceded that of the diaphragm EMG (middle trace, panel B) . This was the only fetus which showed low frequency respiratory modulation.

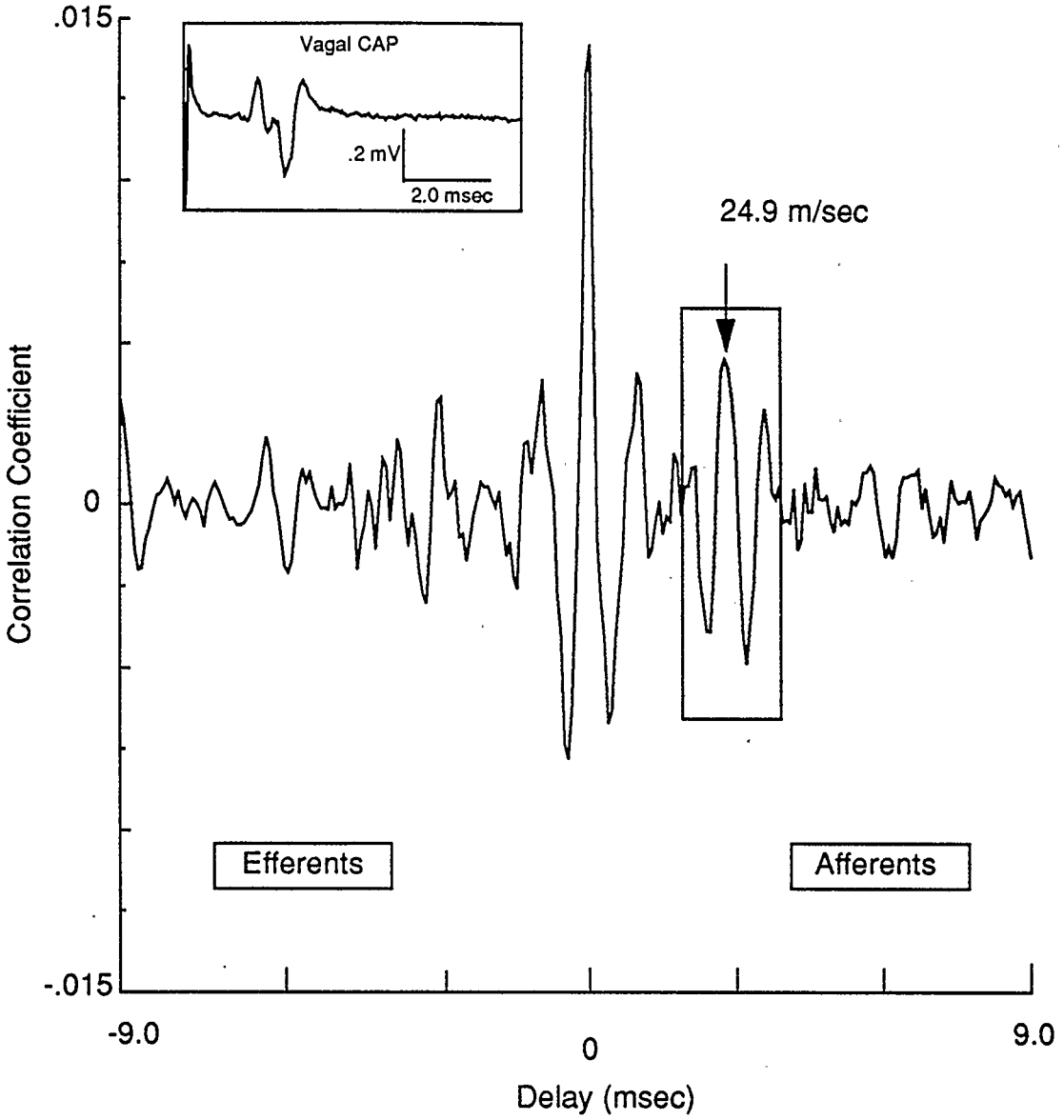

Figure 9. Cross correlation in the fetal lamb. The large peak at 0 msec delay reflected activity that occurred in both recording cuffs simultaneously. A small afferent peak occurred at 2.7 msec. This latency corresponded to a conduction velocity of 24.9 m/sec. The second negative component of the electrically evoked vagal CAP had a similar conduction velocity of 24.8 msec (upper left inset).

shown in Figure 10. This histogram is qualitatively representative of the data from four other fetal lambs. The latency of the afferent peak from each of 17 segments of data analyzed using cross correlation, occurred within a narrow time range with a normal distribution. Thus it was unlikely that the peak was consequence of noise.

Afferent components, though considerably smaller in amplitude than those observed in the neonate, were present in the cross-correlograms of five fetuses. In each fetus the conduction velocity of the afferent component of the cross correlogram was very similar to the conduction velocity of the second component of the compound action potential (see inset Figure 9). The mean conduction velocity of the afferent peak of the cross correlogram in the five fetuses was 26.3 m/sec (± 1.67 S.D). The average conduction velocity of the second component of the CAP in the five fetuses was 26.28 m/sec (± 1.98 S.D). These conduction velocities were not significantly different.

Cross-correlations were also computed for epochs where no fetal breathing was present. Panel B of Figure 11 shows a cross-correlogram of vagal activity when the fetus was not breathing. The afferent activity in Panel B was similar to the afferent component in Panel A, which represented a period of fetal breathing. This suggests that most of the afferent activity was tonic in origin. Cross correlation was also performed as a function of the breathing cycle, as was previously described for the newborn. Figure 12 shows the results of cross correlation during fetal breathing where the results obtained at peak inspiration (A) were compared to the results at end expiration (B). In two fetuses, the amplitude of the afferent peak near peak inspiration was

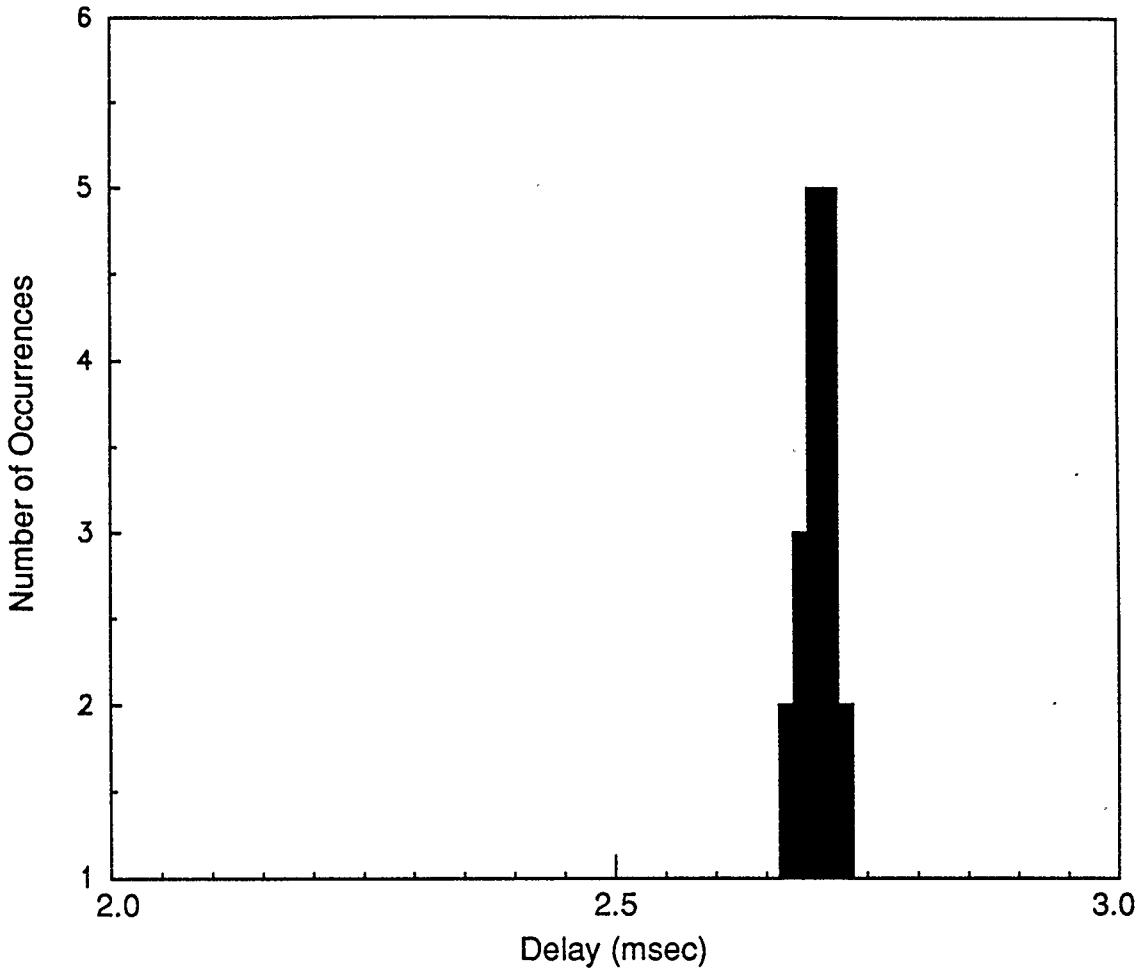

Figure 10. Latency of the afferent peak in the fetus. Cross correlation was performed on 17 segments of data. The latency of the small afferent peak in 17 cross correlograms fell within a 0.1 msec range. The reliability of the latency measurements indicated that the source of the afferent peak in the fetal vagal cross correlogram was unlikely to be the result of a random event.

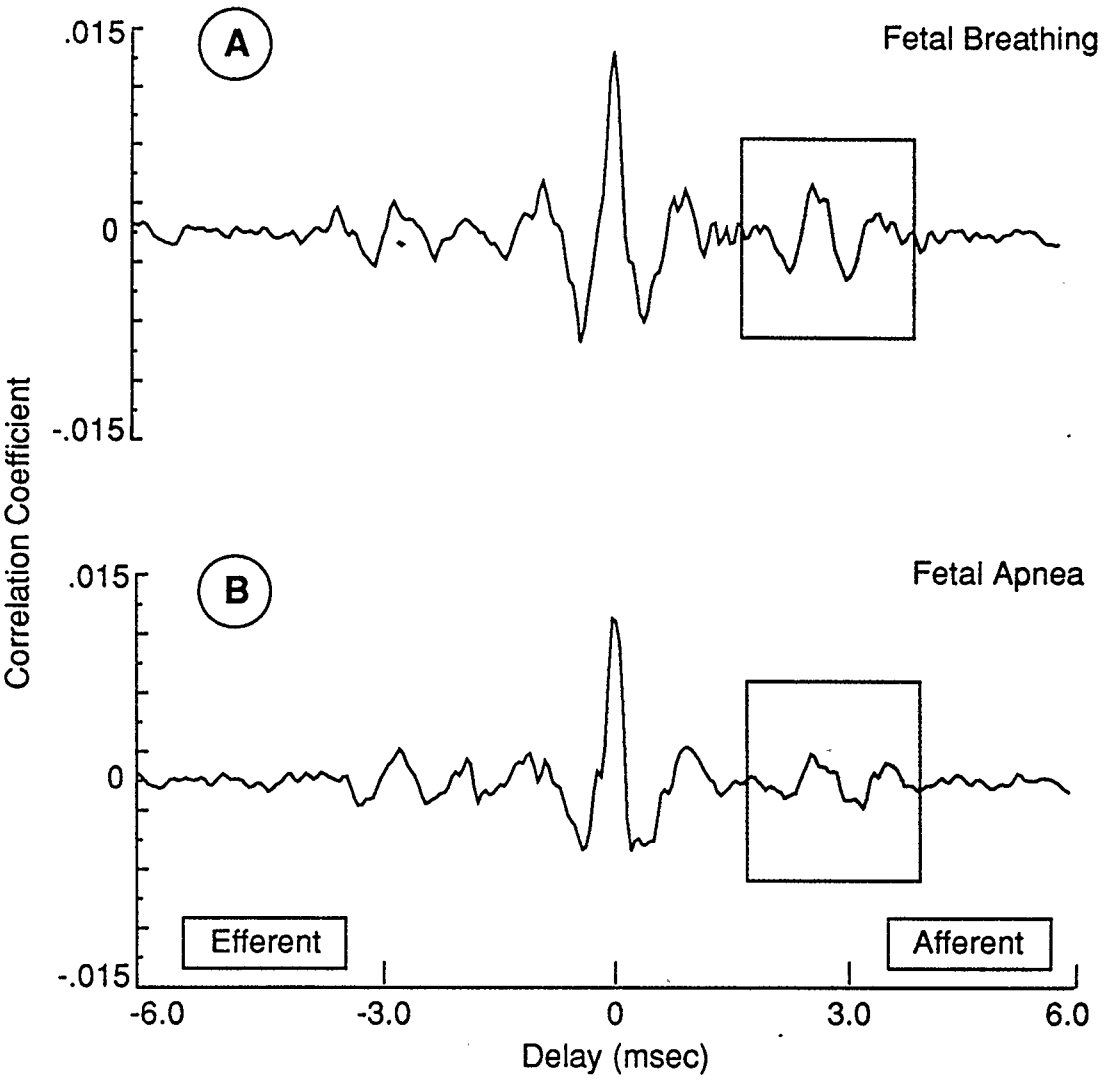

Figure 11. Cross correlation in the fetus: breathing vs. apnea. This figure compares the afferent peak of the cross correlogram during 54 seconds when fetal breathing movements were present and 54 seconds when the fetus was not breathing. The afferent peak of the cross correlogram was larger when the fetus was breathing than when no fetal breathing movements were present. The conduction velocity in each case, was the same (24.9 m/sec) Data are from Fetus C.

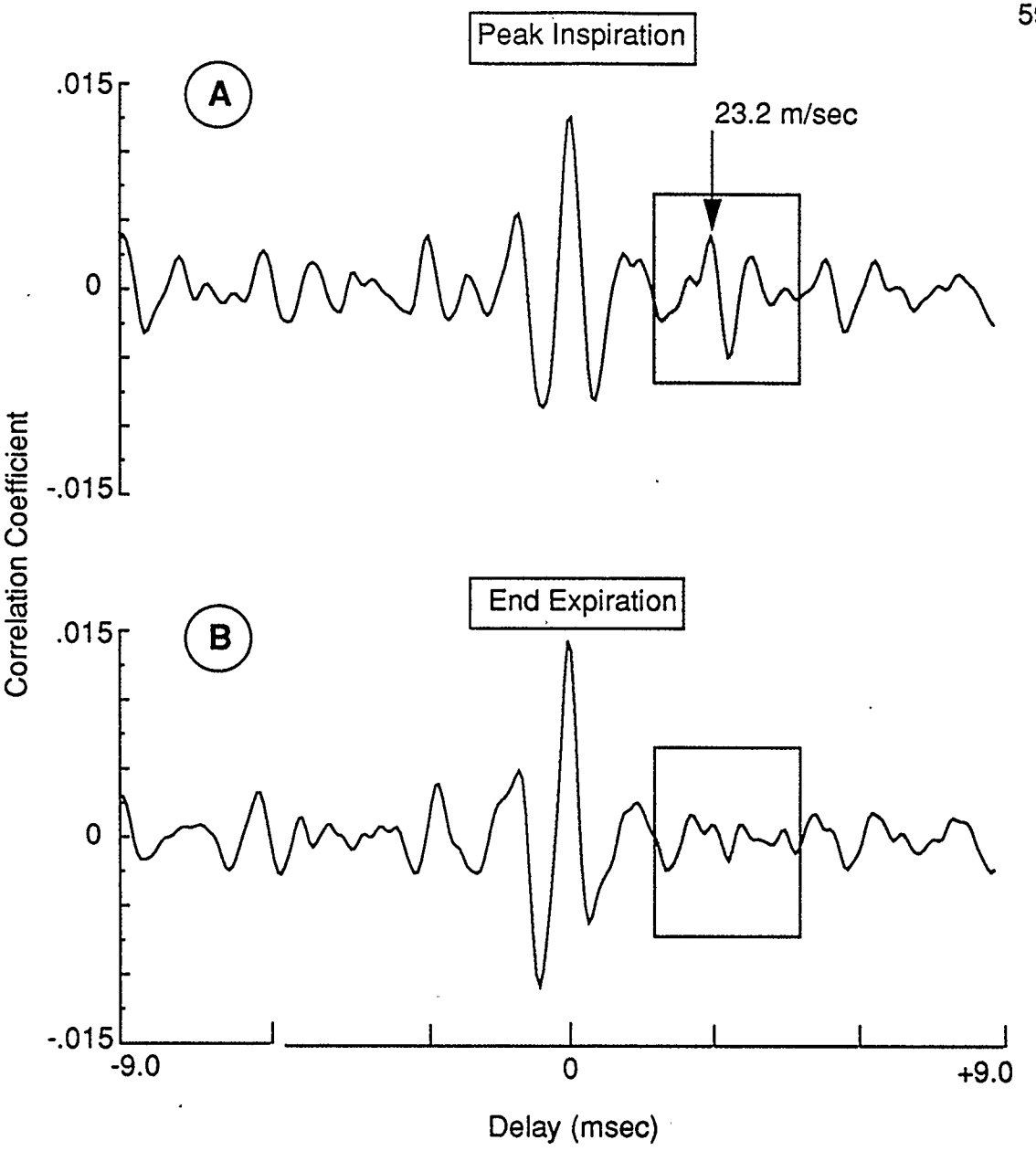

Figure 12. Cross correlation in the fetus:inspiration vs. expiration. The afferent peak of the cross correlogram appeared larger at peak inspiration than at end expiration. Data are from Fetus B at 130 days gestation.

larger than the afferent peak at end expiration. These differences were not as pronounced as in the neonate (Figure 5).

Comparison of Fetal and Neonatal Modulated Vagal Activity

The vagal neurogram recorded from fetal and newborn lambs contained a respiratory modulated signal of afferent origin. The amplitude of respiratory modulation in the five fetuses at 130 ± 1 day gestation was compared to the amplitude of modulation, at the same gain, in eight neonates a week or less old. Figure 13 illustrates that the amplitude of the modulated signal in the newborn lambs was significantly greater than the amplitude of modulation in the fetal lambs.

However a true comparison of modulated activity should be based not just on identical gain for the signals, but on equivalent recording environments. To assess the recording environments, the area of the compound action potentials at maximal stimulus intensity was compared in fetuses and neonates. The only valid comparisons were made between neonates and fetuses in which the conduction distance between the stimulating and recording electrodes was the same. The area of the compound action potential in lamb #014 was 42% smaller than the area of the compound action potential in two fetuses. This suggested that the recording situation in Lamb #014 was inferior to that in the fetuses. However, despite the smaller compound action potential, the amplitude of the modulated neurogram in Lamb #014 was 62% greater than in the fetuses. In another case where conduction distances were

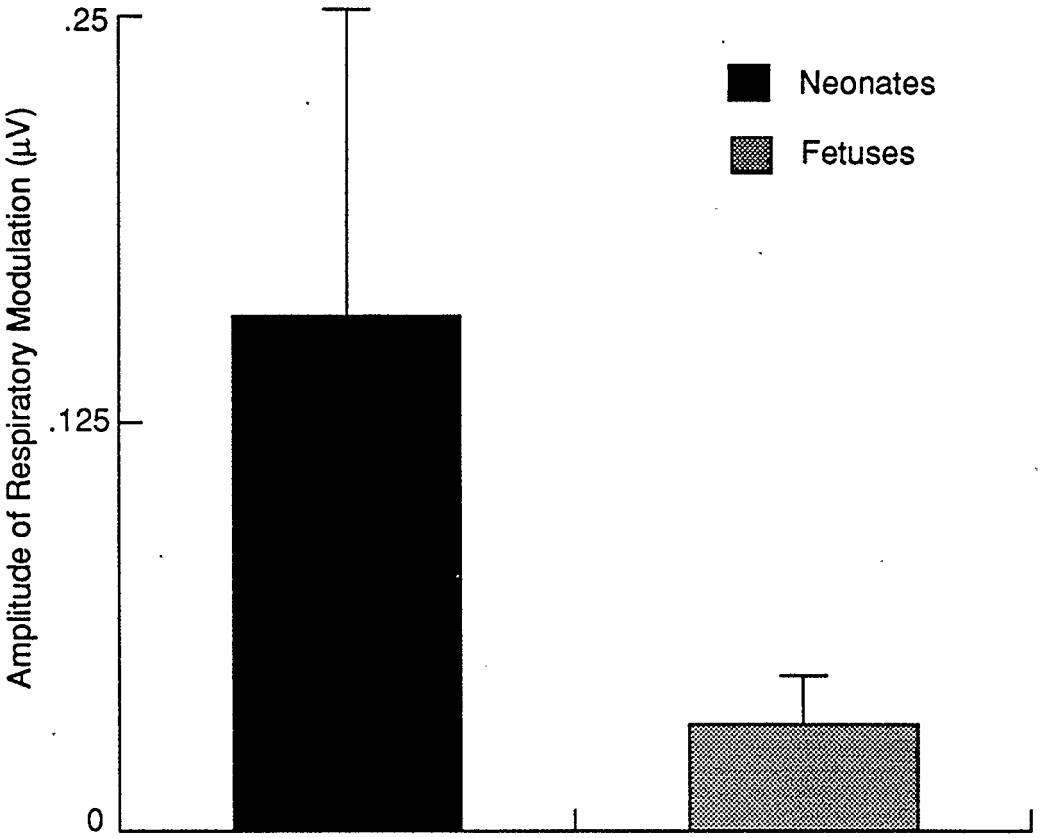

Figure 13. Respiratory modulated vagal activity in fetal and newborn lambs. Comparison of respiratory modulated vagal activity in five fetuses at 131 days gestation and 8 newborn lambs 5 days old.

matched the area of the compound action potential in lamb #003 was 32% smaller than the area of the compound action potential in Fetus A, but the modulated activity in the neonate was still 32% greater than in the fetus. Electrode impedances, which would have affected CAP size were not significantly different in the fetus and the neonate. These results, obtained from only those animals in which conduction distances were matched, suggest that recording environment, assessed via the CAP, cannot account for increased activity in the neonate. Furthermore, the fact that the neonatal CAP was 37% less than the fetal CAP suggests that the amplitude of modulated activity may have been underestimated in the neonate.

Different vagal conduction velocities in the fetus and the neonate could have affected the amplitude of the signal recorded. The size of the neural signal recorded is proportional to the diameter of the axons (somewhere between linear and squared) and axon diameter is proportional to axon conduction velocity (e.g., Stein, 1980). The average conduction velocity of the most active vagal fibres in the neonatal lambs was 12% greater than in the fetal lambs. This increased conduction velocity could explain a neurogram which was 12-25% greater in the neonate than in the fetus. Thus, the differences in conduction velocity between the fetus and the neonate are too small to explain the magnitude of the amplitude differences observed in Figure 13. The differences in the amplitude between the fetus and neonate must therefore have been due to other factors.

Changes in the vagal neurogram after birth

The amplitude of the averaged RLPF vagal signal increased with age in seven lambs. In Figure 14 the amplitude of modulation of the averaged RLPF vagal neurogram in Lamb #003, in whom long term recordings were obtained, is plotted as a function of time. Regression analysis resulted in a correlation coefficient of .80. The slope of the line of best fit through these data points was 1.3 ±.26(SE). As in earlier measurements which compared the recording environment in the fetus and the neonate, the area of the compound action potential was measured for each of the days in Figure 15 as a quantitative index of the quality of a potentially changing recording environment. Although fluctuations in the area of the CAP (Figure 15b) occurred, the fluctuations did not correlate with changes in the amplitude of the modulated neural signal shown in Figure 14. Similarly, changes in breathing frequency (Figure 15a) and electrode impedance (Figure 15c) over the recording period did not show the same developmental trend as that of the RLPF vagal signal.

Long term recording from Lamb #002 showed a similar developmental trend in the amplitude of modulated vagal afferent activity to that seen in Lamb #003. The amplitude of the averaged RLPF vagal neurogram increased with age (figure 16). Again, parallel developmental trends in breathing frequency, CAP area and electrode impedance did not occur (Figure 17).

The validity of CAP area as a tool in assessing the quality of the recording environment is illustrated in Figure 18. In this lamb, the amplitude

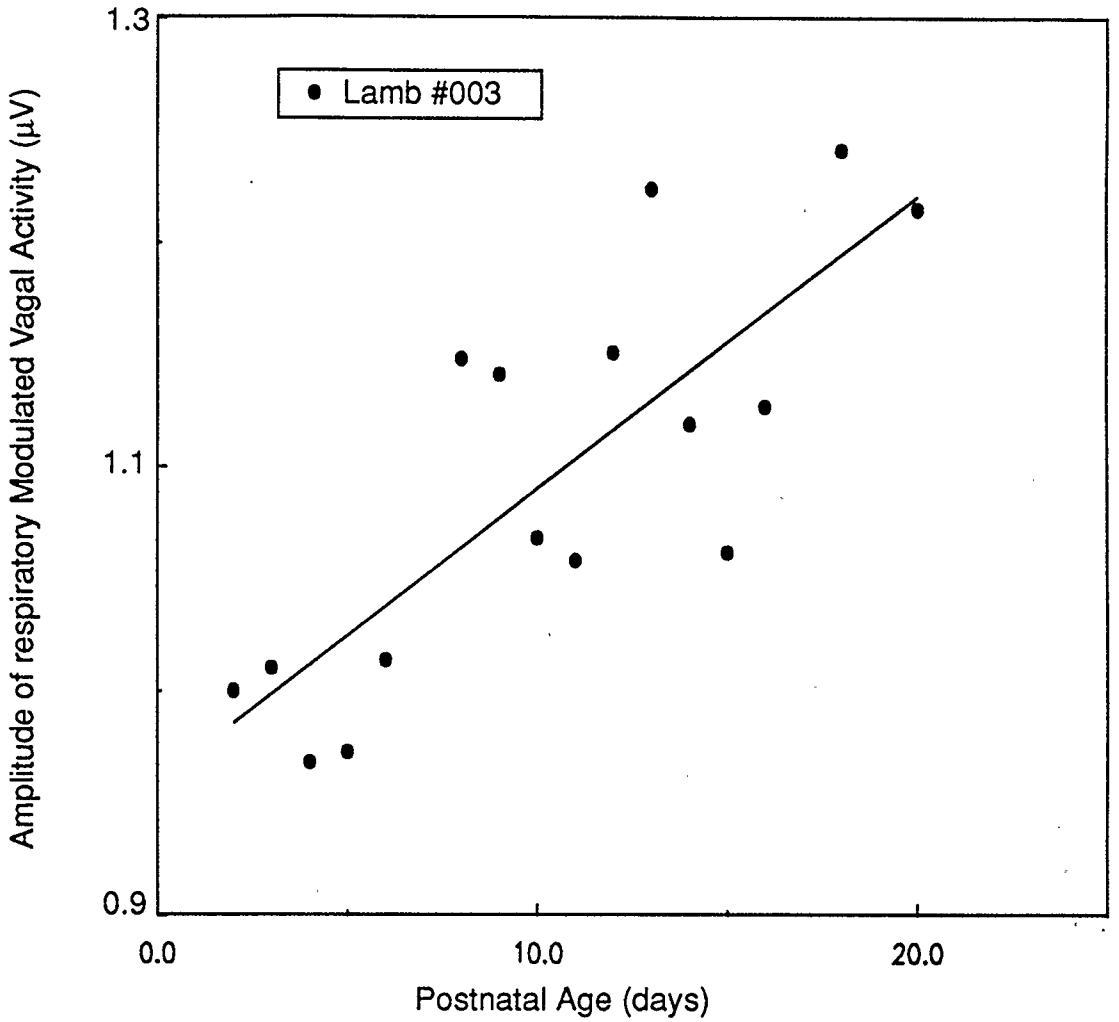

Figure 14. Changes in vagal activity with postnatal age: Lamb #003. The amplitude of respiratory modulated vagal activity increased with increasing postnatal age. The correlation coefficient was .80.

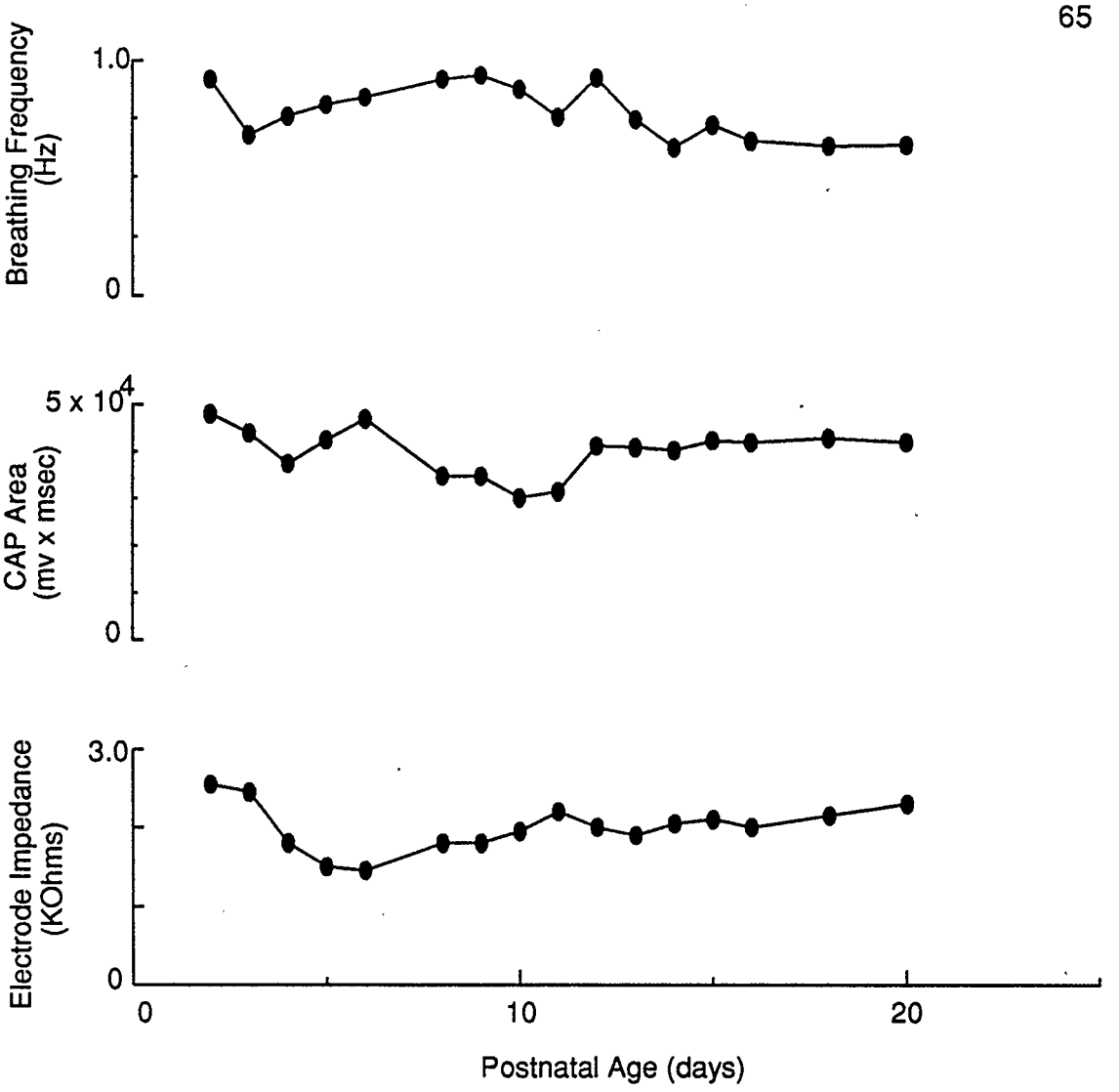

Figure 15. Developmental trends in other variables:Lamb #003. There were no parallel developmental trends in breathing frequency, CAP area or electrode impedance for Lamb #003.

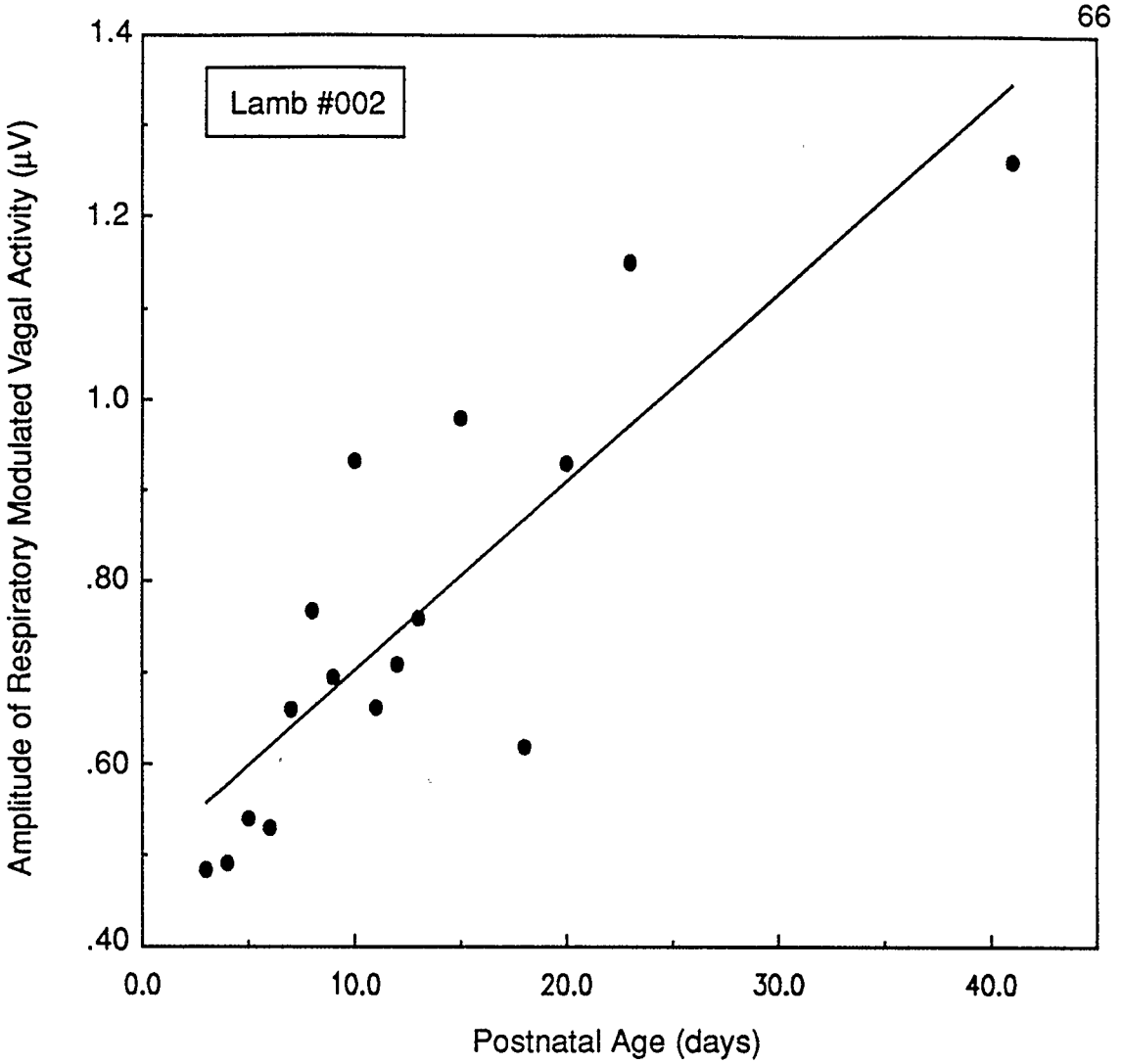

Figure 16. Changes in vagal activity with postnatal age:Lamb #002. The amplitude of respiratory modulated vagal activity increased with increasing postnatal age. The correlation coefficient was.85.

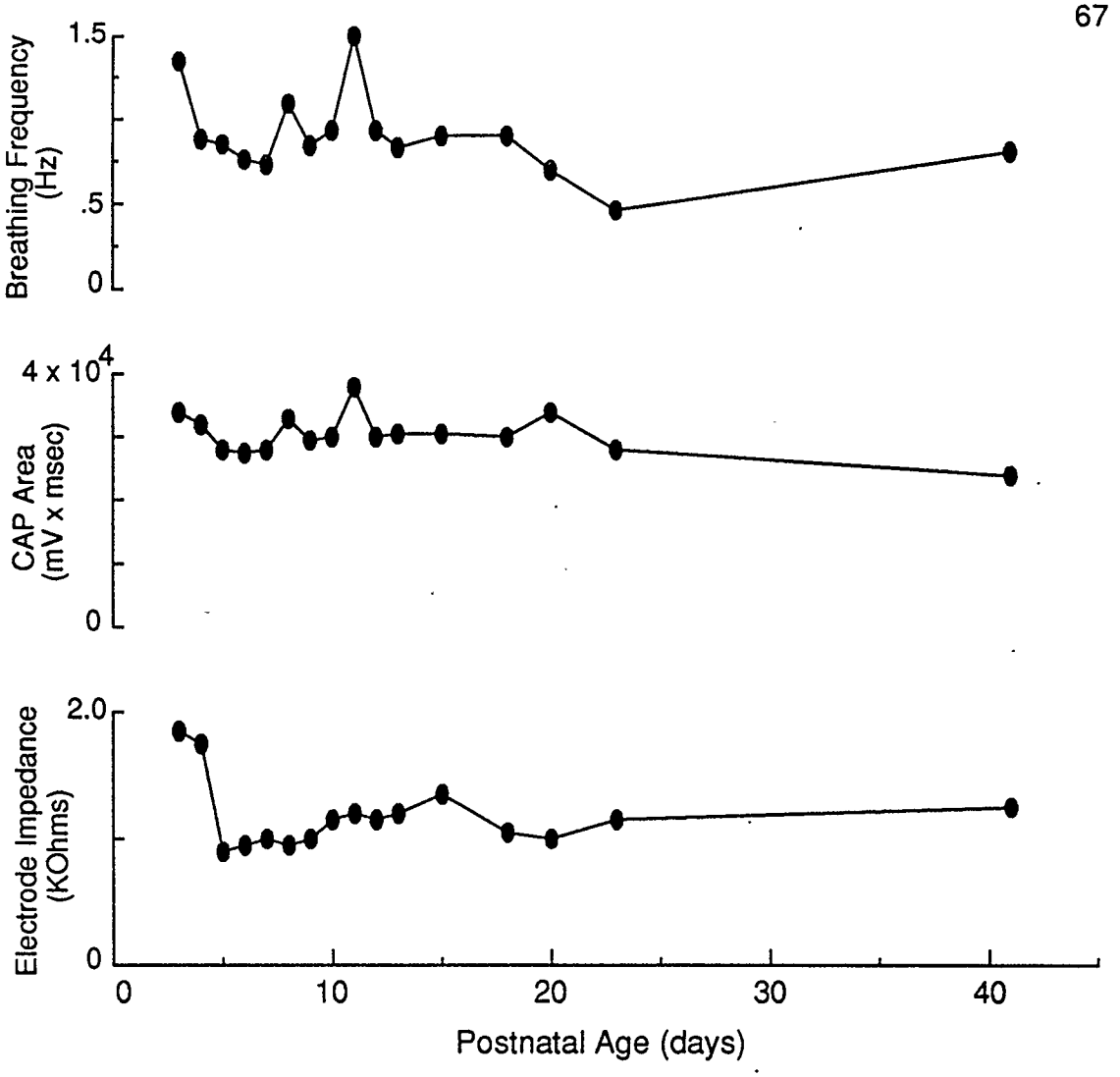

Figure 17. Developmental trends in other variables:Lamb #002. There were no parallel developmental trends in breathing frequency, CAP area and electrode impedance in Lamb #002.

of modulation also increased with time, up to a point. Figure 18 shows a striking example of how a (66%) decrease in the area of the CAP on day 14 corresponded to a large decrease in the amplitude of the modulated neural signal on the same day.

Inter-animal comparisons also showed that the amplitude of the ensemble averaged neurogram increased with age in seven lambs. Population data are presented in summary in Figure 19. Mean RLPF amplitude, CAP area, electrode impedance and breathing frequency were expressed as a per cent of the same variable on day 5. Thus, Figure 19 plots the per cent change on days 10, 12 15 and 20 relative to day 5 (100%). The amplitude of the averaged RLPF vagal neurogram increased with increasing age. No similar developmental trend was apparent for either CAP area or breathing frequency. With respect to impedance measurements, although there appeared to be an increase in electrode impedance with age, there was no significant difference between mean impedance on day 10 and day 20. Furthermore if the impedance measurements were to have had a significant affect, then there should have been significant changes in CAP measurements as well.

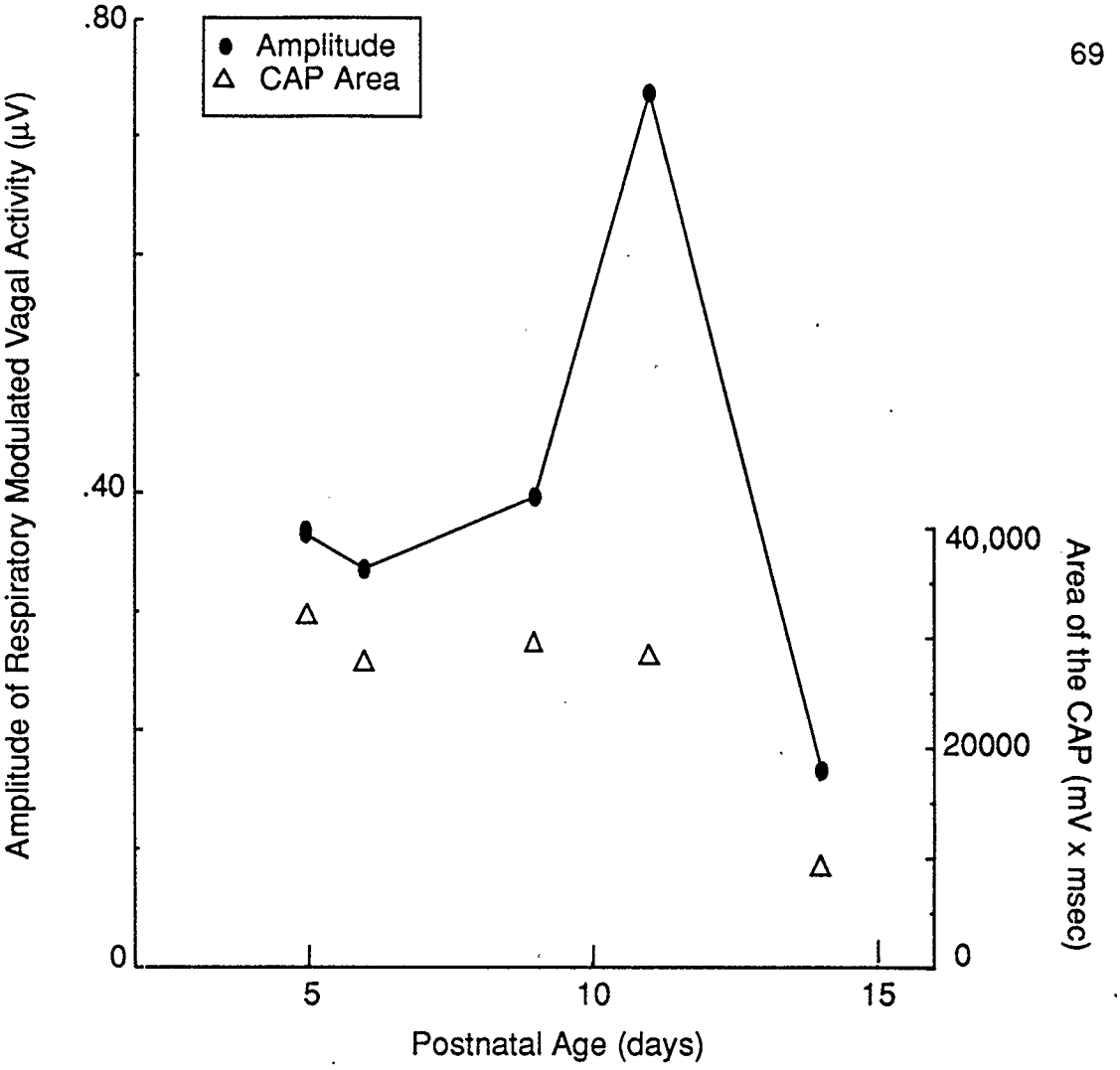

Figure 18. Effect of a change in the CAP.The compound action potential was used as a tool for assessing the quality of the recording environment. The amplitude of respiratory modulated activity increased in Lamb #004 between day 5 and day 11. On day 14, both the area of the CAP and the amplitude of respiratory modulated vagal activity decreased sharply. Data are from Lamb #004.

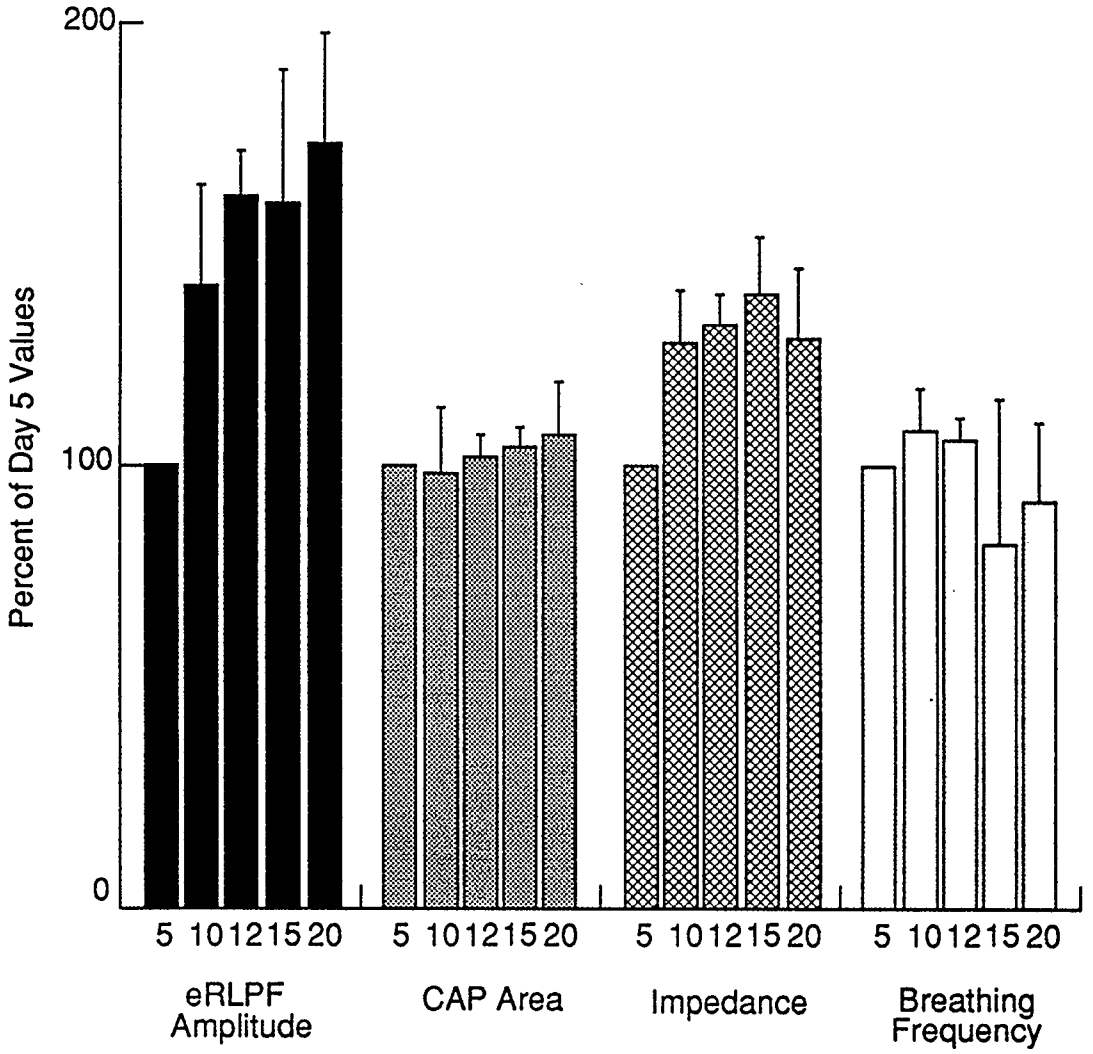

Figure 19. Population data. Amplitude of the RLPF vagal signal, CAP area, electrode impedance and breathing frequency are presented as a per cent of the initial value on day 5. The amplitude of the RLPF signal increased with increasing postnatal age. Although there appeared to be an increase in electrode impedance with age, these changes were not reflected in the CAP and thus were unlikely to have affected the recording of spontaneous neural activity. Not all animals carried through all days. Data included were from were 8 lambs on day 5, 7 on day 10, 3 on day 12, 5 on day 15 and 4 on day 20.

RESPIRATORY RESPONSES TO VAGAL STIMULATION

Effect of Electrical Stimulation on Recruitment of Vagal Fibres

The shape of the vagal compound action potential (CAP) changed as stimulus intensity increased and slower conducting fibre groups were recruited. Representative data are shown in Figure 20. Figure 20 illustrates the CAP at three different intensities of stimulation relative to threshold for the CAP in Lamb # 014. With increasing stimulus intensity the shape of the CAP became more complex. The negative peaks of the CAP represent the contributions of several fibre groups with different conduction velocities.

In Figure 20 the first component (arrow, trace A, 1.0 x threshold) of the CAP represented the activity of fibres with a mean conduction velocity of 32 m/sec. The second component (arrow, trace B, 2.25 x threshold)) represented fibres with a mean conduction velocity of 29 m/sec. The third component of the CAP (arrow, trace C, 5.0 x threshold) corresponded to fibres with a conduction velocity of 22 m/sec.

Quantification of stimulus intensity

In each experiment, the contribution of different vagal fibre groups to the CAP was quantified by plotting the amplitude of each negative peak of CAP as a function of stimulus intensity. Figure 21 quantifies data from Lamb #014. Each point represents a measurement made on an averaged CAP at one stimulus intensity.

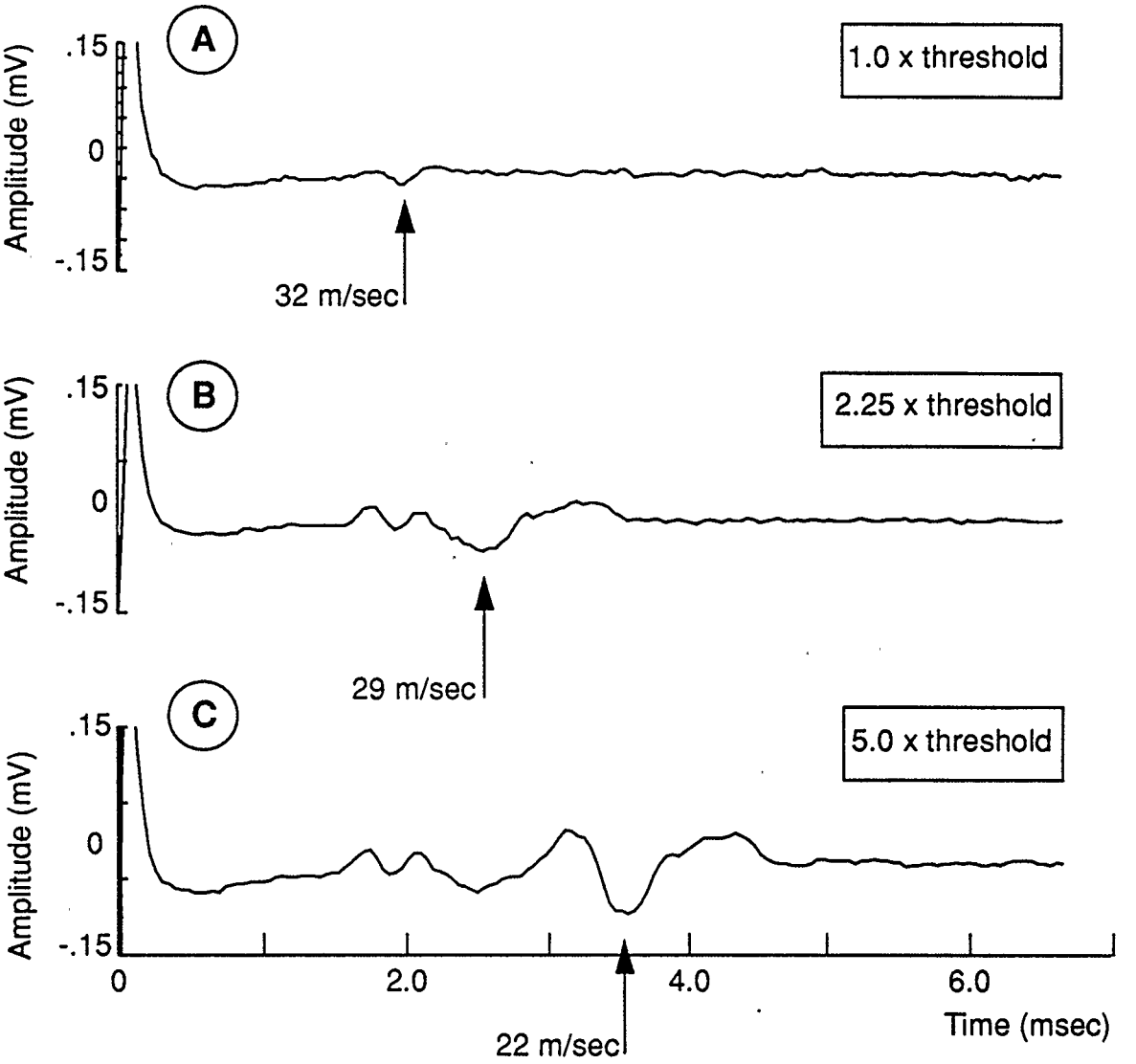

Figure 20. Vagal CAPs at three different stimulus intensities. Each trace was averaged 25 times and is shown at the same gain. As the stimulus intensity increased. the shape of the CAP became more complex. Data are from Lamb #014 and are representative of all lambs.

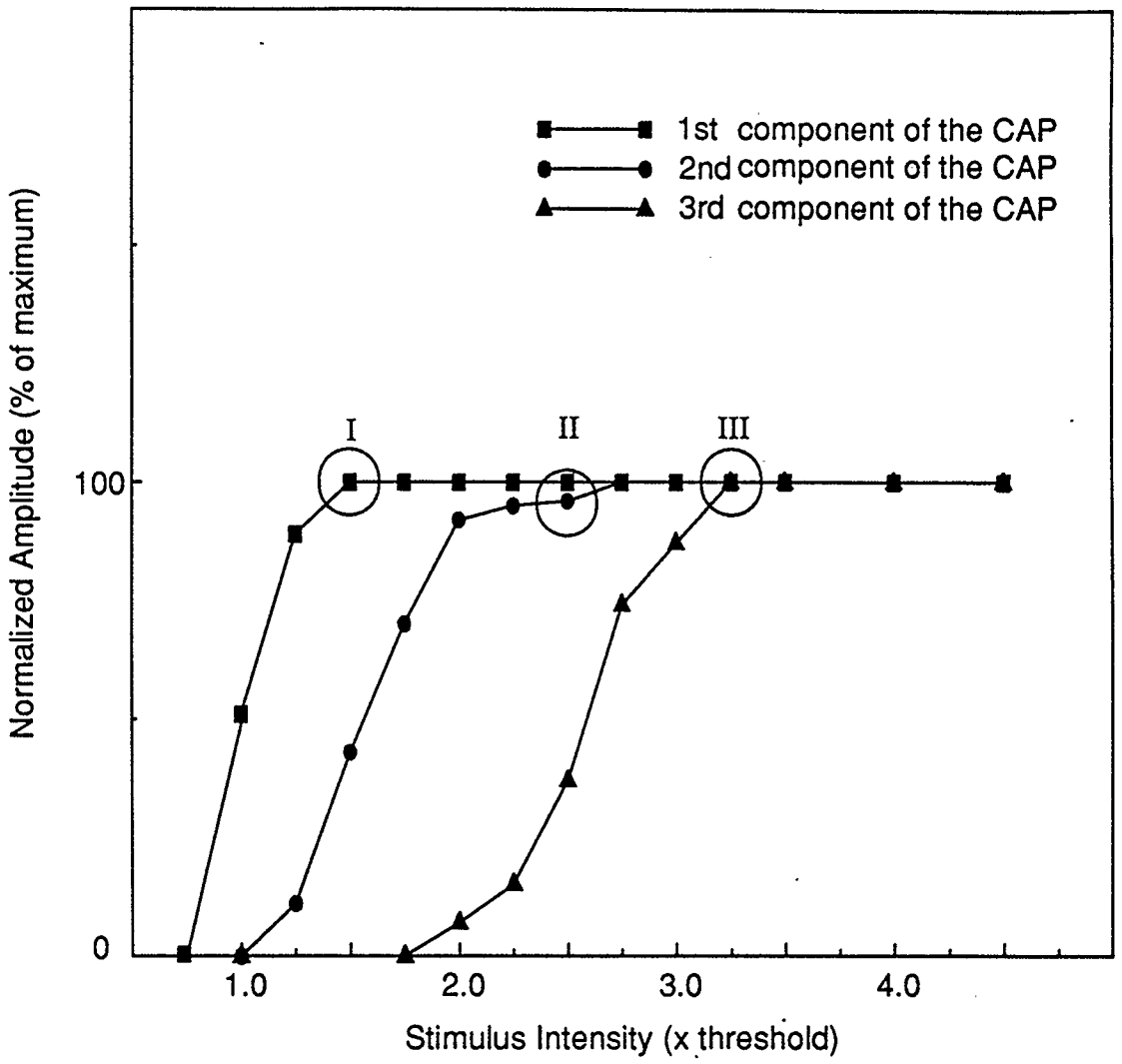

Figure 21.Amplitude of CAP components. The amplitude of each component of the CAP was measured at each stimulus intensity and expressed as a per cent of the maximal amplitude attained. The first component of the CAP appeared at 1.0 x threshold and reached peak amplitude at 1.5 x threshold. (Level I) The second component of the CAP was evoked at 1.25 times threshold. The amplitude of this component reached a plateau near 2.0 x threshold (Level II) The third component of the CAP appeared at 1.75 x threshold and peaked at 3.25 x threshold (Level III).

In Lamb #014, the amplitude of the first component of the CAP increased between 1.0 and 1.25 times threshold, reaching a plateau at 1.5 times threshold. The stimulus intensity corresponding to the plateau amplitude of this component was termed Level I. The second component of the CAP emerged at 1.25 times threshold and gradually increased reaching a plateau at 2.25 times threshold. This stimulus intensity was designated as Level II. The third component of the CAP appeared at 2.0 times threshold, increased in amplitude, and reached an observed maximum at 3.25 times threshold. The stimulus intensity corresponding to the plateau amplitude of the third component of the CAP was termed Level III. Qualitatively similar data were obtained from nine other lambs.

The effects of electrical stimulation of the vagus nerve during inspiration and expiration at different stimulus intensities were characterized by comparing the features of the respiratory cycle during and after stimulation with breathing preceding the stimulus. In the following sections, inspiratory or expiratory duration refers to the duration of a respiratory phase during or after vagal stimulation as a percent of the corresponding respiratory phases preceding the stimulus.

Within-Phase Effects of Vagal Stimulation During Inspiration

Inspiratory Duration

The effects of vagal stimulation during inspiration on inspiratory duration varied depending on the intensity of stimulation used.

At stimulus intensities near threshold for the compound action potential (CAP), vagal stimulation did not alter inspiratory duration. An example of raw data from stimulation at 1.0 times threshold during one experiment in a ten day old lamb is shown in Figure 22. Higher intensities of stimulation (1.25 - 2.75 times threshold) decreased inspiratory duration. Raw data showing one stimulus trial at 2.25 times threshold are shown in Figure 23.

Further increases in stimulus intensity (3.0-5.0 times threshold) prolonged inspiratory duration. Raw data from a stimulus trial at 4.5 times threshold are shown in Figure 24. In Figure 24, vagal stimulation prolonged inspiration and increased the amplitude of the inspiratory burst. In addition the shape of the inspiratory burst was altered. Very early in the burst the EMG shut off briefly. When the burst resumed it displayed the biphasic trajectory characteristic of augmented breaths. The first part of the burst, omitting the very early component just described, was similar to previous breaths. When the stimulated breath attained the amplitude of previous breaths, the inspiratory burst did not shut off, but resumed, increasing the amplitude of the burst.

Figure 25 shows quantification of changes in inspiratory duration as a function of stimulus intensity in Lamb #014. Each filled symbol represents the

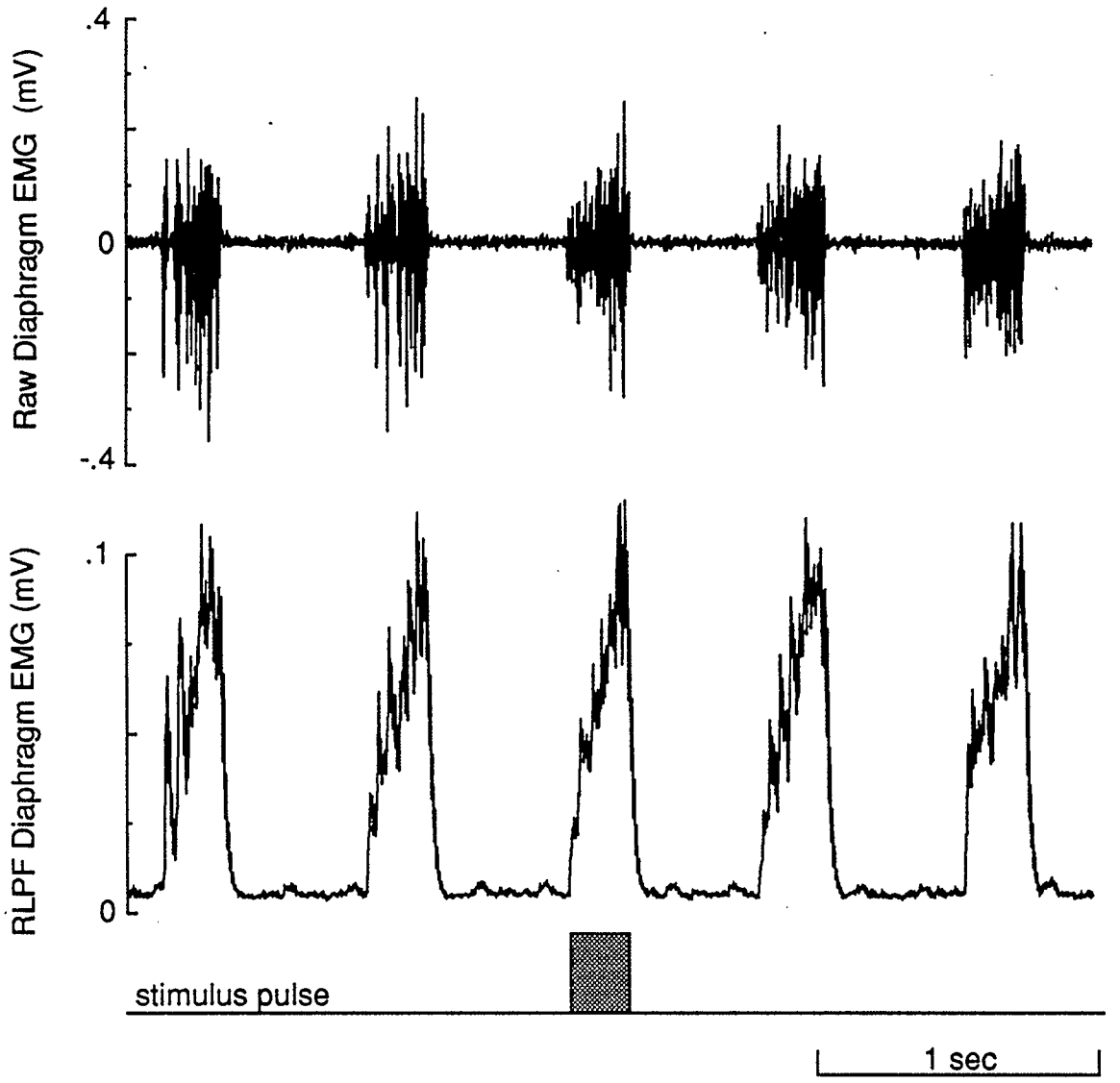

Figure 22. Effect on an inspiratory vagal stimulus at 1.0 times threshold in Lamb #014. The stimulus is denoted by the shaded pulse .Stimulation at this intensity did not appear to have any significant effect..

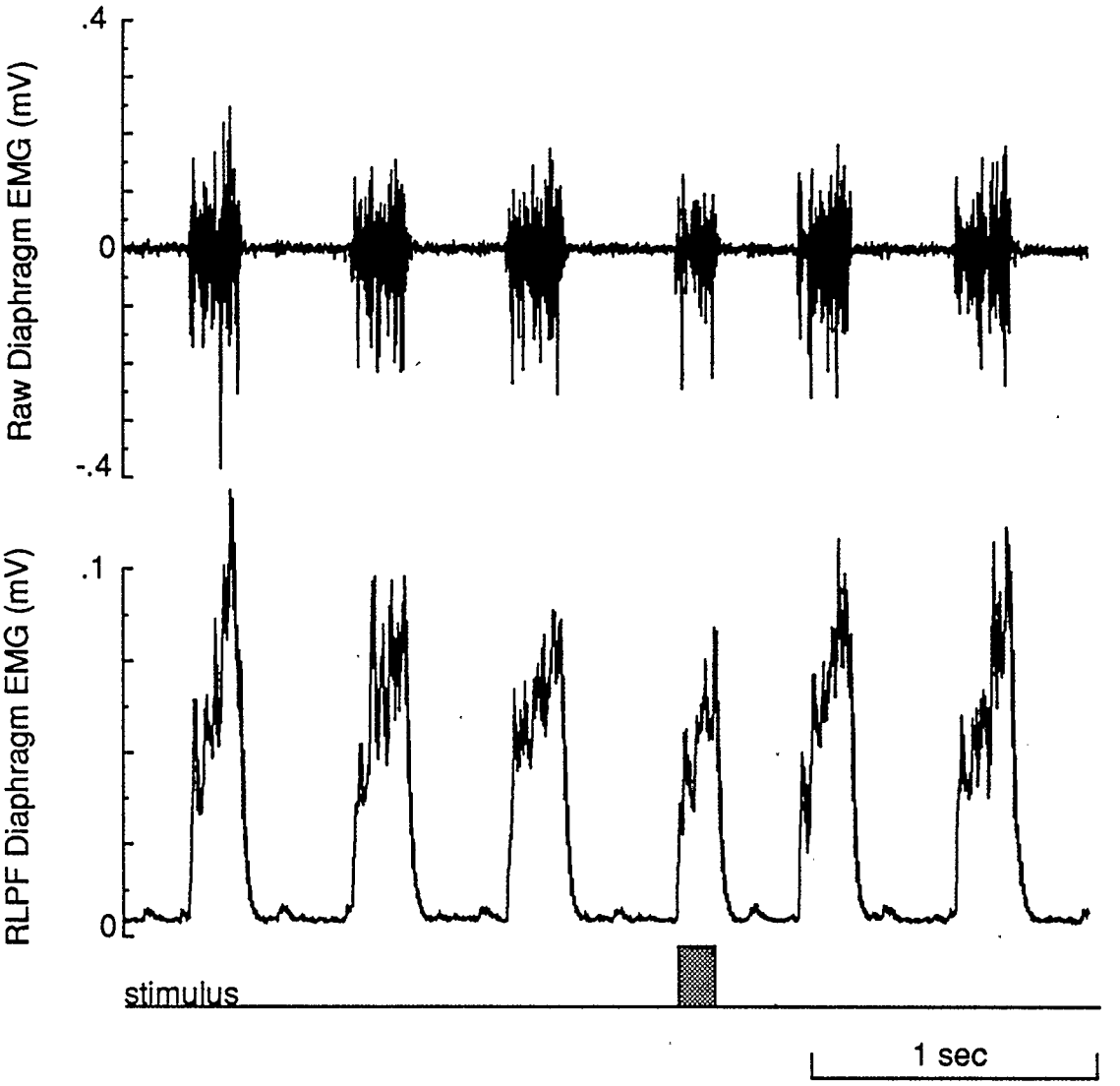

Figure 23. Effect of an inspiratory vagal stimulus at 2.25 times threshold in Lamb #014. The stimulus is denoted by the solid pulse. The stimulus shortened the duration of inspiration and slightly decreased the amplitude of the inspiratory burst. The expiratory phase after the stimulus was shortened compared to previous expiratory cycles.

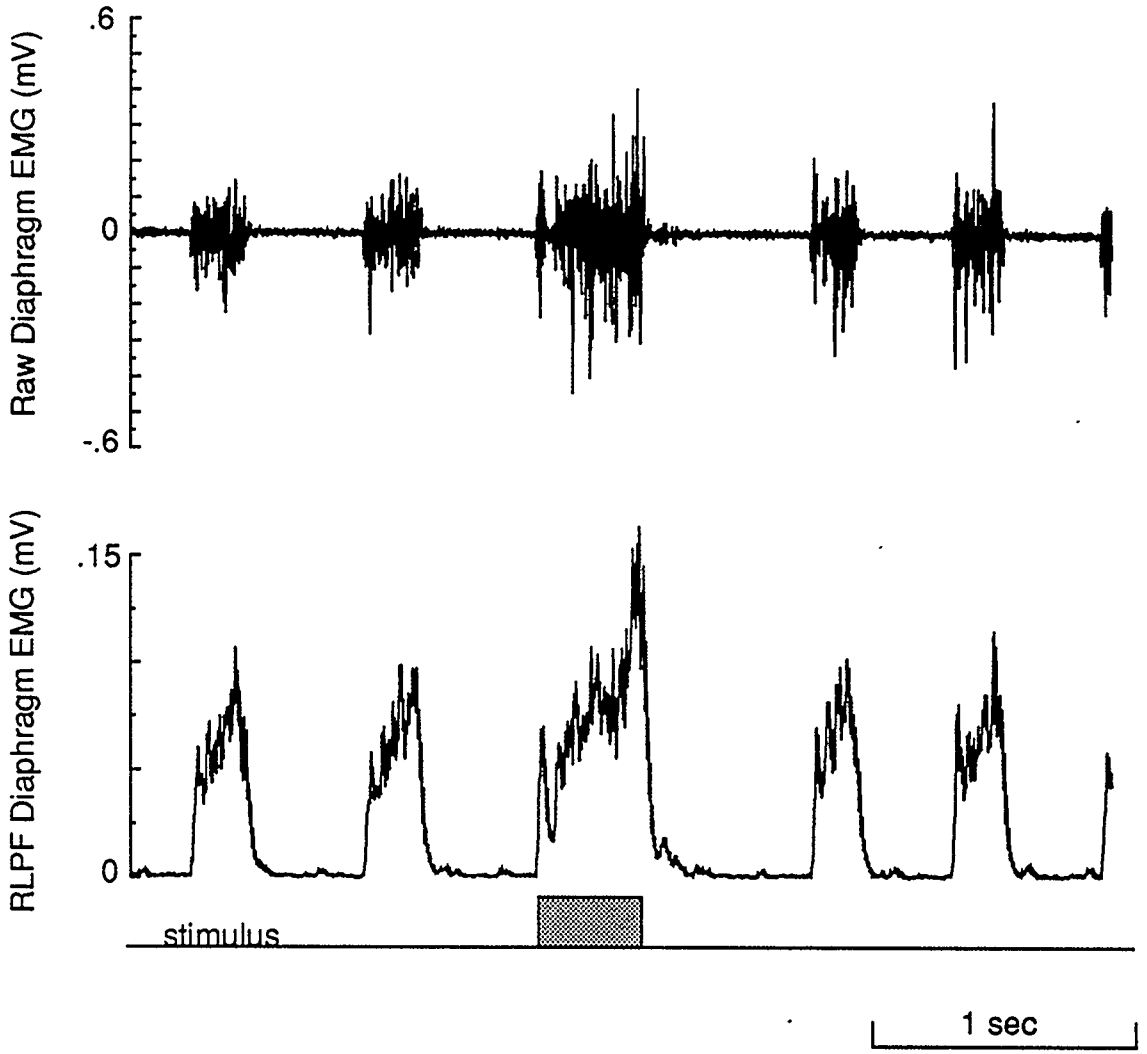

Figure 24. Effect of an inspiratory vagal stimulus at 4.5 times threshold in Lamb #014. Stimulation at this intensity increased the duration of inspiration and the amplitude of the inspiratory burst. Expiratory duration following the stimulus was longer than previous expiratory phases. The duration of the inspiratory phase after the stimulus was shorter than that of inspiratory phases before the stimulus.

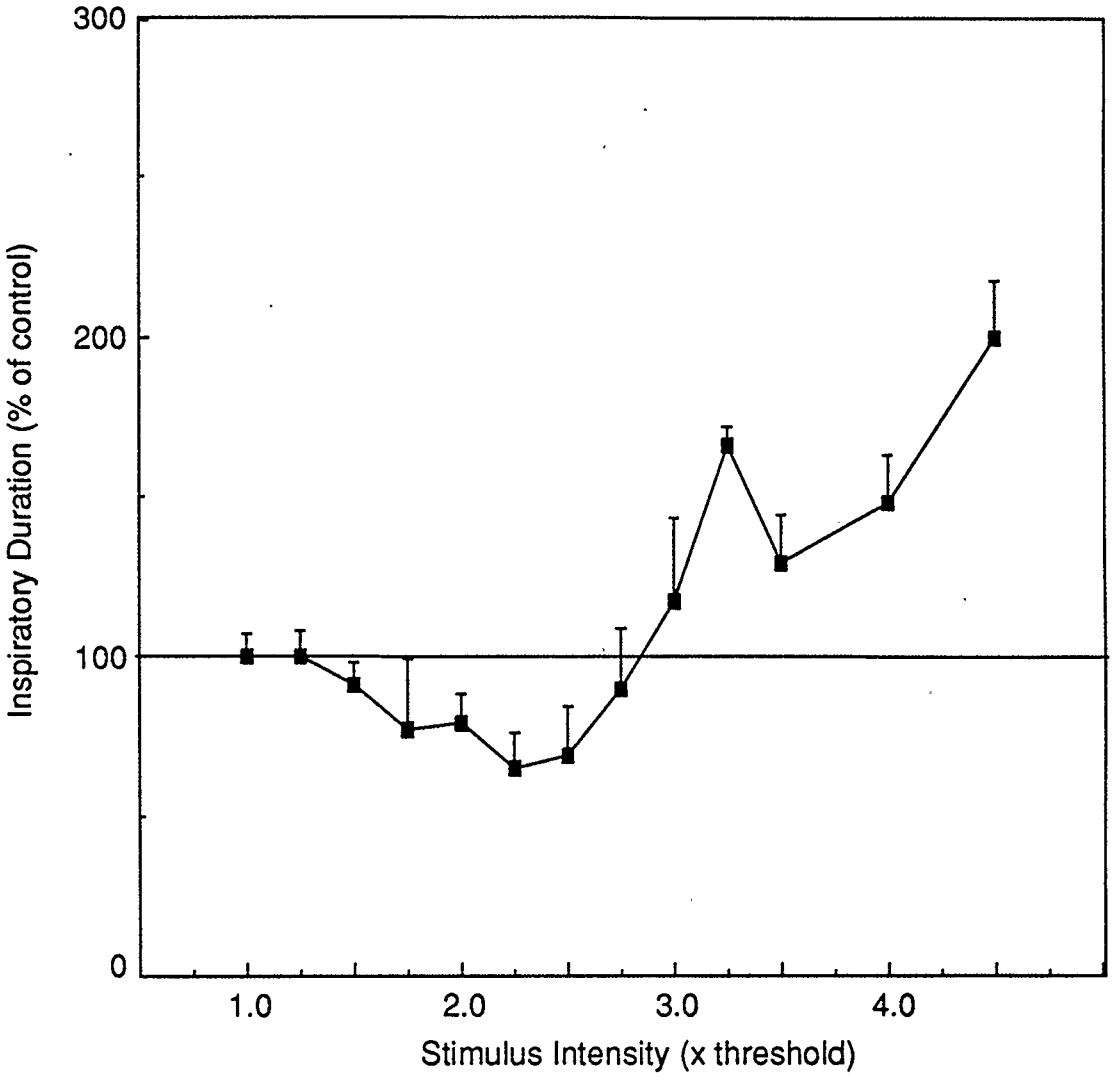

Figure 25. Effect of inspiratoryvagal stimulation on inspiratory duration (% control) in Lamb #014 at 10 days postnatal age. At low stimulus intensities, 1.0 - 1.25 times threshold, there was little effect on inspiratory duration. At 1.5 times threshold, inspiratory duration began to decrease, reaching a low near 2.25 -2.5 times threshold. At 2.75 times threshold inspiratory duration increased reaching a maximum at 4.5 times threshold. A horizontal line was drawn at 100% to emphasize deviation from control values.

mean inspiratory duration (± 1 S.D.) of a minimum of ten stimulus trials, expressed as a per cent of control values. At low stimulus intensities (1.0 - 1.25 x threshold) there was little or no effect on inspiratory duration. As the stimulus intensity increased, mean inspiratory duration gradually decreased, with maximal shortening occurring at 2.25 times threshold.

In Figure 25, inspiratory duration began to increase at 2.75 times threshold. At 2.75 times threshold, stimulation resulted in mean inspiratory duration that was greater than at the previous two intensities. Two of the sixteen stimuli at this intensity evoked augmented breaths. At 3.0 times threshold, stimulation prolonged most breaths but not all prolonged breaths showed an augmented pattern of discharge. At 3.25 times threshold, stimulation consistently prolonged inspiration and resulted in an augmented pattern of diaphragm EMG discharge. The highest inspiratory stimulus intensity (4.5 times threshold) resulted in the greatest observed inspiratory prolongation.

Figure 25 exemplifies the most common response observed. This response was designated as the Type A response. Similar data were obtained from ten lambs. An example from each of ten lambs is provided in Figure 26 where these data are overlayed with some offset so they could be displayed in the same plot. Each lamb showed a decrease in inspiratory duration as the stimulus intensity increased beyond threshold and an increase in inspiratory duration at the highest stimulus intensities used.

Figure 25 showed the effects of electrical stimulation on inspiratory duration as a function of stimulus intensity in Lamb #014. Figure 27 has superimposed on this plot, with a separate right y axis, the amplitudes of the

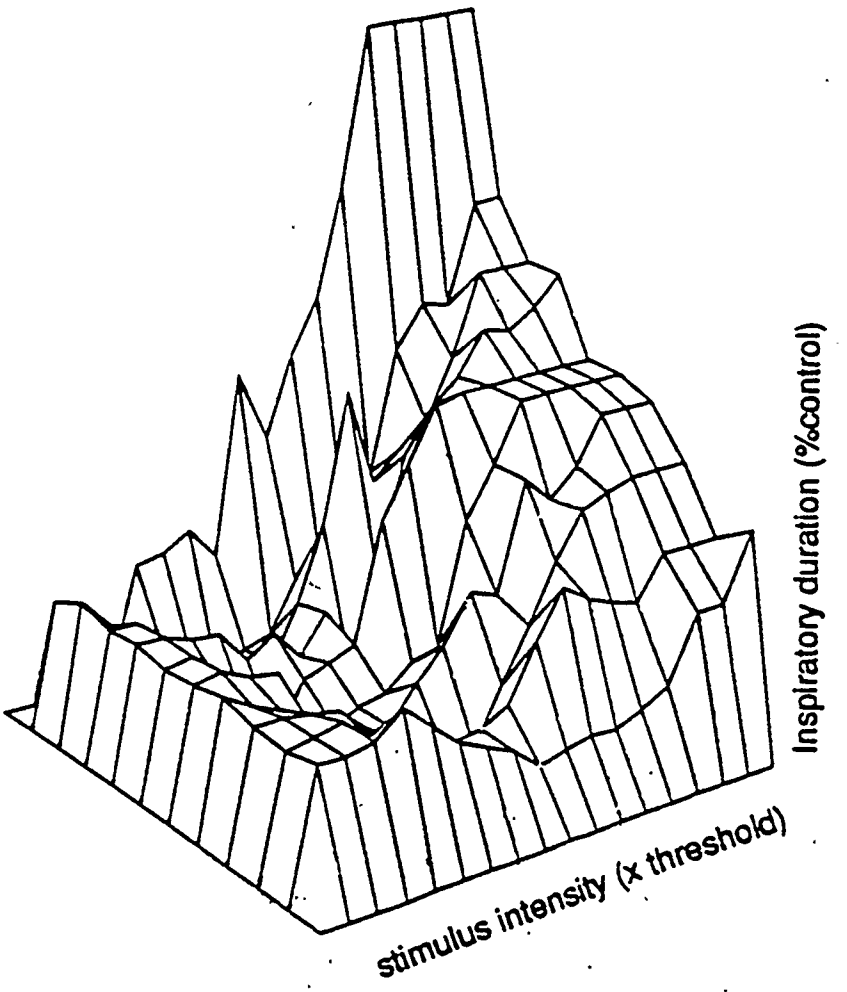

Figure 26. Inspiratory stimulus curves in ten lambs:population data. The inspiratory vagal stimulus decreased inspiratory duration at low intensities and increased inspiratory duration at higher intensities (Type A response). Each line represents the stimulus response curve from a single animal. The three dimensional perspective highlights the homogeneity of theType A response between individual animals.

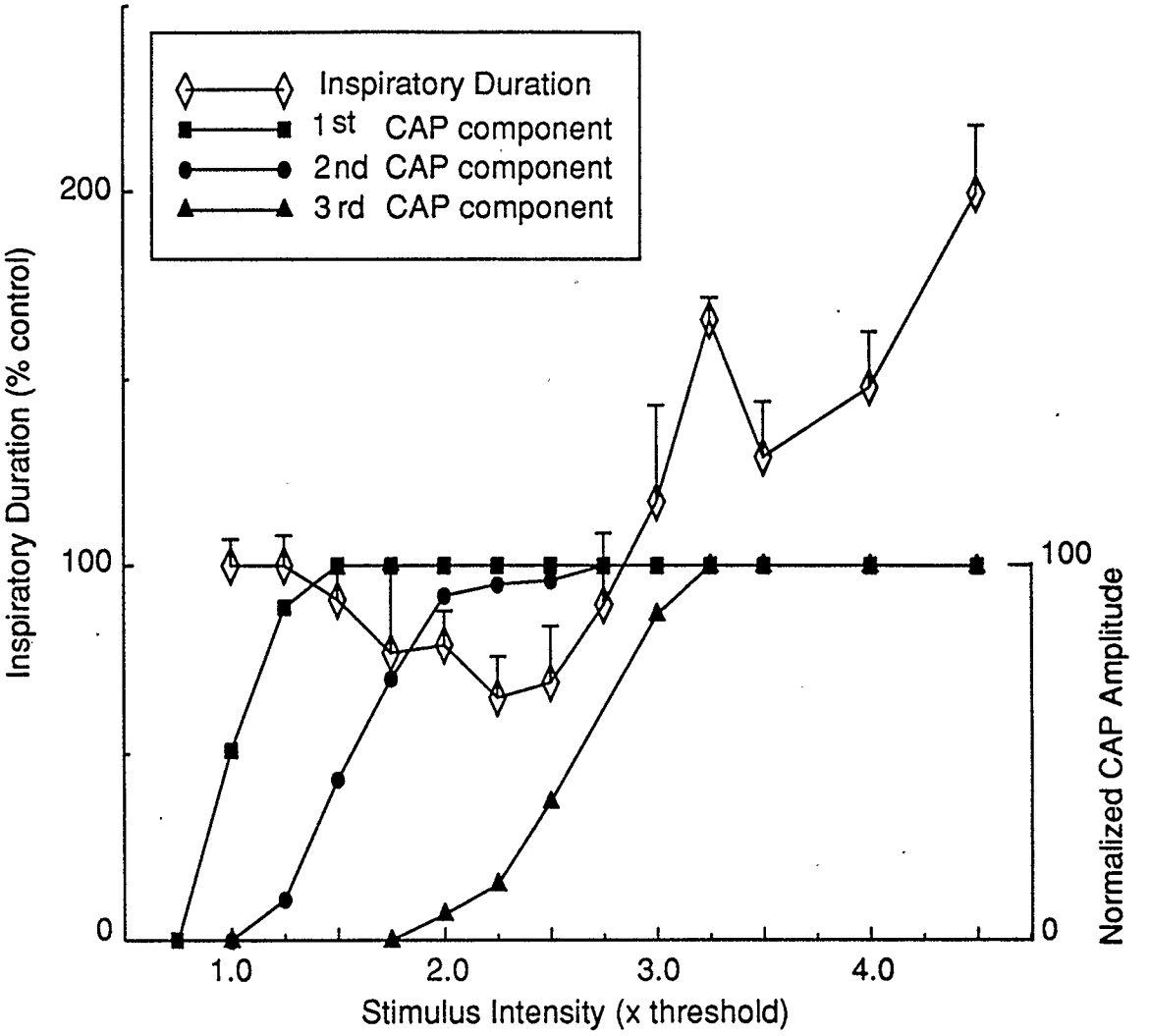

Figure 27. Type A response to vagal stimulation during inspiration. Relationship between inspiratory duration and the amplitude of CAP peaks. Inspiratory duration decreased when the amplitude of the second component of the CAP increased. Inspiratory duration was maximally shortened when the amplitude of the second component of the CAP reached a plateau. Inspiratory duration increased with the increase in amplitude of the third component of the CAP. Maximum inspiratory duration occurred when the amplitude of the third component of the CAP reached a plateau.

negative peaks of the compound action potential from Figure 21. Stimulation at intensities solely corresponding to the first component of the CAP had no apparent effect on the duration of inspiration. The emergence of the second component of the CAP was correlated with a decrease in inspiratory duration. As stimulation intensity increased and the amplitude of the second component of the CAP action potential increased, inspiratory duration decreased further. Beyond a threshold intensity the magnitude of inspiratory shortening appeared to be related to the number of fibres recruited in the second component of the CAP. The maximal decrease in inspiratory duration in Lamb #014 occurred at Level II, the intensity where the amplitude of the second component of the compound action potential had reached a plateau. In lambs with the type A response, the greatest decrease in inspiratory duration always occurred within .25 times threshold of the stimulus intensity designated Level II. The magnitude of inspiratory augmentation corresponding to stimulus intensities including the third component of the CAP was less closely related to intensity and may have reflected a triggered as opposed to a graded response.

Four of the ten lambs did not consistently show the Type A response on different experimental days. In these lambs, during some experiments, vagal stimulation did not decrease inspiratory duration. The open symbols of Figure 28 represent mean inspiratory duration as a function of stimulus intensity in one of these four lambs. Increases in stimulus intensity above threshold did not decrease inspiratory duration and the highest stimulus intensity, 4.5 times threshold, still produced augmented breaths. This response to an inspiratory stimulus, which lacked the decrease in inspiratory duration shown in

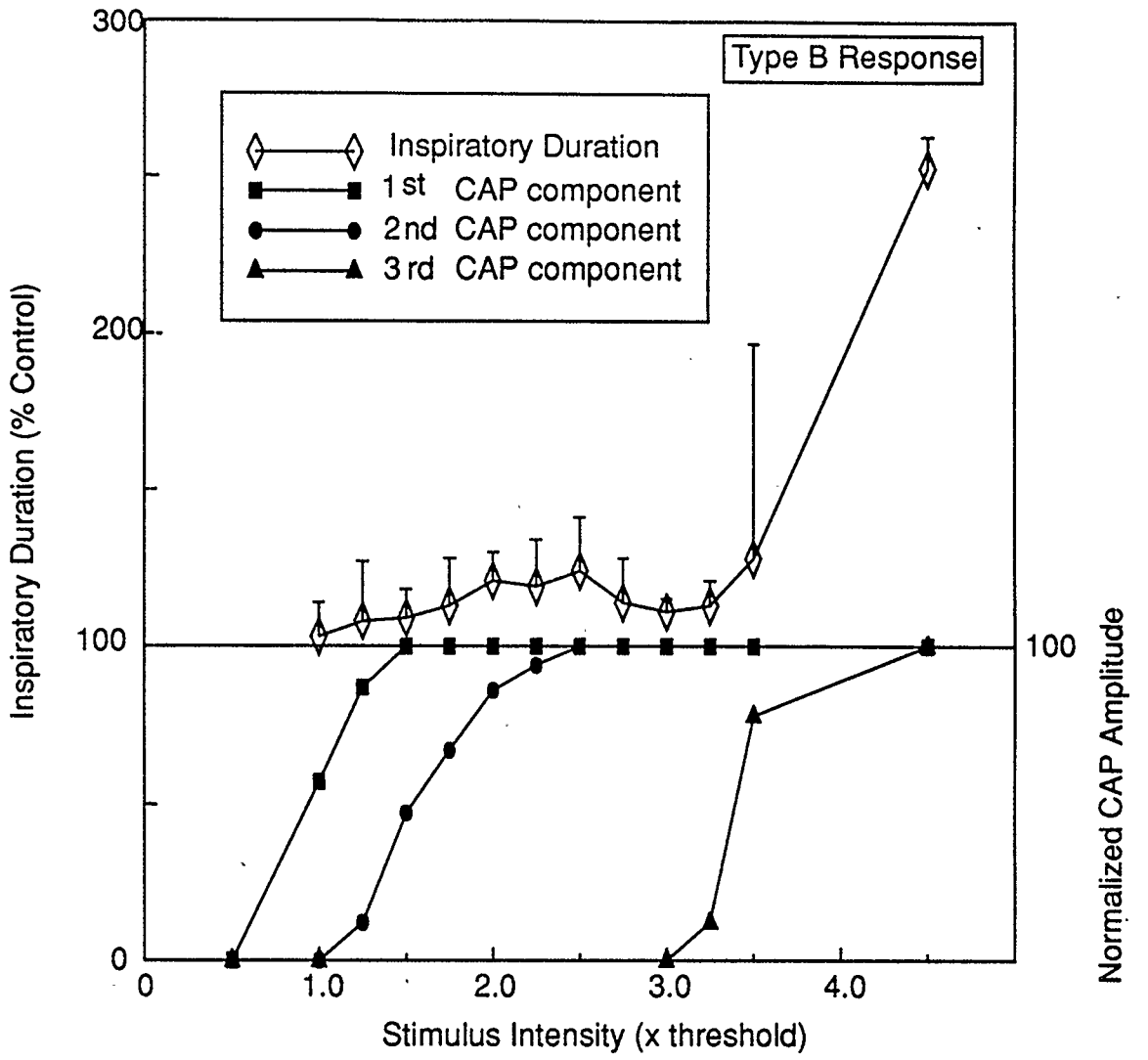

Figure 28. Type B response to vagal stimulation during inspiration. Effect of vagal stimulation during inspiration on inspiratory duration (% control) as a function of stimulus intensity in Lamb #013 (5 days old) and relationship between inspiratory duration and amplitude of CAP components. Although similar intensities of stimulation occurred, inspiratory duration was not decreased at Level II stimulation or any other intensity. Augmentation of inspiration still occurred with Level III stimuli.

Figure 27, was termed the Type B response. The four lambs with the type B response did not show decreased inspiratory duration at Level II or any other intensity of stimulation.

The solid symbols in Figure 28 represent the corresponding CAP amplitudes as a function of stimulus intensity. The CAP plot shows that although equivalent stimulus intensities were administered and no decreases in inspiratory duration occurred, there were clear Level II stimulus effects (2.5 times threshold). An example of raw data from inspiratory stimulation at 2.5 times threshold is shown in Figure 29. Vagal stimulation transiently inhibited the diaphragm EMG early in the burst and inspiratory duration was longer than previous, unstimulated breaths.

Slope of Inspiratory EMG Burst

The slope of the RLPF inspiratory diaphragm EMG during Level II vagal stimulation was compared to the inspiratory slope of unstimulated breaths for both Type A and Type B responses. Figure 30 shows the averaged response data from Lamb #014 at Level II (2.25 times threshold), the intensity which decreased inspiratory duration the most (Type A response). The same data were averaged with respect to two different trigger points indicated by the arrows, at peak inspiration (top panel) and at the beginning of inspiration (bottom panel). Triggering the average at the peak of inspiration provided better resolution of events late in inspiration. Triggering the average at the beginning of inspiration provided better resolution of events early in inspiration.

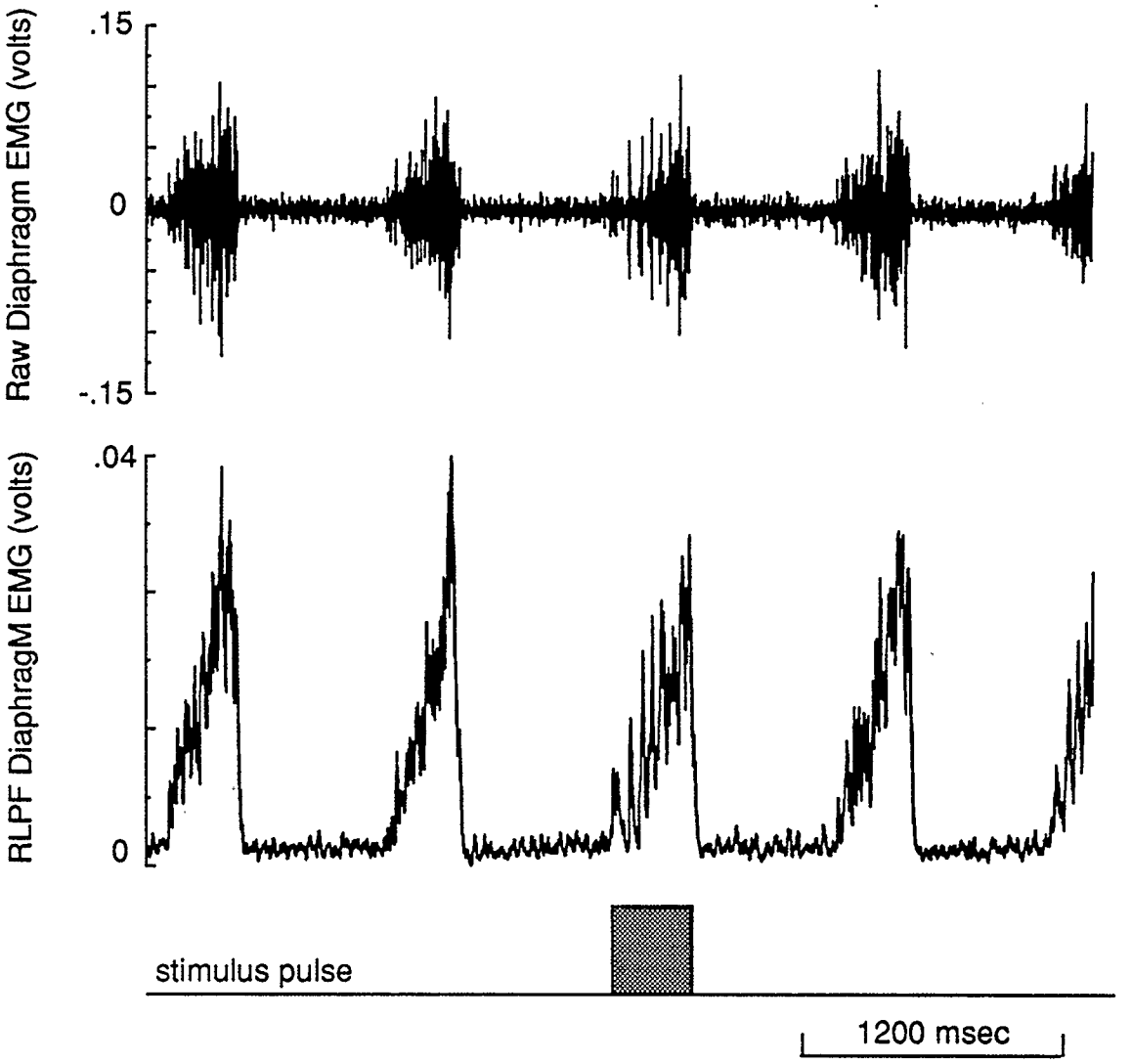

Figure 29. Level II stimulation:Type B response. The effect of an inspiratory Level II stimulus in Lamb #013 at 5 days postnatal age is shown.Transient inhibition occurred early in the EMG burst but net inspiratory duration was longer than control. Expiratory duration after the stimulus was shorter compared to control

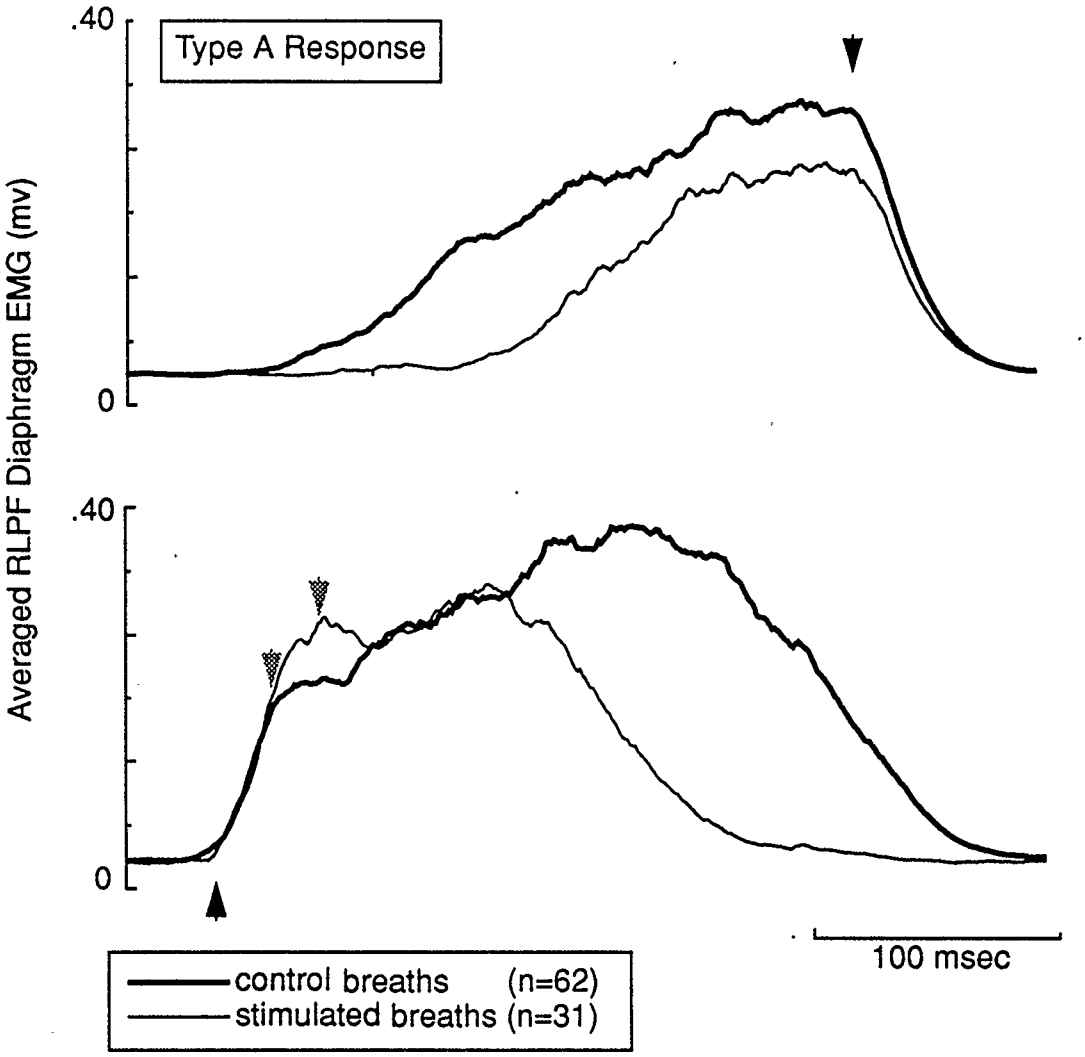

Figure 30. Level II stimulation: averaged Type A response. Averages of the same data were triggered two ways, at the peak of inspiration (top panel, solid arrow) and at the beginning of inspiration (bottom panel, solid arrow) At this intensity, where inspiratory shortening was the greatest, the slope of the inspiratory ramp of averaged stimulated breaths was different than the slope of inspiration of averaged control breaths. Linear regression of the points between the shaded arrows in the lower panel showed that the slope of the stimulated breaths was 80% greater than the slope of the control breaths. Data are from Lamb #014 on postnatal day 10 at 2.25 x threshold.

in each case the bold trace is the cycle triggered average of the control breaths (n=62) and the lighter trace is the average of the stimulated breaths (n=31). The top panel indicates that the slope of the averaged stimulated breaths was steeper than the slope of the averaged control breaths. The bottom panel shows that the slope of the averaged stimulated breaths was the same as the averaged control breaths early in inspiration, increased above control values and then returned to control values. The slope of the averaged stimulated breaths was compared with the slope of the averaged control breaths using linear regression. Linear regression of the points between the shaded arrows showed that the slope of that portion of the averaged stimulated breaths was 80% greater than the slope of the averaged control breaths. The standard error of the regression coefficient (slope) was .46 for stimulated breaths and .21 for the control breaths.

Level II inspiratory stimulus data were also averaged in lambs that did not show decreased inspiratory duration in response to vagal stimulation (Type B response). Figure 31 shows the averaged data from Lamb #013 at 2.5 times threshold. Again, the data were averaged in two ways, at peak inspiration and at the beginning of inspiration. In each case, the bold trace is the cycle triggered average of the unstimulated or control breaths (n=54) and the lighter trace is the cycle triggered average of the stimulated breaths (n=27). The solid arrow indicates at which point the averages were triggered.

In the upper panel of Figure 31 the slope of the averaged stimulated breaths appeared less than the slope of the control breaths. The lower trace

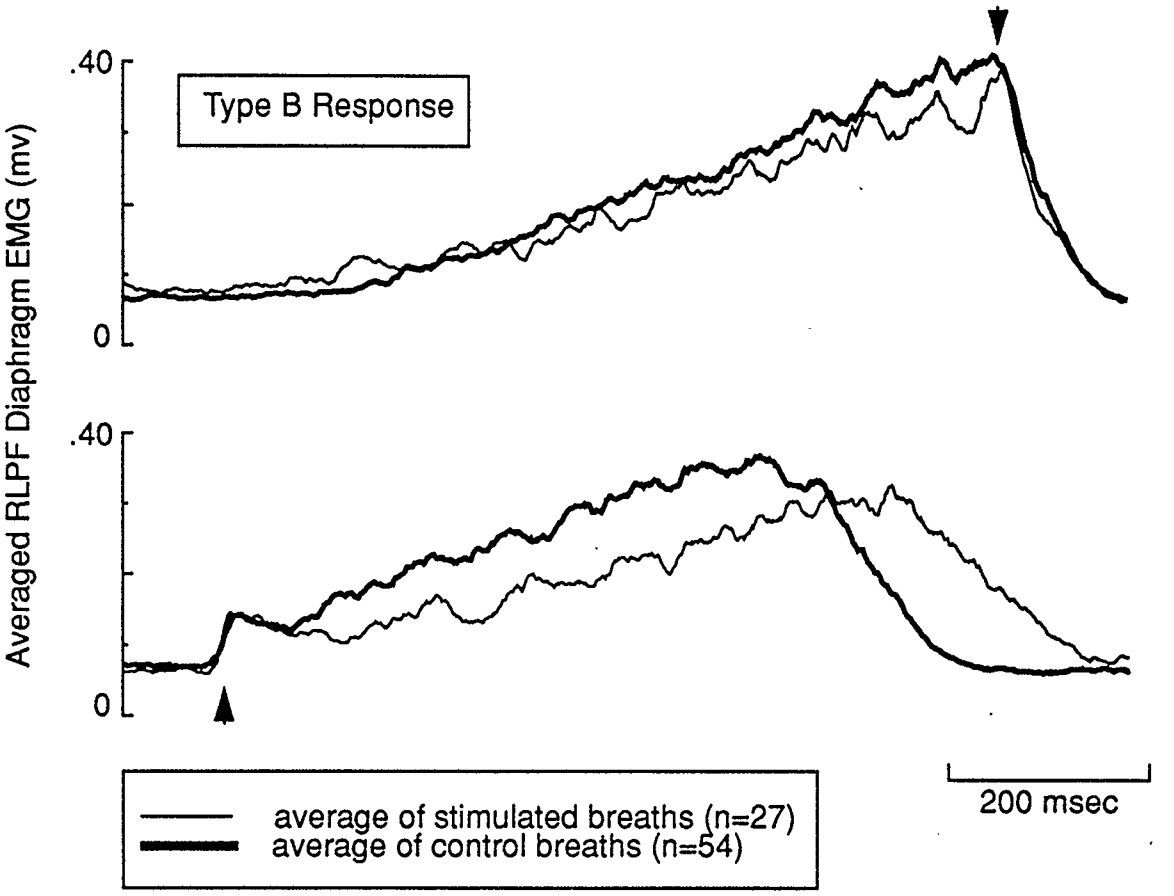

Figure 31. Level II stimulation: averaged Typed B response. The arrows indicate where the average was triggered with respect to time.In the top panel data were averaged with respect to peak inspiratory activity of the diaphragm EMG. In the bottom trace data were averaged with respect to the onset of the diaphragm EMG. The slope of the stimulated breaths was 26% less than the slope of the control breaths.Data are from Lamb #013 on day 5.

shows that both stimulated and control breaths started out the same, but that the slope of the stimulated breaths was 26% less than the slope of the control breaths. A qualitatively similar relationship between control and stimulated breaths was characteristic of all lambs who showed the Type B response.

Data from Level III stimulation are presented in Figure 32 and show that the resulting breath, though it also involved a prolongation of inspiratory duration was considerably different from the inspiratory prolongation of the Type B response, in that it included an augmented component (shaded arrow).

Amplitude of the Inspiratory EMG Burst

The amplitude of the RLPF inspiratory EMG as a function of stimulus intensity had the same shape as the Type A inspiratory duration versus intensity plot. This was true of all lambs with the Type A response (n=10) and the four lambs who showed the Type B response. Figure 33, where amplitude decreased by 24% is a good example; in other lambs the decrease was not as pronounced. At the higher intensities of stimulation, which resulted in increased inspiratory duration, all lambs showed an increase in inspiratory amplitude.

Post-Stimulus Effects of Vagal Stimulation During Inspiration

Qualitatively similar post-stimulus effects were observed in lambs with both Type A and Type B responses. The examples of post-stimulus effects

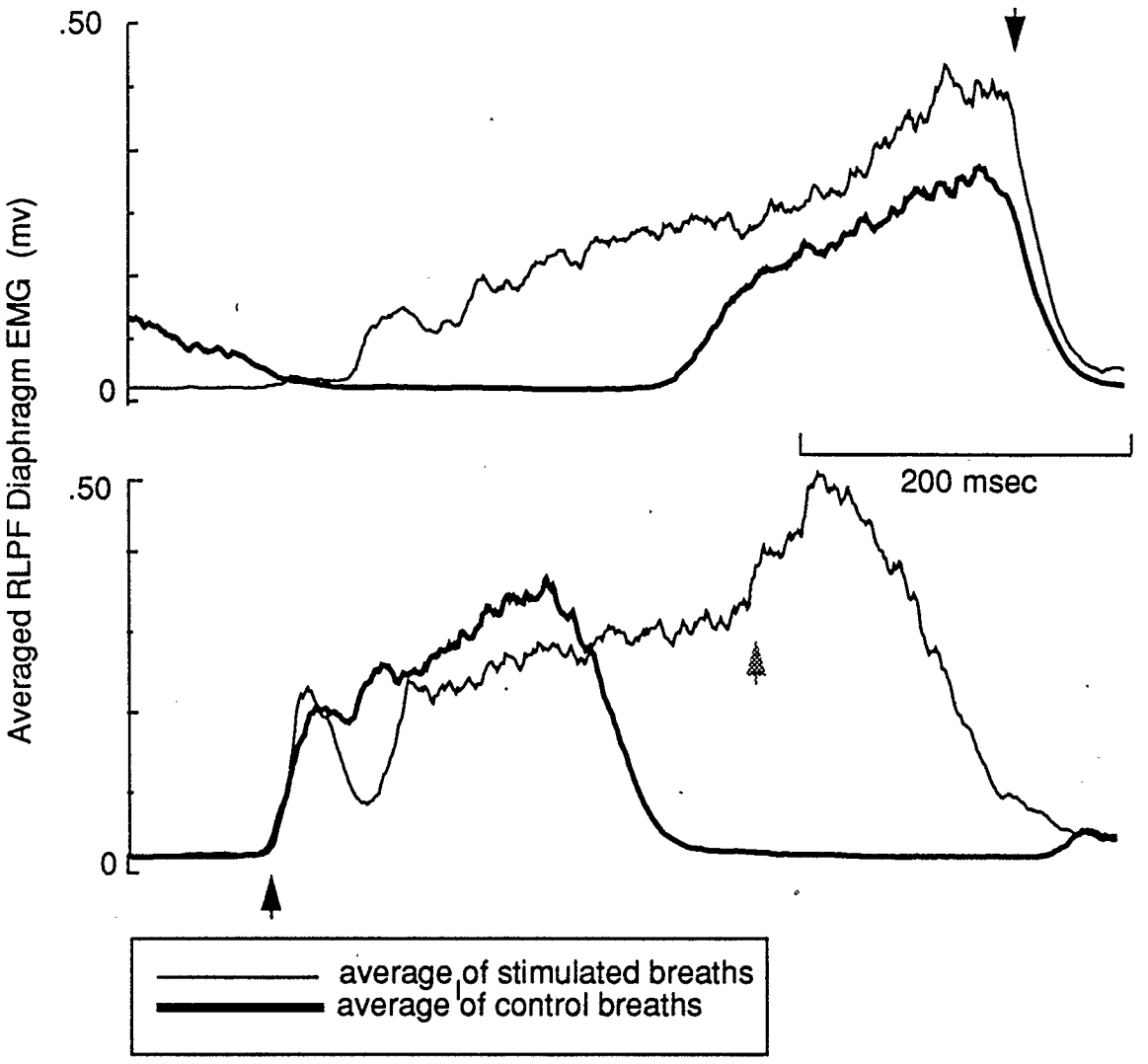

Figure 32. Level III stimulation: averaged response.The Level III inspiratory response was different than the Level II inspiratory response. Although inspiratory duration during Level III stimulation was also longer than control, the shape of the RLPF diaphragm EMG was different than the shape of EMG during a Level II inspiratory stimulus with a Type B response. The level III response included a late increase in the slope and amplitude of the EMG (shaded arrow) Data are from Lamb #013.

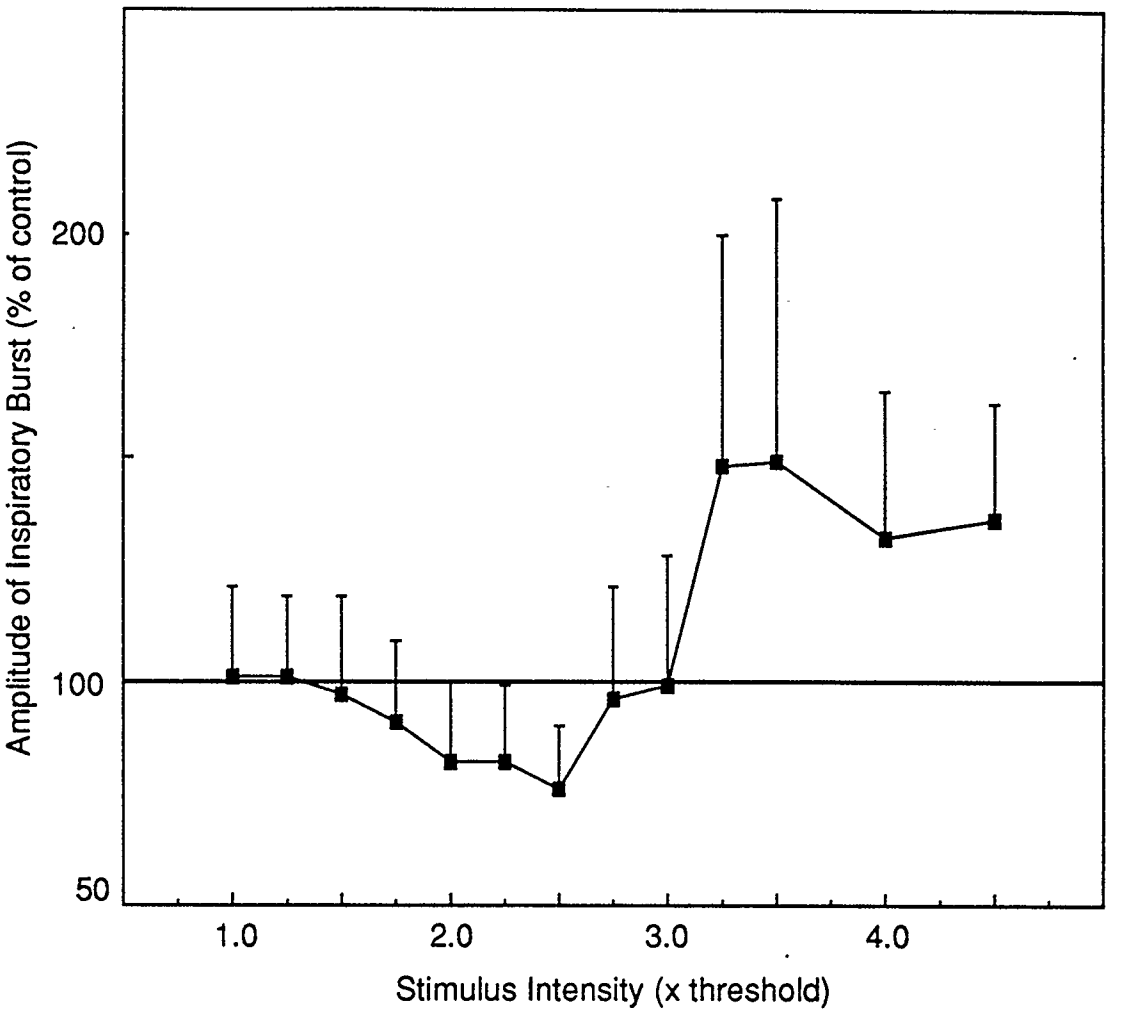

Figure 33. The effect of vagal stimulation during inspiration on the amplitude of the inspiratory burst. Amplitude decreased at intensities between1.5 and 2.5 times threshold. After 3.0 times threshold, amplitude was increased compared to control breaths. Data are from Lamb #014, postnatal day 10.

presented in this section are from Lamb #014 ,the same animal used to illustrate the within phase effects of inspiratory stimulation (Type A response) above.

The raw data of Figure 23 showed that after a vagal stimulus that decreased inspiratory duration the first expiratory phase was also decreased compared to previous expiratory phases. In Figure 24, the expiratory phase following the inspiratory prolonging stimulus was longer than previous expiratory phases.

Figure 34 provides mean values for the duration of the first expiratory phase after an inspiratory stimulus as a function of stimulus intensity in one lamb. The effect of inspiratory stimulation in the first post-stimulus phase had the same shape relative to stimulus intensity as the within phases effect of the inspiratory stimulus (cf Figure 25) Expiratory duration following an inspiratory stimulus was most reduced at 2.5 times threshold, within .25 times threshold of the Level II intensity. Following inspiratory stimuli at intensities greater than 2.5 times threshold, post-stimulus expiratory duration increased with maximal duration occurring between 3.25 and 4.5 times threshold, intensities corresponding to Level III.

The close relationship between the duration of the stimulated inspiratory phase and the first expiratory phase after the stimulus is illustrated by Figure 35. Stimulated inspiratory duration and the duration of the first expiratory phase after the stimulus are plotted for all stimulus trials at all intensities. Linear regression of these data points gave a correlation coefficient of .79. The slope (regression coefficient) was 1.85 $\pm$.08 SE.

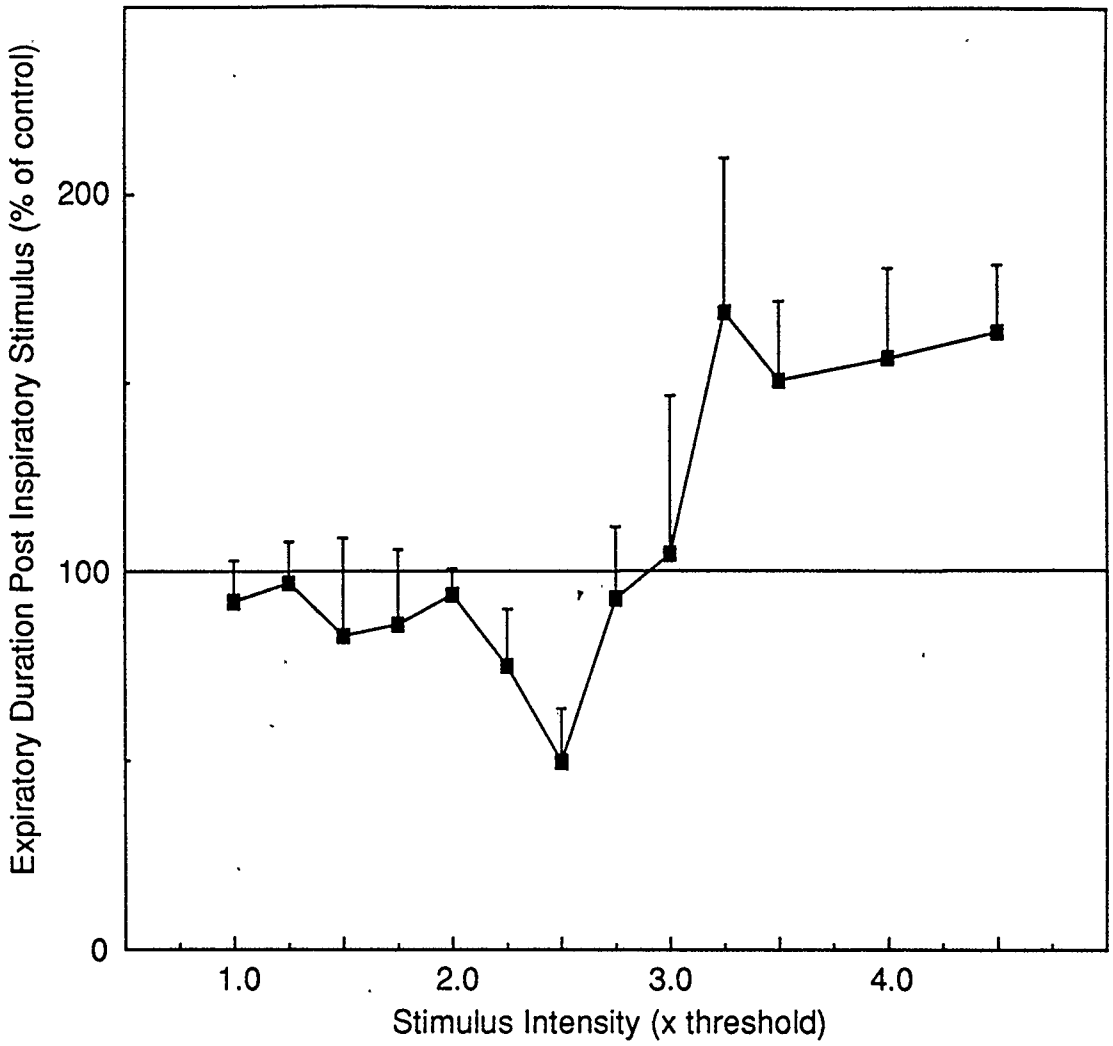

Figure 34. Effect of inspiratory stimulation on post-stimulus expiratory duration. Expiratory duration following an inspiratory stimulus was minimal at 2.5 times threshold. At higher intensities of stimulation, expiratory duration increased. Data are from Lamb #014 on postnatal day 10.

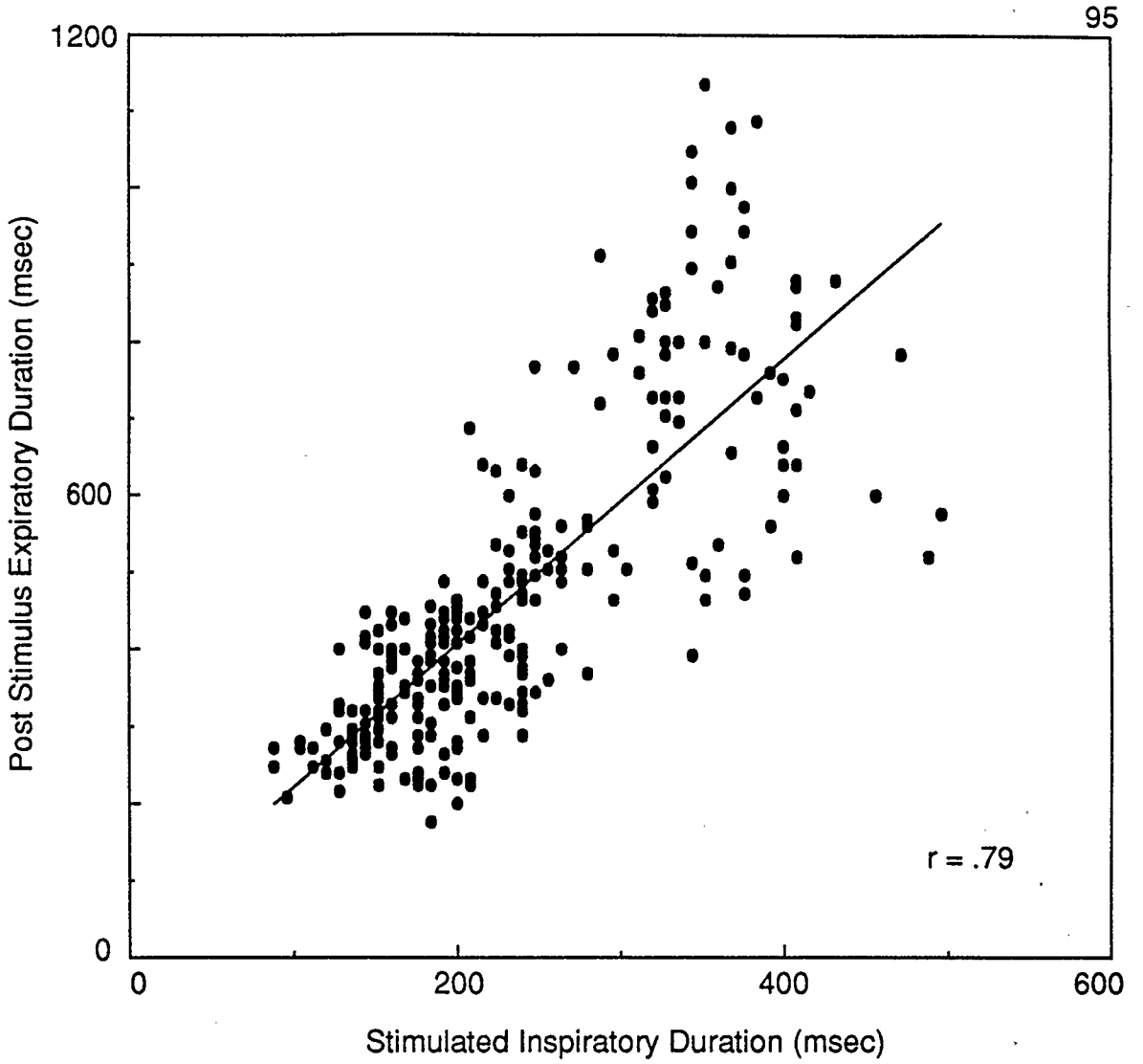

Figure 35. Correlation betwen inspiratory duration and post -stimulus expiratory duration. Expiratory duration after an inspiratory stimulus was well correlated (r=.79) with the duration of the stimulated inspiratory phase. Each filled circle represents 1 stimulus trial at a particular stimulus intensity. All stimulus intensities are shown. Data are from Lamb #014 on postnatal day 10. The slope of the regression line was 1.85 (.085 SE).

Subsequent post-stimulus phase effects were less clear. Data from Lamb #014 in Figure 36 show that the mean duration of the first inspiratory phase following an inspiratory stimulus varied as a function of stimulus intensity. The raw data of Figure 24 (4.5 times threshold) show that the first inspiratory phase following the inspiratory prolonging stimulus was shorter than inspiratory phases before the stimulus. However as seen in Figure 36 mean inspiratory duration post-stimulus, as a function of stimulus intensity, was quite variable in Lamb #014. A clear relationship between mean inspiratory duration post-stimulus and stimulus intensity was seen in only three lambs (#010, #011 and #015). In these lambs mean duration of the first inspiratory phase following an inspiratory stimulus was clearly decreased at high stimulus intensities (Figure 37). More subtle differences may have occurred with inspiratory stimuli at lower intensities. In lambs #011 and #015, mean post-stimulus inspiratory duration may have been slightly increased after 2.0 times threshold.

Within-Phase Effects of Vagal Stimulation During Expiration

Vagal stimulation during expiration had different effects on expiratory duration depending on the intensity of stimulation used.

At low stimulus intensities there was no effect on expiratory duration. An example of raw data recorded during stimulation at 1.0 times threshold is shown in Figure 38.

Raw data from one stimulus trial at 2.25 times threshold are shown in

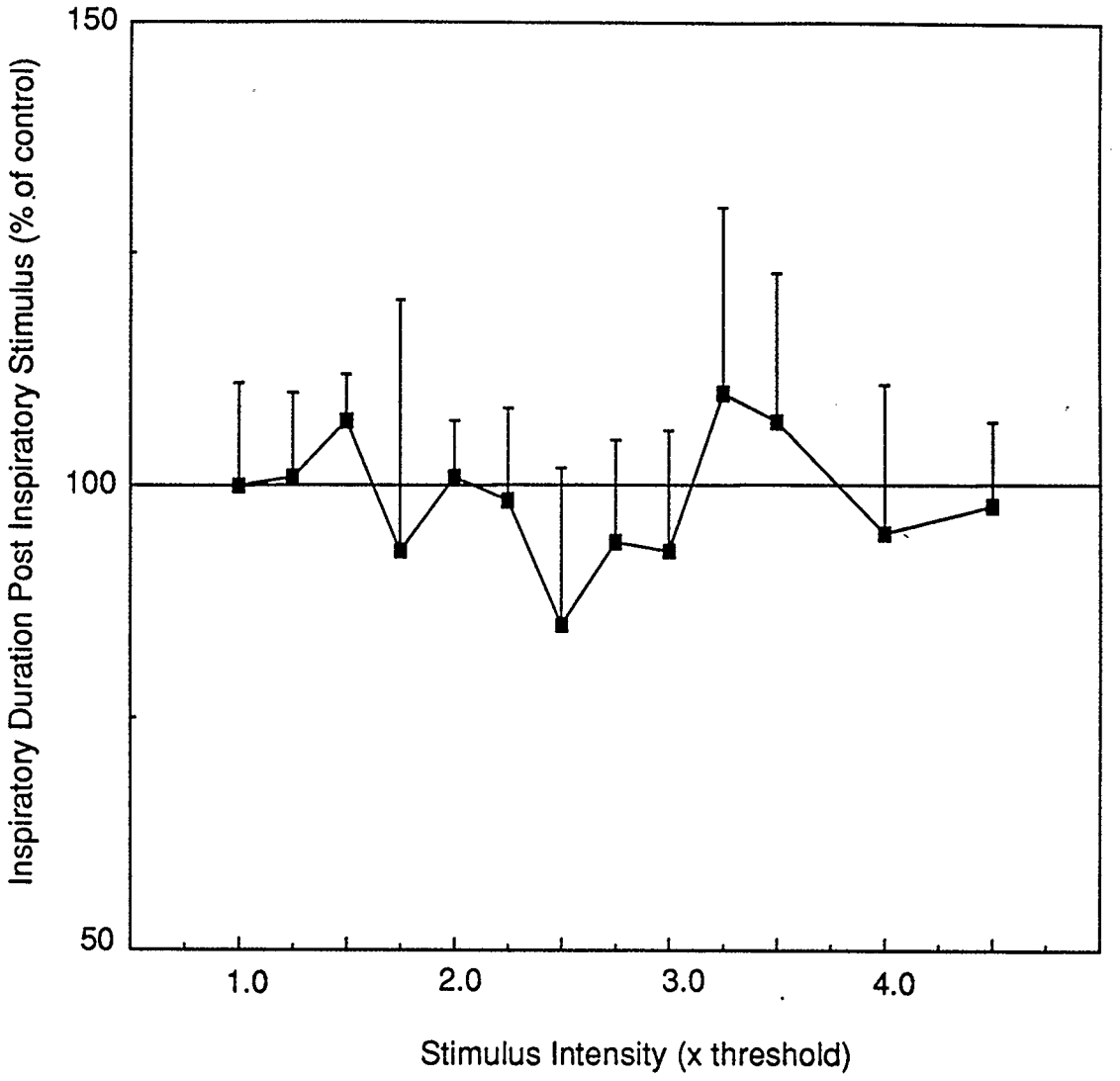

Figure 36. Effect of inspiratory stimulation on post-stimulus inspiratory duration. Inspiratory duration of the breath following an inspiratory stimulus was variable and did not appear to be related to the intensity of stimulation. Data are from Lamb #014, postnatal day 10.

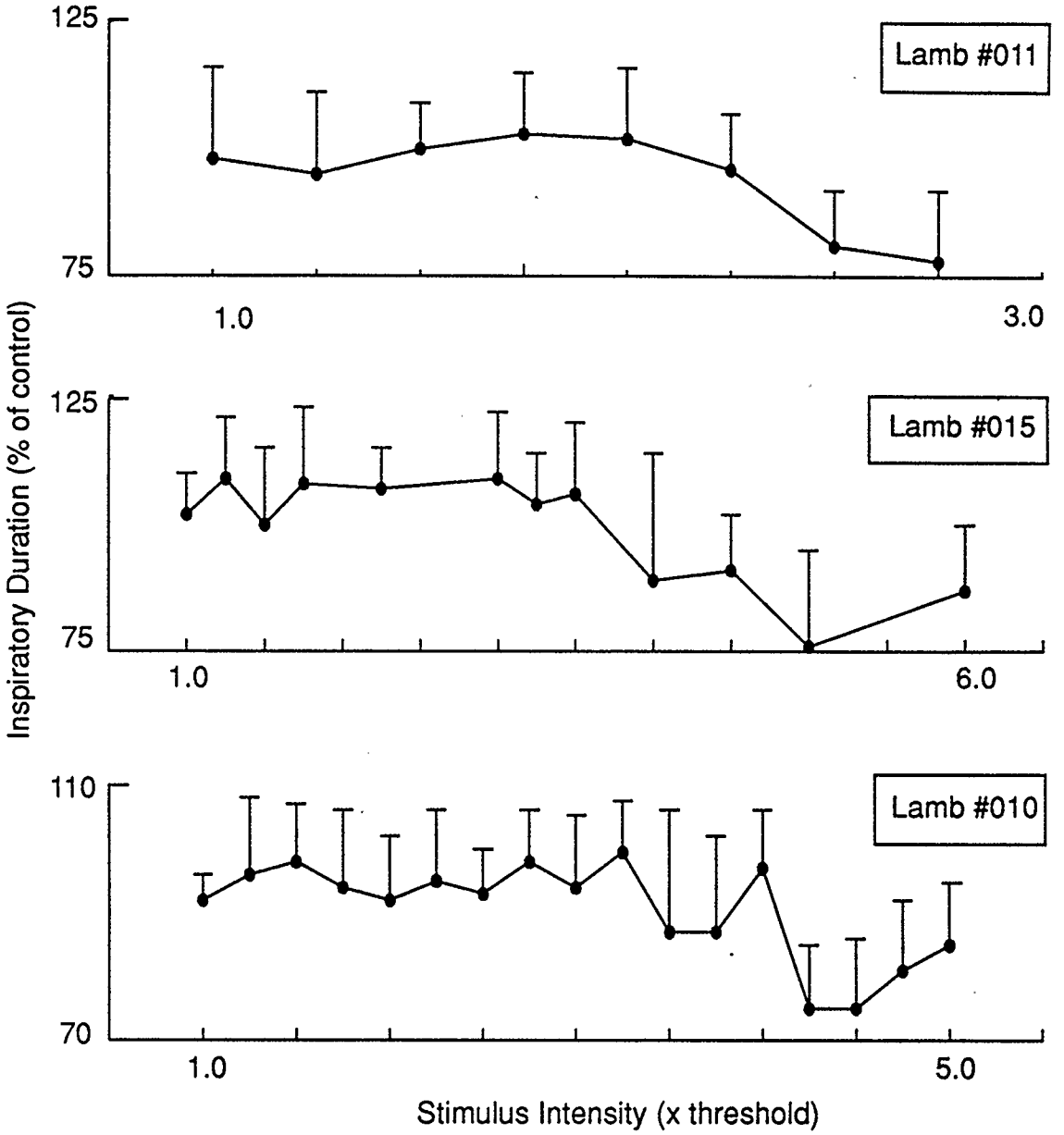

Figure 37. Post-stimulus inspiratory duration in three lambs.Mean duration of the first inspiratory phase following an inspiratory vagal stimulus was plotted as a function of stimulus intensity. In the top two traces there was a slight increase and then a more pronounced decrease in duration as stimulus intensity increased. In the bottom trace only the decrease in duration at the higher intensities of stimulation is apparent. In the other lambs inspiratory duration was highly variable.

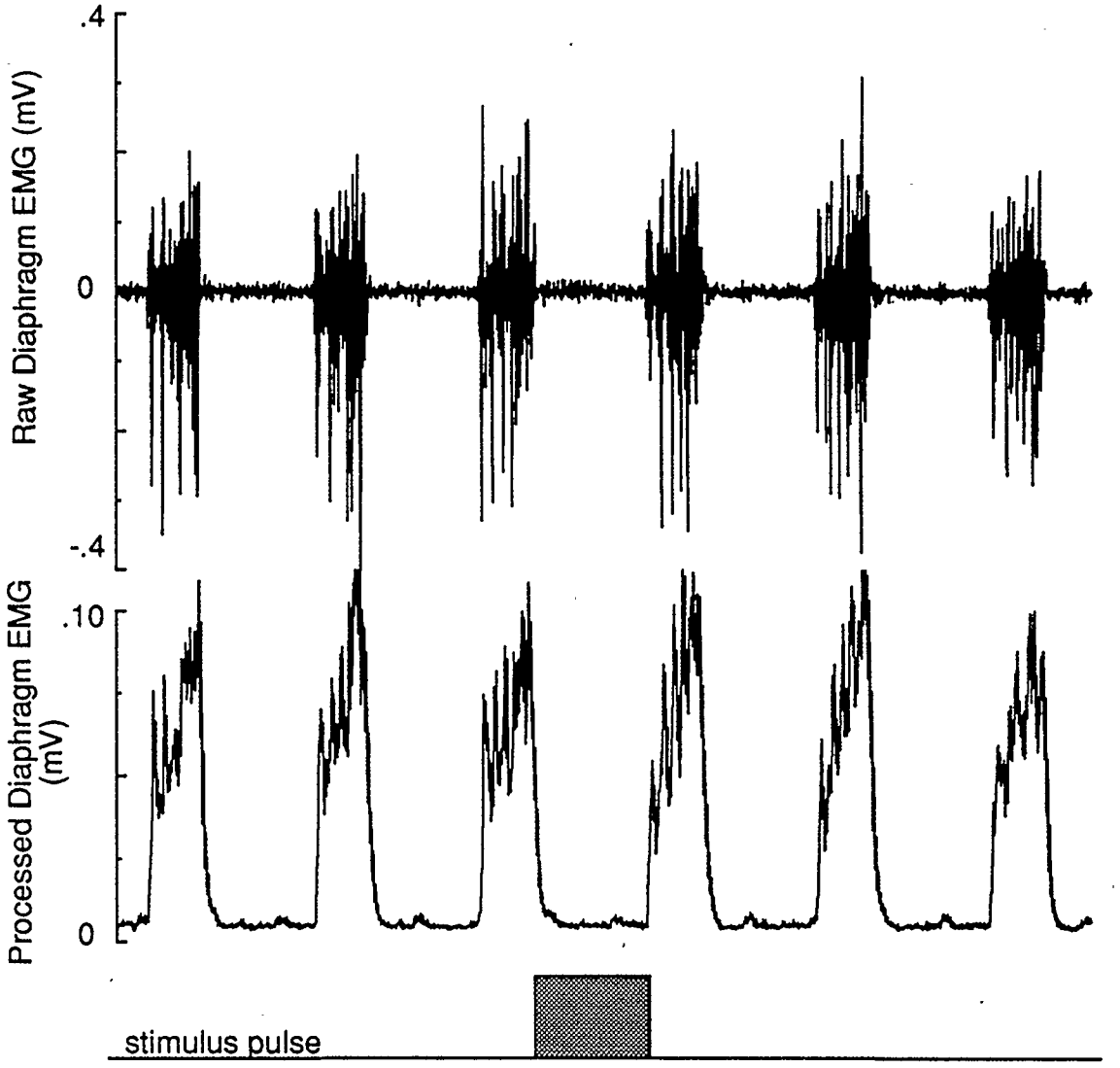

Figure 38. Effect of an expiratory vagal stimulus at 1.0 threshold in Lamb #014. Stimulus at this intensity had little effect.

Figure 39. Expiratory duration was prolonged during the stimulus. Inspiratory duration following the stimulus was longer than previous inspiratory cycles and the next expiratory phase was shortened compared to unstimulated expiratory phases. This response was typical of all lambs, regardless of whether they showed a Type A or Type B inspiratory response.

Raw data from one stimulus trial at 4.5 times threshold are illustrated in Figure 40. In Figure 40, vagal stimulation shortened the duration of expiration. There was also increased EMG during the stimulated phase and in the next expiratory phase.

The effect of vagal stimulation on expiratory duration was dependent on the intensity of stimulation (Figure 41). At low stimulus intensities (1.0-1.25 times threshold) expiratory duration was relatively unaffected by vagal stimulation. Stimulation at higher intensities (1.5-3.0 times threshold) revealed a gradual increase in duration of expiration, with maximum prolongation occurring at 3.0 times threshold. Expiratory prolongation reached a plateau between 2.25 and 2.75 times threshold although the maximum observed value occurred, with large variability, at 3.0 times threshold. Figure 41 shows that mean expiratory duration decreased sharply at 3.25 times threshold and fell below control levels. Mean expiratory duration between 3.25 and 4.5 times threshold showed little variability.

The effects of vagal stimulation on expiratory duration were consistent in all lambs on all experimental days. The superimposed traces of Figure 42 represent expiratory stimulation data from each of ten lambs. Level II stimulation influenced expiratory duration to a greater extent than it influenced

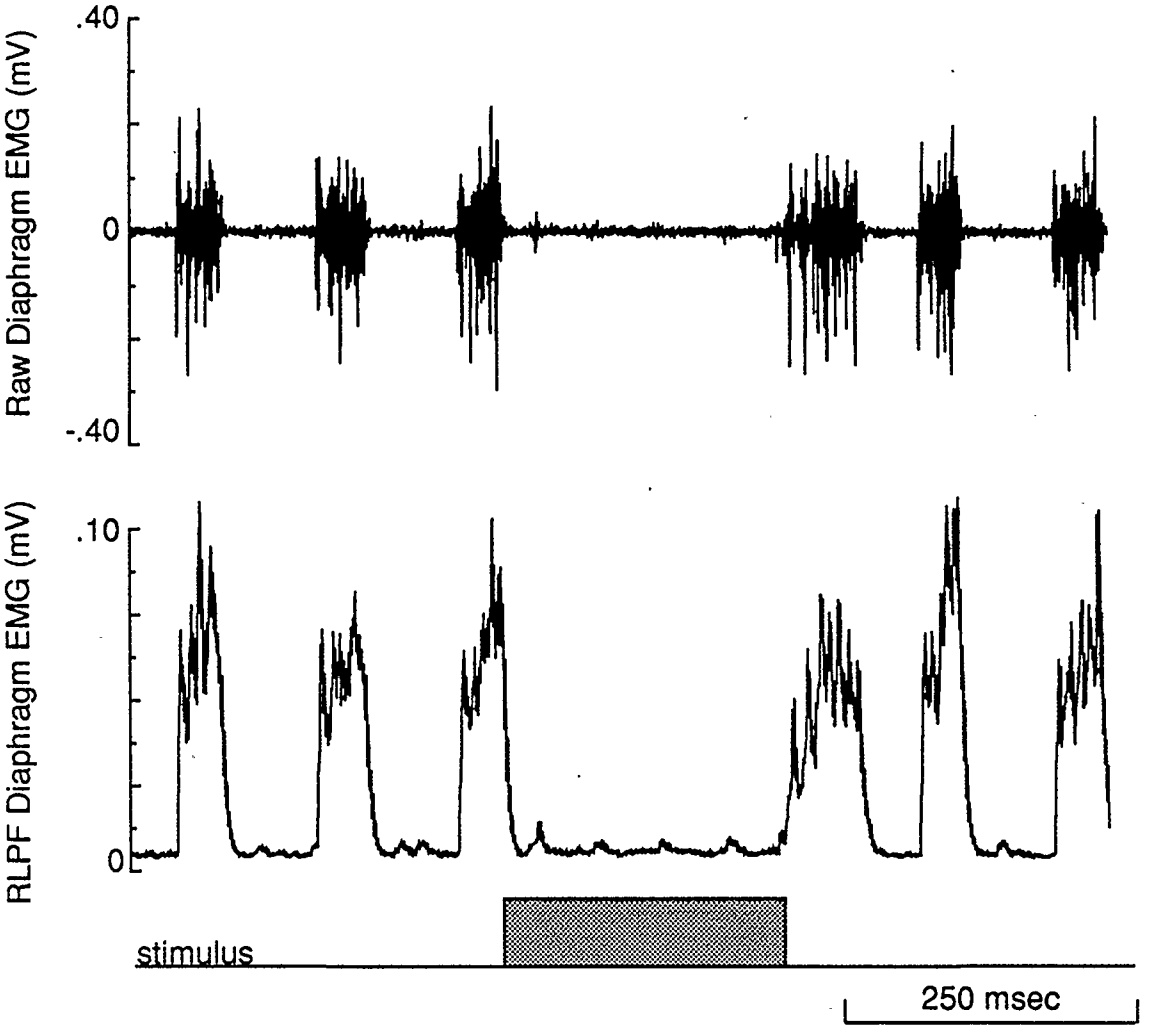

Figure. 39. Effect of an expiratory vagal stimulus at 2.25 times threshold in Lamb #014. The stimulus is indicated by the solid pulse. Vagal stimulation increased expiratory duration. The inspiratory phase after the stimulus was longer than control values before the stimulus; the expiratory phase following the stimulus was shorter compared to pre stimulus values.

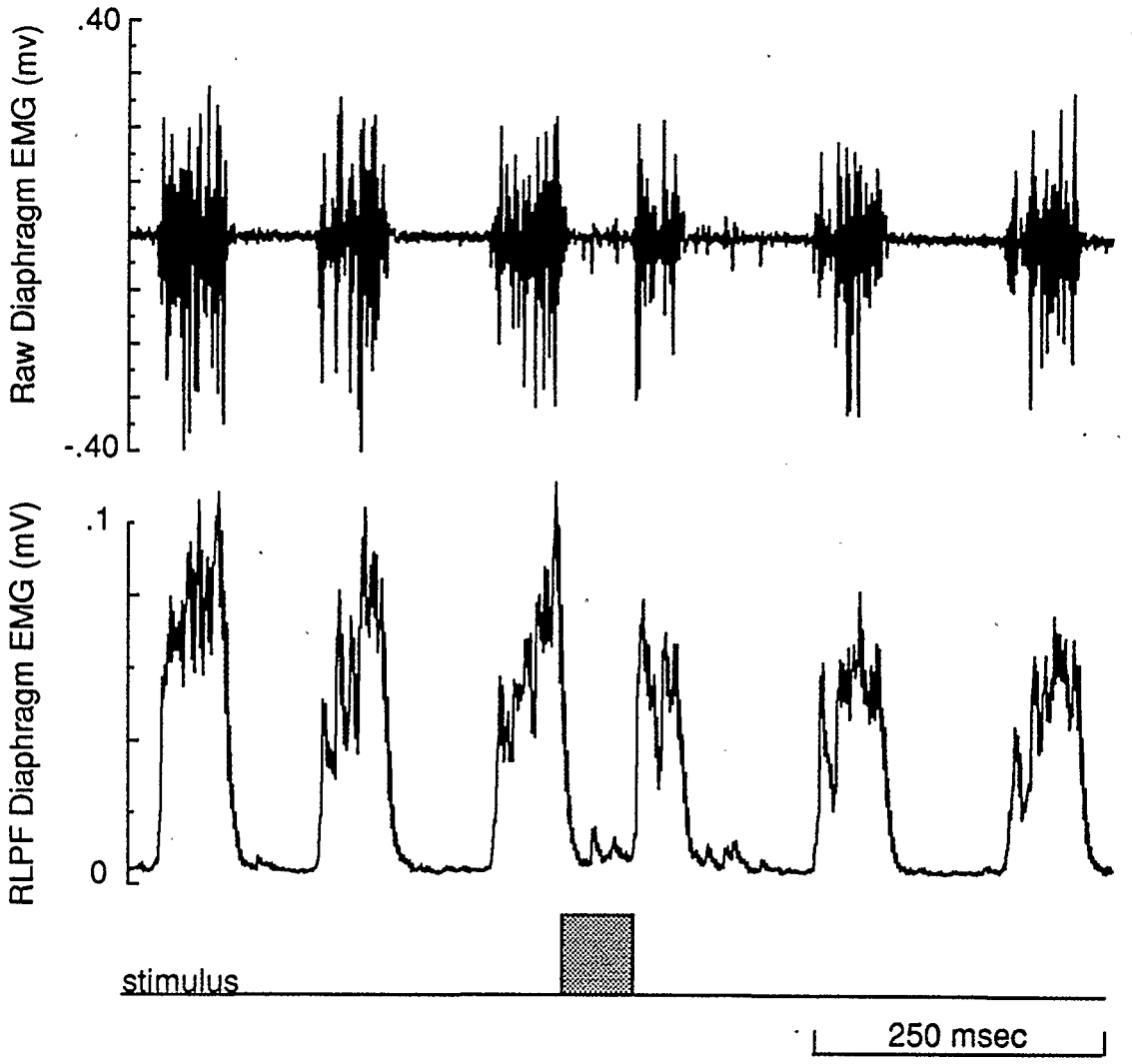

Figure 40. Effect of an expiratory vagal stimulus at 4.5 times threshold in Lamb #014. Vagal stimulation at this intensity shortened expiratory duration. The inspiratory phase after the stimulus was of shorter duration and amplitude and the next expiratory phase was longer than controls. The stimulus also appears to have increased tonic EMG activity.

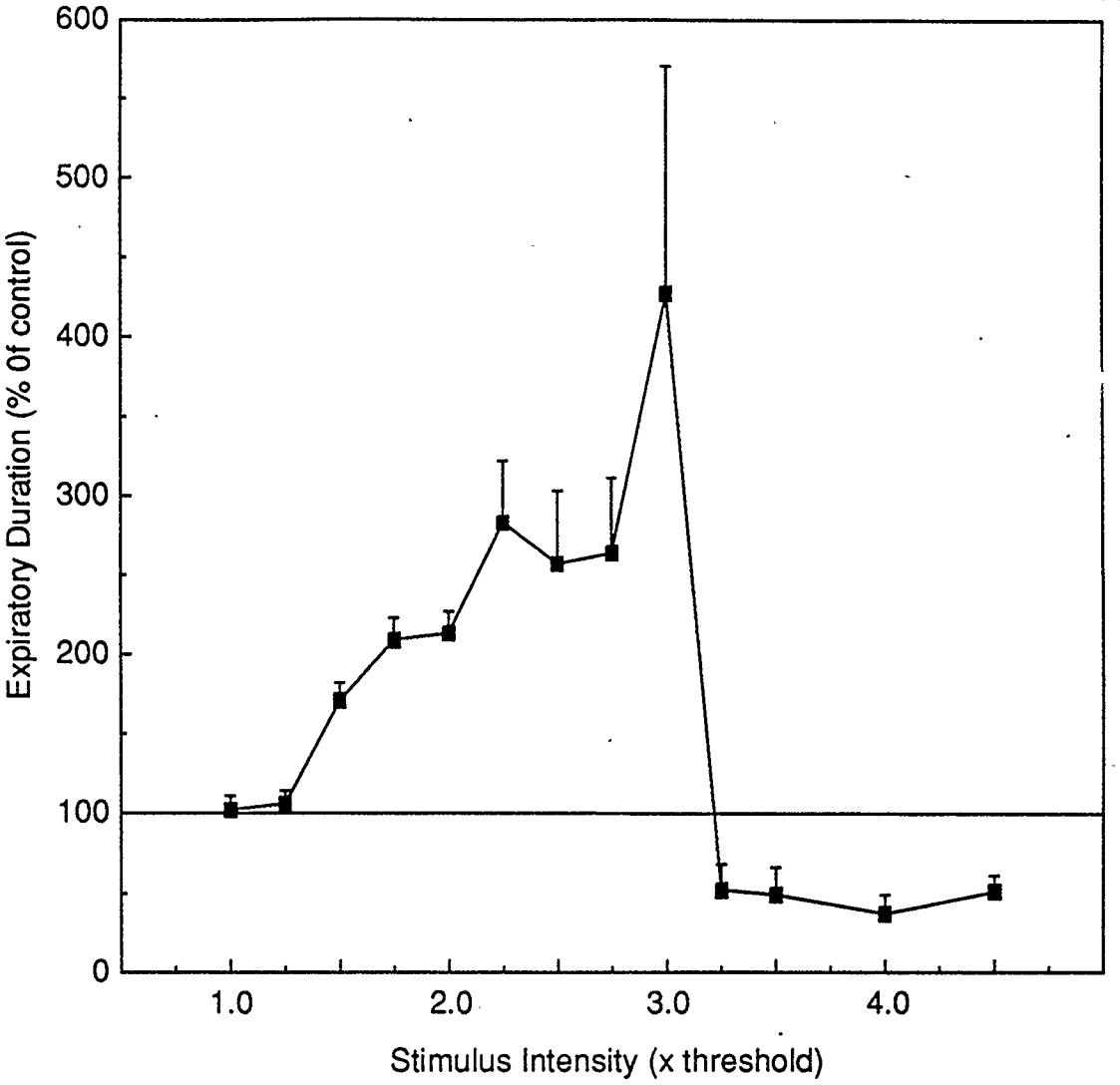

Figure 41. Effect of vagal stimulation during expiration on expiratory duration (% control) in Lamb #014. At 1.0 and 1.25 times threshold, vagal stimulation had no affect on expiratory duration. Expiratory duration increased at 1.5 times threshold. Maximum expiratory duration occurred at 3.0 times threshold. At 3.25 times threshold, expiratory duration decreased below control values.

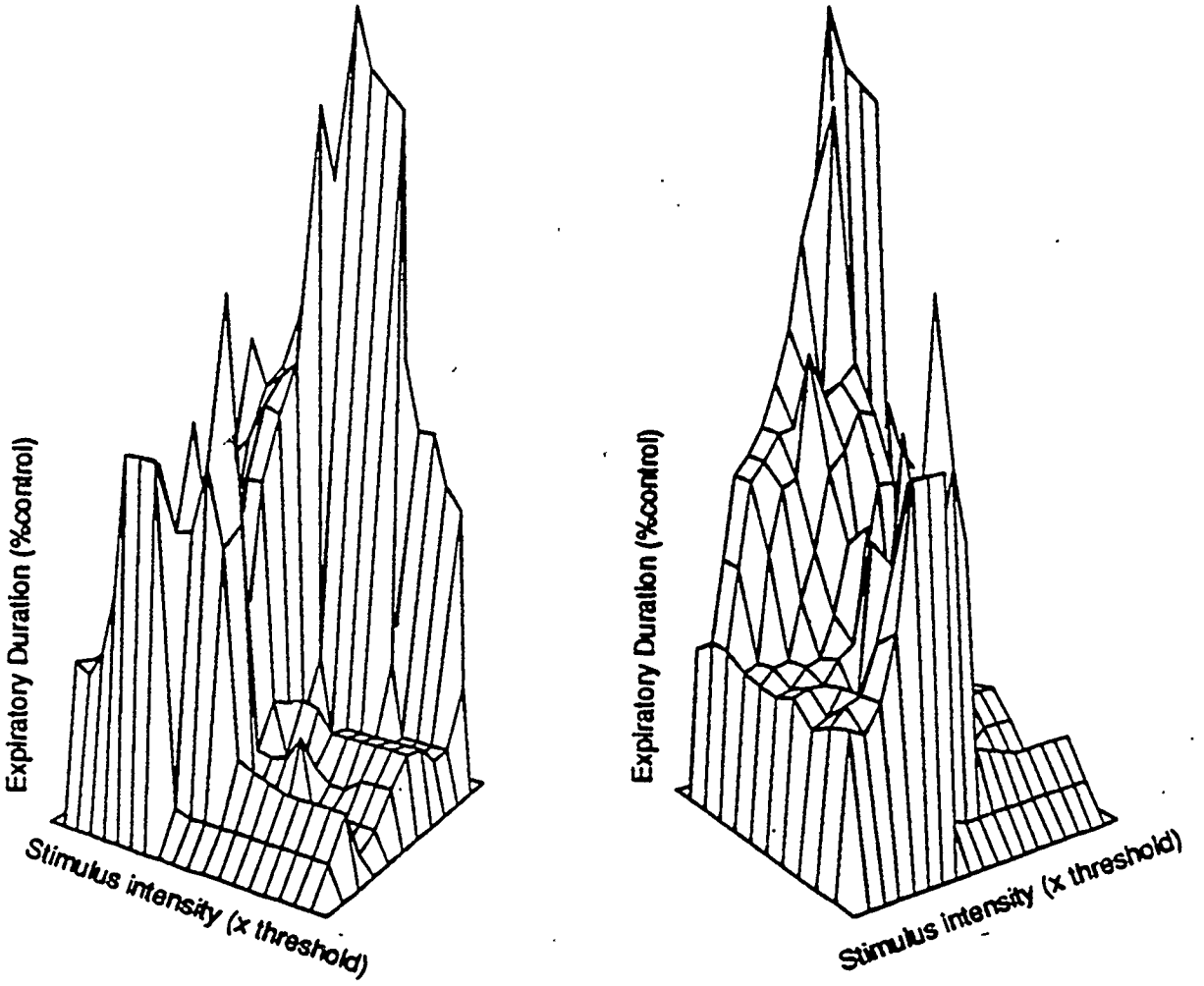

Figure 42. Expiratory stimulus curves in ten lambs:population data. The expiratory vagal stimulus prolonged expiratory duration at low intensities and decreased expiratory duration at higher intensities. Each line represents the stimulus response curve from a single animal. The two, three dimensional perspectives highlight the homogeneity of the response between individualism animals.

inspiratory duration.

Figure 41 showed the effects of electrical stimulation on expiratory duration as a function of stimulus intensity in Lamb #014. Figure 43 has superimposed on this plot, with a separate right y axis, the amplitudes of the negative peaks of the compound action potential from Figure 21. Stimulation at intensities solely corresponding to the first component of the CAP had little or no effect on the duration of expiration. The emergence of the second component of the CAP was correlated with an increase in expiratory duration. As stimulation intensity increased and the amplitude of the second component of the CAP action potential increased, expiratory duration increased further. Beyond a threshold intensity the magnitude of expiratory prolongation appeared to be related to the number of fibres recruited in the second component of the CAP. Maximal increases in expiratory duration in Lamb #014 occurred at Level II, the intensity where the amplitude of the second component of the compound action potential reached a plateau. All lambs showed maximum expiratory prolongation within .25 times threshold of the stimulus intensity designated Level II. The magnitude of expiratory shortening corresponding to stimulus intensities including the third component of the CAP was less closely related to intensity and may have reflected a triggered as opposed to a graded response.

Post-Stimulus Effects of Vagal Stimulation During Expiration

Expiratory stimulation produced post-stimulus effects in lambs with Type

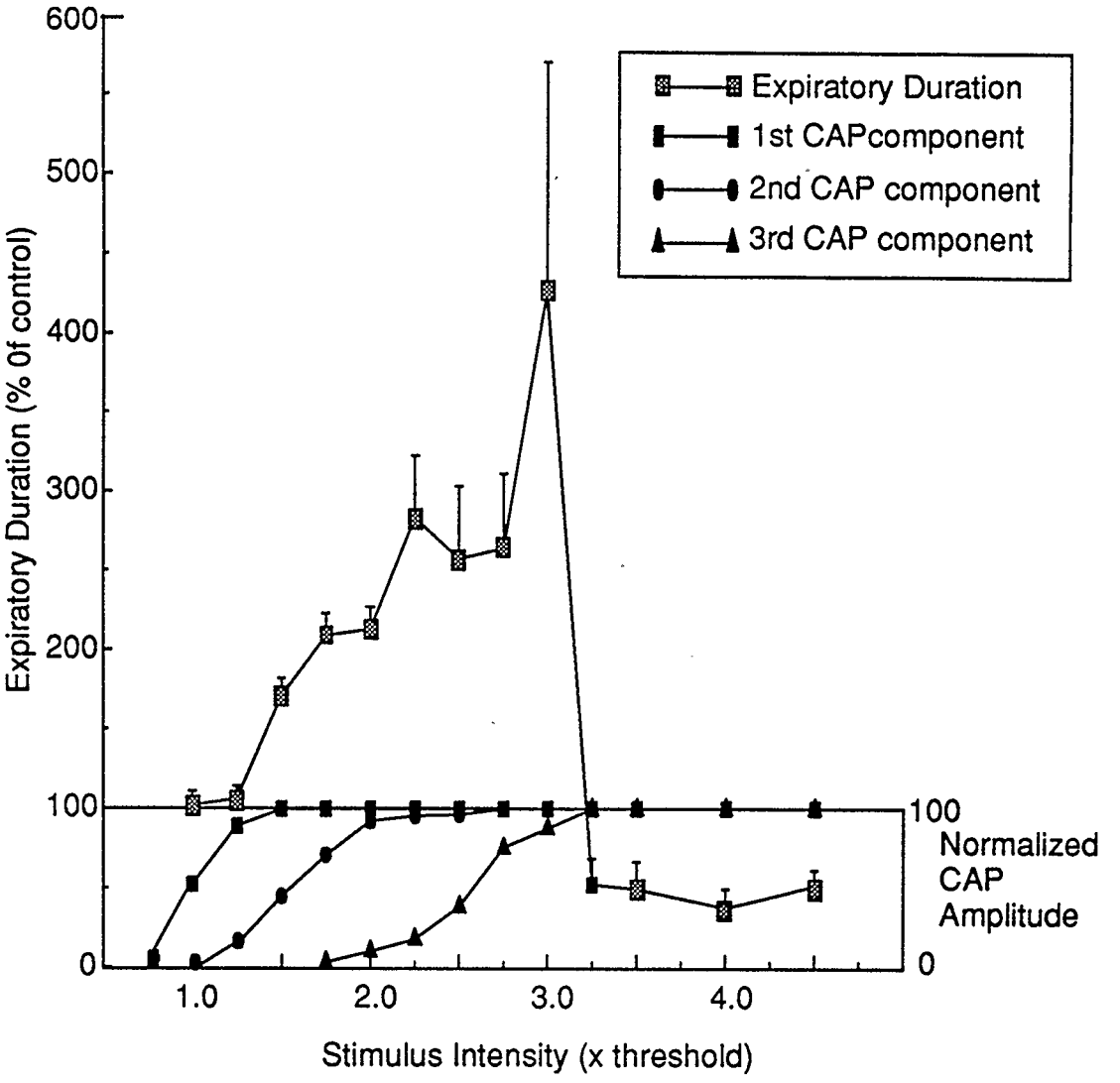

Figure 43. Relationship between expiratory duration (shaded symbold) during expiratory vagal stimulation and CAP amplitude (solid symbols) in Lamb #014. At 1.0 and 1.25 times threshold, vagal stimulation had no affect on expiratory duration. Expiratory duration increased at 1.5 times threshold. There was an increase in the amount of prolongation at 3.0 x threshold despite the fact that the amplitude of the second component of the CAP had reched a plateau. It is possible that the amplitude of the CAP was underestimated due to the increased contributions of more slowly conducting fibres. At 3.25 times threshold, expiratory duration decreased below control values.

A and Type B inspiratory responses. In Figure 39 inspiratory duration in the first breath following an expiratory prolonging stimulus was increased while the first expiratory phase following the stimulus was decreased compared to pre-stimulus expiratory phase duration. After an expiratory shortening stimulus as in Figure 40, the duration of the first inspiratory phase after the stimulus was decreased and the duration of the next expiratory phase was increased.

Mean inspiratory duration after an expiratory stimulus is plotted in Figure 44 as a function of stimulus intensity. Between 1.5 and 3.0 times threshold, inspiratory duration was increased compared to control values. Prolongation of mean inspiratory duration plateaued between 2.25 and 3.0 times threshold. At 3.25 times threshold mean inspiratory duration decreased sharply and fell below control values. A comparison with Figure 41 shows that these post-stimulus effects were smaller in magnitude but occurred in parallel to the within phase effects of vagal stimulation on expiratory duration.

The close relationship between stimulated expiratory phase duration and the duration of the next inspiratory phase are described in Figure 45 where quadratic regression yielded a correlation coefficient square of .81.

Post-stimulus effects on the duration of the expiratory phase following an expiratory stimulus are shown in Figure 46. Expiratory duration was decreased at 1.75 and 2.0 times threshold and increased after 2.5 times threshold. This plot is nearly the mirror image of the plots which described the within phase effects of expiratory stimulation (Figure 41) and the post-stimulus effects in the first phase after the stimulus (Figure 44).

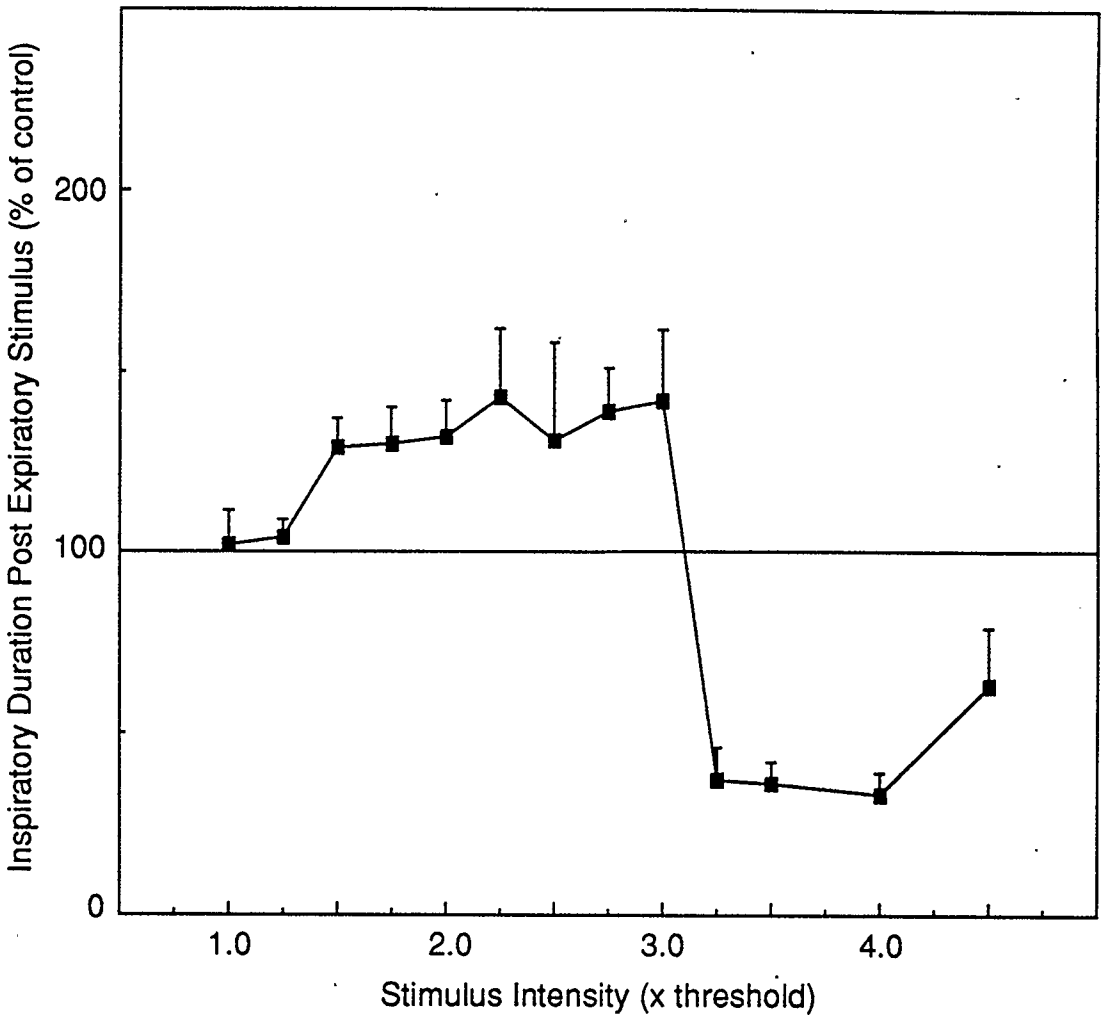

Figure 44. Effect of expiratory stimulation on post-stimulus inspiratory duration. Inspiratory duration following an expiratory stimulus was increased between 1.5 and 3.0 times threshold but decreased sharply at 3.25 times threshold. Data are from Lamb #014.

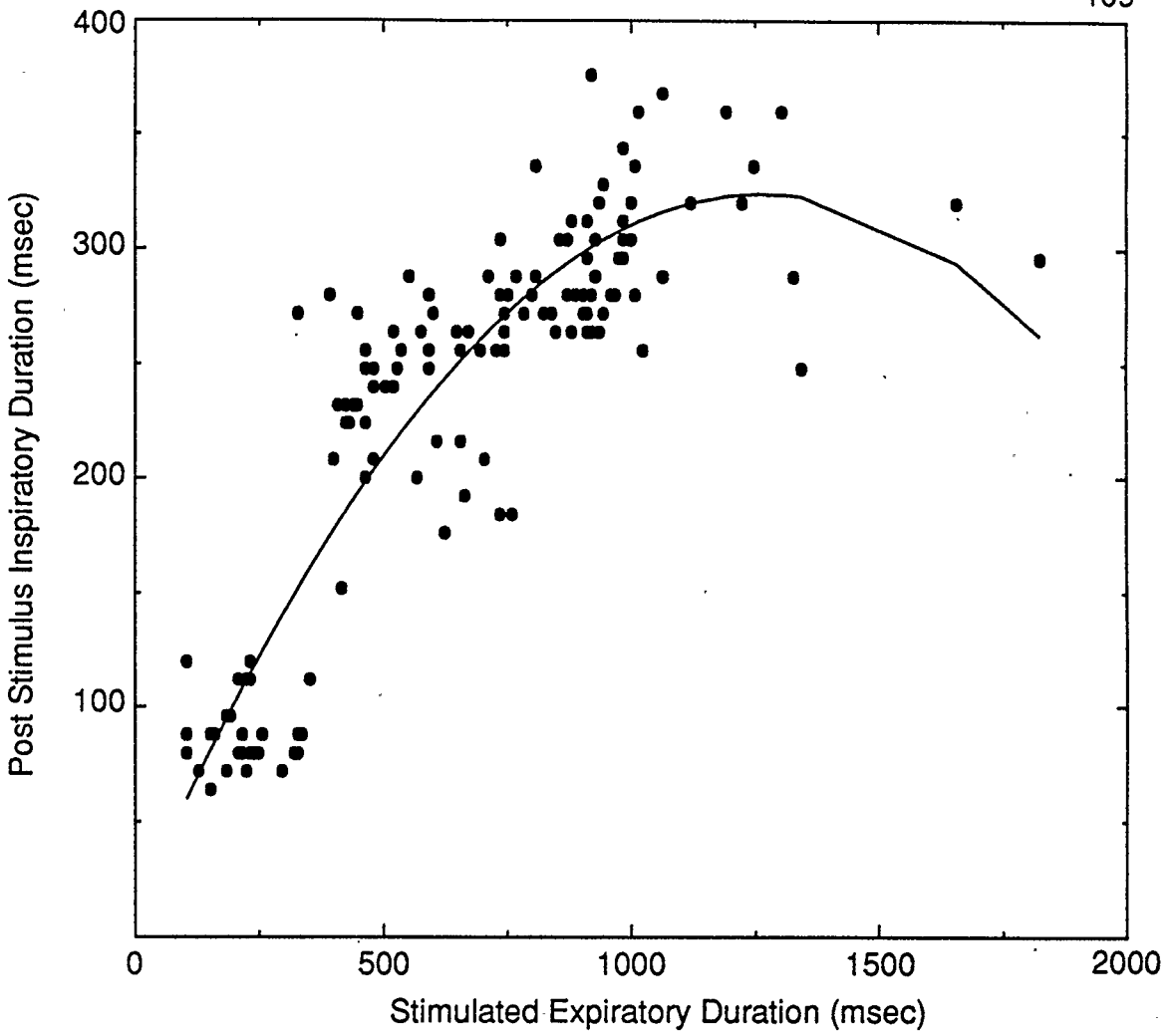

Figure 45. Correlation between expiratory duration during vagal stimulation and post-stimulus inspiratoryduration. Curvilinear regression yielded an R square of .81.

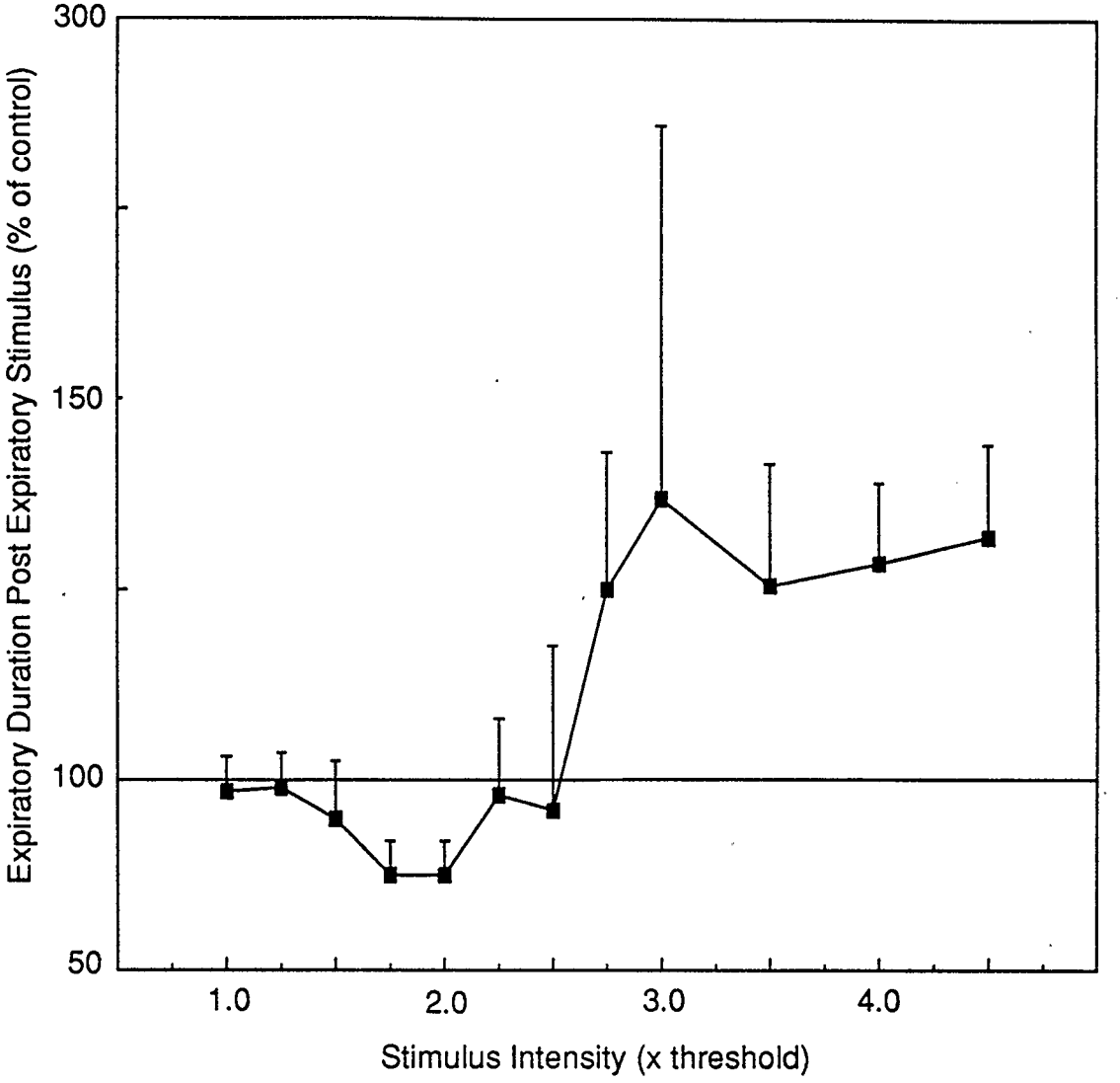

Figure 46. Effect of expiratory stimulation on post-stimulus expiratory duration. Expiratory duration following an expiratory stimulus was unaffected at 1.0 and 1.25 times threshold but increased at 1.75 and 2.0 times threshold. Between 2.75 - 4.5 times threshold, expiratory duration increased. Data are form Lamb #014 on postnatal day 10.

Relationship Between Inspiratory and Expiratory Effects

Within-phase effects

The data from Lamb #014 showed that the dependence of expiratory duration on stimulus intensity nearly mirrored that of inspiratory duration as a function of stimulus intensity. The two curves are plotted together in Figure 47. These results are typical of lambs with the Type A response. Changes in duration of expiration at stimulus between 1.5 and 3.0 times threshold were of greater magnitude than inspiratory effects at the same intensities of stimulation. The greatest mean expiratory prolongation occurred at 3.0 times threshold but had considerable variability. Mean expiratory duration may have plateaued at 2.25 times threshold; there were no significant differences in mean expiratory duration at 2.25, 2.5, or 2.75 times threshold. Expiratory shortening and inspiratory prolongation both occurred after 3.0 times threshold. The effects at high stimulus intensities were the same for lambs with the Type B response.

Post-stimulus phase effects

Post-stimulus effects occurred after both inspiratory and expiratory stimulation. Plotted against stimulus intensity, mean expiratory duration in the first phase following an inspiratory stimulus (Figure 48b) had the same shape as the within phase effects of the inspiratory stimulus (Figure 48a). Similarly,

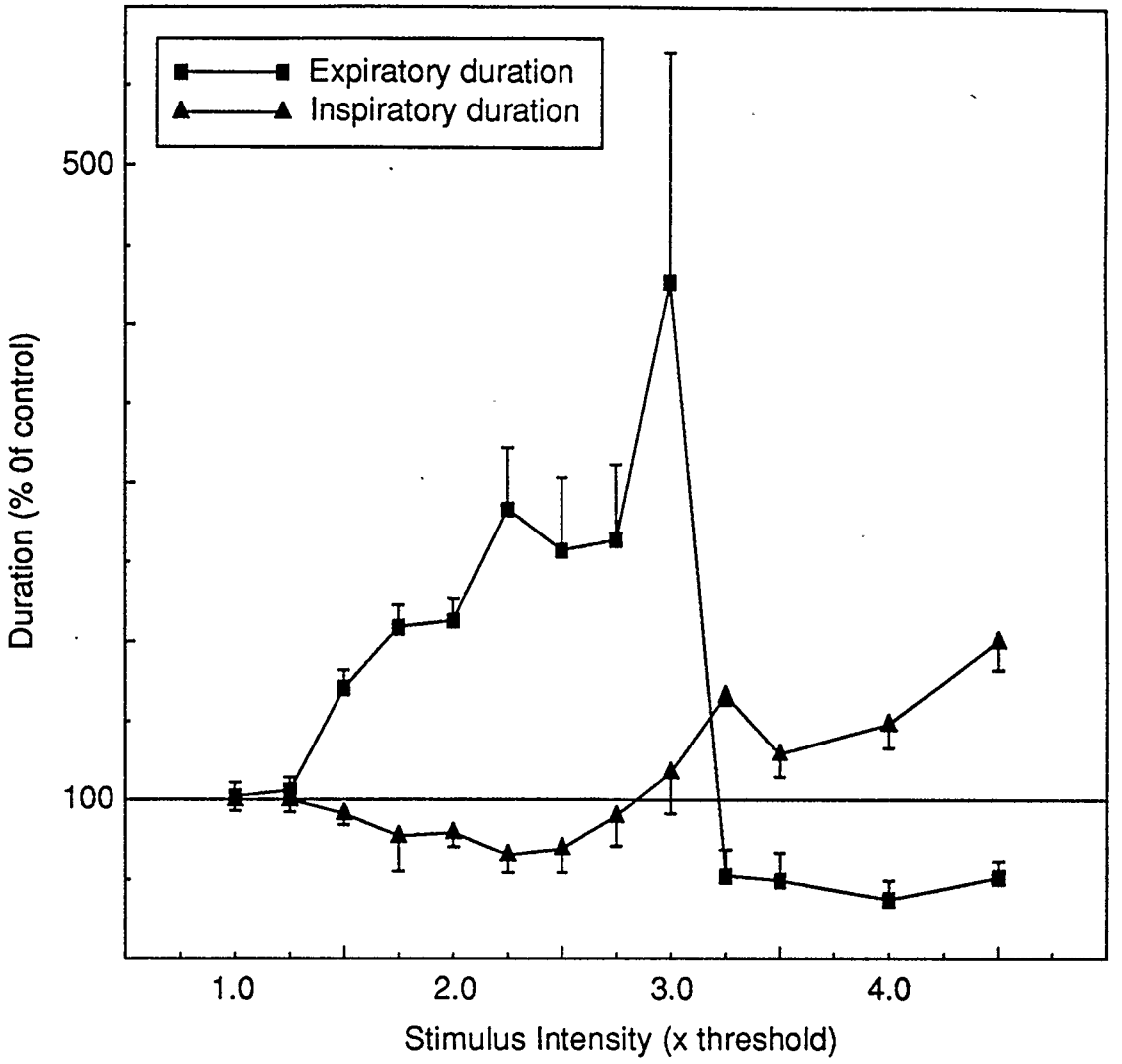

Figure 47. Relationship between within phase effects of inspiratory and expiratory vagal stimulation. Both inspiratory and expiratory duration deviated from control values at 1.5 times threshold. Data are from Lamb #014, postnatal day 10.

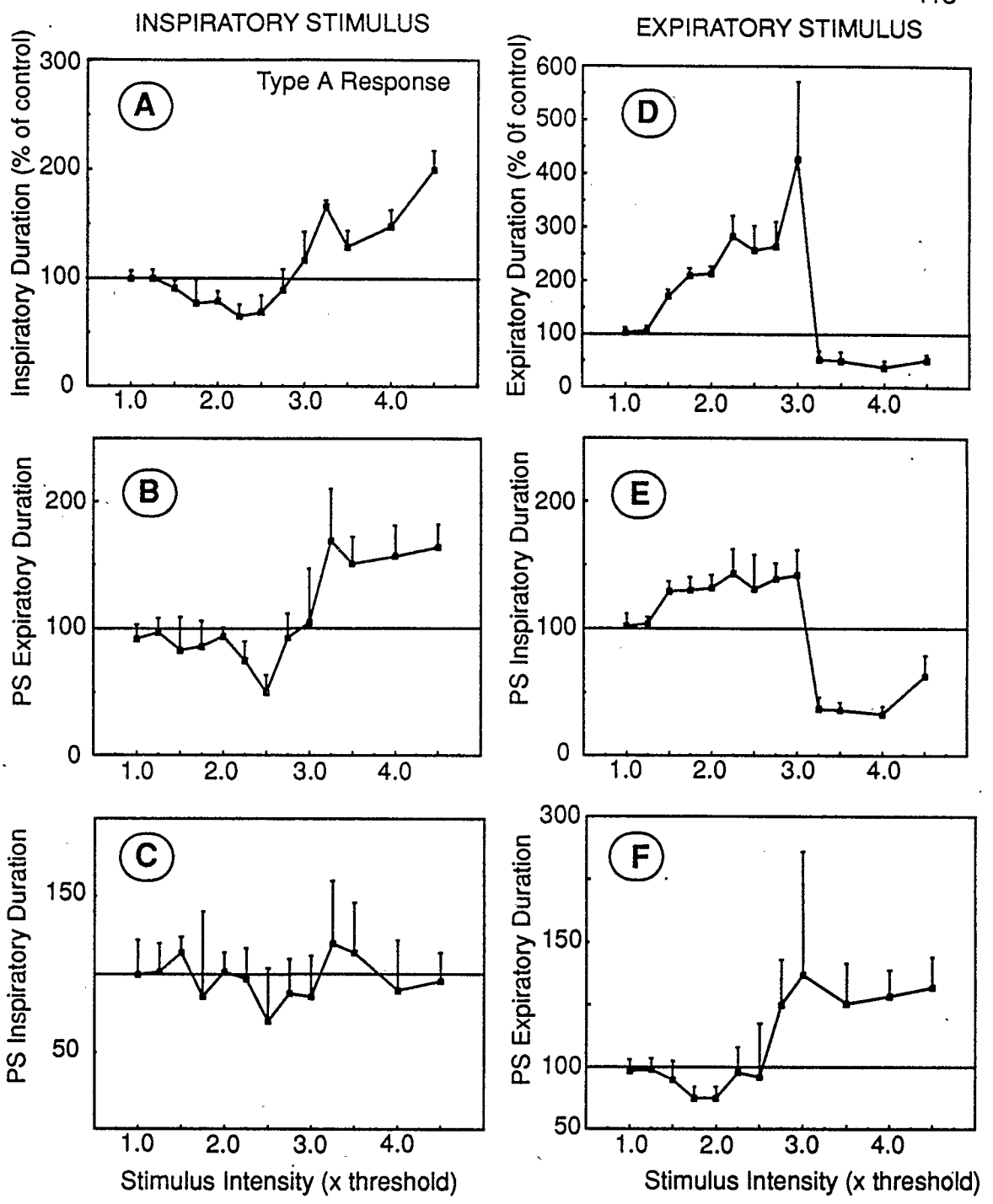

Figure 48.Summary of wlthin phase and post stimulus phase effects. The left column summarizes the within phase and post stimulus effects of an inspiratory stimulus while the right hand column summarizes the within phase and post stimulus effects of an expiratory stimulus. See text for details.

mean inspiratory duration after an expiratory stimulus (Figure 48e) as a function of stimulus intensity had the same shape, but effects of lesser magnitude, as the within phase effects of an expiratory stimulus (Fig 48d). These post-stimulus effects on inspiratory duration (Figure 48e) were opposite in effect to the within phase effects of an inspiratory stimulus (Figure 48a). The post-stimulus effects of inspiratory and expiratory stimuli in the first phase after the stimulus are near mirror images (Figure 48b cf Figure 48e).

Mean post-stimulus effects in the second phase after a vagal stimulus were clear after an expiratory stimulus (Fig 48f) and had a profile which, except for differences in magnitude, was a near mirror of the within phase effects of the expiratory stimulus (Fig 48d). In most cases, post stimulus effects in the second phase after an inspiratory stimulus were not clear (Figure 48c).

Dependence of Within-Phase Effects on Previous Phase Duration

Inspiration Duration

Equivalent strength inspiratory stimuli produced two responses, one which included a decrease in inspiratory duration (Type A) and one which did not include a decrease in inspiratory duration (Type B). Since lambs with both types of responses showed expiratory prolongation, the differences in inspiratory responses were investigated.

For lambs with the type A response, at Level II stimulus intensity where a maximal decrease in inspiratory duration occurred, the relationship between

control inspiratory duration and stimulated phase duration was analyzed to see if the effect of Level II inspiratory vagal stimulation was correlated with the duration of inspiration preceding the stimulus. The data were drawn from seven lambs (age 5-28 days) in which there was sufficient variability in breathing frequency to observe a trend.

In four of seven lambs (#012,#013,#014,#015) a negative slope described the relationship between stimulated inspiratory duration and control inspiratory duration. This means that, for these lambs, vagal stimulation during inspiration had a greater effect when control inspiratory times were long than when control times were short. An example of this relationship is shown in Figure 49. Each point on the graph represents a single stimulus trial at the same stimulus intensity. In three of these lambs on different experimental days this relationship was not clear.

Since stimulated inspiratory duration appeared in some instances to be related to control inspiratory duration (Figure 49), this offered a potential explanation for the lack of decreased inspiratory duration (Type B response) during Level II inspiratory stimulation in some lambs. However, Figure 50 which pools mean inspiratory duration from Level II inspiratory stimulation data from all lambs on days 5, 10, 15 and 20, shows that the Type B (no decreased inspiratory duration) response occurred over a range of control inspiratory times. Linear regression of these data points gave a correlation coefficient of .22 with a regression coefficient (slope) of .00045 (±.0004 SE).

Figure 51 plots mean inspiratory duration (at Level II) as a function of control inspiratory duration versus control expiratory duration (Ti/Te ratio). This

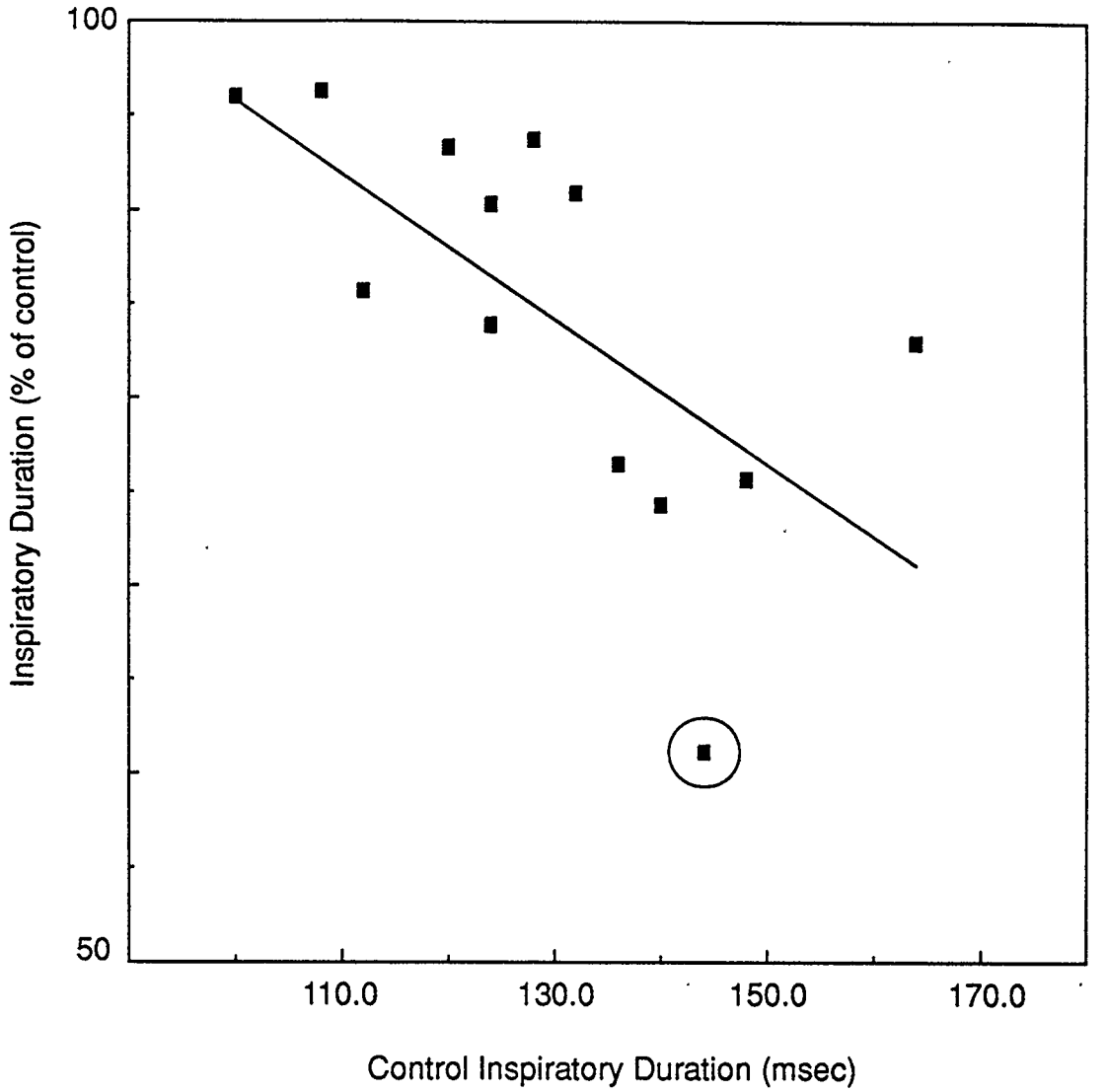

Figure 49. Control inspiratory duration influenced inspiratory duration during Level II stimulation. In Lamb #015, on postnatal day 19, maximum inspiratory shortening occurred at 1.25 times threshold. At this intensity the amount that inspiration was shortened as a result of vagal stimulation was negatively correlated to control inspiratory time. The longer the control inspiratory time, the more inspiration was shortened by vagal stimulation. The correlation coefficient when the circled point was included was -.65; without the circled point, the coefficient was -.67.

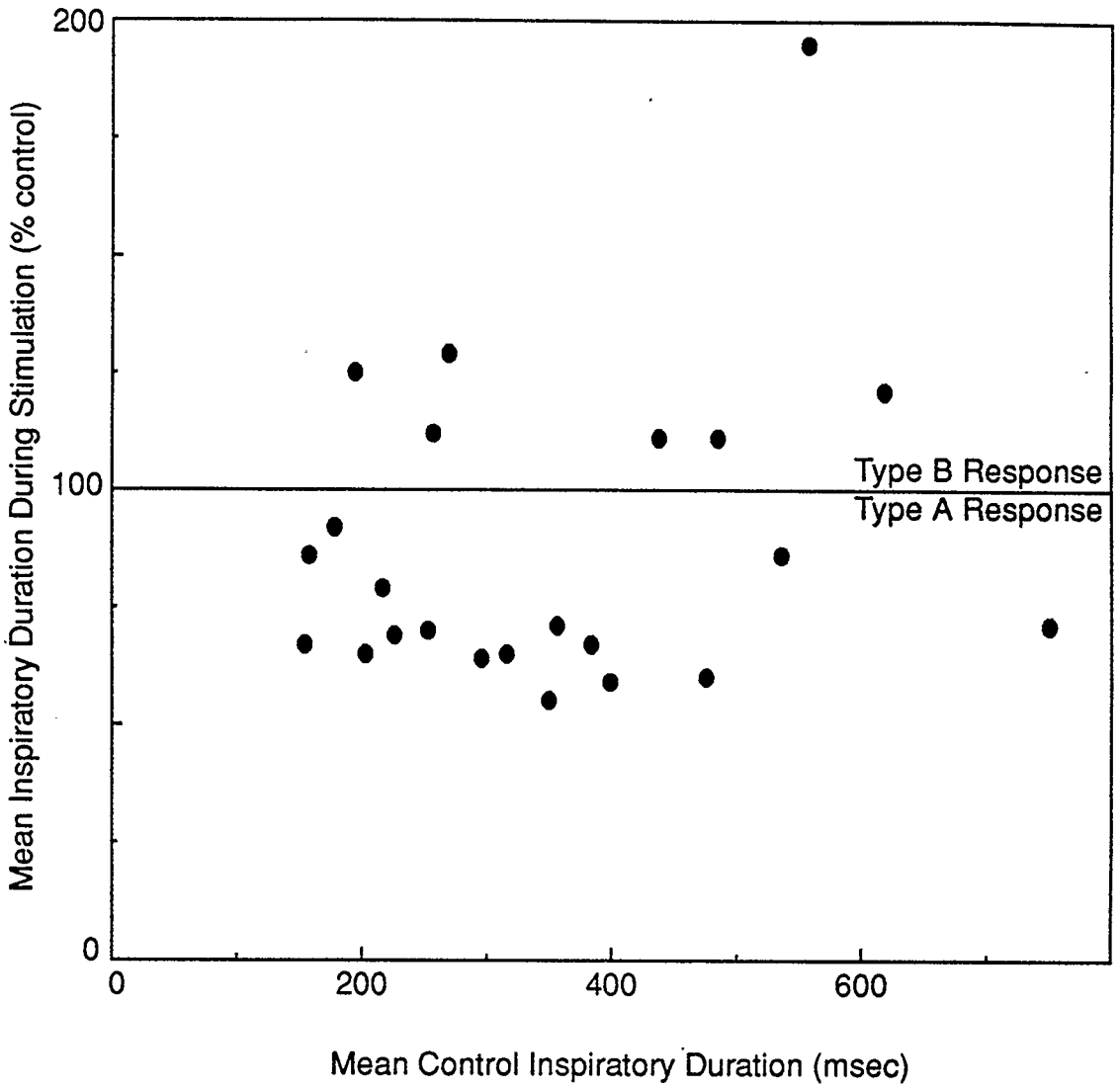

Figure 50. Type A and Type B responses were not influenced by control inspiratory duration. Inspiratory lengthening in response to a level II inspiratory stimulus (points in clear area) occurred over a range of control inspiratory times. Each point represents mean inspiratory duration during Level II stimulation in a single animal on one day.

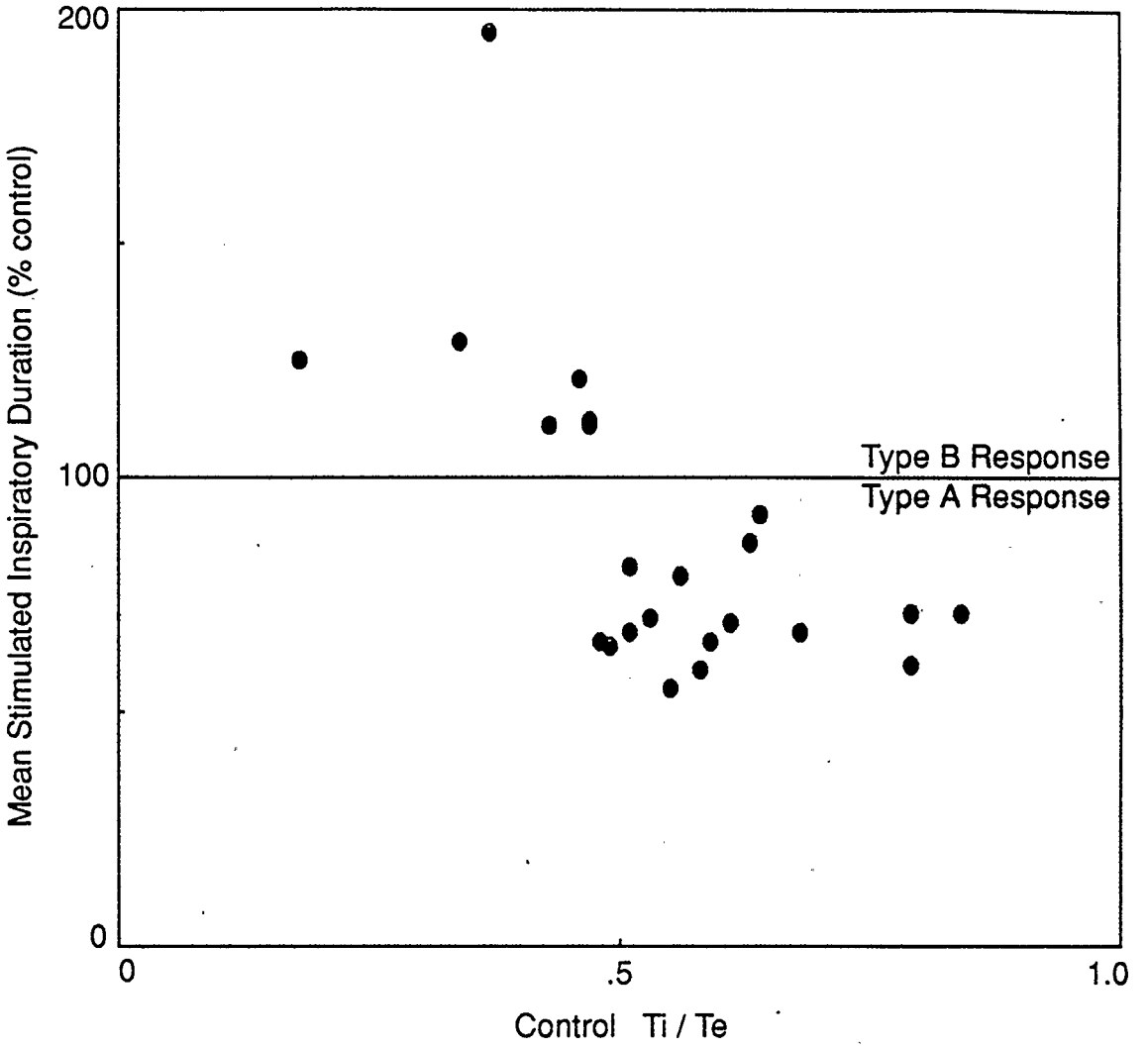

Figure 51. Type A and Type B responses were influenced by Ti/Te. These are pooled data at level II stimulation on days 5, 10, 15 and 20. Mean inspiratory duration during level II stimulation is plotted against the Ti / Te ratio of control breaths. Inspiratory lengthening occurred when Ti/Te ratios were small (<.47). Each point represents mean inspiratory duration during Level II stimulation in one lamb on one day.

figure shows that mean inspiratory duration during level II stimulation was prolonged at low control Ti/Te values (<.47) and decreased at high control Ti/Te ratios (>.47). Linear regression of these data points found a correlation coefficient of -.62. The regression coefficient (slope) was -1.36 (±.37 SE).

The results of this analysis suggest that if inspiratory duration is short relative to expiratory duration, a level II inspiratory stimulus may be ineffective in decreasing inspiratory duration. Differences in the Ti/Te ratio could explain the variability in the response to level II stimulation observed in lambs.

Expiratory Duration

The amount of expiratory prolongation observed during level II expiratory stimulation may also have been related to the duration of control expiratory phases.

In four of seven lambs (#007,#013,#014,#015) a negative slope described the relationship between stimulated expiratory duration and expiratory control duration. This relationship is illustrated by Figure 52 where each point represents a stimulus trial at Level II intensity. With longer control expiratory duration, stimulated expiration duration was prolonged less than when control expiration was shorter. In Lamb #012 there was no correlation between stimulated expiratory duration and control expiratory duration. There was also no relationship in Lamb #014 on a different experimental day.

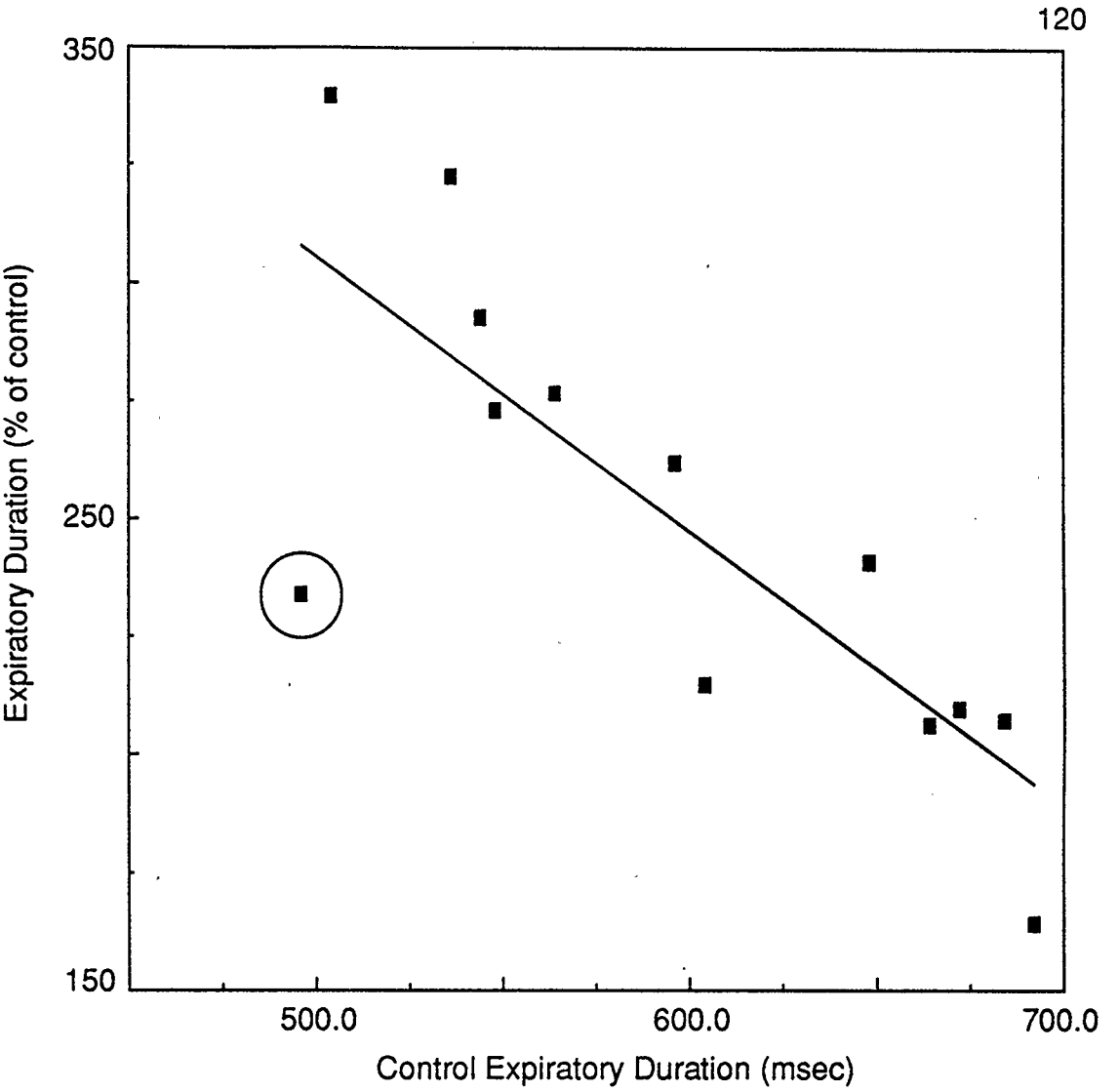

Figure 52. Control expiratory duration influenced expiratory duration during Level II stimulation. In Lamb #015, on postnatal day 14, maximum expiratory prolongation occurred at 1.5 times threshold. At this intensity, the amount that expiration was prolonged compared to control breaths was negatively correlated to control expiratory time. The greater the control expiratory time, the less expiration was affected by vagal stimulation.The correlation coefficient when the circled point was included was -.80. Without the circled point the correlation coefficient was -.94.

EFFECT OF LEVEL II STIMULATION DURING DEVELOPMENT

The previous section described the respiratory reflexes evoked by graded electrical stimulation of the vagus nerve in unanesthetized newborn lambs. Level II stimulation evoked specific, reproducible respiratory reflexes resembling the inspiratory inhibitory and expiratory prolonging reflexes originally described by Breuer and Hering (1868). This section describes the effects of a Level II stimulus on inspiration and expiration in lambs as a function of age.

Developmental changes were studied using two approaches: intra-lamb and population comparisons. With intra-lamb comparisons, changes in reflex effects during development were studied within single lambs. With population comparisons, results from all lambs were pooled for each age studied. The advantage of intra-lamb comparisons is that the baseline differences between lambs do not influence the results, while the advantage of the population approach is that even data from lambs studied on only one day can be included, thus increasing the size of the data base.

Variation with Increasing Age: Intra-Lamb Comparisons

In individual lambs, there were significant changes in respiratory reflexes evoked by Level II stimulation during development. Figure 53 shows that in three lambs, the effects of Level II inspiratory stimulation on mean inspiratory duration showed three different developmental trends. In Lamb #007 the

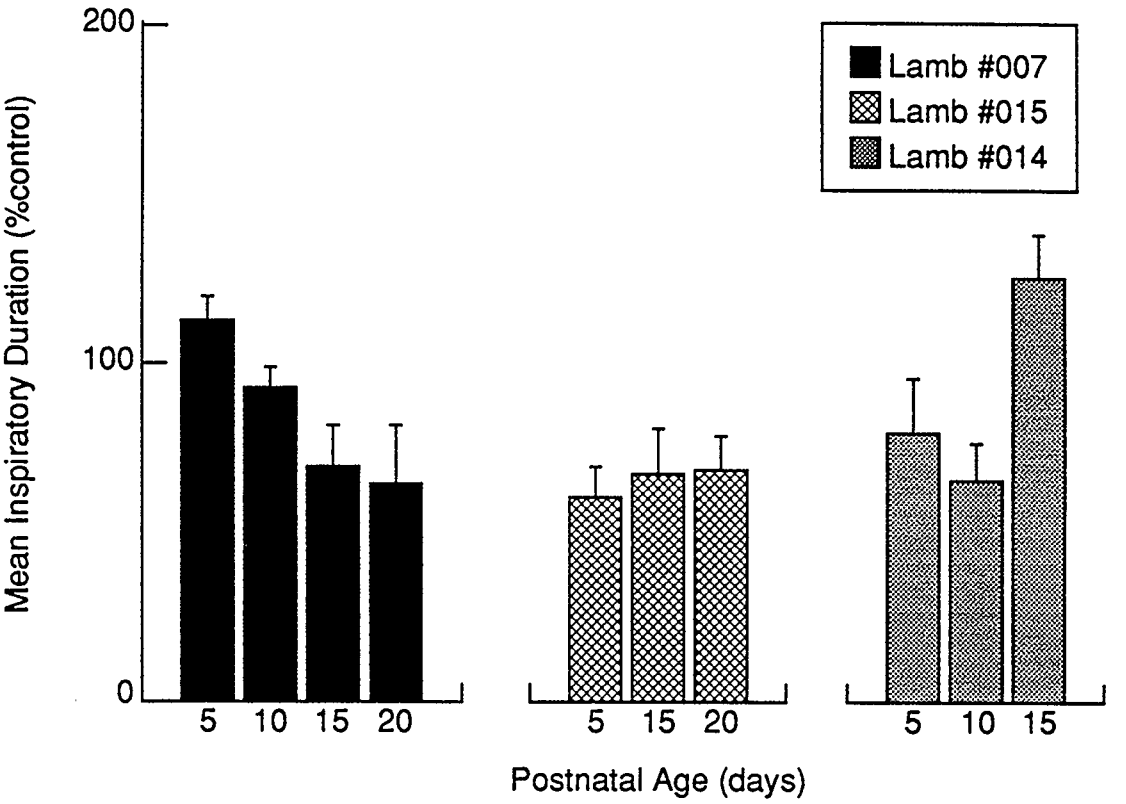

Figure 53. Changes in inspiratory duration as a function of age in three lambs. The effects of a Level II inspiratory stimulus varied as a function of age in Lamb #007 and #014. In Lamb #007, the effects of the stimulus increased with increasing postnatal age; inspiratory duration decreased with age. In Lamb #014, the effects of the stimulus decreased with increasing age; inspiratory duration increased with age. However, Lamb #015 showed no changes in inspiratory duration as a function of age.

effects of Level II stimulation in decreasing inspiratory duration increased with age. In Lamb #014 (shaded bars) there was also an increase in the effectiveness of the Level II stimulus with age; mean inspiratory duration was decreased more on day 10 than on day 5. On day 15 however, mean inspiratory duration was significantly longer than on previous days. In Lamb #015 (hatched bars), there was no significant change in the effect of the Level II stimulus on mean inspiratory duration with age.

Table 1 summarizes the significant developmental trends in inspiratory duration for individual lambs (column 1).Two lambs showed significant increases in inspiratory duration, two showed significant decreases and the remaining three did not show significant changes. Thus, although the inspiratory reflex effects of Level II stimulation significantly changed during development in individual lambs, there were no consistent trends between lambs.

Other measurements of reflex effects exhibited a similar developmental pattern. Table 1 also shows the significant changes in other variables that occurred during inspiratory (inspiratory amplitude, post-stimulus (PS) expiratory duration and post-stimulus inspiratory duration) or expiratory (expiratory duration, post-stimulus inspiratory duration and post stimulus expiratory duration) stimulation. As with inspiratory stimulation, significant changes generally occurred in opposite directions in different lambs. For example, post-stimulus expiratory duration following a Level II expiratory stimulus increased with age in two lambs but decreased with age in three other lambs. Only expiratory duration and post stimulus inspiratory duration (during inspiratory

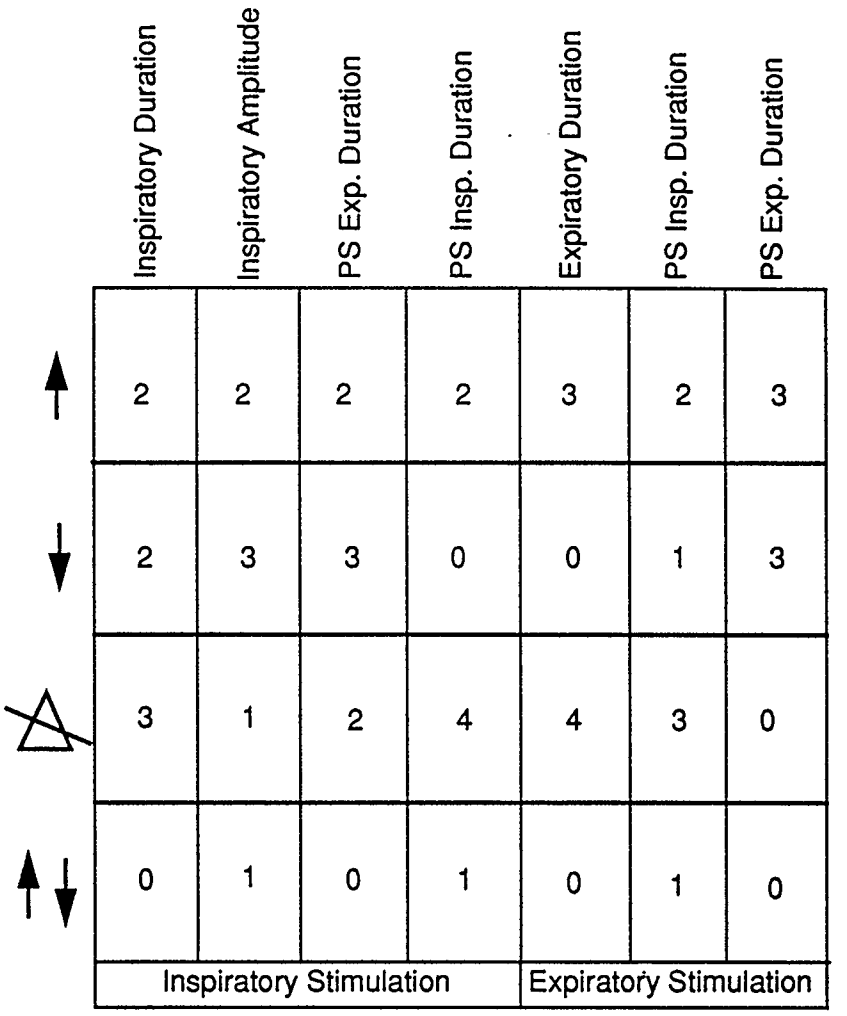

Table 1. Distribution of the developmental effects of Level II stimulation

stimulation) exhibited uni-directional changes. Thus, the myriad effects of Level II stimulation show significant changes with development, however different lambs typically show different patterns.

Interestingly, there was no clear correlation between developmental changes in the effects of stimulation during inspiration and stimulation during expiration. For example, lambs with significant increases in expiratory duration with age could show either significant increases (n=1) or decreases (n=2) in mean inspiratory duration during inspiratory Level II stimulation with age. Similarly, from a developmental perspective, within phases effects were not correlated with post-stimulus effects. For example, during inspiratory stimulation decreases in post-stimulus expiratory duration were associated with either an increase (n=1) or a decrease (n=2) in inspiratory duration (within phase effect). Thus, although the data are limited, the results suggest that the different inspiratory and expiratory reflex effects evoked by Level II stimulation mature independently.

Figures 54 and 55 summarize the developmental changes for inspiratory and expiratory Level II stimulation based on intra-lamb comparisons. For each lamb, the mean data are expressed as percentage of the mean data value for the same variable obtained on day 5. This method of expressing the data normalized for systematic differences between animals. Consequently the day 5 value was always 100%. The results, which are consistent with the individual examples described above, show that overall, there were no significant developmental trends in most of the variables measured with both inspiratory and expiratory stimuli. Only post-stimulus expiratory duration following an

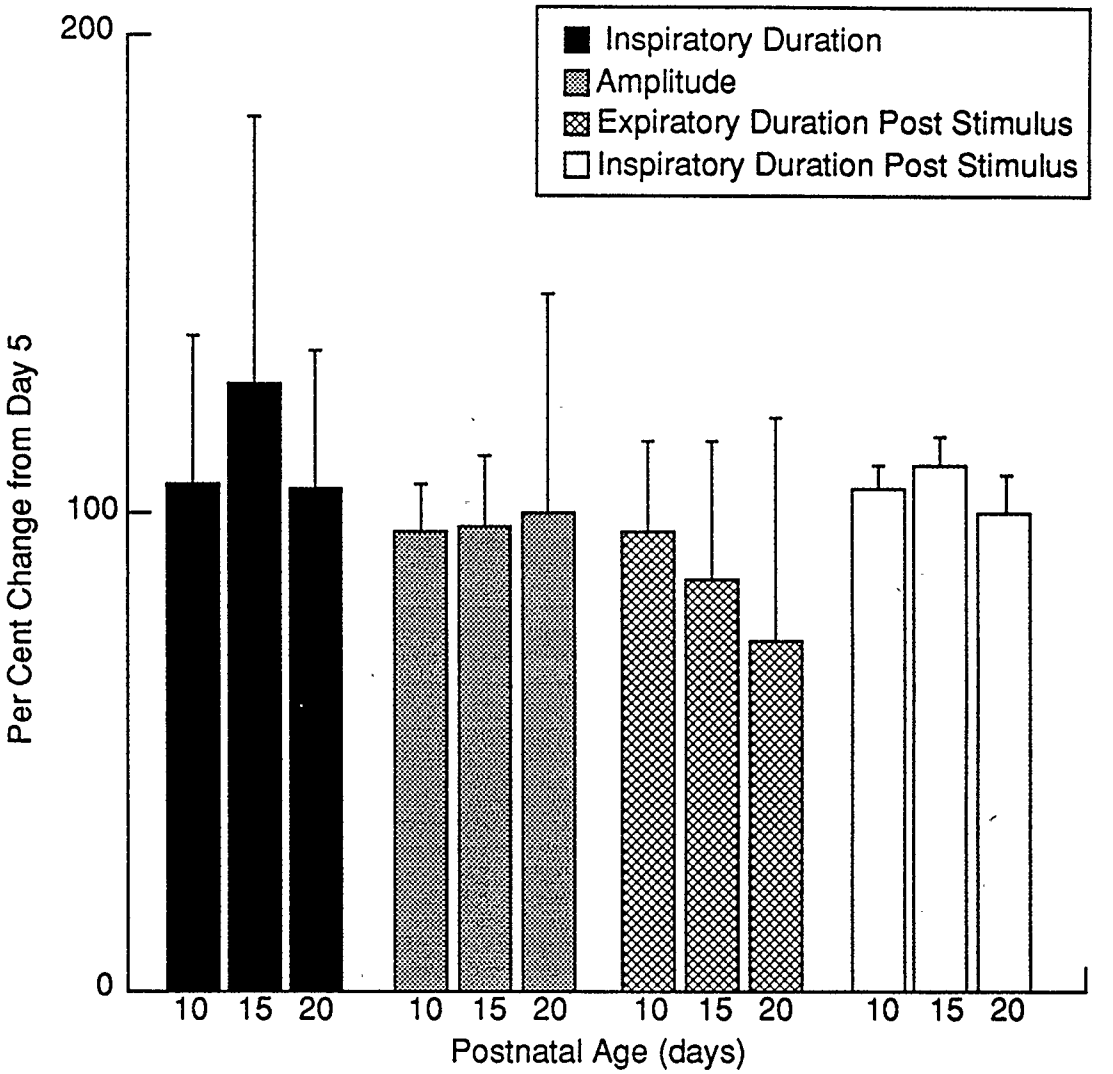

Figure 54. Per cent change in inspiratory variables during Level II stimulation as a function of age. The mean per cent change in measured variables on Days 5, 10 and 15 is expressed relative to initial measurements on Day 5 (100%). There were no significant differences in any of the measured variables as a function of age.

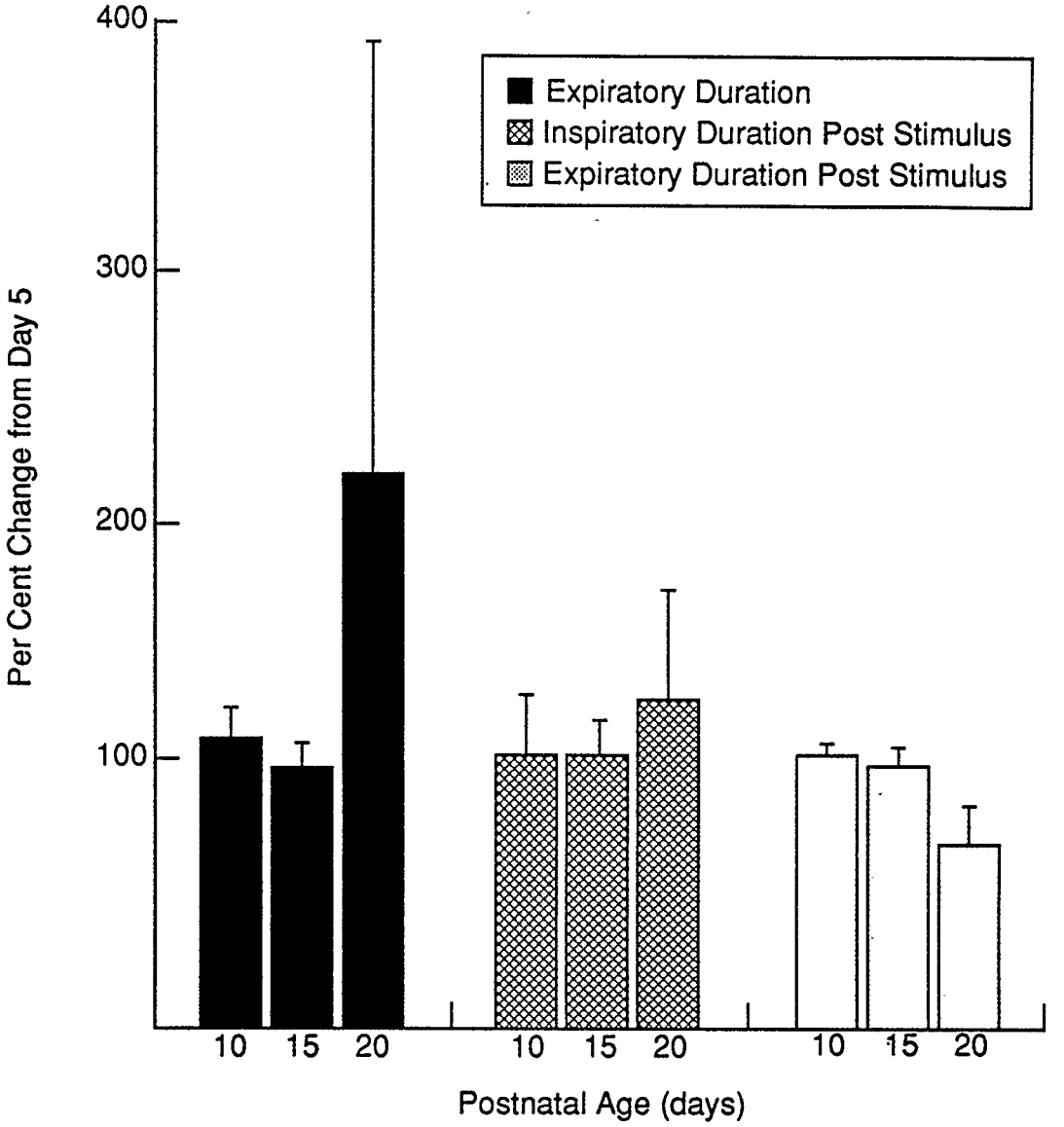

Figure 55. Per cent change in expiratory variables during Level II stimulation as a function of age.The mean per cent change in measured variables with an expiratory Level II stimulus on Days 10, 15 and 20 is expressed relative to initial measurements on Day 5. The only significant difference in the response to Level II expiratory stimulation with age was in expiratory duration post stimulus.

expiratory stimulus showed a significant decrease with increasing age. Thus, although individual lambs can show significant developmental changes in respiratory response to Level II stimulation, there was no overall developmental trend between days 5 and 20.

Although there were no significant changes with age, changes in the efficacy of electrical stimulation might have masked a developmental trend. This possibility was excluded because the CAP was monitored during all experiments and a range of stimulus intensities were used.

Variation with Increasing Age: Population Comparisons

To complement the intra lamb studies, developmental changes in the population data were investigated. Figures 56 and 57 show the effects of age on the same measurements as in Figures 54 and 55. Figures 56 and 57 differ however, in that the actual values of the respiratory measures were used so that additional points from other lambs, with only one recording day, could be included. Displayed this way, there still were no developmental trends in any of the four variables displayed. Thus, no developmental trends were revealed by either intra-lamb or population approaches.

Variation Within a Single Age

Given the relatively great variability, especially in inspiratory duration, variability in individual measurements within animals the same age was examined. Figure 58 shows that on day 5 mean inspiratory duration during Level II stimulation differed significantly between animals. Lambs #007 and

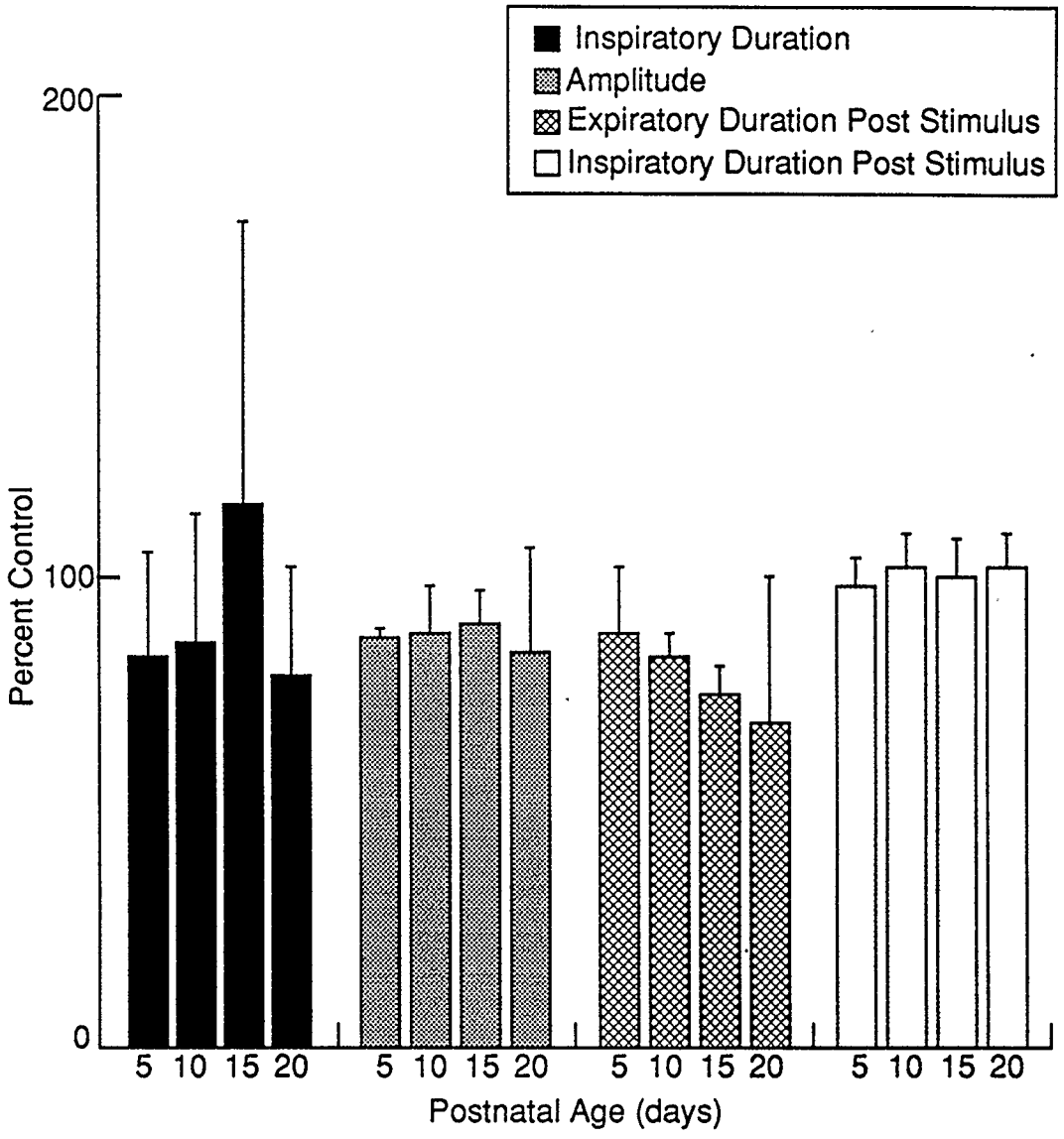

Figure 56. The effects of a Level II inspiratory stimulus in lambs at four different ages. There were no significant differences in mean inspiratory duration, amplitude or post stimulus phase durations from day 5 to day 20. The number of lambs on days 5, 10, 15 and 20 was 8, 7, 4 and 4 respectively.

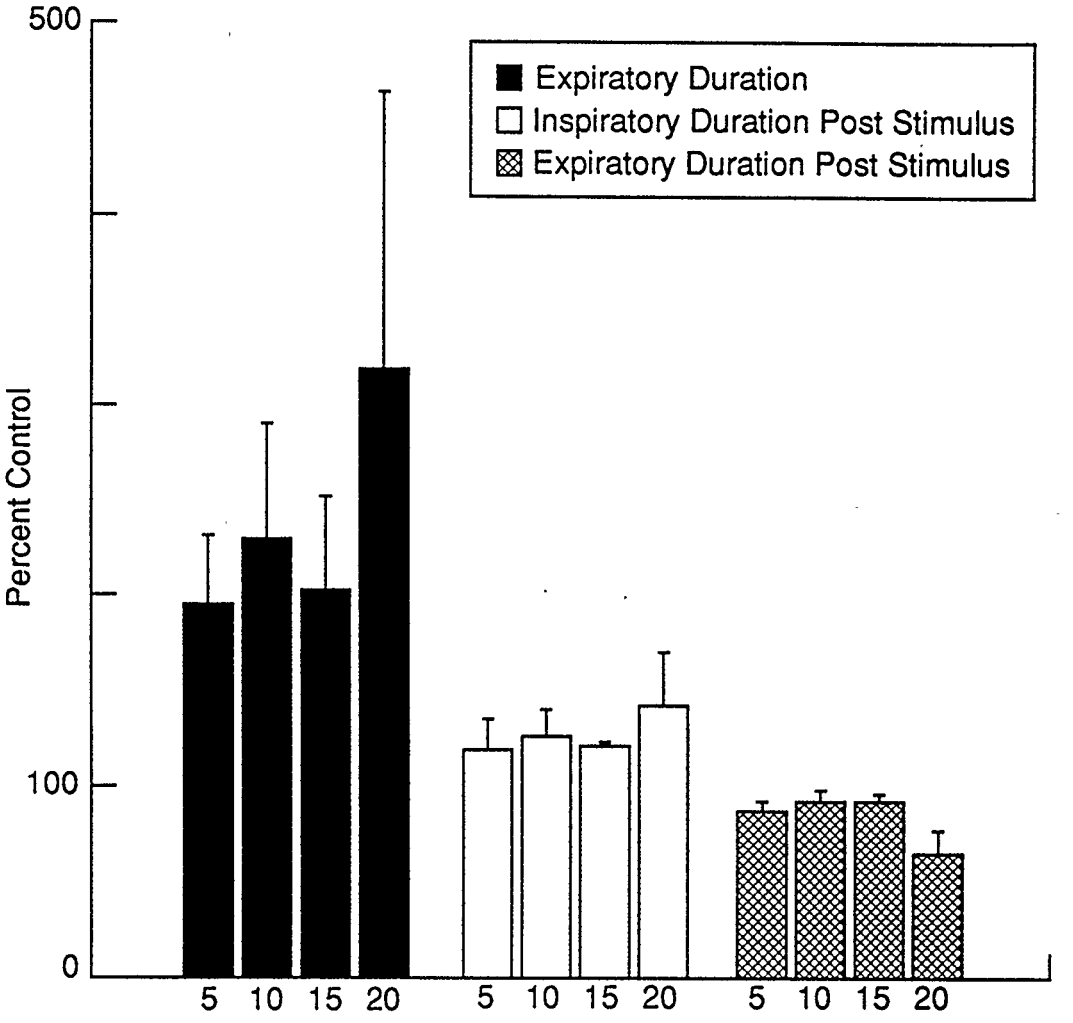

Figure 57. The effect of a level II expiratory stimulus in lambs of different ages. There were no significant differences in mean expiratory duration or mean inspiratory duration following an expiratory stimulus as a function of age. There were significant differences in mean expiratory duration following an expiratory stimulus. The n on days 5, 10, 15 and 20 was 8, 7, 4 and 4.,

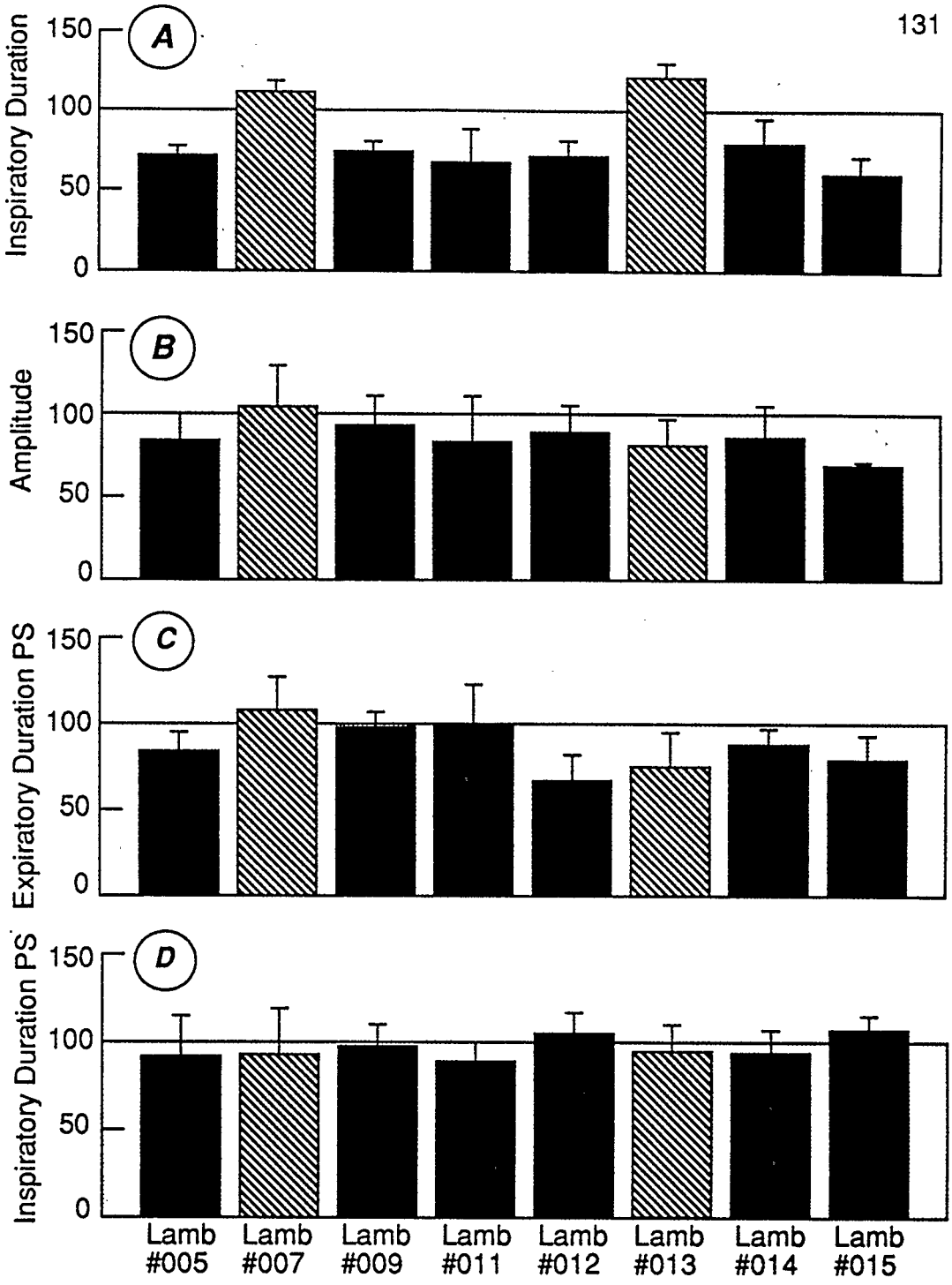

Figure 58. Effects of an inspiratory Level II stimulus on inspiratory duration, amplitude, and post stimulus phase durations in eight newborn lambs 5 days old. Values are expressed as % of control (100%).Lambs #007 and #013 did not show clear inspiratory shortening.

#013 showed increased inspiratory duration while the remaining lambs showed decreased inspiratory duration. There were also differences between lambs in inspiratory amplitude (Fig. 58, panel B) ,and post-stimulus expiratory durations (Fig. 58, panel C). Responses to Level II stimulation during expiration also varied (Figure 59). Although expiratory prolongation was a consistent response to the stimulus in all lambs at all ages there were significant differences in mean expiratory duration (Fig. 59A) and post-stimulus inspiratory duration between lambs (Fig.59B)

Variability in the response to inspiratory and expiratory Level II stimuli between similarly aged lambs occurred on subsequent recording days as well. Figures 60-65 illustrate the variability in measurements obtained with 10 ,15 and 20 day old lambs. In ten day old lambs (Fig. 60) there were significant differences in mean inspiratory duration (panel A) and amplitude of the inspiratory burst (panel B) during Level II inspiratory stimulation, as well as in mean expiratory duration (figure 61, panel A) during Level II expiratory stimulation. Similarly, in fifteen day old lambs (Figure 62) there were also significant differences in mean inspiratory duration (panel A) and amplitude of the inspiratory burst (panel B) during Level II inspiratory stimulation as well as significant differences in mean expiratory duration (Figure 63, panel A) during Level II expiratory stimulation. On day 20, (Figure 64) there were significant differences in mean inspiratory duration (panel A), amplitude of the inspiratory burst (panel B), and post-stimulus expiratory duration (panel C) during Level II inspiratory stimulation and significant differences in mean expiratory duration

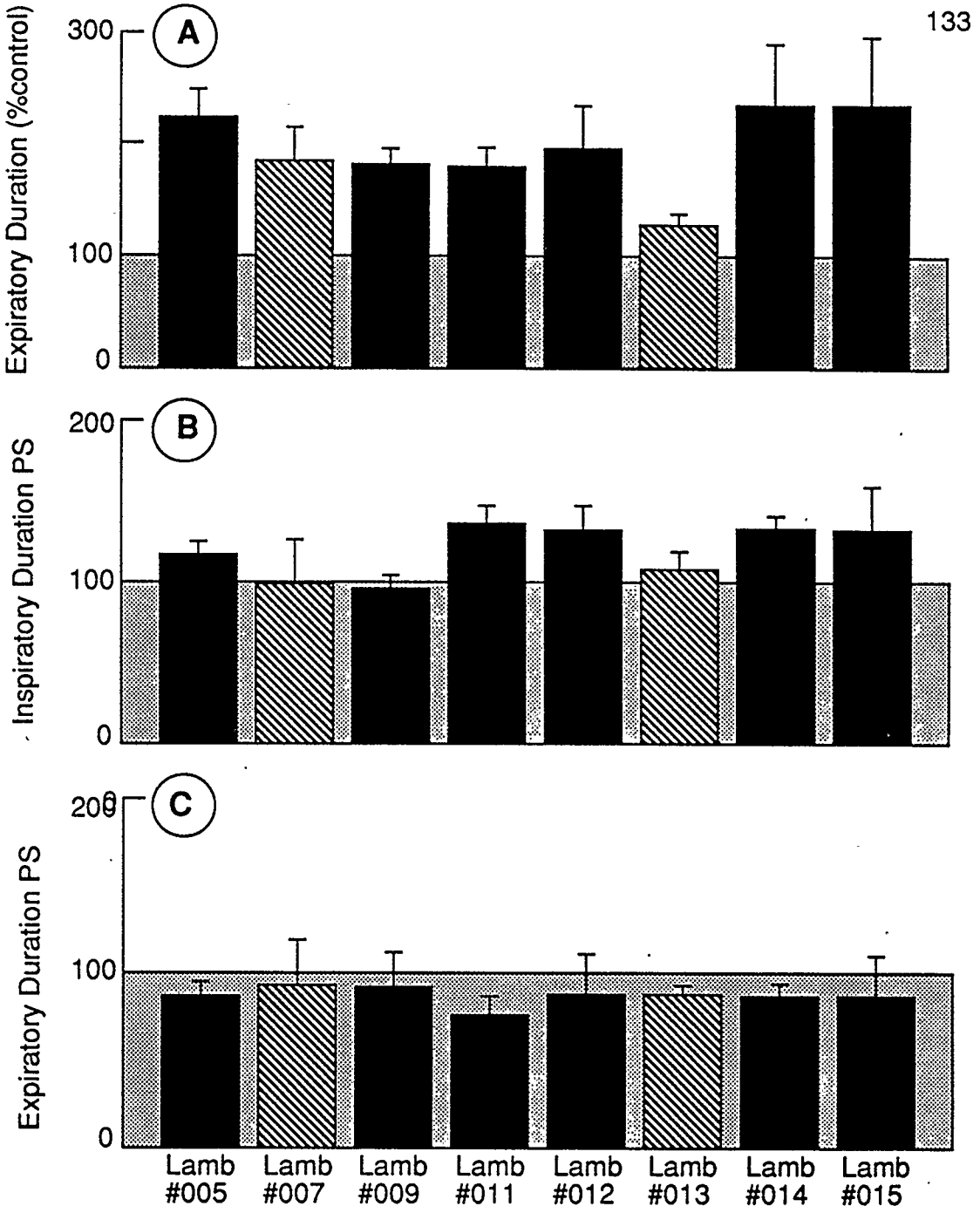

Figure 59. Effect of an expiratory Level II stimulus on expiratory duration and post stimulus phase durations in eight 5 day old newborn lambs. Values are expressed as % of control (+/- 1 S.D.) There were significant differences in mean expiratory duration and in post stimulus inspiratory duration between lambs ($p<.01$)

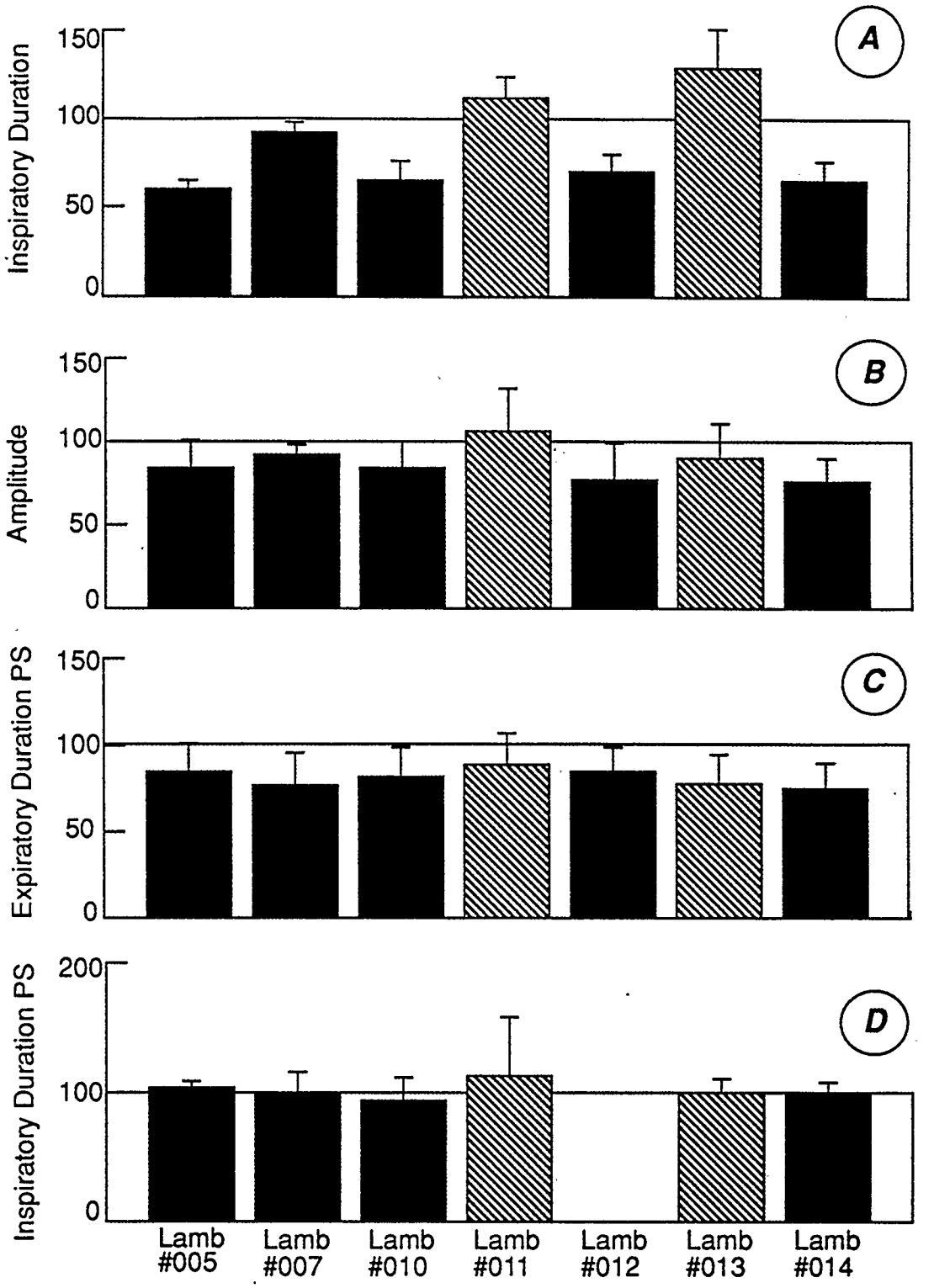

FIgure 60. Effect of an inspiratory Level II stimulus in seven 10 day old lambs. Lamb #011 and #013 did not show inspiratory shortening. Data from lambs with the Type B response are described by the diagonally striped bars.

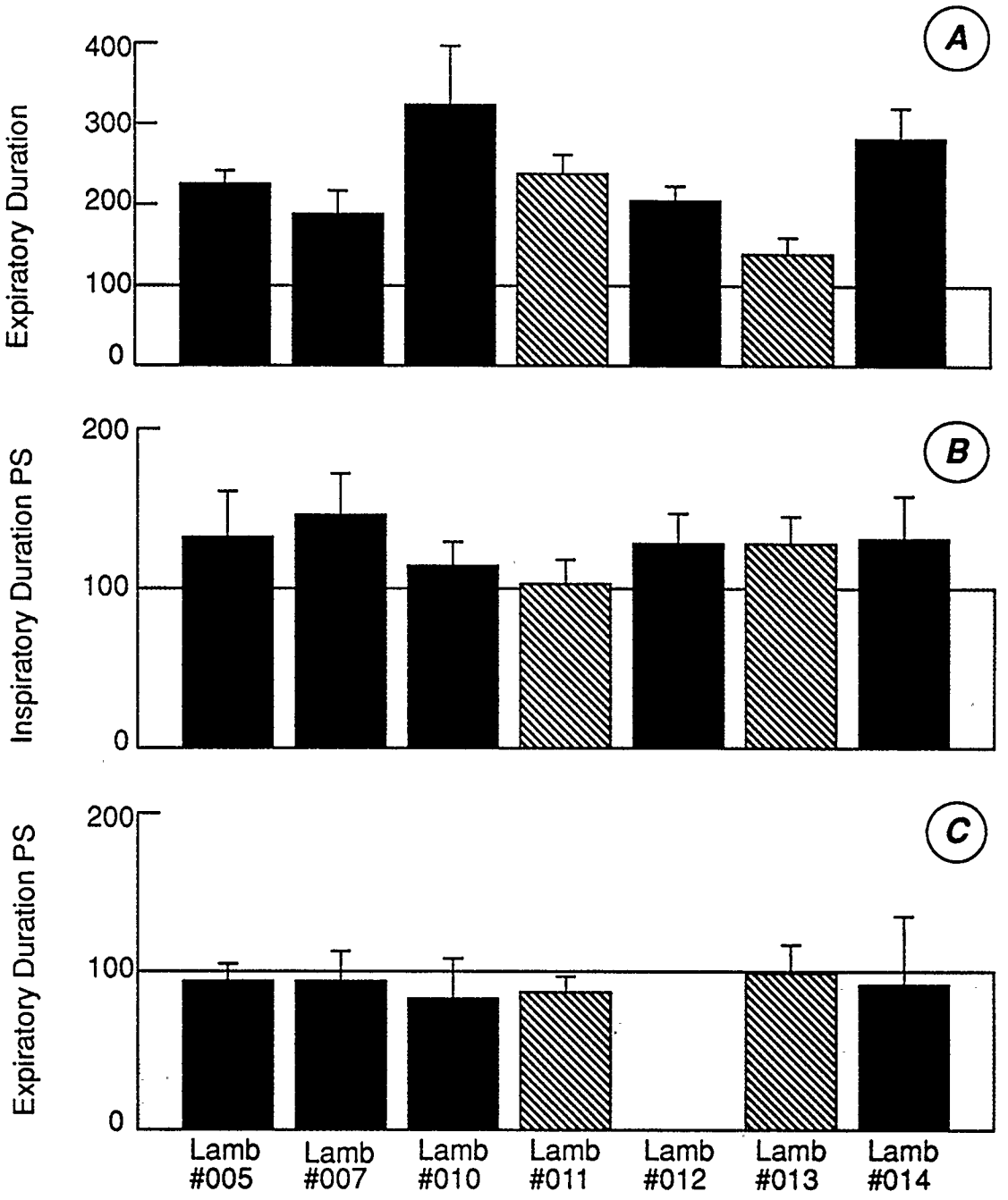

FIgure 61. Effects of an expiratory Level II stimulus on Day 10.There were significant differences in mean expiratory duration as a result of the stimulus (panel A).There were no significant differences in post stimulus (PS) phase durations (panel B and C).

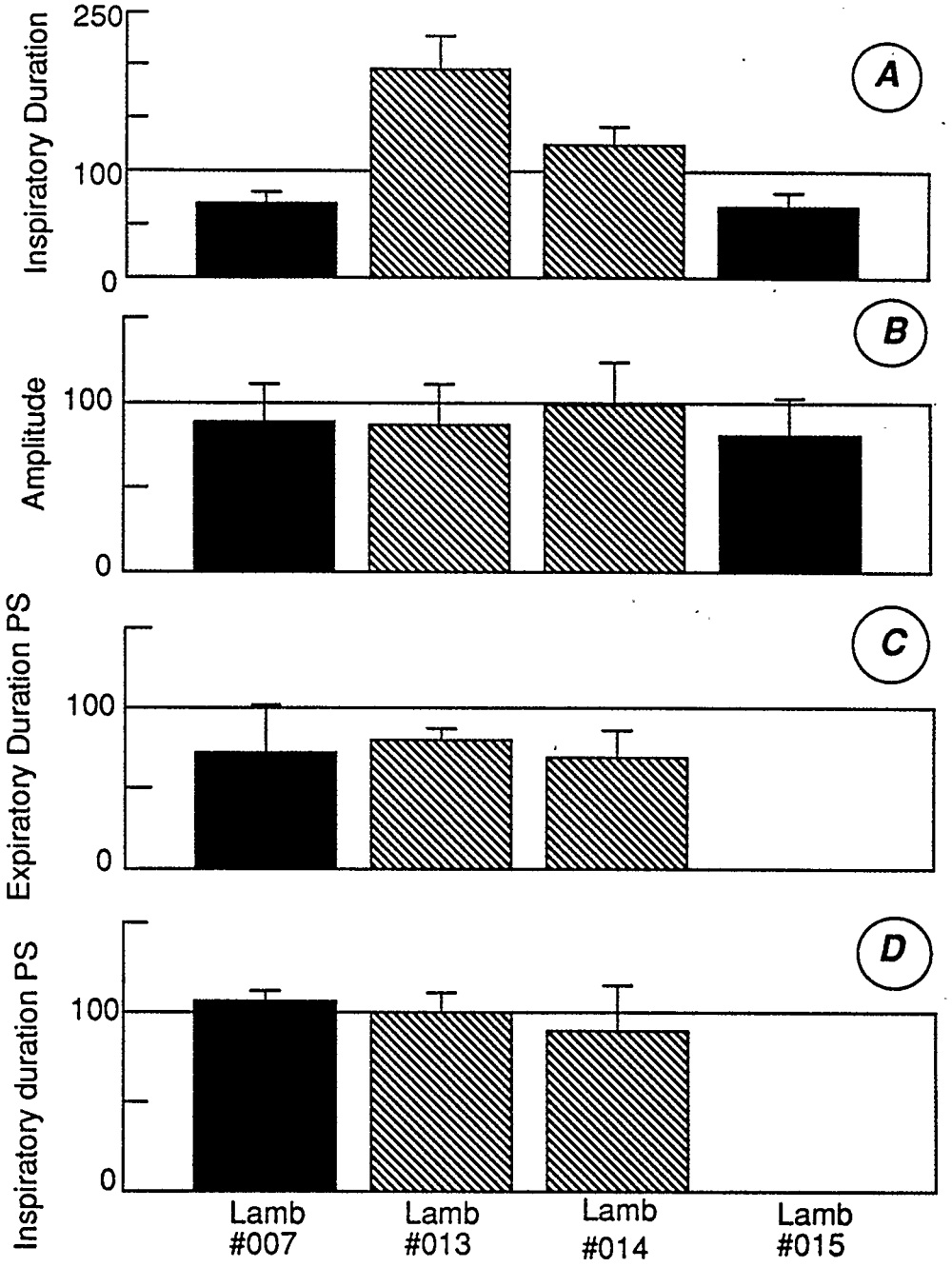

Figure 62. Effect of an inspiratory Level II stimulus on four fifteen day old lambs. There were significant differences in mean inspiratory duration. Two lambs, #013 and #014 showed inspiratory lengthening ins response to the level II stimulus. There were no significant differences between lambs in post stimulus phase duration. Post stimulus data were not available in Lamb #015.

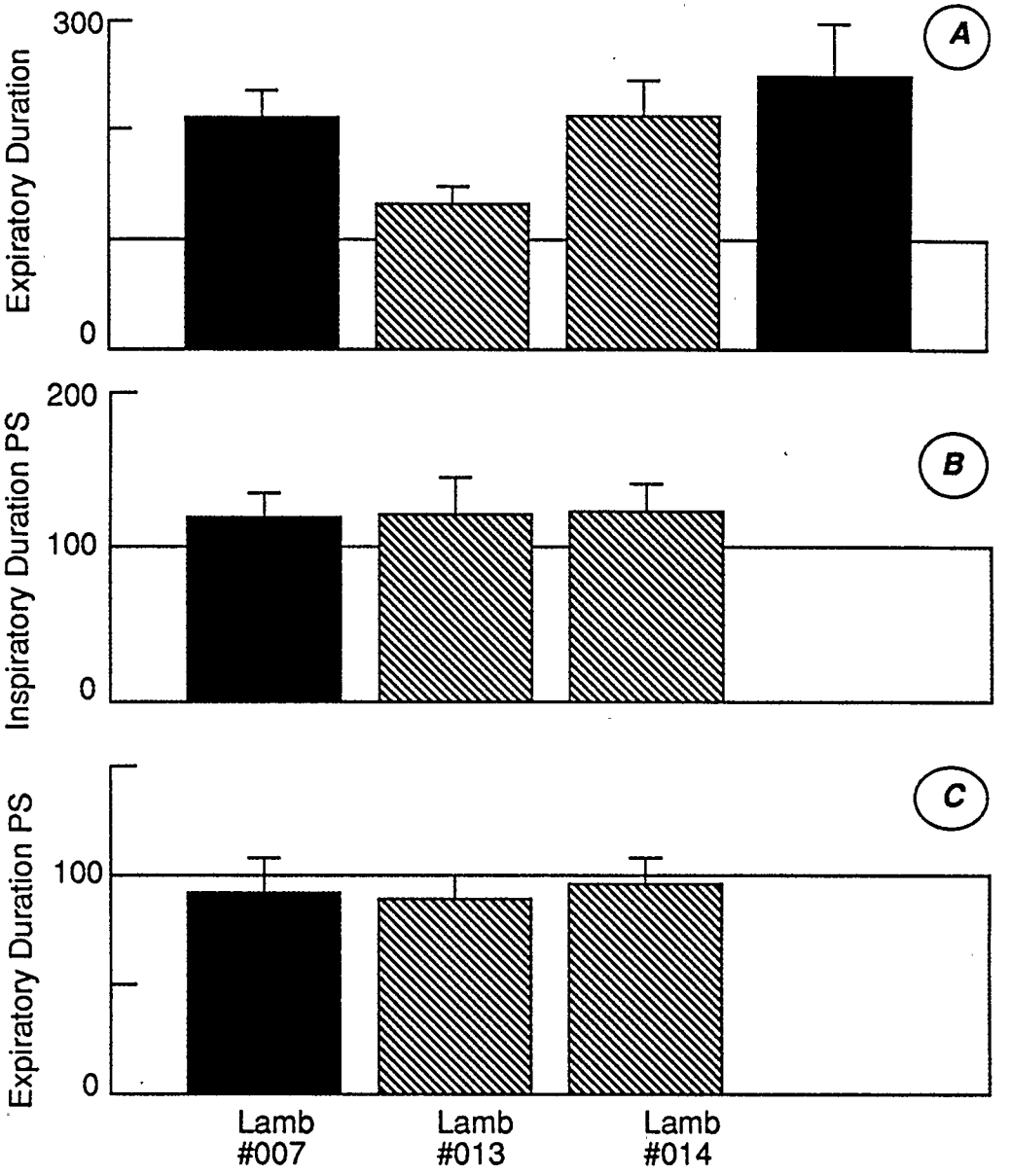

Figure 63. Effects of an expiratory Level II stimulus in four 15 day old lambs. Expiratory duration was prolonged in each lamb, but there were significant differences in mean expiratory duration. There were no significant differences in post stimulus phase durations.

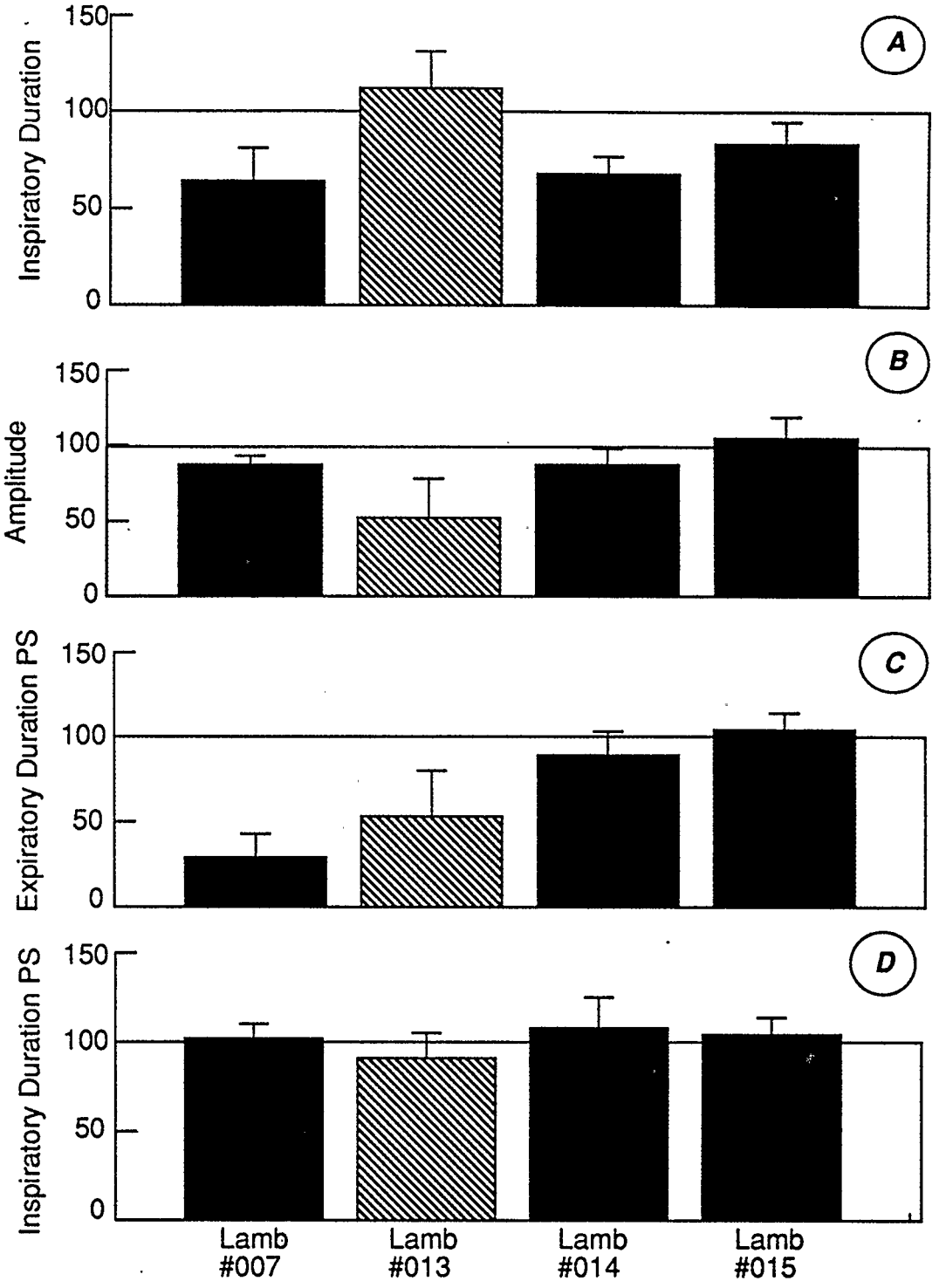

Figure 64. Effects of an inspiratory Level II stimulus in four 20 day old lambs.There were signficant differences in mean inspiratory duration, amplitude and in post stimulus expiratory duration.

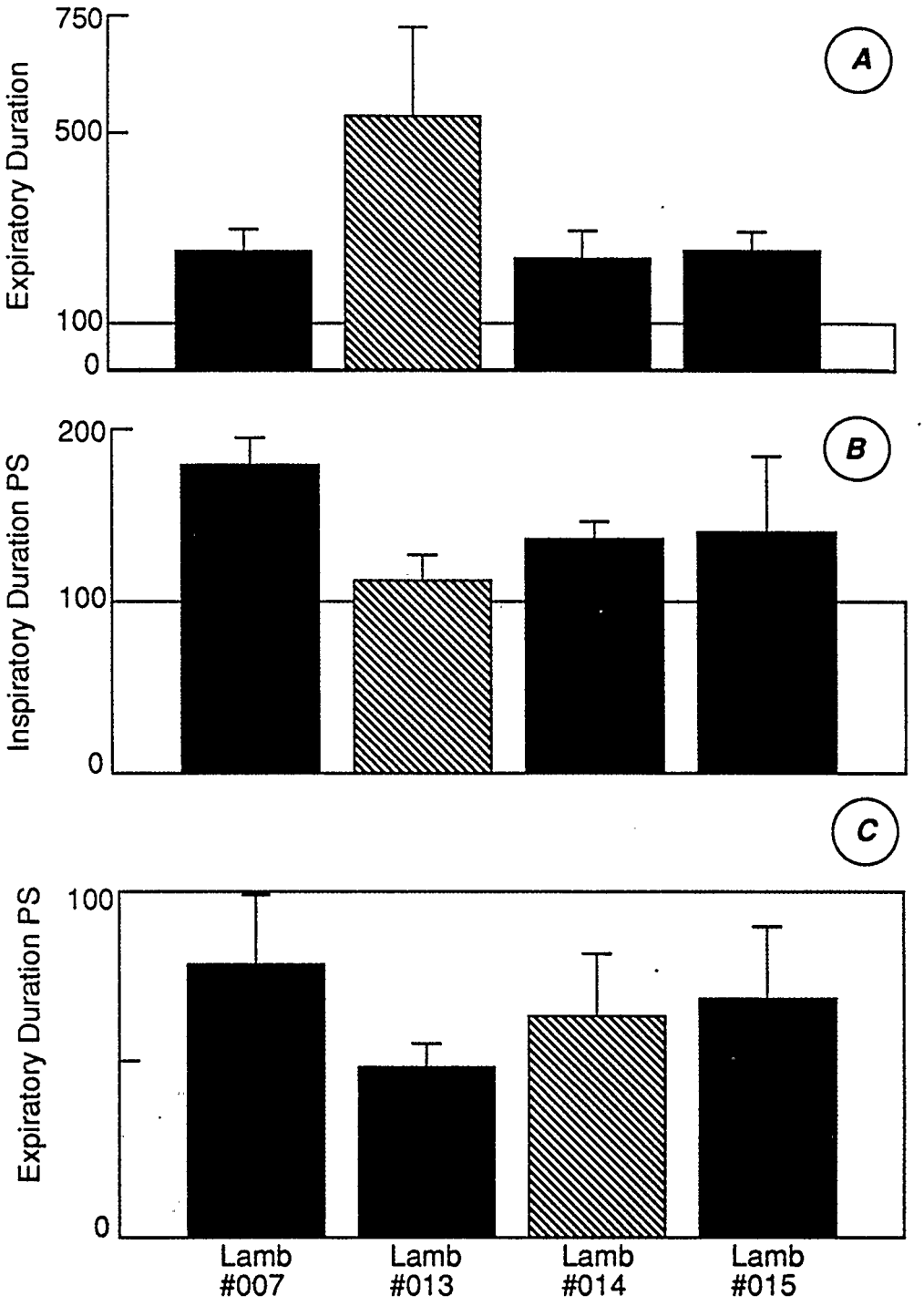

Figure 65. Effect on an expiratory Level II stimulus in four 20 day old lambs. There were significant differences in mean expiratory duration and post stimulus inspiratory duration.

(Figure 65, panel A) and post-stimulus inspiratory duration (Figure 65, panel B) during Level II expiratory stimulation.

Because mean inspiratory duration varied in both magnitude and direction, all values for inspiratory duration were pooled and presented in the histogram in Figure 66. This histogram shows that the distribution of the variability in the response was far from gaussian. Except for two measurements, the distribution was strongly bimodal, with peaks occurring at approximately -30% and +20%.

Dependence on Ti/Te

The above results show that significant differences can occur within single lambs during development or between similarly aged lambs. Figure 67 shows that in Lamb #014 changes in mean inspiratory duration (top panel) were matched by inverse changes in the Ti/Te ratio (bottom panel) but were not well correlated with Ti alone (middle panel).

For the entire population, Figure 68 shows that changes in Ti/Te were closely correlated with statistically significant changes in inspiratory duration at different ages. Thus, the variability in direction of the inspiratory response appears correlated with the Ti/Te ratio.

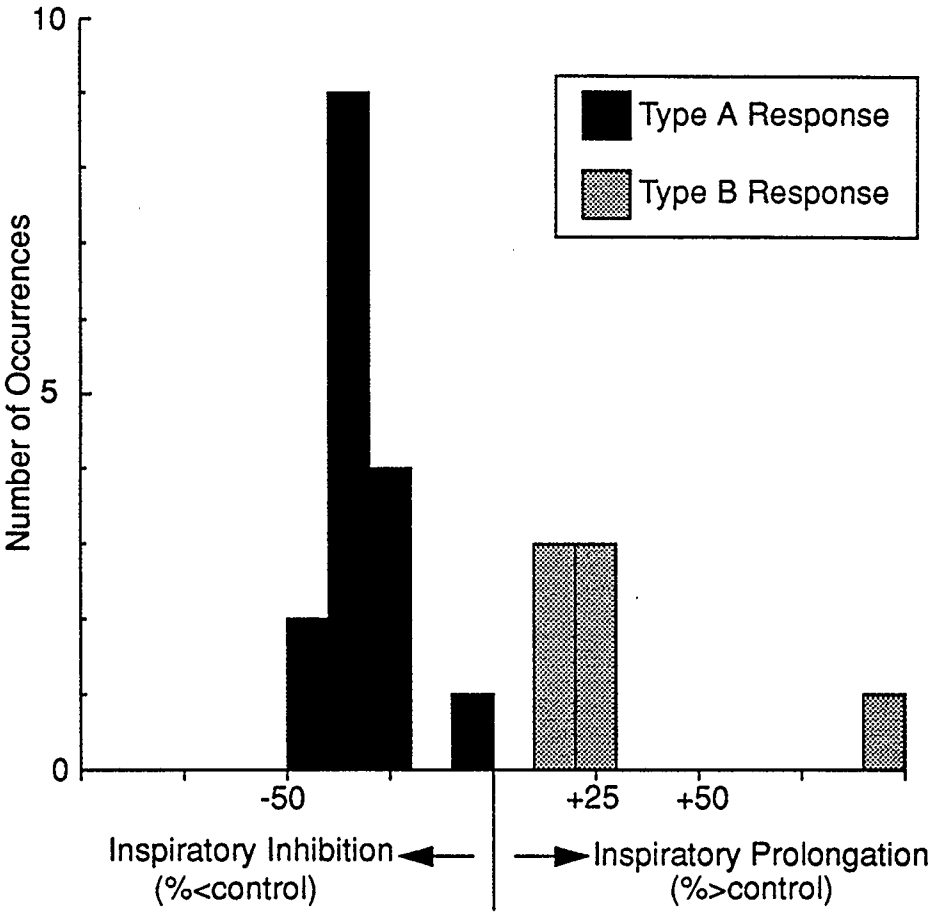

FIgure 66. Bimodality of the response to an inspiratory Level II stimulus. Lambs with the Type A response showed inspiratory inhibition while lambs with the Type B response showed inspiratory prolongation.

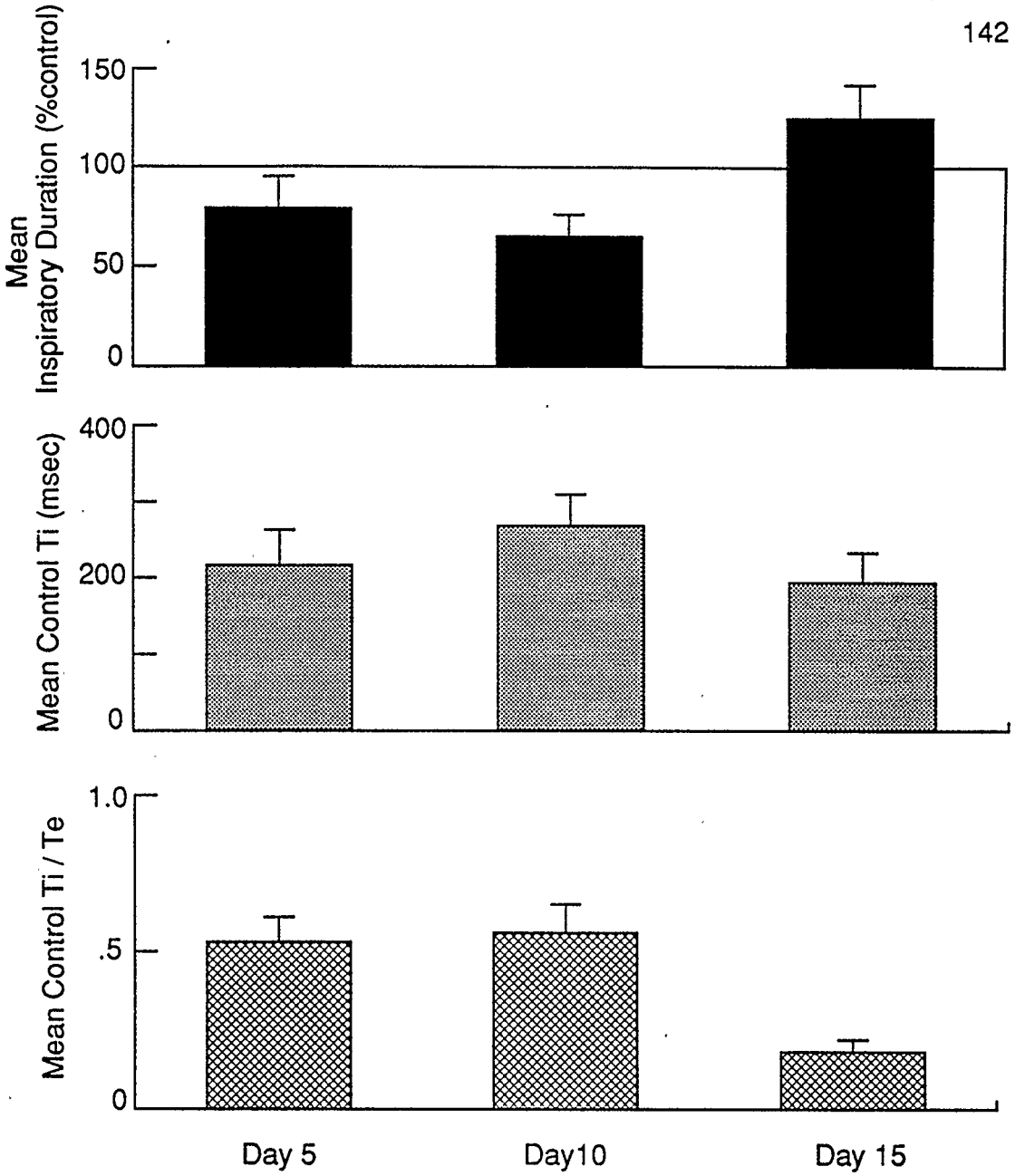

Figure 67. Relationship between changes in inspiratory duration as a function of age and other respiratory variables. On day 15, level II stimulation did not shorten inspiration. This response could not be attributed to a change in inspiratory duration (Ti) since there was no significant difference in inspiratory duration between the three days. However there was a significant decrease in the duration of the inspiratory portion of the respiratory cycle (Ti / Te). Data are from Lamb #014.

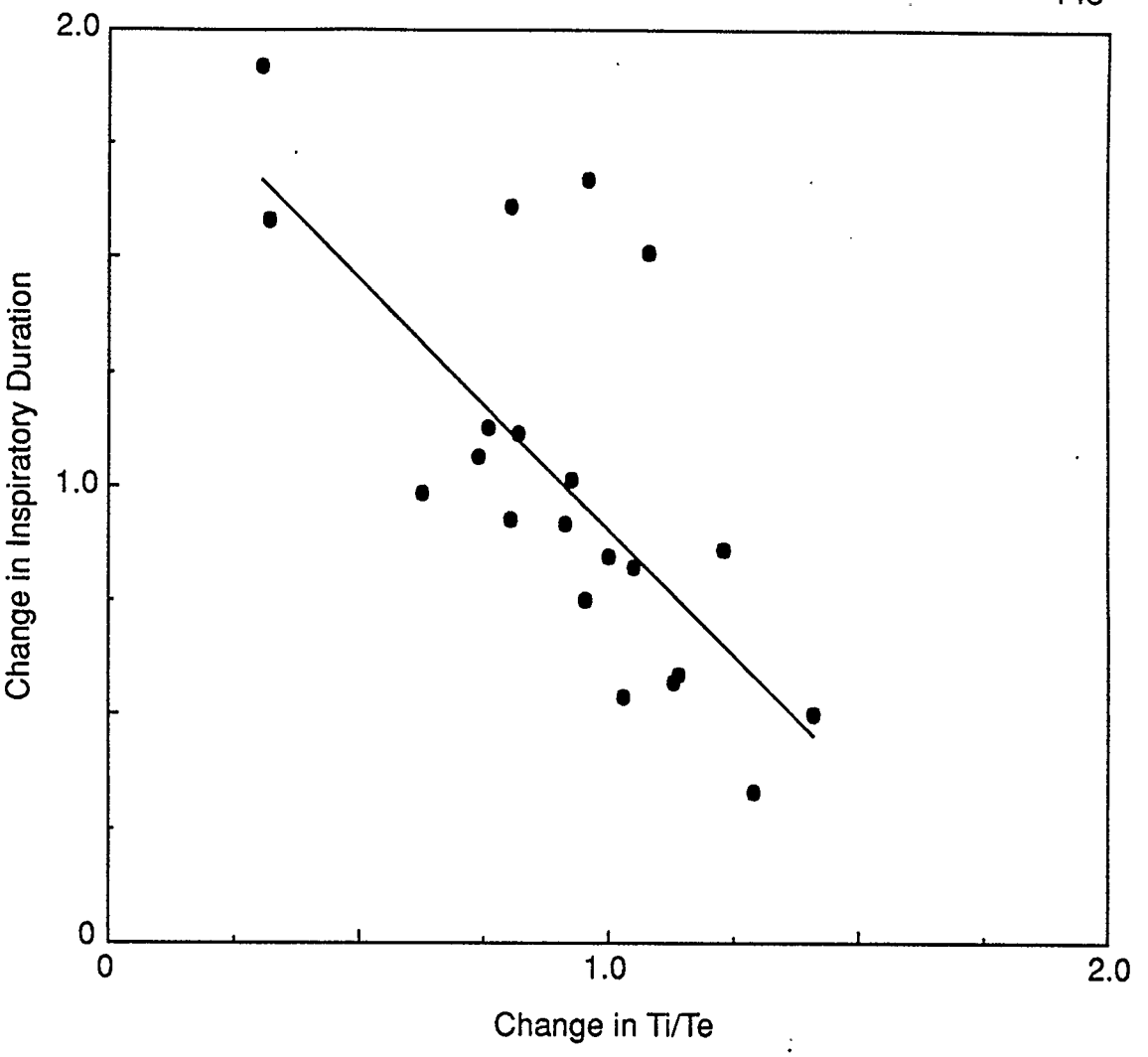

Figure 68. Changes in inspiratory duration during Level II stimulation are inversely correlated with changes in Ti/Te with age. Each point in this figure reflects the change in inspiratory duration during stimulation versus the change in Ti/Te ratio between two different ages in a single lamb. The x value is the ratio of Ti/Te on the later postnatal day to the Ti/Te value on the earlier postnatal day. Similarly, the y value is the ratio of inspiratory duration on the later postnatal day to inspiratory duration on the earlier postnatal day. For each lamb, one point was computed for each possible pair of days where there was a change in inspiratory duration.

DISCUSSION

The goal of these experiments was to independently characterize SAR activity and SAR mediated reflex activity in unanesthetized fetal and newborn lambs. The results of these experiments provided the following new information:

1) respiratory modulated vagal afferent traffic was detectable during spontaneous fetal breathing movements at 130 days gestation.

2) this respiratory modulated vagal afferent activity in the fetus at 130 days was of lesser magnitude than in the 5 day old neonate.

3) respiratory modulated vagal afferent activity increased in awake newborn lambs with increasing postnatal age.

4) graded electrical stimulation of the vagus nerve during inspiration and expiration elicited distinct respiratory reflexes which were dependent on stimulus intensity.

5) vagal stimulation had effects beyond the phase in which the stimulus was administered; these post-stimulus effects were also finely graded with stimulus intensity.

6) similarly aged newborn lambs responded differently to the same vagal stimulus because of differences in breathing pattern.

7) there were no developmental changes in the central components of the Breuer-Hering inspiratory-inhibitory or expiratory-prolonging reflex.

VAGAL ACTIVITY IN FETAL AND NEWBORN LAMBS

Fetal Recordings

The characteristics of vagal neural activity in the unanesthetized fetus in utero have not been previously documented.These studies have shown that respiratory modulated vagal activity accompanies fetal breathing movements in the sheep fetus at 130 days gestation. This respiratory modulation was identified as of afferent origin using cross correlation analysis.

The present results provide indirect evidence that the respiratory modulated vagal afferent activity in the fetus originated from slowly adapting pulmonary stretch receptors (SAR) in the airways. Activity from SAR afferents, recorded from the cut end of the vagus, dominates the vagal neurogram in other species (Paintal,1953; Patberg, 1984). Like SAR activity, the respiratory modulated activity in the fetus increased shortly after the beginning of inspiration and peaked after peak diaphragmatic EMG activity. Similar afferent respiratory modulated activity from intact vagal nerve recordings in newborn lambs has been identified as mostly SAR in origin based on the characteristics of the activity and estimates of possible contributions from other large vagal fibres (Ebly, 1986).

Cross correlation analysis identified the conduction velocity of the afferent activity that contributed to the vagal neurogram. The average conduction velocity of the afferent component of the cross correlogram was

26.3 (± 1.67) m/sec in the five fetuses. This conduction velocity was lower than the conduction velocities reported from three single fibre recordings (38, 43, and 46 m/sec) from exteriorized fetuses (Ponte & Purves, 1973).

Although SAR are tonically active at functional residual capacity in adult animals (Paintal, 1973), in the newborn, depending on the species, there is little or no discharge at FRC (e.g., Schweiler, 1968). In the unanesthetized sheep fetus cross correlation analysis revealed vagal afferent activity at end expiration during fetal breathing and during periods when the fetus was not breathing. This afferent activity had the same conduction velocity as the afferents which were active during fetal breathing, thus it is likely that these tonically active afferents represented tonic SAR activity (Paintal, 1966).

During fetal breathing, the amplitude of the afferent peak of the cross correlogram was only slightly greater than the amplitude of the cross correlogram when the fetus was not breathing. This means that the phasic afferent component was relatively small. A small phasic SAR component is consistent with the small volume fluctuations which occur during fetal breathing movements. Since the volume of fluid normally held in the fetal lung is small (Normand et al.,1971) and volume excursions during fetal breathing measured per minute or hour are also small (Maloney et al., 1975; Harding,1980) the stimulus to the receptors may be minimal. Large volume changes, despite the high compliance of the fluid filled fetal lung (Polgar & Weng, 1979) may not occur because the larynx provides substantial resistance to fluid flow to the lungs during normal fetal breathing (Fewell & Johnson, 1983). The lack of a

substantial difference between the phasic and tonic levels of SAR afferent activity could be attributed to low stimulus levels at the receptor site.

The significance of fetal breathing movements is unclear (Jansen & Cherniak, 1991). Fetal respiratory movements are important for the normal development of the lungs (e.g.,Fewell et al., 1981). The results of my experiments have shown that at 130 days gestation, fetal breathing movements are accompanied by respiratory modulated afferent activity. While modulated afferent activity may be present when fetal breathing movements first occur earlier in gestation and through term at 147 days, the data from these experiments represents only fetuses at 130 days gestation. The source of this respiratory modulated afferent activity is most likely SAR.

The significance of SAR activity in the fetus is also unclear. Dawes et al., (1972) claimed that vagotomy did not affect fetal breathing movements. Since gas exchange does not occur in the lung, there may be no reason for SAR to regulate the pattern of respiration. On the other hand, since peripheral chemoreceptors are not receiving information about events in the lung, the CNS's only source for information about lung events may be from SAR mechanoreceptors. More recently, Hasan & Rigaux (1990) have reported that vagotomy does affect fetal breathing by decreasing the duration of fetal breathing periods. In contrast to the adult animal, vagotomy in the fetus decreased inspiratory duration during fetal breathing (Hasan, personal communication).

Fetuses born without vagi may be unable to breath after birth. Rigatto et al., (1988) found that chemodenervated (bilateral sinal, superior laryngeal

and vagal section) fetuses were unable to sustain breathing at birth. The chemodenervated (sinal section only) fetuses in the study of Jansen et al., (1981) were able to sustain breathing at birth. Though nerve section may have compromised the airways in the former study, vagotomy in fetal sheep (Hasan, personal communication) resulted in newborns who were unable to sustain breathing. Herrington et al., (1971) have shown that lambs which were vagotomized before delivery had drastically reduced respiratory rate which they compensated for with increased tidal volumes.

Loss of sensory information from vagal afferents could affect development in the CNS. During certain phases of development, some cells depend on afferent information for their survival. Removal of afferents can increase cell death in brainstem nuclei. Some cells become less sensitive to the removal of afferents during later phases of development (Parks, 1979; Levi-Montalcini, 1949) thus it is possible that vagotomy in the fetus at an earlier gestational age may have more drastic effects neonatally because it would be depriving the central nervous system of sensory input from the lungs earlier in development. Vagal afferent activity from SAR, associated with fetal breathing movements, may be important for the formation or facilitation of synapses which are necessary for breathing at birth. Synaptic formation (e.g., Kalil, 1990) and interactions (e.g., Shatz, 1990) in the developing nervous system are activity dependent. Stabilization of synaptic connections requires that they be electrically active during development (Changeux & Danchin, 1976). If vagotomy occurs before sufficient electrical activity has occurred, synaptic connections necessary for breathing after birth may be compromised.

Comparison of Fetal and Neonatal Recordings

The characteristics of the signals recorded from the intact vagus nerve in the unanesthetized fetal and the neonatal lamb were compared. In both the fetus and the neonate the averaged RLPF vagal neurogram increased during inspiration and decreased during expiration. Cross correlation analysis in each case yielded an afferent peak. The afferent peak of the cross correlogram had the same conduction velocity as the second negative component of the CAP.

Analysis of whole nerve recordings obtained from the fetal vagus yielded results that were similar to those obtained from the neonatal vagus, but smaller in magnitude. The amplitude of SAR respiratory modulated vagal activity in the fetus was significantly less than the amplitude of SAR respiratory modulated vagal activity in the 5 day old neonate. These findings are in contrast to the findings of Ponte & Purves (1973) who found that multifibre recordings of SAR activity in the exteriorized fetus had the same frequency of discharge as those obtained from the adult sheep.

Several factors may have contributed to the differences in the magnitude of presumed SAR activity between the 130 day old fetus and the 5 day old newborn. It is important to exclude the possibility that differences in the recording configuration were responsible for the observed differences in the magnitude of the signals. Analysis of compound action potentials in fetuses and neonates showed that there were difference in the quality of recording, but that these differences were opposite in direction to those which would have

explained the results. That is, despite a better recording situation in the fetus, the signals recorded from the neonate were still much larger. Thus the differences in the magnitude of the signal recorded in the fetus and the neonate were not due to artefactual differences in recording the signal.

There are striking differences in the breathing milieu of the fetus and the neonate. In the intrauterine environment, the breathing movements of the fetus, unlike those of the neonate, are unrelated to gas exchange. The fetal lung, which is filled to 40% of total lung capacity with liquid (Scarpelli et al., 1975) and has a high compliance (Peters, 1969; Polgar & Weng, 1979). Only small volume excursions occur with each fetal breathing movement (e.g., Maloney et al., 1975). Since SAR activity is related to lung volume and inversely related to lung compliance (Sellick & Widdicombe, 1970) it is possible to predict that SAR activity in the fetus will be low. The neonate breathes much larger tidal volumes than the fetus .The neonatal lung is filled with air and the air-surfactant interface of the neonatal lung makes it much less compliant than the fetal lung (Mortola et al., 1985). Low compliance means that greater pressures are required to inflate the lungs to the same volume. Thus, based on the inspired volume and compliance of the newborn, we can predict that SAR activity will be greater in the newborn than in the fetus.

If myelination in the 130 day old fetus is not complete this also suggests a reason for a smaller signal. Recent evidence from Hasan et al., (1991) suggests that by 135-137 days gestations the fetal sheep has the same proportion of myelinated vagal fibres as the adult sheep. Since large axons are the first to be myelinated in development (Ritchie, 1984) at 130 days gestation

there is a good chance that SAR fibres, which are large, are already myelinated.

The average conduction velocity of the afferent peak of the cross correlogram was slower in fetuses at 130 days than in the 5 day old newborn lambs (26.3 m/sec vs. 33.3 m/sec). The greater conduction velocity in the neonate could be due to increased myelination or increased nerve fibre diameter. Conduction velocity is proportional to the diameter of the nerve fibre and the extracellular current recorded is proportional to the diameter or squared diameter of the nerve. Hence, the difference in conduction velocity would account for a signal that was 12-25% greater in the neonate than in the fetus. Since the amplitude of the signal in the neonate was almost five times as large as the amplitude of the signal in the fetus, the increased conduction velocity in the neonate does not sufficiently explain the difference in the amplitude of the signals.

Lastly, differences in behavioral state between the fetal and newborn lambs may have also contributed to the observed differences in SAR activity. Vagal recordings from neonates took place during quiet wakefulness while fetal recordings occurred during fetal breathing movements in REM sleep. In adult animals sleep related differences in vagal afferent activity have been suggested (Sullivan et al., 1978). Finer et al., (1976) have suggested that stretch receptor feedback is reduced in infants during REM sleep. During REM sleep resting lung volume decreases by approximately one tidal volume; this could lead to a decrease in the firing rate of SAR (Henderson-Smart & Read, 1979). If similar changes in resting lung volume also occur with changes of

state in the fetus then SAR activity in the fetus might have been somewhat greater during periods of fetal wakefulness.

The timing of SAR activity relative to the peak activity of the diaphragm EMG was different in the fetus and in the neonate. The peak of the averaged RLPF vagal neurogram followed the peak in the averaged RLPF diaphragm EMG by an average of 130 msec in the fetus and by an average of 80 msec in the newborn lamb. A delay in peak SAR activity with respect to peak diaphragm EMG activity is reasonable because of the time it takes for the electrical activity of the diaphragm to translate to force and because of the resistance and compliance characteristics of the respiratory system. Several factors may contribute to the greater delay in peak SAR activity relative to peak diaphragm EMG in the fetus. First, the upper airway provides resistance to flow, this resistance causes a delay in the stimulus to SAR which are located in the airway smooth muscle. Secondly, in premature infants, the airway is more compliant than in a term infant (Bhutani et al., 1981). If the airway of the fetal lamb had characteristics similar to the premature infant, this increased compliance may have contributed to the increased delay in the peak activity of the RLPF vagal neurogram. Finally, it is also possible that the twitch-contraction time of diaphragm muscle fibres could decrease with age; therefore it would take less time for diaphragm EMG to translate to pressure.

Neonatal Recordings

The amplitude of respiratory modulated vagal afferent activity, increased as a function of age in the newborn lamb. There are several possible explanations for an observed increase in respiratory modulated SAR activity with increasing age in the newborn. First, I will rule out the possibility that the increase in amplitude observed was merely a function of an improvement in the ability of the electrodes to record a signal and second, I will discuss the physiological factors which might account for developmental increases in SAR activity.

An increase in the amplitude of SAR modulated activity could be due strictly to an improvement in the recording configuration of the electrodes. The voltage recorded from the electrodes is the product of the extracellular current produced by the nerve and the impedance (generalized resistance) of the recording electrodes. Therefore, increases in the voltage recorded could be due to increases in extracellular current, increases in the impedance of the electrodes, or increases in both. Stein et al., (1978) have argued that impedance measurements from similar electrodes remain constant over time - except for an increase which may occur in the first few days due to the replacement of fluid in the cuff with higher resistance connective tissue. Although small fluctuations in the impedance of the electrodes occurred, there was no consistent relationship between these fluctuations and the amplitude

of the modulated neurogram implying that changes in impedance did not contribute to the changes in SAR activity observed.

The increase in the amplitude of the RLPF neurogram with age could reflect an increase in the discharge frequency of the receptors or the recruitment of additional fibres. Increases in the single fibre activity of SAR with development have been observed in puppies (Fisher & Sant'Ambrogio, 1982), kittens (e.g., Marlot & Duron, 1979), rabbits pups (Schweiler, 1968) and opossums (Farber et al., 1984). In these species, which are less mature at birth than the lamb, the increase in activity may have been at least partially attributable to increases (growth) in the diameter of nerve fibres (e.g., Farber, 1984). In the newborn lamb, the number of myelinated fibres is already comparable to the adult level at birth (Hasan et al, 1991). The CAP recorded in newborn lambs did not change significantly in magnitude or conduction velocity. Since the CAP represents the algebraic sum of all the action potentials that can be evoked at a particular stimulus intensity and reflects the number and size of vagal action potentials, comparison of CAPs at maximal stimulation levels would have revealed whether significant changes in number or myelination of axons had occurred.

The lungs, which were very compliant when they were fluid filled in the fetus, are less compliant per unit volume in the newborn than in the adult of some species (e.g.,Fisher & Mortola, 1980; Shaffer et al., 1985). The lungs become more compliant with increasing age due to changes in the arrangement and physicochemical properties of elastin and collagen fibres in the lung (Polgar & Weng,1979). If SAR activity were inversely related to lung

compliance as reported by Sellick & Widdicombe (1970) low lung compliance in the newborn compared to adult would predict greater SAR activity in the newborn because greater pressures would be required to inflate the lungs. Since SAR discharge is linearly related to transpulmonary pressure, the results from this study, if we assume that the characteristics of the receptor remained the same, are consistent with an increase in transpulmonary pressure with age.

Although changes in transpulmonary pressure could explain the observed increase in SAR activity with age, in children, transpulmonary pressures can be the same as those generated in the adult (Peters, 1969). If we extrapolate these data to the newborn lambs, then it is possible that similar transpulmonary pressures were generated on each recording day, thus it is possible that the observed increase in SAR activity with age cannot be attributed to gradual increases in transpulmonary pressure with age.

Newborns use grunting or post inspiratory activity of the diaphragm to maintain an elevated end expiratory lung volume (Mortola, et al., 1985). This may reduce the amplitude of tidal volume excursions in young lambs. As the animal matures, and end expiratory lung volume is no longer elevated larger tidal volumes become possible.

Sant'Ambrogio (1987) has suggested another possible reason for increases in SAR activity with increasing age. He suggests that there is a different mechanical coupling between the membranous backwall of the trachea and the more compliant U-shaped cartilages that leads to a lower mechanical tension at the receptor site in the newborn than in the adult. This would

explain why the SAR signal in the newborn is small despite relatively potentially large transpulmonary pressures.

If the lambs had not been sufficiently recovered from surgery during the first recording days, pain, in particular that from the incision for the placement of the diaphragm EMG electrodes, might have caused them to take smaller breaths. A smaller respiratory effort would have resulted in smaller inspired volume producing a smaller stimulus to the receptor. Less receptor stimulation would have resulted in fewer vagal actions potentials and a smaller RLPF modulated signal. However, this seems unlikely because the changes in the amplitude were gradual and continued far longer than the expected recovery period.

Another consideration is that the axons may have been traumatized during the surgical manipulations used to implant the nerve cuffs. If this had been the case the CAP recorded would have increased in magnitude as the injured axons recovered and resumed normal activity. Since the CAP did not show a gradual increase in magnitude with increasing age this rules out the possibility that axons were traumatized, increased in number or grew in diameter. Furthermore, if the nerve cuff recording electrodes damaged the nerve in some way by becoming tighter with growth and blocking some axons, these changes were also not indicated by the area of the compound action potential except in the one example provided (Lamb #004). If nerve damage had occurred, the findings from this study would be an underestimate of the magnitude of changes that actually occurred.

EFFECTS OF VAGAL STIMULATION

The effects of graded electrical stimulation of the vagus nerve have not been previously described in conscious newborn animals. Electrical stimulation was used in order to characterize the central responses to SAR input because it was simple to administer and had the potential to be extremely reproducible.

Interpretation of the stimulus intensity

Preferential stimulation of functionally homogeneous vagal afferents using electrical stimulation of the whole vagus nerve is not feasible because of the overlap in conduction velocities of vagal fibres, which arise from and innervate a variety of, as well as the same, organ systems.

Previous studies using whole nerve stimulation were done in animals in which less vagal conduction distance was available than in the newborn lamb. In the newborn lamb, the length of the cervical vagus allowed large distances between recording and stimulating electrode; thus providing more conduction distance for the dispersion of vagal action potentials. As a result, the vagal compound action potential that evolved as stimulus intensity increased had a more complex shape than has been previously reported in previous studies using electrical stimulation (cf. Feldman & Gautier, 1976).

In the newborn lamb, the vagal CAP consisted of three components. These three components arose from different populations of vagal fibres and

overlap in the conduction velocity of functionally different fibres occurred (Paintal, 1966). When the amplitude of each component of the CAP in the newborn lamb was plotted as a function of stimulus intensity there was a good correlation between the effects of electrical stimulation and the shape of the CAP.

However, while the amplitude of the individual CAP components shows the range of stimulus intensities over which similarly conducting fibre groups were recruited, this amplitude may not have completely reflected the number of fibres being recruited at the highest intensity for each component. For example, in Figure 43, further expiratory prolongation occurred beyond the intensity where the amplitude of the second component had plateaued. It is possible that a further increase in the amplitude of this component was obscured by the recruitment of slower conduction velocity axons. The positive deflections of action potentials from slower conduction velocity axons may have partially cancelled the negative deflections of faster conduction velocity axons which would have contributed to the second component of the CAP. Thus the amplitude of the second component at 3.0 times threshold in Figure 43 may have been underestimated. However, the increase in expiratory prolongation at 3.0 times threshold could also be explained by some subtle change in state (Frankstein, 1970; Nadel et al., 1973; Younes et al., 1974)

In these experiments, the responses to electrical stimulation at an intensity corresponding to a particular component of the CAP, could only be attributed to the activity of a particular type of receptor strictly by inference, considering the characteristics of both the receptor and the response induced

by stimulation (Sant'Ambrogio & Remmers, 1985). The first component of the CAP represented vagal fibres with the fastest conduction velocity. Stimulation at intensities corresponding to this component had little affect on inspiratory and expiratory duration. Paintal (1966) recorded from single vagal fibres and reported that the fastest conducting fibres in the vagus were motor fibres from the recurrent laryngeal (RL) nerve. In lambs, stimulation at Level I, the intensity where the amplitude of the first component of the CAP was maximal should have revealed the maximal effects of stimulating these fast conducting fibres. In spite of the possibility of exciting laryngeal receptors through laryngeal muscle contractions, stimulation failed to evoke a respiratory response.

The second component of the CAP probably included a group of afferent fibres from presumed SAR. The effects of stimulating at the intensity where this component was maximal (Level II) were consistent with the reflex effects of SAR stimulation described in the literature (e.g., Gautier et al., 1981). In addition the conduction velocity of the second component of the CAP was virtually identical to the conduction velocity of the respiratory modulated afferent component of the cross correlogram, strongly suggesting that it too represented respiratory modulated vagal afferents.

The third component of the CAP had a slower conduction velocity than the presumed SAR component and probably included a population of fibres corresponding to rapidly adapting or irritant receptors (IR). The effects of stimulation at intensities when this component was present were consistent with the reflex effects of IR stimulation described in anaesthetized animals (e.g., Knox, 1973).

Although the identity of the three components of the vagal CAP is purely by inference, in the remaining text these components of the CAP will be referred to as the presumed "RL" component, the presumed "SAR" component and the presumed "IR" component even though they have not conclusively been identified as such in the present work. It is also important to note the extreme unlikelihood of any one of these components representing a completely functionally homogeneous group of fibres, rather each component represents a fibre group with similar conduction velocities. The fibres could have different origins. That is, the so called "RL" component is likely to contain some of the more rapidly conducting "SAR" as well as other fibres, and so forth. Furthermore, beyond the very lowest intensities of stimulation, more than one group of fibres were stimulated at each stimulus intensity. Level II stimulation, which corresponded to the maximal amplitude of the "SAR" component also stimulated "RL" motor fibres. Level III stimulation, which corresponded to the maximal amplitude of the presumed "IR" component stimulated the two other more rapidly conducting fibre groups as well. Stimulus intensities below Level III, where the "IR" component was smaller may not have produced IR effects because a threshold stimulation level, in terms of number of fibres, had not been achieved.

Thus, despite the overlap in conduction velocity of functionally different vagal fibres and contributions from non respiratory vagal fibres it was nevertheless possible to attribute reproducible, well defined respiratory responses to a selective response of the central nervous system to the stimulation of particular, similarly conducting, vagal fibre groups.

"SAR" Stimulus Effects

The inspiratory effects of "SAR" stimulation were dependent on the prestimulus breathing pattern. If inspiratory duration was greater than 47% of expiratory duration the effects of "SAR" stimulation were graded and inspiratory duration decreased as the amplitude of the "SAR" component (ie.,number of fibres recruited) of the CAP increased (Type A response). The decrease in inspiratory duration with "SAR" stimulation was consistent with the results of studies using electrical stimulation of the cut end of the vagus (e.g., D'Angelo, 1979; Feldman & Gautier, 1976) and lung inflation (Olinsky et al., 1974) to excite "SAR" and evoke the inspiratory inhibitory Breuer-Hering reflex. Previous studies using vagal stimulation did not use finely graded increments in stimulus intensity. This study has shown that in unanesthetized newborn lambs decreases in inspiratory duration with "SAR" stimulation are finely graded in response to increasing "SAR" stimulus intensity.

The amount that inspiration was decreased by a Level II or plateau "SAR" stimulus depended on the duration of inspiration in the breaths preceding the stimulus. When prestimulus inspiratory duration was long, the "SAR" stimulus was more effective in decreasing inspiratory duration than when prestimulus inspiratory duration was short. Similar findings were described by von Euler and Trippenbach (1976). These investigators found that when breathing frequency increased, larger volume stimuli were needed to terminate inspiration.

A potential explanation for a greater "SAR" stimulus requirement with shorter inspiratory times is presented in the work of Boyd and Hillenbrand (1937). These early studies define the inspiratory terminating threshold as the minimum number of volleys or stimulation time required to terminate inspiration. Using vagal stimulation during inspiration at a frequency of 120 Hz, these workers found that threshold for inspiratory termination was never less than 30 volleys. Thus, if stimulation frequency and current intensity are constant then breaths with shorter inspiratory durations receive fewer stimuli than breaths of longer duration and hence, can achieve less of an "SAR" mediated decrease in inspiratory duration.

When inspiratory duration was less than 47% of expiration "SAR" stimulation during inspiration did not decrease inspiratory duration but rather increased it (Type B response). This seemingly aberrant phenomenon of inspiratory prolongation at intensities of stimulation which were expected to produce inspiratory shortening has similarities to findings by other investigators (D'Angelo, 1979; Younes & Polacheck, 1985; Bruce et al 1982). Younes and Polacheck (1985) administered brief inspiratory stimuli which resulted in inspiratory prolongation, with decreased amplitude and slope of inspiratory output. However, these previous findings of inspiratory prolongation with an inspiratory stimulus are not strictly comparable because the stimulus employed was either very brief or intentionally below the threshold required to terminate inspiration. In the experiments with newborn lambs, the failure of the "SAR" stimulus to decrease inspiratory duration (Type B response) in some lambs however, was not the result of a subthreshold stimulus because further

increments in stimulus intensity still did not decrease inspiratory duration. Furthermore, the vagal stimulus used was the same strength and relative duration (throughout inspiration) as the "SAR" stimuli which were effective in decreasing inspiratory duration. Thus, maximal "SAR" stimulation during inspiration resulted in two different responses even in similarly aged lambs, whereas previous work in anesthetized animals showed differences in the response to inspiratory stimulation occurred when the characteristics of the stimulus were altered.

The two effects in response to electrical stimulation of "SAR" during inspiration are suggestive of parallel central processing pathways with opposite effects; one pathway decreases the duration of inspiration while the other pathway increases the duration of inspiration in response to the same stimulus (Figure 69A). The effects of the two pathways are superimposed, however normally, the effects of the inspiratory prolonging pathway are completely overshadowed by the enormity of the effect of the inspiratory inhibitory pathway (Younes & Polacheck, 1981). In the experiments performed by Younes and Polacheck (1985), the inspiratory prolonging pathway was revealed with prolonged stimulation over many breaths either as a consequence of buildup in that pathway or adaptation of the inspiratory inhibitory pathway. These investigators suggest that the simultaneous activation of pathways of different strength with different time courses are responsible for the within phase and post-stimulus effects of "SAR" stimulation.

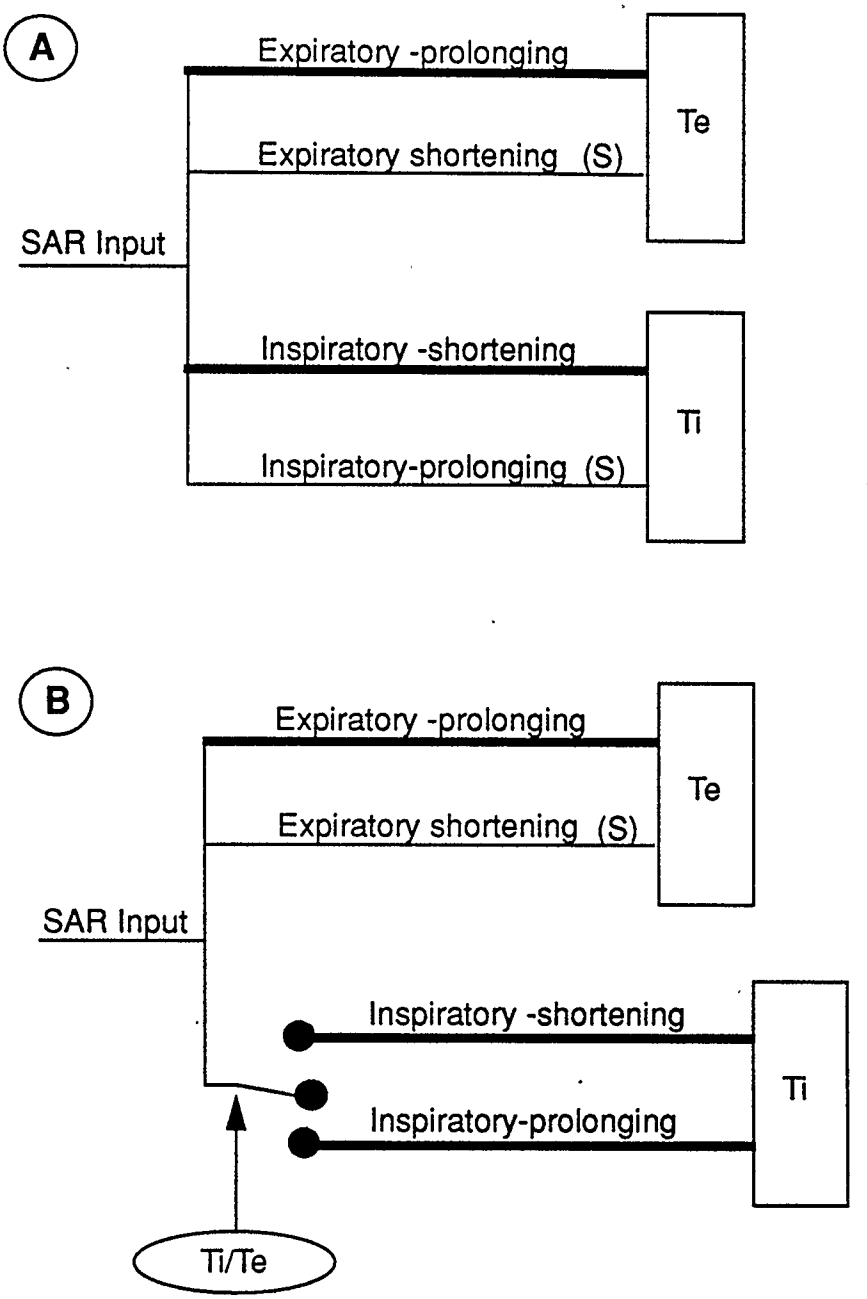

Figure 69. Two models for the processing of SAR input. Model A is suggested by the work of Younes & Polachek (1985). Model B may explain the finding in the awake newborn lamb. (S) is indicative of a pathway with a slow time constant.

My experiments have also shown inspiratory prolongation in response to maximal SAR stimulation during inspiration. The failure of the inspiratory SAR stimulus to decrease inspiratory duration (Type B response) in unanesthetized newborn lambs was related to the prestimulus ratio of inspiratory to expiratory duration (Ti/Te). When the Ti/Te ratio was low, the inspiratory SAR stimulus was ineffective in terminating inspiration. When the Ti/Te ratio was high, the inspiratory "SAR" stimulus decreased inspiratory duration. Thus the results of my experiments have revealed a variable which determines which of the two pathways - inspiratory inhibitory or inspiratory prolonging - dominates.

Furthermore, the form of the dependence on the Ti/Te ratio revealed by my data suggests that the two pathways do not shift gain as a function of Ti/Te ration, but switch to one or the other. We can then suggest a single functional pathway whose sign can be switched by the Ti/Te ratio; thus Ti/Te ratios greater than .47 produce inspiratory shortening, and Ti/Te ratios below .47 produces inspiratory prolongation. Figure 69B shows a simple representation of such a pathway. The functionally single pathway actually consist of two pathways which are mutually inhibitory; only one pathway can be operational at any particular time. One pathway is stronger and inhibits the other pathway. Changes in the Ti/Te ratio flip the switch from one pathway to the other. This is a potential explanation for why equivalent "SAR" information does not have equivalent effects depending on the breathing pattern.

The post-stimulus effects of inspiratory SAR stimulation were also graded with the intensity of stimulation. Decreased inspiratory duration in response to within phase "SAR" stimulation was followed by decreased post-stimulus expiratory duration. That is, the expiratory duration of the first phase following the inspiratory stimulus was decreased, despite the fact there was no stimulus during that phase. Since the within-phase effect of "SAR" stimulation during expiration was expiratory prolongation, the post stimulus effects of an inspiratory "SAR" stimulus on expiratory duration occur in the same direction as the within phase effect of the inspiratory stimulus, but in the opposite direction to the within phase effect of an "SAR" expiratory stimulus.

A post-stimulus decrease in expiratory duration occurred even in the lambs who did not show a decrease in inspiratory duration with "SAR" stimulation (Type B response). Similar results are described after a brief subthreshold SAR stimulus, however post-stimulus expiratory duration was decreased less (-3.86%) than it was after a stimulus which was threshold for decreasing inspiratory duration (-21.4%) (Younes & Polacheck, 1985). Data from the unanesthetized lambs used in my research show that the post-stimulus effects after a Type B response (inspiratory prolonging with "SAR" stimulation) could be as strong as the post-stimulus effects after a Type A response. This confirms earlier finds of Younes and Polacheck (1981) suggesting that the post-stimulus expiratory duration is related to the presence of SAR input and not necessarily to inspiratory inhibition. Since the "SAR" inspiratory stimulus used in the lambs was of longer duration and had greater post-stimulus effects than those described by Younes & Polacheck (1985), this

suggests that the post-stimulus effect, though not dependent on inspiratory shortening, is correlated to the magnitude of SAR input.

The post-stimulus effects of the inspiratory "SAR" stimulus can also be explained using the model presented in Figure 69B. If the "SAR" stimulus simultaneously activates inspiratory and expiratory pathways then the presence of post-stimulus effects reflects the sustained activation of the longer time course expiratory shortening pathway, once the dominant effects of the stimulus have decayed. This model does not explain the increase in post-stimulus inspiratory duration seen by Younes & Polacheck (1985). In newborn lambs post-stimulus effects on mean inspiratory duration in the second phase after an "SAR" inspiratory stimulus were not clear although single stimulus trials occasionally showed an increase in inspiratory duration.

The effects of an expiratory "SAR" stimulus had the same sign regardless of the Ti/Te ratio. An expiratory "SAR" stimulus, regardless of whether the inspiratory "SAR" response was Type A or Type B, always prolonged expiration. This is not inconsistent with the circuit described in Figure 69B but suggests that the processing of expiratory "SAR" information occurs before the switch which determines inspiratory inhibition or inspiratory prolongation (Figure 69B).

Prolongation of expiratory duration in response to within-phase "SAR" stimulation was followed by increased inspiratory duration. Increased inspiratory duration is a response in the same direction as the within phase effects of the expiratory stimulus but is opposite in direction to the within phase effects of inspiratory "SAR" stimulation during inspiration. Post-stimulus expiratory

duration was shortened, this effect is opposite to the within phase effects of the expiratory "SAR" stimulus. Both post-stimulus responses were graded with increasing "SAR" stimulus intensity.

In order to explain both the within phase and the post-stimulus phase effects of expiratory stimulation we assume the following: 1) that there are two parallel expiratory pathways with opposite effects; one prolongs expiration and one shortens expiration 2) normally the expiratory prolonging pathway is the more powerful of the two so that an "SAR" expiratory stimulus results in expiratory prolongation; 3) the switch (Figure 69B) always activates the inspiratory prolonging pathway because the expiratory "SAR" stimulus resulted in a low Ti/Te ratio, hence post-stimulus inspiratory duration is prolonged; and 4) expiratory shortening pathways, whose effects are normally overshadowed by the expiratory prolonging pathway have a longer time course which is manifest after the expiratory prolonging effects have decayed.

In my experiments the amount of expiratory prolongation with "SAR" stimulation depended on the duration of the prestimulus expiratory phase. Thus, when the prestimulus expiratory duration was long, the expiratory "SAR" stimulus had a smaller effect than when pre-stimulus expiratory duration was short. Other variables which can influence the magnitude of expiratory prolongation will be discussed later. Contrary to reports in the literature based on experiments in anesthetized adult animals (Feldman & Gautier,1976; Younes & Polacheck, 1985), these experiments in unanesthetized newborn lambs showed that the effects of an inspiratory SAR stimulus on mean post-stimulus expiratory duration were not significantly different in magnitude than

the effects of an expiratory "SAR" stimulus on mean post-stimulus inspiratory duration.

"IR" Stimulus Effects

Although the original intent of these experiments was not to characterize the effects of "IR" stimulation, the presence of "IR" effects at the higher intensities of stimulation was extremely useful in delineating the extent of the "SAR" response. In contrast to the responses to "SAR" stimulation, the magnitude of the augmented response with "IR" stimulation -- which occurred in all lambs -- was less closely correlated with intensity and may have reflected a discrete triggered response rather than a continuously graded reflex (Cherniack, et al 1981). Because a reflex is a response which is graded with respect to the intensity of the stimulus, the refractoriness of the within phase "IR" inspiratory response suggests a somewhat more complicated response. During the refractory period for augmented breaths Sellick and Widdicombe (1970) found that spontaneous irritant receptor discharge was decreased. Davies and Roumy (1982) found that paralysed rabbits did not display a refractory period, indicating that the refractoriness was not purely a central mechanism. Some of the variability I observed may have been a result of the refractoriness of the response. In unanesthetized newborn lambs augmented breaths could be evoked roughly every ten seconds, though it is possible that there was still a relative refractory period for <u>maximal</u> augmented responses.

Previous reports describe IR inspiratory refractory periods as long as two minutes (Roumy, 1977).

In unanesthetized newborn lambs, post-stimulus effects of "IR" stimulation had the same directional characteristics as the post-stimulus effects of "SAR" stimulation. That is, the effects of stimulation in the first post-stimulus phase were in the same direction as the within phase effects of the stimulus while the effects in the second post-stimulus phase were opposite to the within phase effects of the stimulus. After an "IR" evoked augmented inspiration, expiratory duration was lengthened and the following inspiratory phase was decreased. This means that the post stimulus responses to "IR" stimulation occur in the same direction as the post-stimulus effects of "SAR" stimulation. The effects of a stimulus on the post-stimulus phase are in the same direction as the effects of the stimulus within the stimulated phase, but are opposite to the effects the stimulus would have if it were given within that respiratory phase. This may reflect a strategy the respiratory system adopts regardless of the type of perturbation it receives (cf., Younes et al., 1987).

The expiratory post-stimulus effects of "IR" stimulation observed conflict with those of Davies and Roumy (1982) who reported that, in anesthetized rabbits, the first expiratory phase after a triggered augmented breath is shortened. My data and those of others (e.g., Knox, 1973) suggest that shortened expiratory response is more consistent with the post-stimulus effect of inspiratory SAR stimulation.

Unlike the inspiratory response to within phase "IR" stimulation, the expiratory effects of "IR" stimulation had no refractory period (Davies & Roumy,

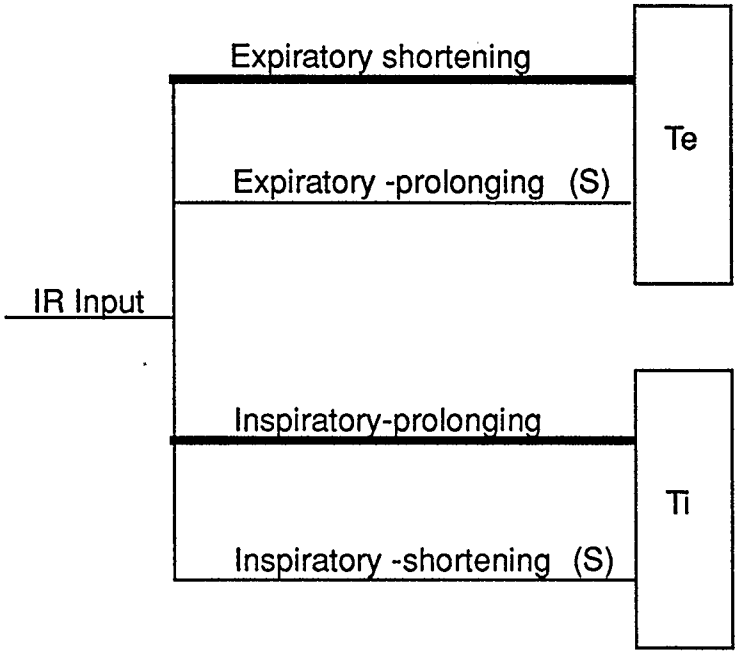

Figure 70. Model explaining IR processing. (S) again denotes pathways with a slow time constant.

1982), smaller variability within each intensity of stimulation and fewer changes with increasing stimulus intensity. After "IR" evoked expiratory shortening, the post-stimulus inspiratory phase was shortened and the next expiratory phase was prolonged.

The within phase and post-stimulus effects of inspiratory and expiratory IR stimulation are consistent with the parallel pathways suggested in Figure 69A (Younes & Polacheck, 1981) with the modifications shown in Figure 70. The dominant pathways (thick lines) are the inspiratory augmenting and expiratory inhibitory pathways. All four pathways are activated simultaneously by any IR stimulus. In the case of an inspiratory "IR" stimulus, the dominant effects on inspiration would be produced by the relatively short lived, inspiratory augmenting pathway. The expiratory inhibitory pathway would be excited simultaneously, but it's effects would be short lived as well and disappear with the removal of the stimulus. The longer lasting effects of the expiratory prolonging and inspiratory inhibitory pathways would not be apparent until the stimulus is removed and the dominant (initial) effects of the stimulus have decayed. The sustained activity of these opposing pathways explain the post-stimulus prolonged expiratory phase and shortened inspiratory phase. Contrary to previous reports (Davies & Roumy, 1982), the effect of maximal "IR" stimulation on shortening expiratory duration in newborn lambs was not greater than the effects of a maximal "SAR" stimulus in prolonging expiration. Davies and Roumy (1982) also suggested that if SAR and IR fibres are stimulated simultaneously, SAR effects would dominate. In my experiments stimulation at intensities corresponding to the "IR" component of the CAP also

stimulated the fibres contributing to the "SAR" and "RL" components as well, yet "IR" responses dominated.

The stimulation data suggests that the respiratory system of the newborn lamb monitors and maintains the Ti/Te ratio. Since gas exchange is a consequence of both inspiratory and expiratory durations, it can be affected by alteration in the Ti/Te ration. The nervous system appreciates that Ti and Te should be matched, because Ti and Te appear to be well correlated after a perturbation of either phase. Thus the nervous system has mechanisms for keeping the Ti/Te ratio constant. The results presented here suggest that reflexes evoked from vagal afferents contribute to regulating the Ti/Te ratio.

The post-stimulus effects of electrical stimulation reflect central integration of vagal inputs or memory. Thus the events in one respiratory phase can condition the characteristics of the following phase (Benchetrit & Bertrand, 1975). The results of experiments in lambs have shown that these effects are present in the newborn and are graded in response to increasing stimulus intensity.

The post-stimulus effects of vagal stimulation observed in newborn lambs may illustrate a coordinated response or rule that the respiratory controller follows in the face of a respiratory phase perturbation. If one respiratory phase is affected by a stimulus, then the subsequent phase will be affected in the same way (direction), whereas the second phase after the stimulus is affected in the opposite direction. Although these post-stimulus effects have been described previously for SAR (Younes & Polacheck, 1985) and pontine stimulation (Younes et al., 1987), the extension of this rule to the

post-stimulus effects of an entirely different receptor group (IR) has not previously observed.

The purpose of post-stimulus effects may be to offset the within phase effects of a vagal stimulus on respiratory phase duration. This may reflect a strategy to maintain an appropriate Ti/Te ratio in response to a perturbation. For example, if the expiratory prolonging SAR reflex effects were accompanied by the inspiratory inhibitory (amplitude and duration) effects, then minute ventilation would decrease. Ventilation is maintained because Ti is longer after the expiratory stimulus.

Proprioceptive feedback, especially from vagal afferents, might explain some of these observations in the unanesthetized animal. For example, the "SAR" stimulus shortened inspiratory time. Consequently, a lower lung volume was achieved. This meant that there less difference between peak inspiratory lung volume and end expiratory lung volume, hence end expiratory lung volume could be achieved more quickly, shortening the necessary expiratory time. However, Younes & Polacheck (1985) found the same SAR post-stimulus effects in animals that had been denuded of proprioceptive feedback. Thus some portion of the post-stimulus effects are central in origin. Post-stimulus effects of IR stimulation have been noted in fluorocarbon perfused, denervated, paralysed guinea pigs (Cleland, personal communication), suggesting again, a central origin for these effects.

DEVELOPMENT OF SAR REFLEX ACTIVITY

My experiments were designed to measure changes in the central component of the SAR mediated inspiratory inhibitory and expiratory prolonging BH reflexes in newborn lambs. These studies of the BH reflex differed significantly from those done in the past in that previous studies examined the strength of inspiratory and expiratory components of the BH reflex as a whole; they gave a stimulus which excited SAR to an unknown extent, the SAR discharge was transmitted to brain via vagal fibres and produced a respiratory effect. Analysis of the amplitude of vagal CAP components over the range of stimulus intensities used in each experiment identified the stimulus intensity at which the SAR component was maximal. Since this intensity could be reliably identified in every experiment, the central effects of maximal SAR stimulation could be compared between similarly aged animals, between lambs at four different ages, and in the same lamb on different days.

Significant differences in the sign of within phase effects of equivalent inspiratory SAR stimuli were apparent in similarly aged lambs and in lambs at different ages. This difference was related to breathing pattern. If the Ti/Te ratio was high, the SAR stimulus decreased inspiratory duration (inspiratory inhibitory BH reflex). If the Ti/Te ratio was low, the SAR stimulus did not decrease inspiratory duration. Instead inspiratory duration was lengthened. In addition, the amount that inspiratory duration was decreased was dependent on the duration of prestimulus inspiratory phases.

The dependence of the sign and magnitude of stimulus effect on breathing pattern complicated the developmental aspect of the study. Since breathing pattern in spontaneously breathing newborns may change as a function of development, developmental studies of inspiratory SAR reflex strength may be more difficult than expected. In newborn lambs, breathing frequency decreases with increasing age (Johnson et al., 1979). If the decrease in frequency results from a lengthening of expiratory duration, due for example, to an increase in FRC with growth, the Ti/Te ratio would decrease. My results suggest that with a sufficiently low Ti/Te ratio, the inspiratory inhibitory component of the SAR reflex would not exist and the presence of the reflex would only be apparent if post-stimulus effects were measured. Increased expiratory duration as a result of increased FRC means that larger changes in volume feedback are required to produce a given decrease in inspiratory duration (Finker & Iscoe, 1984). This is presumably because increasing FRC increases the tonic level of SAR activity.

In infants however, Fisher et al., (1982) report that inspiratory and expiratory duration increase proportionally with age, so that Ti and Ti/Ttot do not change substantially. In kittens (Duron & Marlot, 1979) inspiratory time becomes longer with age. My results suggest that an increase in inspiratory time alone could account for an increase in the strength of the inspiratory inhibitory SAR reflex, however a decrease in the strength of the reflex with increasing age in kittens is suggested by the work of Trippenbach et al., (1979).

Thus, although my results showed that there were no significant differences in the central component of the inspiratory SAR reflex as a function of age, it is possible the two responses to equivalent SAR stimuli may have obscured an age-related change. However if we assume that there was indeed no change in the central component of the inspiratory BH reflex with age, and combine this with the finding that modulated SAR activity increased with age, the net effect would be an increase in reflex efficacy. An increase in the strength of the inspiratory SAR reflex is consistent with the work of Gerhardt and Bancalari (1981).

Although the response to expiratory SAR stimulation was consistent, there were differences in the amount of expiratory prolongation observed between similarly aged lambs. Many factors can influence the magnitude of expiratory prolongation with an SAR or lung volume stimulus (Younes et al.,1974). In my experiments, the amount of expiratory prolongation was related to the duration of prestimulus expiratory phases. When prestimulus expiratory duration was long, the SAR stimulus prolonged expiratory duration less than when prestimulus expiratory duration was short. Thus, if newborn lambs change their breathing frequency with age and expiratory duration increases, the amount of reflex expiratory prolongation, if dependent solely on prestimulus expiratory duration, would decrease as a function of increasing age. Such a finding would be consistent with the results of lung inflation during expiration in infants (Bodegard et al., 1969) and rat pups (Smejkal et al., 1985).

As mentioned previously however, while the initiation of expiratory prolongation is clearly a vagally mediated event, the duration of expiratory

prolongation can be affected by several factors. The magnitude of expiratory prolongation in response to lung inflation or electrical stimulation of SAR also depends on the pre-existing partial pressures of CO2 and O2 in the blood (Younes et al., 1974). Frankstein (1970) found that in cats, the apnea resulting from electrical stimulation of the vagus nerve did not occur if the cat was distracted during the stimulus. This suggests that the central processing of vagal input arising during expiration can be altered by changes in state. Similarly, Nadel et al., (1973) found that in dogs, the expiratory prolongation resulting from lung inflation was abolished by auditory or visual distraction, exercise and increased body temperature. Therefore it would seem that measurements of the duration of expiratory prolongation may be of limited usefulness for characterizing expiratory SAR reflex strength.

Keeping in mind the limitations mentioned above, my finding that there were no age related differences in the central component of the expiratory prolonging component of the reflex is awkward to interpret. Nevertheless if we assume that tonic levels of SAR activity increase with increasing FRC as the animal grows, this, combined with my finding of no change in the central component of the reflex would result in a net increase in the strength of the reflex with age. Such a result is consistent with previous work by Cross et al., (infants, 1960) and Gautier & Mortola (rats and rabbits, 1981) who reported increases in expiratory duration during lung inflation with increasing age.

There was a significant difference as a function of age in one of the post-stimulus effects of the expiratory SAR stimulus. Expiratory duration post-

stimulus appeared shorter than it had on previous days. This result suggests that the "memory" of the stimulus became more effective as the animal gets older. Hence, the gain of the expiratory shortening pathway illustrated in the model in Figure 69B may have increased with age.

In conclusion, developmental studies of the SAR reflex in spontaneously breathing animals need to consider the effects of many variables. Similarly aged animals can show different responses. The same animal can show responses of opposite sign or varying magnitude on different days depending on the breathing pattern. Breathing pattern itself may change as a function of age. Without considering these factors carefully, accurate measurements of SAR reflex strength in the spontaneously breathing developing animal may be impossible.

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