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Effects of Chemoreceptor and Baroreceptor Inputs on the Termination of Inspiration

Dexter Franklin Speck
Loyola University Chicago

EFFECTS OF CHEMORECEPTOR AND BARORECEPTOR INPUTS ON THE TERMINATION OF INSPIRATION

by

Dexter Franklin Speck

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BIOGRAPHY

Dexter F. Speck is the son of Bursie V. Speck and Myrna Y. Speck. He was born June 4, 1955 in Altoona, Pennsylvania. He attended elementary school and high school in the Huntingdon Area public school system. In September, 1973, Dexter entered Wheaton College in Wheaton, Illinois. He graduated cum laude from Wheaton College with a Bachelor of Science degree in Biology.

The author began his graduate studies in July of 1976 in the Department of Physiology, Loyola University Medical Center. During the past three years, he has worked under the direction of Dr. Charles L. Webber, Jr. On March 1, 1980, Dexter married Pamela Jo Pickens of Moberly, Missouri. Pamela is currently employed at Central DuPage Hospital as a surgical technologist.

In July, the author will begin two years of postdoctoral study at Northwestern University Medical Center in Chicago where he will be studying under the direction of Dr. Jack L. Feldman.

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ABSTRACTS

1. SPECK, D.F. and C.L. Webber, Jr. Effect of thoracic dorsal rhizotomy on respiratory parameters in quiet breathing. Fed. Proc. 38: 368, 1978.

2. SPECK, D.F. and C.L. Webber, Jr. Time-course of inspiratory off-switch activation by intercostal afferent stimulation. Fed. Proc. 38: 1229, 1979.

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CHAPTER I

INTRODUCTION

The mechanisms that determine respiratory rhythmicity have fascinated investigators for many years. Although the genesis of this oscillating neural cycle is still not completely understood, sufficient experimental evidence has been accumulated to permit the theoretical construction of several different models of respiratory rhythmicity. Most of these models subdivide the respiratory cycle into four specific processes: expiration, the onset of inspiratory activity, the augmenting phase of inspiration and the termination of inspiratory activity. These processes act together to determine the duration of both inspiration and expiration.

This study was designed to investigate the influences exerted on the inspiratory terminating process by several known respiratory modulating stimuli. A technique for assessing the inspiratory-to-expiratory switching threshold has been developed and subsequently utilized to demonstrate the effects of both chemoreceptor and baroreceptor inputs on the switching system. The influences of chemoreceptor activities have been assessed by altering the alveolar concentrations of both carbon dioxide and oxygen, while the modulatory effects of baroreceptor inputs have been clarified by manipulating the pressure within the carotid sinus.

CHAPTER II

LITERATURE REVIEW

A. The Inspiratory Off-Switch

Inspiratory activity is characterized by a distinct onset, a phase of gradual augmentation and an abrupt termination. This termination of inspiration appears to be the key timing event in the generation of the rhythmic nature of breathing. It directly determines the depth and duration of inspiration, as well as indirectly influencing the expiratory duration. The termination of inspiration is thought to be accomplished through the function of an "inspiratory terminating off-switch." (34).

1. CONCEPTUAL DEVELOPMENT OF THE SWITCHING SYSTEM

The effects of lung inflation on inspiratory processes were initially and clearly demonstrated by Hering and Breuer in 1868 (35, 108). In their classical experiments they showed that both inspiratory-facilitatory and expiratory-facilitatory reflexes could be elicited from the lung. The inhibition, or termination, of inspiration and the subsequent promotion of expiration was due to the degree of lung expansion: the larger the lung volume, the greater the inhibition of inspiration. Using a different type of intervention, Boyd and Maaske (31) also demonstrated the inhibition of inspiratory activity. These investigators showed in sodium barbital anesthetized dogs that adequate central

stimulation of the vagus nerves would cause a decrease in the duration of inspiration. If the stimulation parameters fell below a certain threshold, there was no noticeable change in the pattern of respiration. Therefore, these investigators were able to determine a specific "inhibitory threshold". This threshold was expressed as the minimal number of afferent volleys necessary to terminate inspiration. Boyd and Maaske observed that the "inhibitory threshold" increased as the vagal stimulation was begun earlier in inspiration.

A similar experiment performed in cats by Larrabee and Hodes (135) revealed that during late inspiration, only a few stimulations of the superior laryngeal nerve were sufficient to cause the premature termination of inspiration. However, when the same nerve was stimulated shortly after the onset of inspiratory activity, many more stimuli were required before the duration of inspiration could be affected. Stimulations that were subthreshold did exert a conditioning effect upon any subsequent afferent volleys. The results of this study were explained by the postulate that the respiratory pattern was influenced by some dynamic process, a "central excitatory state", which developed progressively during inspiration and then gradually subsided during expiration. When this "central state" attained a certain critical threshold level, inspiratory activity was terminated. The afferent volleys used in this experiment contributed the difference between the level of the threshold and the level of the "central state". Therefore the progressive decline in the number of stimulus volleys was due to the gradual augmentation of the "central state".

The initial observations of Hering and Breuer (35, 108) were greatly elaborated upon by the work of Clark and von Euler (41). In their 1972 experiments, they examined the relationships seen between tidal volumes and inspiratory durations in both man and cat. Both species breathed from a closed system which was initially filled with 100% O_2. As rebreathing continued, the concentration of carbon dioxide slowly increased; thereby increasing both the tidal volume and the respiratory frequency. Several experiments involving the use of artificially imposed inflations in the cat were also performed. The data obtained from both types of protocols indicated that the volume of lung inflation required to terminate the inspiratory phase of respiration decreased as a hyperbolic function of the time after inspiratory onset. This relationship depended upon the intactness of the vagus nerves. In the discussion of these results, Clark and von Euler suggested that the nature of the falling threshold with time must be due to the properties of the central respiratory mechanisms. A simple model was then proposed to explain the normal control of the depth and rate of breathing. Both of these parameters were defined by the rate of lung inflation and the slope of the decay of the "inspiratory characteristic". The rate of inflation, in turn, depended mainly upon the chemical drive for respiration. The ideas and data contributed by these previously mentioned studies have since been utilized in the construction of several more substantial models of respiratory control (34, 50, 52, 76, 85) which have been further described in some recent review articles (49, 198, 199). These models focus primarily upon the factors that appear to

influence the termination of inspiratory activity. These influences include a wide variety of mechanoceptors, chemoreceptors, and central processes. A specific brainstem mechanism, the so-called "inspiratory off-switch", has been suggested as the integrating system through which all of these influences interact to determine the duration of each inspiration. It is currently postulated that the neural activity that contributes to this inspiratory terminating process develops progressively and then, after reaching a threshold level, initiates an inspiratory-to-expiratory phase switching mechanism which serves to promptly terminate the inspiratory process. Based on this hypothesis, it is obvious that the duration of the inspiratory phase is related to two variables: (1) the time course of neural activity augmentation in all of the systems that excite the switching mechanism and (2) the actual threshold of the inspiratory off-switch.

2. EXCITATORY INPUTS TO THE SWITCHING MECHANISM

The time course of increase of neural activity within the switching system is dependent upon many different inputs. One of the major sources of input to the hypothesized switching system is inspiratory in character and is central in origin. This excitatory input has been called the central inspiratory excitation (CIE) process by von Euler et. al. (77). The CIE increases progressively with inspiration and resembles the augmenting nature of the phrenic discharge. Although it is not conclusively known which medullary cells generate the rising CIE, virtually all of the inspiratory modulated cells of the nucleus tractus solitarius (NTS) and the nucleus retroambigualis (NRA) retain their normal firing

pattern after the elimination of peripheral sensory feedback. Thus it may be concluded that neurons in these populations either generate or receive the CIE from another medullary population. Since most of these NTS inspiratory neurons also have spinal projections to the phrenic nucleus (77, 78), measurements of the integrated phrenic inspiratory activity are often used as estimates of the CIE (80, 83).

The rate of rise, or slope, of this CIE can be influenced by many different factors, the best studied of which are temperature and carbon dioxide concentration (32, 33, 43, 44, 80, 190). The elevation of either of these influences causes an increase in the slope of the integrated phrenic signal. This enhanced activity may also be observed in recordings from the inspiratory neurons of the medulla. The peak amplitude of the integrated phrenic activity also increases in response to carbon dioxide (189).

In the intact animal, one very important extramedullary determinant in the timing of inspiratory termination is contributed by the pulmonary stretch receptors whose afferent projections are carried in the vagus nerve (1, 123). As was previously mentioned, moderate lung inflation imposed during the inspiratory phase of respiration produces a shortening of inspiration (41, 88). Conversely, the prevention of lung expansion during inspiration by either tracheal occlusion or by cessation of respirator ventilation in a paralyzed animal causes a lengthening of that phase (11, 86, 87, 100). This relationship between lung volume and inspiratory duration is often displayed graphically as a curve of the tidal volume (V_T) versus the time of inspiration (T_I) (41, 99, 100).

Graphs such as these indicate that the volume threshold for inspiratory termination decreases progressively with time from the onset of inspiration (33, 41, 88). Unilateral vagotomy decreases the amount of pulmonary stretch receptor activity that reaches the brainstem. This experimental intervention has been shown to shift the V_T versus T_I relation to the right (99) so duration is increased. With bilateral vagotomy the inspiratory duration is greatly prolonged and actually becomes independent of physiological changes in lung volume.

The slope, or the rate of rise, of the integrated phrenic recording is ordinarily not affected by the excitation of the pulmonary stretch receptors in cats (48, 50, 60, 87). Individual phrenic fibers (136) and medullary inspiratory neurons (45, 77) also show no change of activity resulting from enhanced vagal input. The slope is likewise unaltered by afferent vagal electrical stimulation that is of sufficient strength to prematurely terminate inspiration (116). Under certain conditions there may occasionally be a graded inhibitory effect of lung inflation observed near the end of the normal inspiratory duration (6, 200). The evidence for this graded inspiratory inhibition suggests that inspiratory termination occurs over a variable period during which time, lung volume inhibits phrenic output in a graded and substantially reversible fashion. The extent to which the inflation reflex serves only as a trigger to terminate inspiration depends on the experimental conditions (200). This conclusion is supported by the observation that volume information beyond the first 300 to 600 milliseconds of inspiration influences both the inspiratory duration and the shape of the integrated phrenic signal in the anesthetized dog (57).

Pulmonary stretch receptor activity does elicit changes in the activity of several different types of brain stem inspiratory-modulated neurons (see 50). Afferents from the vagus nerve have been shown to terminate within the intermediate and caudal portions of the nucleus tractus solitarius (NTS) (3, 55, 114, 160). Various studies (14, 45, 77) have demonstrated the existence of respiratory-modulated neurons in the ventrolateral NTS that increase their firing rate in response to lung inflation. These cells also increase their discharge during inspiration. Neurons responding to both of these criteria are the R-beta cells of von Baumgarten (14) and the inspiratory-vagal (IV) cells of von Euler et. al. (77). Such neurons comprise approximately 40% of the inspiratory-modulated cells in the NTS. These neurons may be driven by single shocks to the vagus nerve with a one-to-one response. The latency of response to vagal driving ranges from 2.1 to 8.0 milliseconds (77). The longer latencies may possibly be due to the very slow conduction velocities observed in the terminal branches of the vagal afferents. Therefore, the vagal afferents are thought to synapse directly on these neurons.

It is interesting to note that these IV neurons are easily excited by lung inflation during the inspiratory cycle; however, a much larger inflation is required to elicit a similar response during the expiratory phase of respiration (16, 50). In addition, von Euler et. al. (77) have demonstrated that the threshold for driving the inspiratory-vagal cells from the vagus decreases during the course of inspiration. It takes a much larger vagal stimulation current, which

activates more pulmonary stretch receptor axons, to drive the inspiratory-vagal neurons early in inspiration than late in inspiration. This characteristic is similar to that observed with the Hering-Breuer reflex. Therefore it is through these neurons that the Hering-Breuer excitation of such neurons during expiration might be due to either excitation of such neurons during expiration might be due to either inhibition or disfacilitation (50).

Another type of respiratory-modulated neuron found in the nucleus tractus solitarius is the pump unit or P cell (16, 50). The activity of these cells is related only to the volume of lung inflation. Their discharge patterns are not affected by the central respiratory activity and they respond similarly to inflation during either inspiration or expiration. Since these neurons may also be activated by superior laryngeal nerve stimulation, they are thought to be second-order afferent neurons (16) from the pulmonary stretch receptors.

Stimulation of the vagus nerve may excite several different types of receptors in addition to the pulmonary stretch receptors. Although the direct effects of the activation of these afferents is not conclusively known, they do appear to influence respiratory rhythmicity (61, 155). The irritant receptors (rapidly adapting stretch receptors) are assumed to increase the depth of inspiration (123, 136). It has also been assumed that activation of the irritant receptors causes a hyperpnea and a decrease in the duration of inspiration (178). However, recent studies have indicated that the reflex effects of the irritant receptors may be the same as those of the slowly adapting pulmonary stretch receptors (61, 155). In the study of Winning & Widdicombe (190),

stimulation of either the lung irritant receptors or the type J receptors, which are alveolar nociceptive structures, elicited a decreased inspiratory duration without affecting the rate-of-rise of phrenic inspiratory activity.

A third major excitatory input to the suggested off-switching system is contributed by the pneumotaxic center. This functional center which was first described by Lumsden (141) is located within the caudal midbrain and the rostal pons. Neural recordings from this region in vagotomized cats have demonstrated a high concentration of respiratory-modulated neurons in the nucleus parabrachialis medialis (NPBM) and the Kolliker-Fuse nucleus (22, 23, 47). Electrical stimulation of these areas provokes a phase-switch of the respiratory cycle (22, 47). If the stimuli are delivered to the more dorsal portions of the NPBM, it is possible to promote the onset of a premature inspiration. Stimulation of the ventral sites of the pneumotaxic center elicits a premature expiration. The voltage threshold for this termination of inspiration by electrical stimulation of the ventrolateral NPBM decreases progressively as the stimuli are applied later in the inspiratory phase (47, 82, 83).

Additional support for the hypothesis that the NPBM neurons contribute an excitatory input to the offswitching system is provided by several lesion experiments. Such studies have demonstrated that destruction of the NPBM makes it more difficult to terminate inspiration. After lesion of the NPBM, the whole Hering-Breuer volume threshold curve is shifted upward in a parallel manner. The lesions do not cause any significant change in the characteristic hyperbolic time course (80,

124). It thus appears that the off-switch threshold is raised whenever there is a decrease in the amount of NPBM activity (22, 80, 88). This effect is clearly demonstrated by the combined lesion and stimulation experiments of von Euler and Trippenbach (83). In this study electrodes were placed bilaterally in both NPBM so that each produced inspiratory termination upon stimulation. The excitability changes of the off-switch mechanism were then determined by stimulating on the one side before and after lesioning the contralateral NPBM. A significant increase in the voltage threshold for inspiration-to-expiration switching was observed after the unilateral lesion. Such lesions in the NPBM do not affect the rate of rise of inspiratory activity (83), neither do they influence the responsivity to changes in carbon dioxide levels (80).

In animals with intact vagus nerves very few active respiratory-related units are found within the pneumotaxic center (174). However, in recordings from vagotomized cats, many such neurons may be located (22, 23). This discrepancy may be explained by the hypothesis that the pulmonary stretch receptor input inhibits the respiratory modulation of pneumotaxic center neurons (86, 87). Through the use of a cycle triggered pump which inflates a paralyzed animal coincident with phrenic inspiratory activity, it may be demonstrated that whenever the inflation of the lungs is prevented, tonically discharging pontile neurons change to a phasic pattern of respiratory modulation (87). The normal tonic discharge is not due to a direct inhibition or excitation of the NPBM neurons by the vagal input, since neither electrical stimulation of the vagus nerve (22) nor increases in lung inflation (86, 87) directly affect the firing

patterns of these pneumotaxic center neurons. It is currently believed that the pulmonary afferents cause a presynaptic inhibition of a phasic input to the NPBM. This phasic respiratory modulation is thought to originate from medullary neurons, possibly the inspiratory-alpha neurons in the ventral respiratory group (49, 50). In addition to this excitatory pathway from the medulla, the pneumotaxic neurons also receive a powerful excitatory input from the spinal somesthetic and trigeminal systems (22). Through this connection, it is possible that respiration could be synchronized with sensorimotor activity (112).

The discharge pattern of the pneumotaxic center neurons may become phasic even in the intact animal. This may occur under certain neural influences such as "reticular arousal" (51, 113). Indeed, Sieck and Harper (184) have demonstrated that the discharge rates of NPBM neurons, as well as their phase relationship to the respiratory cycle, varied depending on the sleepwaking state. During the awake state in cats, recordings from pneumotaxic neurons displayed an intermediate firing rate that had only a slight amount of variability. When the cats entered a state of quiet sleep there was a significant decrease in the neuronal discharge rate and in the variability. However, during rapid eye movement (REM) sleep, there was a significant increase in both the variability of the discharge and the frequency of the neuronal firing. These findings are consistent with the observation of a faster and more irregular respiratory rhythm during REM sleep. The data therefore support the hypothesis that the pneumotaxic center may under certain circumstances contribute a phasic input to the genesis of the respiratory pattern in the conscious intact cat. Despite this evidence

for phasic pneumotaxic activity, it must be concluded that the primary role of the NPBM neurons is to provide a tonic input which has an excitatory, threshold lowering effect on the inspiratory off-switch mechanism.

Additional influences on the inspiratory off-switch mechanism include the superior laryngeal afferents (16, 97, 116, 135) and the intercostal muscle afferents (163, 182). Somatic afferents such as the abdominal muscle afferents in the lumbar nerves (182) may potentially serve to activate the off-switch. Stimulation of cutaneous afferents in the superficial radial nerve and muscle afferents in the hamstring nerve also has an effect on the timing of respiratory phase switching (117) and inspiration is prematurely terminated by stimulation during the early portions of that phase. Stimulation of the sympathetic chain at any thoracic segment also results in the inhibition of respiration in both the primate and the canine before and after vagotomy (127). However, sympathetic stimulation does not alter the depth or timing of inspiratory activity; but instead seems only to prolong expiratory duration and thereby decreases the respiratory frequency. Although the specific origin of these afferent fibers has not been determined, the conduction velocities of the sympathetic afferents causing respiratory inhibition were found to be within the A-delta fiber range. The inhibition of respiration is not thought to be part of a general response to peripheral stimulation since continual stimulation of the femoral and brachial nerves elicits the opposite response, an increase in the breathing frequency (127). Similarly, it has been demonstrated that the reflex

enhancement of ventilation that occurs after squeezing or stretching the gastrocnemius muscle may be due to the activation of non-medullated C fibers, as well as the Group I and Group II fibers (118).

3. LOCALIZATION OF THE INSPIRATORY OFFSWITCH MECHANISM

It is postulated that the various inputs to the off-switch all interact to determine the inspiratory duration. However, there is very little evidence to indicate either the anatomical site or the mechanism of this interaction. It has been observed that respiratory rhythmicity may persist after the elimination of all sensory input. In the decerebrate cat, Wang and his co-workers (191) sectioned the ninth, tenth, eleventh, and twelfth cranial nerves, the spinal cord at C6, and the C1 - C6 dorsal roots. Since these preparations continued to breathe rhythmically, these authors concluded that, while sensory input does influence respiration, the respiratory pattern may be generated solely by the neurons in the brainstem. Other experiments (110, 175, 191) have confirmed this conclusion that the minimal neural substrate necessary for any sort of respiratory rhythmicity is located in the rostral medulla. Since the termination of inspiration is essential for the generation of a respiratory cycle, it may be concluded that the off-switching mechanism must also reside within this region.

There are two distinct groups of respiratory related units in the medulla, the dorsal respiratory group associated with the nucleus tractus solitarius (NTS) and the ventral respiratory group (VRG) which includes the nucleus ambiguus (NA) and the nucleus retroambigualis (NRA). The axons of the NTS neurons are widely distributed within the ventral respiratory group, but the VRG cells do not send axons to the NTS

(143, 144). Therefore, it may be concluded that the NTS is the most likely site for rhythm generation. Unfortunately, the evidence for this is not clear. Oberholzer and Tofani (154) reported that bilateral destruction of the NTS in animals with prior vagotomy causes only slight changes in the respiratory pattern. However, Koepchen et. al. (126) observed that NTS lesions produced apneusis. The studies of Brodie and Borison (36) also demonstrated that ablation of the rostrodorsal medulla resulted in apneusis. After a subsequent, more ventrally placed lesion, the apneustic pattern converted to one of gasping. These studies show that a form of respiratory rhythm can be generated by the VRG alone, but normal patterns require both the ventral and the dorsal respiratory groups.

The inspiratory vagal cells (IV) are found within the NTS (77). The discharge of these units augments during inspiration in both the intact cat and the vagotomized cat. When the vagal input is disrupted, the firing frequency of the IV cells increases at a slower rate. Although the duration of inspiration is longer, the final firing rate of these cells is similar whether there is vagal input or not. This suggests that the inspiratory off-switch mechanism functions at a fixed level of firing of the IV cells (199). It is tempting to believe that the IV cell output may itself be the off-switch that triggers the cessation of inspiratory activity. However, most authors (34, 49, 50, 76) believe that there is a separate off-switch population interposed between the IV cells and the inspiratory neurons.

One possible candidate for this separate off-switching system is suggested by the work of Cohen and Feldman (49, 50). Certain neurons

in the NTS have a discharge pattern that begins late in inspiration, increases to a peak frequency and then rapidly decays during expiration. Their peak frequency consistently coincides with the inspiratory-to-expiratory transition, regardless of the duration of inspiration. This correlation of maximum discharge rate with the termination of inspiration occurs independently of vagal input. These neurons may either form a special subpopulation or they may simply be late-firing IV cells. In either case, their discharge pattern is consistent with the hypothesis that they may play a part in the termination of inspiration.

B. Intercostal Respiratory Afferents

The intercostal musculature contains many sensory receptors including bare nerve endings, golgi tendon organs, Pacinian corpuscles, and both the primary and secondary muscle spindle endings. In the cat, the secondary muscle spindle afferents are the most plentiful of the proprioceptive endings (9). All of these sensory nerve endings are evenly distributed in both the internal and external intercostal muscles and are most numerous in the more rostral rib interspaces (111). Caudal to the seventh rib the receptors are somewhat less numerous. Recordings from these thoracic afferents demonstrate a phasic spontaneous activity with peak discharge frequencies occurring during inspiration (56). The lowest threshold afferent fibers are known to synapse directly with the intercostal alpha motorneurons (71, 177).

The activation of these intercostal muscle spindle afferents may be elicited by artifical respiratory movements (56). During spontaneous respiration, spindle activation is due to a mismatch between the contractions of the intrafusal and extrafusal muscle fibers, which are in

turn related to the discharges of the alpha motorneurons and the fusimotor neurons (176), respectively. Both of these efferent fibers show maximal activation during inspiration and respond to stresses such as hypercapnia, hypoxia, and lung inflation. These responses demonstrate that the fusimotor neurons are subject to a supraspinal control similar to that exerted on the alpha motorneurons (176).

The activity of these intercostal muscle afferents plays an important role in the spinal regulation of the depth of breathing. This control involves both intercostal-intercostal and intercostal-phrenic reflexes (69, 75). Such reflexes originate from both the internal and external intercostal muscles of the caudal thoracic segments and are facilitatory to tidal volume (64, 66, 115) especially in elastic loaded or low compliance situations (179). In the spinal cat, similar reflex effects are obtained in response to stimulation of either the internal or the external intercostal nerve. The axons of the tendon organs and the secondary muscle spindles contribute to the reflex in the internal nerve, whereas in the external nerve, only the secondary endings seem to be involved (66).

In addition to their involvement in the spinal reflexes, the respiratory muscle mechanoreceptors have also been implicated in various extra-segmental and supraspinal reflexes. The best technique for demonstrating these reflexes is direct stimulation of the intercostal muscle afferents. Hess (109) observed that prolonged stretch of the intercostal musculature could increase the duration of expiration and therefore decrease the respiratory rate. Inspiratory duration may also be influenced by mechanical interventions such as chest compression,

intercostal muscle stretch, and rib vibration (163). All three of these stimulations may prematurely terminate inspiratory activity and also lengthen the expiratory duration. Since these effects on respiratory timing are similar to those elicited by either lung inflation (32, 41, 99, 136) or, pneumotaxic center stimulation (47, 83), various investigators (83, 101, 165) have suggested that the intercostal muscle afferents may provide an excitatory input to the inspiratory off-switch mechanism.

Each type of mechanical manipulation used by Remmers (163) provides independent evidence that the muscle spindles are the receptors that are responsible for the reflex effects. Rib vibration at 300 Hz is a very selective stimulus for the activation of muscle spindles (81). Likewise, the dependence of phrenic inhibition on the rate of stretch rather than on the degree of developed tension suggests a spindle mechanism (163). Additional evidence is contributed by the observed changes in reflex responsivity after succinylcholine and BA 28882 (2, 4-di (diethylamino) - 6 - (phenylacetylhydrazine)-1, 3, 5-triazine). Succinylcholine has been demonstrated to augment the muscle spindle responses to stretch (162) and should therefore increase the spindle discharge evoked by chest compression. This fact explains the enhancement of the inhibitory effectiveness of chest compression after succinylcholine paralysis. On the other hand, BA 28882 appears to exert a suppressive effect on the muscle spindles exclusively (15). After BA 28882 chest compression is not as effective in inhibiting inspiratory activity (163).

Electrical stimulation of the intercostal nerves may also be utilized to elicit the premature termination of inspiration (65, 164, 165, 182). Analysis of dorsal root potentials and the subsequent cal-

culation of conduction velocities indicate that both Group I and Group II afferent fibers participate in the inhibition of phrenic activity that occurs after stimulation of the external intercostal nerve (164, 165, 182). Remmers (165) suggests that the Group II afferents carry sensory information from the secondary muscle spindle endings. The receptors from which the Group I fiber activation arises are not known but are presumed to be the golgi tendon organs (182). With internal intercostal nerve stimulation, only the Group I afferents appear to be responsible for the termination of inspiration (182). These Group I afferents may include cutaneous receptors as well as muscle afferents.

Bilateral superficial sections of the lateral cervical cord block the inspiratory-inhibitory response to chest compression (166). These lesions also disrupt the ryhthm of breathing and often cause apneusis in vagotomized, decerebrate cats (129, 166). These results indicate that section of the lateral columns interrupts the ascending portion of the supraspinal inspiratory-inhibitory reflex. Since the spinoreticular tract is located within the lesioned areas, it is possible that the fibers involved in the reflex travel in this tract. Histological studies after ventrolateral cordotomies have demonstrated the terminal degeneration of spinoreticular afferents in both the medulla and the pons (79, 142).

There is no change in either the rate of respiration or the depth of breathing following ventrolateral cervical cord lesions if the vagus nerves are intact (129). This finding suggests that the spinal pathway plays a less important role in the normal regulation of the respiratory

pattern than either the vagal afferents or the pneumotaxic center. Such a conclusion is supported by the absence of any changes in the respiratory patterns of cats after thoracic dorsal rhizotomies (185, 186). These results support those of Coombs (54, 158) who used deafferentation and brain sectioning techniques to conclude that the thoracic afferents synapse with midbrain structures and thereby generate reflexes that may be important in the control of respiration. In her study, section of the thoracic dorsal roots diminished costal respiration, but did not alter the respiratory frequency. More recently some investigators have demonstrated that bilateral section of the thoracic dorsal roots causes an increase in the spontaneous respiratory rate (92, 181). Other experiments dealing with the ventilatory response to mechanical loading have also indicated the existence and the importance of supraspinal reflexes derived from the respiratory muscle mechanoreceptors (30, 37). Therefore, the physiological importance of these afferents as determinants of respiratory pattern is still not conclusively known.

The respiratory effects of the ventrolateral cervical cord lesions may also be related to the interruption of the ventral spinocerebellar tracts which contain fibers originating from many different muscles (166). Stimulation of the intercostal nerves is known to elicit evoked potentials in the cerebellum (42). Cerebellar evoked potentials may be recorded specifically from the surface of the ipsilateral intermediate cortex and the lateral margins of the vermis of the anterior lobe. These evoked responses have a latency of less than seven milliseconds and are eliminated by transection of the spinocerebellar tract (42).

Although not commonly appreciated, the cerebellum appears to have some connections with the brainstem respiratory system. Stimulation of the anterior lobe of the cerebellar cortex has an inhibitory effect on respiration in the decerebrate cat (150). This effect includes a marked inhibition of the phrenic inspiratory activity (65). Cerebellar depression induced by occlusion of the cerebellar arteries results in a fall in respiratory rate and an increase in respiratory depth. In many cases, the cerebellar depression may lead to a pattern of apneusis (96). Cerebellectomy may also cause apneusis which disappears after a short time period (107). Ablation or cooling of the anterior lobe of the cerebellum produces a significant reduction in the hyperpnea due to natural stimulation of muscle receptors (156). The results from all of these studies suggest that in the cat the anterior lobe of the cerebellum exerts a tonic, predominately inhibitory influence upon the inspiratory mechanisms of the lower brainstem. Since the intercostal afferents are known to project to the cerebellum (42) and the reflex events elicited by their activation are similar to those elicited by stimulation of the anterior lobe, it appears possible that the intercostal inspiratory-inhibitory reflex may be mediated through the spinocerebellar tracts.

A recent study by Shannon (182) demonstrates that the major effect of intercostal nerve stimulation is either a premature termination or a transient reduction in the phrenic activity. Extracellular recordings within the nucleus tractus solitarius revealed that the response of the inspiratory alpha cells and most of the beta cells to intercostal nerve stimulation resembled the response seen in the gross phrenic activity;

when the phrenic discharge was inhibited, the inspiratory neurons were also inhibited. A few IV cells appeared to be excited by the stimulation of the lower intercostal afferents. The results of this study support the concept that the inhibitory effect of the intercostal afferents on phrenic activity is part of a generalized reflex which links the intercostal muscles to the other respiratory muscles (115, 164, 182). This link involves the medullary respiratory neurons and is consistent with the idea that the intercostal muscle afferents may contribute an excitatory input to the inspiratory off-switch mechanism (83, 101, 165).

C. Chemoreceptor modulation of respiration

Total ventilation is increased subsequent to exposure of experimental animals to either hypercapnia or hypoxia. The enhanced ventilation under such conditions is due to an increase in both the rate and the depth of breathing. In the anesthetized cat, the response to hypoxia and hypercapnia is similar. These responses involve an increase in the tidal volume and the simultaneous decrease in both inspiratory and expiratory duration (93). In contrast, the inspiratory duration is not significantly altered by hypoxia in the conscious cat (93). This qualitative difference between the effects of hypercapnia and hypoxia on breathing patterns is supported by other studies (40, 89, 171). Such differences may be due to indirect peripheral responses that can affect the respiratory system or they may be caused by a specific central effect of hypoxia or hypercapnia on the mechanisms that are responsible for the termination of inspiration (93).

1. RESPONSE TO HYPOXIA

The peripheral arterial chemoreceptors are the mediators for the immediate increase in ventilation that is produced by an oxygen deficiency. These receptors have been localized in the carotid bodies at the bifurcation of the common carotid artery and in the aortic bodies found in the ascending arch of the aorta. The various stimuli to these receptors, the reflex respiratory and cardiovascular responses to stimulation, and the gross morphology have been extensively studied and reviewed (24, 161). The mechanisms for the transduction of chemical stimuli into nerve action potentials are still unknown, although they are intrinsically coupled to chemoreceptor metabolism since metabolic blockers such as DNP, CN and oligomycin can activate chemoreceptor discharge. Most investigators feel that the peripheral chemoreceptors are activated primarily by decreases in the arterial partial pressure of oxygen (20, 24), although some evidence indicates that the oxygen content or oxygen delivery rate may be the determining factor rather than the partial pressure (59, 192).

Afferent impulses from the carotid and aortic chemoreceptors are conveyed to the brainstem by the IX and X cranial nerves, respectively. Anatomical studies using degeneration techniques indicate that the primary afferent fibers of the sinus nerve project predominantly to the intermediate area of the nucleus tractus solitarius (NTS) (55). Similar results are obtained using transganglionic transport of horseradish peroxidase. This anatomical evidence is supported by several recent neurophysiological studies which have demonstrated the existence of

short latency evoked potentials recorded from the region of the NTS after stimulation of the carotid sinus nerve (40, 62, 148). Davis and Edwards (63) have reported that carotid chemoreceptor afferents are connected monosynaptically with units of the NTS and the nucleus ambiguus. However, based on the phrenic nerve responses to carotid sinus nerve stimulation, Berger and Mitchell have concluded that the carotid sinus nerve afferents do not directly excite the inspiratory units of the NTS (19).

Examination of single-fiber or whole-nerve chemoreceptor activity shows a progressive increase in impulse activity as the arterial partial pressure of oxygen decreases below 600 mm Hg, with the most rapid rise in activity occurring as partial pressures fall below 200 mm Hg (26, 132). When the arterial oxygen pressure decreases below 30 mm Hg the chemoreceptor discharge begins to fail (132). In contrast to the continual increase in chemoreceptor discharge as the arterial partial pressure of the oxygen decreases, ventilatory responses to hypoxia do not appear until a threshold of approximately 70 mm Hg. Chemoreceptor discharge is also enhanced with increases in the carbon dioxide and hydrogen ion concentration (26). Changes in carotid sinus pressure may exert a transient effect upon chemoreceptor activity (26). When the pressure within an isolated carotid sinus segment is raised, chemoreceptor activity decreases significantly within the first 5 to 10 seconds. Shortly thereafter the activity stablilizes at a level which is not different from control. This observation indicates that alteration of carotid sinus pressure within the range of 60 to 160 mm Hg has no

lasting effect upon chemoreceptor activity (25). Alterations in carotid body venous flow from 10 to 60 microliters per minute also have no influence on chemoreceptor activity (25). However, other investigations have demonstrated an increase in chemoreceptor impulse activity after hemorrhage (134).

Under normoxic conditions, there is a small amount of respiratory drive contributed by the oxygen sensitivity of the peripheral chemoreceptors as indicated by the depression of breathing that results after the sudden inhalation of 100% oxygen (67, 133). This depressant effect is often followed by a slight augmentation of respiration. After section of the carotid sinus nerve, the respiratory depression is eliminated and the respiratory augmentation is enhanced (94, 145, 168). These observations suggest that normoxia exerts a significant central depression of ventilation which is counteracted by the peripheral chemoreceptor drive that exists with normoxia. Thus the normal ventilation is a function of the degree of normoxic depression of the inspiratory medullary neurons and the excitatory input contributed by the peripheral chemoreceptors (94). Severe medullary hypoxia will reduce the ventilatory response to both hypoxia and hypercapnia, regardless of the amount of excitatory input (38).

Direct examination of hypoxia-induced changes in the medullary neuronal discharge indicates that inspiratory activity is enhanced by hypoxia if the carotid sinus nerve is intact (13, 153). Hypoxia also depresses the activity of the expiratory neurons under these conditions (153). A recent study by St. John and Wang (173) revealed that some inspiratory units are faciliated and others are depressed by isocapnic

hypoxia. After carotid sinus nerve section hypoxia causes a decrease in the respiratory-related discharge of all units. These investigators conclude that peripheral chemoreceptor afferents produce a discrete and unequal excitation of the medullary respiratory units. These observations also support the concept that the ventilatory responses to hypoxia are the net result of peripheral chemoreceptor activation and a direct depression of the brainstem respiratory complex (94).

The respiratory responses elicited by chemoreceptor activation depend upon the existing phase of respiration (7, 29, 73, 74). This differential effect is most easily demonstrated by electrical stimulation of the carotid sinus nerve (29, 73), although it may also be shown by direct chemical stimulation of the carotid bodies (7, 74). Stimulation of the carotid sinus nerve during inspiration reflexly increases the inspiratory air flow and the phrenic discharge (73). These responses are accompanied by an increase in the tidal volume or the peak level of integrated phrenic activity only if the stimulus is applied during the latter half of inspiration. Such a stimulus also causes a lengthening of both inspiratory and expiratory duration, while stimulation early in inspiration decreases the inspiratory duration. Stimuli applied during expiration prolong that expiration without affecting the nature of the subsequent inspiration (73). These results suggest that the peripheral chemoreceptor input may be gated and that such input is processed immediately and is not "stored" or "remembered".

Various studies have indicated that the vagus nerves may be partially responsible for the gating of chemoreceptor input. The vagal stretch reflex can inhibit the carotid chemoreflex drive of ventilation during hypocapnic hypoxia (121). Similarly, bilateral vagotomy enhances the ventilatory responses to transient hypoxia (68). Cardiovascular responses to baroreceptor and chemoreceptor reflexes are also inhibited by afferents from the lung (191). Changes in heart rate after carotid chemoreceptor activation are blocked by maintained lung inflation. It is interesting to note that these reflexes are also blocked by the central inspiratory excitation even if the lung volume is not changed (91).

The observation of identical inspiratory durations in conditions of isocapnic hypoxia and normoxia despite changes in the depth of breathing (58, 157) suggests an effect by hypoxia upon the threshold of the inspiratory off-switch neurons (157). Similarly, the hypoxia-induced changes in the duration of apneusis may indicate an effect exerted by the arterial partial pressure of oxygen on the off-switch threshold (171). These effects of oxygen remain to be clearly demonstrated.

2. RESPIRATORY MODULATION BY CARBON DIOXIDE

Alteration of the arterial carbon dioxide concentration has a dramatic effect on both tidal volume and respiratory frequency. These effects are mediated through the peripheral chemoreceptors and the central chemoreceptor. Analysis of the transient ventilatory response to a step change in the inspired level of carbon dioxide has been used to determine the relative contributions of the central and peripheral

sensors. The rapid, early response to a step change is associated with the fast-acting peripheral arterial chemoreceptors and the slower response is initiated by activation of the central chemoreceptors (18, 95). This type of study has indicated that the peripheral chemoreceptors account for 12 to 18% of the total ventilatory response to carbon dioxide. A more sophisticated approach used by Berkenbosch et. al. (21, 103) examined the ventilatory response to carbon dioxide in cats which were prepared with an isolated perfusion of the pons and medulla. This preparation permitted the independent stimulation of either the central or the peripheral chemoreceptors. From these experiments, it was concluded that the peripheral chemosensitivity arose almost exclusively from the carotid bodies and contributed one-third to one-half of the total hypercapnic ventilatory response (21, 103).

Peripheral carbon dioxide chemosensitivity may include structures other than the carotid bodies and the aortic bodies. For example, pulmonary stretch receptor afferent discharge is diminished by elevated carbon dioxide levels (10, 130, 131, 152). Inhalation of 8% carbon dioxide in oxygen causes a 20% decrease in the pulmonary stretch receptor discharge of the cat (131). All pulmonary fibers responding to carbon dioxide are also sensitive to mechanical stimulation; no receptors which are exclusively sensitive to carbon dioxide have been found in the lung (130, 131). The physiologic importance of the carbon dioxide sensitivity of the pulmonary stretch receptor remains to be established although the hyperpnea elicited by exercise and hypercapnia may be partially due to the reduction in pulmonary stretch receptor activity that accompanies

the increase in alveolar carbon dioxide levels in these situations (12).

After peripheral chemodenervation, the slow ventilatory response to increased carbon dioxide suggests that the central sensor does not instantly equilibrate with the arterial blood. It is hypothesized that central chemosensitivity does not arise from the brainstem respiratory neurons, but from a separate chemosensitive zone located near the ventral medullary surface (147). Bilateral focal cooling of this chemosensitive region causes changes in respiration that resemble those seen in hypocapnia (39). Pokorski (159) recorded from single units in this chemosensitive zone and observed cells that responded very quickly and consistently to the topical administration of either carbon dioxide in saline, bicarbonate ion, or hydrogen ion. Individual units responded to all three of these stimulations with either a consistent increase or decrease in their firing rate (159). However, a similar study by Lipscomb (138) failed to locate any such neurons within the chemosensitive region. These investigators did observe cells located in the region of the normal respiratory areas that responded to chemostimulation (138).

Mitchell and Herbert (146) recently examined the effect of changes in carbon dioxide on the membrane potential of respiratory neurons. They found that the direct effect of increased carbon dioxide was a relative hyperpolarization of the membrane potential of both inspiratory and expiratory neurons. In the absence of any synaptic input, this direct effect would cause a decreased excitation. Since increased carbon dioxide concentrations enhance the activity of these neurons, it may be concluded that the indirect effect of excitatory synaptic inputs outweighs

the direct cellular depression elicited by carbon dioxide (146).

The pneumotaxic center has been implicated in the frequency response that occurs in hypoxia or hypercapnia. After lesion of the nucleus parabrachialis medialis in cats, frequency responses are eliminated but tidal volume alterations still occur (172, 173). The tidal volume response to hypercapnia is unaltered while the tidal volume response to hypoxia is increased. Therefore, the hypoxia-induced minute ventilation is maintained after pneumotaxic center lesions. Because of the significant suppression of minute volume changes with hypercapnia but not with hypoxia following pneumotaxic center ablation, St. John (169) has concluded that the pneumotaxic center is an integral component of the central chemoreceptor controller system.

In the intact animal, the ventilatory response to hypercapnia involves an increase in both the rate and depth of breathing. The increased tidal volume precedes the changes in respiratory frequency (33, 194). However, after bilateral vagotomy, respiratory frequency remains stable regardless of chemical drive (33, 125, 188). Shannon (180) has reported a slight but significant increase in respiratory frequency induced by hypercapnia after vagotomy. The extravagal mechanism responsible for the shortening of inspiratory duration is not dependent upon afferents from the chest wall or diaphragm. St. John (170) has also concluded that neither the total duration of the respiratory cycle nor the respiratory frequency remains constant as ventilation increases in mid-collicular decerebrate cats with bilateral vagotomy. He has determined a "reversal point" for respiratory frequency;

if the initial frequency is below this "reversal point", hypercapnia increases the rate of breathing; if the initial frequency is greater, hypercapnia decreases the respiratory frequency. At this time, the best explanation for the effects of carbon dioxide on the rate and depth of breathing arises from the models of the inspiratory off-switch (33, 34). Hypercapnia increases the discharge of the inspiratory alpha cells (the CIE) which causes an increased depth of inspiration as well as an enhanced input to the switching system. Hypercapnia also elevates the threshold of the inspiratory off-switch mechanism. Through these two effects, the respiratory rate and depth responses to carbon dioxide may be regulated.

D. Baroreceptor modulation of respiration

It is well known that the carotid sinus and the aortic arch baroreceptors initiate reflexes which regulate the cardiovascular system. These reflexes also have an effect upon the respiratory control system. In addition to these two classical baroreceptor locations, many other vascular beds may have some degree of sensitivity to changes in the blood pressure (128). The cardiovascular reflexes associated with baroreceptor activation have been extensively reviewed but the respiratory reflexes have not been fully investigated (119, 120, 193). Activation of the baroreceptors elicits an inhibition of both heart rate and blood pressure. These effects are accompanied by decreases in the tidal volume and respiratory frequency. Intravenous administration of epinephrine results in a dramatic increase in systemic blood pressure and the subsequent reflex decrease in respiratory frequency due to a

prolongation of expiratory duration. After bilateral section of the vagus nerves and the carotid sinus nerves in decerebrate cats, the increased blood pressure is accompanied by an increase in ventilation (197). This clearly demonstrates that neural activity in the carotid sinus and vagus nerves mediates the apnea that results from increased systemic blood pressure (4, 8, 197). Conversely, carotid occlusion (84) and hemmorhage both elicit an increase in ventilation (70, 134). After carotid sinus denervation, hemorrhage causes a decrease in respiration (70). These reflex changes in arterial pressure have been purported to alter the arterial chemoreceptor discharge (59, 134). However, more recent evidence indicates that changes in the arterial blood pressure between 60 and 160 mm Hg have little effect on the resting discharge of the carotid chemoreceptors (25). Thus, changes in arterial pressure may reflexly alter ventilation by means of baroreceptor inputs into the respiratory controller (28). Heistad et. al. (106) observed that activation of carotid baroreceptors by elevating the pressure within one carotid sinus may inhibit the ventilatory response to contralateral carotid chemoreceptor stimulation. This finding suggests a central interaction of chemoreceptor and baroreceptor reflexes which affects ventilation as well as the previously demonstrated cardiovascular responses (105). Interaction of these reflexes may occur in the paramedian reticular nucleus of the medulla (149).

The cardiovascular responses elicited by baroreceptor stimulation are dependent upon the timing of the respiratory cycle (91). Heart rate responses to moderate baroreflex stimuli are inhibited by inspir-

ation, but the responses to intense stimuli are not influenced by the phase of respiration (72). This respiratory-baroreceptor interaction appears similar to the respiratory-chemoreceptor interactions (73, 91) which were described above. Both vagal activity and central inspiratory activity inhibited the chemoreceptor reflexes. It is suggested that similar respiratory-baroreceptor interactions may also be involved in the processing of the ventilatory response to baroreceptor activation.

Brief baroreceptor stimulation has no noticeable effect on the pattern of breathing and ventilation on anesthetized dogs (102). However, Biscoe and Sampson report that a single shock of the carotid sinus nerve causes a brief depression of phrenic activity after a latency of 5 to 10 milliseconds (27). Higher intensity stimulation or a brief tetanus evokes a burst of phrenic action potentials. The enhanced phrenic activity is thought to be due to chemoreceptor fiber stimulation, while the decreased phrenic discharge may be attributed to the activation of baroreceptor fibers. Bishop (28) has reported an immediate augmentation of the diaphragmatic and abdominal activity in response to deactivation of the baroreceptors by bilateral occlusion of the common carotid arteries. From these results she suggests that carotid baroreceptors inhibit both inspiratory and expiratory neurons. The inhibition of inspiratory neurons by baroreceptor activity is confirmed by extracellular recordings from single respiratory units which reveal a decrease in the discharge frequency of inspiratory neurons after activation of the baroreceptors (190). However, in contrast to Bishop's conclusions (28), the expiratory neuronal discharge is prolonged by baroreceptor stimulation (90).

Intracellular potential recordings from respiratory neurons by Richter and Seller (167) indicate that the discharge frequencies of inspiratory neurons decrease after either aortic nerve stimulation or elevation of the carotid sinus pressure. During the decrease in firing rate, the membrane potential is hyperpolarized and the degree of hyperpolarization varies within the respiratory cycle. The hyperpolarizing potentials may indicate the existence of a direct inhibitory connection of baroreceptors with the inspiratory neurons (167). Some non-respiratory medullary neurons have been located which respond to baroreceptor stimulation with a hyperpolarization of their membranes. These neurons may belong to the reticular activating system which is thought to exert a tonic activation on the medullary respiratory neurons (46). Baroreceptor stimulation during expiration has no effect on the resting membrane potential of expiratory neurons. However, the membrane potential of expiratory neurons is depolarized when baroreceptor stimulation is applied during the period of spontaneous membrane hyper-polarization (i.e., inspiration). The depolarizing shifts of the membrane potential may therefore be explained by a disinhibition of expiratory neurons when the inspiratory neurons are hyperpolarized and inhibited (46). This indicates that the baroreceptor afferents have no direct synaptic connections with the expiratory neurons, but may influence expiratory neurons indirectly through the inspiratory neurons.

Antidromic electrical stimulation experiments within the medulla of the cat demonstrate that myelinated primary afferents of the carotid sinus nerve terminate within the immediate vicinity of the nucleus tractus solitarius (139). Extracellular recordings from the brainstem

reveal that both baroreceptor activation and carotid sinus nerve stimulation enhance the activity of neurons in the ipsilateral nucleus tractus solitarius, the nucleus ambiguus and the parahypoglossal area. The latency of these responses suggests the existence of polysnaptic pathways for the conduction of baroreceptor inputs to the nucleus tractus solitarius (139). Although it is well documented that baroreceptor afferents project to recognized respiratory areas and influence respiratory patterns, there have been very few studies which have attempted to examine the effects of these afferents in the control of breathing. One such study (98) examined the depth and frequency responses during baroreceptor activation by inflation of an aortic balloon. In this study, an elevation of the blood pressure from 100 to 190 mm Hg did not affect the tidal volume versus inspiratory duration plot. This finding suggests that the volume-related vagal control of inspiratory duration is uninfluenced by changes in arterial pressure (98). However, other observations have indicated that baroreceptor activity may affect the excitability of the inspiratory off-switch (182). This hypothesis remains to be clearly demonstrated.

CHAPTER III

METHODS

A. Surgical Preparation

Mongrel cats of both sexes were used for these experiments. Cats ranged in weight from 1.6 to 5.4 kilograms. With the exception of 3 cats which were anesthetized with sodium pentobarbital (35 mg/kv, i.v.; Holmes Serum Co., Inc.), all animals were anesthetized with halothane (Fluothane, Ayerst Laboratories, Inc.). These cats were placed in a wooden box and a mixture of halothane and oxygen was administered through a face funnel with a Drager Narkovet apparatus at a flow rate of 3 to 6 liters per minute. Anesthesia was usually complete within 5 to 10 minutes. The cat was then placed on its back on a waterfilled heating pad (GormanRupp Industries Model K13).

A midline incision, begun at a point several centimeters rostral to the transverse vein, was extended to a point slightly caudal to the larynx. At this time, the proper connections were made between the tracheal cannula and the halothane gas machine in order to create a closed rebreathing system. A balloon reservoir was periodically filled with 100% oxygen and carbon dioxide was continually absorbed from the system by a filter containing Sodasorb (W.R. Grace & Co.).

Another incision was made in the femoral triangle and the artery and the vein were catheterized with PE 100 polyethylene tubing. Catheter tips were advanced into the abdominal aorta and the inferior vena cava,

In some animals the femoral vein was catheterized bilaterally. The arterial catheter was connected to a pressure transducer (Statham P23Db) and systemic blood pressure was recorded on a Grass Model 7 polygraph. The level of the mean arterial pressure provided an indication of the depth of the halothane anesthesia; most cats were maintained adequately anesthetized at a mean pressure of approximately 90 mm Hg.

The cervical vagus nerves were then located and looped with a loose ligature of umbilical tape which marked them for easy identification at a later time. In most cats the aortic depressor nerves were separated from the vagus nerves. Bilateral dissection immediately under the transverse vein exposed the external carotid arteries. These vessels were ligated at the point where they were crossed by the hypoglossal nerves. This procedure decreased the blood flow through the circle of Willis to the cerebral cortex and therefore greatly facilitated the decerebration technique.

The cats were then repositioned to the prone position. Earbars were inserted and the head was fixed in a David Kopf stereotaxic frame. The skin and muscles overlying the sixth and seventh ribs were removed with the aid of a cautery. The musculature was removed in a similar manner from the left side of the skull, the T1 spinal process, and a segment of the lumbar vertebrae.

The stereotaxic frame was then tilted downward so that the head was not greatly elevated above the heart. This procedure, although increasing the amount of cranial bleeding, was found to be beneficial because it prevented the entrance of air into the venous sinuses and

the subsequent trapping of air emboli in the lung to which cats are very sensitive. A hole approximately one-half inch in diameter was placed in the skull with a trephine. This hole was positioned in the left caudal portion of the skull in such a way that no major cerebral sinuses were damaged. Bone wax was used to help control the bleeding that sometimes occurred from the skull. A vacuum suction developing a subatmospheric pressure of approximately 25 centimeters of water was initiated and the dura was cut. The bulk of the cerebral cortex was removed, thereby visually exposing the superior and inferior colliculi. Using the tentorium as a guide, a blunt spatula was inserted between the colliculi at an angle of approximately 45 degrees and advanced until it touched the floor of the skull. While holding the spatula in this position and stabilizing the brainstem, the remainder of the nervous tissue rostral to the midcollicular section was removed. The basilar artery was then clamped with a sterling silver McKenzie brain clip (J. Sklar Mfg. Co.). A small piece of Surgicel absorbable hemostat (Surgikos, Johnson & Johnson) was rolled into a ball and forced into the sella turcica, thereby impeding the blood flow through the Circle of Willis. A second piece of Surgicel was gently placed over the surface of the cut brainstem. This entire decerebration technique required about five minutes to complete. Occasionally it was necessary to pack the skull with additional Surgicel in order to control the bleeding.

The halothane concentration in the rebreathing system was immediately decreased and the cat was carefully monitored for several minutes. Most cats continued to breathe rhythmically throughout the

decerebration procedure. After the intracranial bleeding had been completely stopped, the anesthesia was removed entirely. The preparation was then allowed to stabilize for about 30 minutes before it was moved to another stereotaxic device located in a large Faraday cage. A clamp was tightly fixed to the T1 spinal process and another clamp grasped the lumbar vertebrae. The cat was then suspended by these two clamps in the stereotaxic frame. Earbars were reinserted and the head was once again stabilized. A bilateral vagotomy was performed and the breathing pattern invariably slowed and deepened. Any cat that developed an obvious plateau phase in the integrated phrenic signal after vagotomy and continued in that manner for several minutes was sacrificed. Approximately 90% of all decerebrated animals did not become apneustic, but continued to breathe eupnically. Only these cats were used for subsequent data collection.

A small water circulating heating pad was tied loosely to the cat's ventral surface and a rectal thermometer (Yellow Springs Instr. Co., Inc. TeleThermometer 43TW) was inserted. Body temperature was maintained at 38 $\pm$ 1°C by adjusting the temperature of the circulating water bath. The cat was then paralyzed with a four mg/kg injection of gallamine triethiodide (Flaxedil, American Cyanamid Co.) administered through the femoral venous catheter. Artificial ventilation was initiated with a respirator (Harvard Apparatus Co., Inc., Model 661). The tidal volume and frequency of the respirator was adjusted to achieve a ventilation that would stabilize the end-expiratory $\%CO_2$ (measured with a Beckman LB1 CO_2 Gas Analyzer) at a level of approximately 4%. A continuous intravenous infusion of gallamine (4 mg/kg/hr) was then begun

in order to maintain a constant degree of paralysis throughout the experimental protocol.

The C5 and C6 rootlets of the right phrenic nerve were isolated via a dorsal approach and a pool of mineral oil was formed over the nerve before it was cut. The cut central end was positioned across a bipolar platinum-iridium electrode. Occasionally it became necessary to desheath the phrenic nerve to facilitate the recording signal. Electrical activity was amplified by a W.P. Instruments Model DAM-5A Differential Preamplifier and a Grass Dual P9 A.C. Preamplifier connected in series (passband - 300 Hz). The amplified and filtered phrenic signal was displayed on an oscilloscope (Tektronix D13), rendered audible (Grass AM7 Audio Monitor) and routed through a custom-built processing circuit that was a modification of the circuit used by Cohen (44). This processor performed a half-wave rectification and the subsequent "leaky" integration (moving average) of the rectified phrenic recording. The time constant of the resistance-capacitance integration was 200 milliseconds.

The processor also generated an inspiratory/expiratory gating signal which was determined from the onset and the termination of the phrenic inspiratory activity. The gating signal was high during inspiration and low during the period of phrenic quiescence. This signal was used to trigger a tachograph (Grass, Model 7P44B) which monitored the instantaneous respiratory frequency and a stimulator (W.P. Instruments, Series 800) which was used for intercostal nerve stimulation. Both outputs from the processor (the gating signal and the integrated phrenic recording) were displayed on the storage oscilloscope and were also

recorded on the polygraph. The entire preparation is illustrated in Figure 1.

The muscle layers between the sixth and the seventh ribs were carefully removed to expose the T6 intercostal nerve which is located along the caudal edge of the sixth rib against the parietal pleura. The pleura was usually perforated during this procedure. A short segment of the mixed intercostal nerve was dissected free and sectioned approximately 3 to 5 centimeters distal to its junction with the spinal column. The central end was positioned across a bipolar electrode and stimulated during midinspiration with a 3mA stimulus train (200Hz, 0.1 msec duration, 3-pulses). If this stimulation failed to elicit a premature termination of inspiration, the intercostal nerve was repositioned on the stimulating electrode. After a suitable response was elicited, the electrode and the nerve were stabilized and covered with mineral oil. Additional mineral oil was administered throughout the experiment.

Each animal experiment involved the determination of the minimal stimulus strength required to elicit an immediate transition from inspiration to expiration at several delays after the onset of inspiratory activity. Figures 2 and 3 demonstrate the nature of these phase transitions. Inspiratory duration is shortened and the amplitude of the integrated phrenic signal is decreased. Changes in the spontaneous respiratory frequency (Figure 3) also provided a sensitive criteria for the determination of premature phase switching, since effective stimulations shortened both the inspiratory and the expiratory durations.

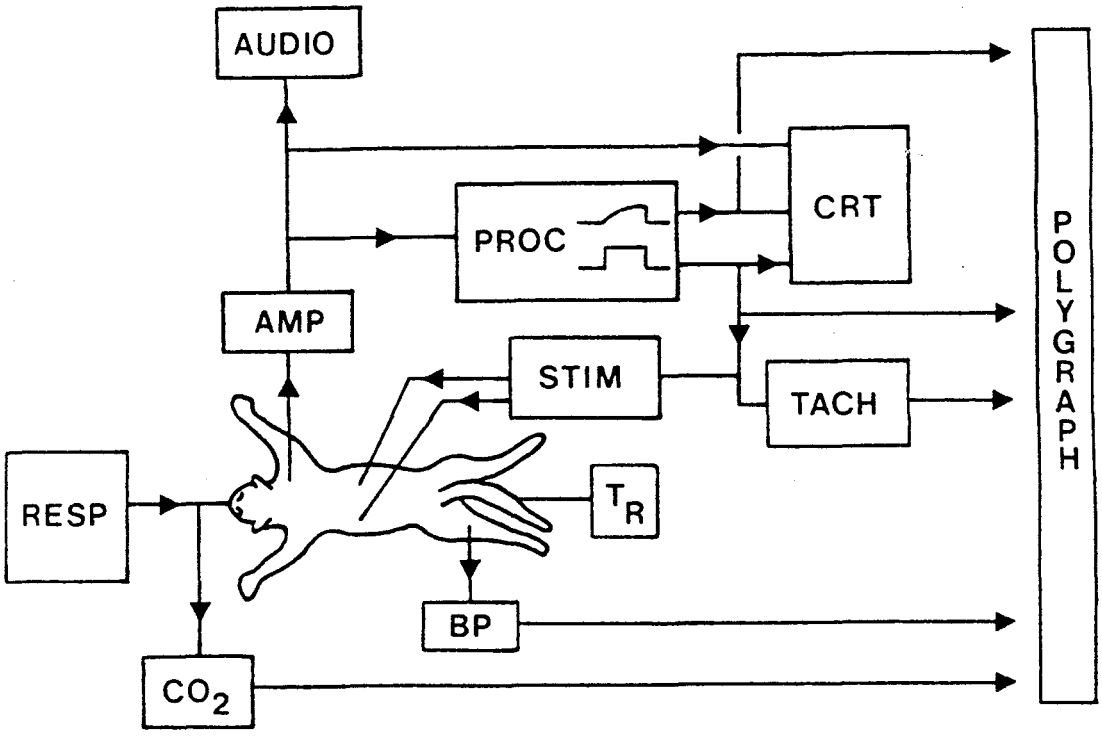

Figure 1. Instrumentation of the cat for the demonstration of inspiratory off-switch thresholds. Rectal temperature (T_R) and systemic blood pressure (BP) are monitored and displayed on a Grass Model 7 Polygraph. The cat is artifically ventilated (RESP) to a specific end-expiratory % CO_2 which is determined by a gas analyzer (CO_2). The right phrenic nerve is placed on a bipolar electrode and the signal is amplified (AMP), rendered audible (AUDIO), processed by a custom-built circuit (PROC), and displayed on an oscilloscope (CRT). The integrated phrenic signal and the gating signal are displayed on the oscilloscope and the polygraph. The gating signal triggers a tachograph (TACH) and a stimulator (STIM) which delivered a stimulus train to the T6 intercostal nerve.

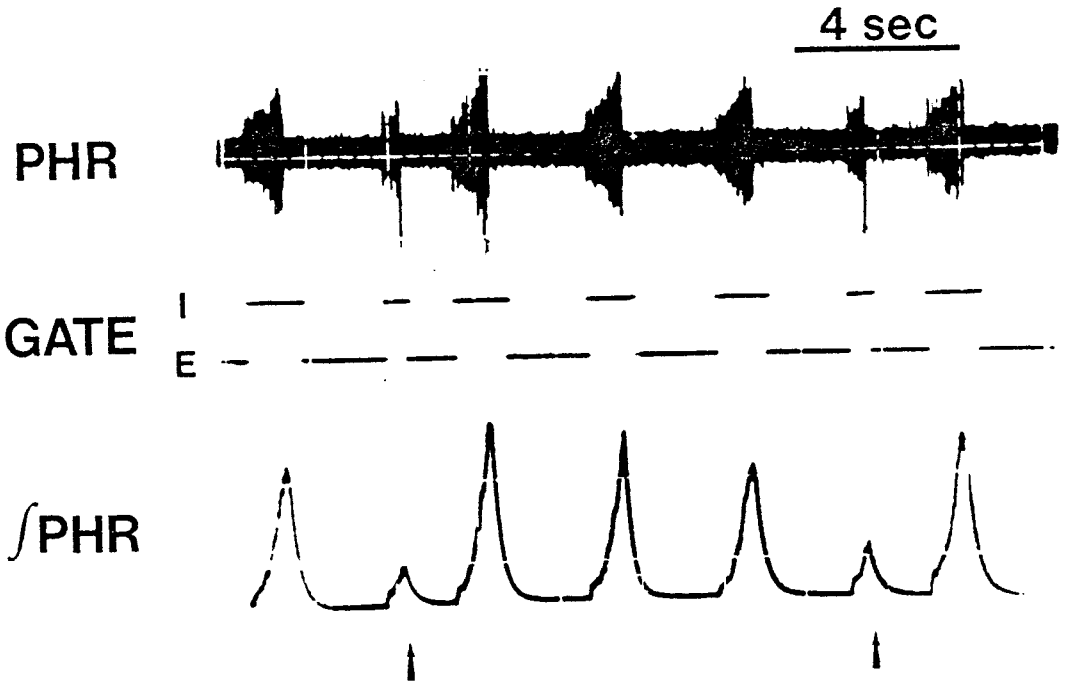

Figure 2. Example of an oscilloscope tracing during the determination of an inspiratory off-switch threshold. Channels include the raw phrenic neurogram (PHR), the inspiratory/expiratory gating signal (GATE), and the integrated phrenic activity (PHR). At the arrows the left intercostal nerve was stimulated with a threshold stimulus delivered 600 milliseconds after the onset of inspiration. Note the abrupt termination of phrenic activity and the attenuation of the integrated phrenic signal.

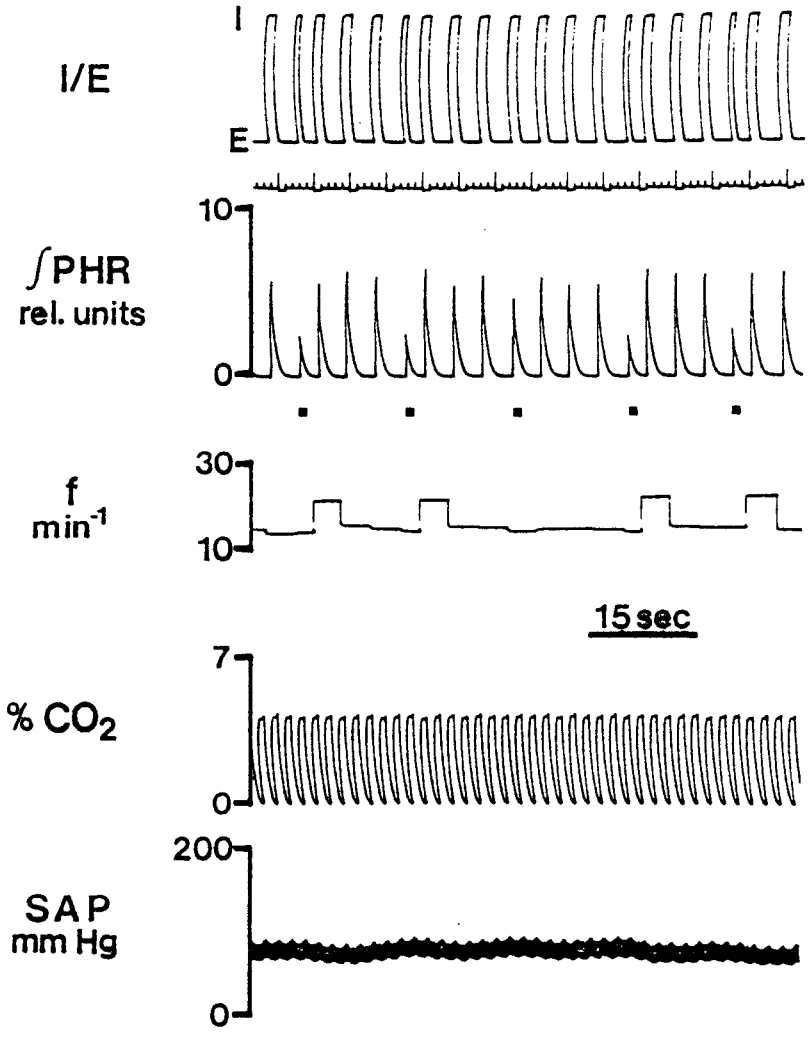

Figure 3. Example of the polygraph record during the determination of an inspiratory off-switch threshold. At the times indicated by the boxes, the left intercostal nerve was stimulated at a delay of 600 milliseconds. Successful activation of the inspiratory off-switch is evidenced by a step increase in the spontaneous respiratory frequency (f), a decrease in the integrated phrenic amplitude (PHR), and a premature termination of inspiration seen in the I/E gating signal. The third stimulus did not elicit a phase switch. Throughout the stimulations, end-expiratory % CO_2 and systemic arterial pressure (SAP) remain constant.

The stimulus intensity was adjusted over a range of 0.001 mA to 5 mA (W.P. Instruments, stimulator, series 800) until the threshold current was obtained. This threshold intensity was defined as the minimal current necessary to elicit a phase switch in at least 50% of the stimulated cycles. All intercostal nerve stimulations involved a brief stimulus train of only 3 pulses. These pulses were 0.1 msec in duration and they were delivered at a frequency of 200 Hz. The stimulus trains were triggered by the onset of inspiratory activity (positive gain of the inspiratory/expiratory gating signal) and any desired delay could be introduced between the trigger and the following stimulus. To guard against neural fatigue or facilitation, every fourth inspiratory cycle was stimulated in most animals. This stimulation pattern was changed occasionally in those animals which had either very rapid or very slow respiratory rhythms. In all cases, trains of stimuli were separated by at least 12 seconds.

B. Experimental Design and Statistics

Eight different experimental series were performed to examine the inspiratory off-switch excitability under various experimental conditions. The protocol for each experimental group involved the determination of threshold stimulus strengths before, during, and after some sort of perturbation. Each experimental series contains the data obtained from ten successful individual cat preparations. Experiments were judged as successful for only those animal preparations which fulfilled two criteria. First, it was necessary that the cat maintained a rhythmic breathing pattern throughout the experiment. Second, it was required

that repeatable thresholds for inspiratory termination could be elicited at delays of 300 milliseconds after the onset of inspiration.

In each cat, nerve stimulations were administered at ten different delays ranging from 100 to 1,000 milliseconds after the initiation of inspiratory activity. Since 10 cats were utilized for each group, a 10 x 10 Latin Square was used to provide a perfect randomization of the order of delays. This technique assured that the results obtained in these experiments were not due to systematic experimental error or preparation deterioration. For each experimental preparation, the threshold stimulus strength was determined at each of the ten delays. These observations comprised the control measurements. The experimental conditions were then altered and a second set of stimulus strengths were obtained. Whenever possible, the perturbation was then removed and a third set of data was determined. This recovery phase guaranteed that any changes seen during the perturbation were actually due to that perturbation, and not due to the instability and deterioration of the animal preparation.

All statistical comparisons involved paired data obtained from the same animal. Therefore a two-tailed Students paired t-test was used. Some of the experimental groups involved numerous comparisons with the same control. In these studies, the multiple comparison tables devised by Dunnett (195) were used in conjunction with the Students paired t-test. These tables compensate for the increased alpha error introduced when one uses the same data in more than one Students t-test. P values of less than 0.05 were considered statistically significant.

C. Experimental Groups

In the 10 cats comprising Group I, both the left and right intercostal nerves were stimulated. The inspiratory-terminating thresholds were determined for the independent stimulation of the left and the right T6 intercostal nerve and for the simultaneous stimulation of both nerves. The sequence of these three different types of stimulation was randomized and the order of selected delays within each stimulation sequence was determined by the Latin Square as previously described.

The experiments of Group II examined the importance of other somatic afferents on the inspiratory phase switching mechanism. The left superficial radial nerve and the left sciatic nerve were isolated and mounted across a bipolar electrode. These nerves were stimulated individually at delays of 600 msec after the onset of inspiration with stimulus trains identical to those used for intercostal nerve stimulation. Current strength and/or the duration of the stimulus train was then increased as needed in an effort to determine an off-switch threshold for the stimulation of these nerves. Intercostal stimulations were also performed in each cat to assure the responsivity of the animal.

Groups III, V, VI, and VII examined the effects of changes in the alveolar gas concentration upon the excitability of the inspiratory off-switch. In two of these series (Groups III and V), threshold strengths were compared at different levels of end-expiratory % CO_2. The carbon dioxide levels in these particular studies were increased from 4% to 5% by decreasing the tidal volume of the respirator. It is assumed that

such a decrease in ventilation would cause a decrease of approximately 1% in the alveolar oxygen concentration. Therefore, the effects of this perturbation could be due to the alteration of both oxygen and carbon dioxide levels. The response to a 1% elevation of alveolar carbon dioxide concentration should greatly exceed the response elicited by the slight reduction of oxygen within the alveoli. Group VI was conducted to examine the effects of oxygen exclusively. In these preparations, the respirator frequency and tidal volume was adjusted so that the end-expiratory % CO_2 could be maintained at approximately 4% throughout all of the experimental phases. Off-switch excitability was determined while the cat was ventilated with: (1) room air, (2) a hypoxic gas mixture, (3) a hyperoxic mixture, and (4) room air. In half of the experiments, the administration of the hyperoxic gas mixture preceded the hypoxic gas. Hypoxic (17% O_2) and hyperoxic (45% O_2) gases were obtained by mixing 100% O_2 with 100% N_2 in a large plastic bag. Oxygen concentration was then analyzed with a Beckman OM-11 oxygen gas analyzer.

Group VII, the last of the studies involving alteration of the alveolar gas concentration, required the independent alteration of oxygen and carbon dioxide. A hypoxic gas mixture identical to that described above was used. A hypercapnic normoxic gas (4% CO_2, 21% O_2, balance N_2) was also mixed and analyzed with a Beckman LB-2 CO_2 Gas Analyzer and the Beckman OM-11 O_2 Gas Analyzer. Inhalation of this mixture provided a normoxic hypercapnic stimulus and eliminated the problem of simultaneous changes in oxygen and carbon dioxide that had been associated with the previous studies. The experiments of Group VII

also investigated the role of the peripheral chemoreceptors in the response to altered carbon dioxide and oxygen concentrations. In each of these cats, the carotid sinus nerve was located with the aid of a stereomicroscope and looped with a loose silk ligature before the decerebration was performed. While the carotid sinus nerves were intact, threshold curves were determined under four different experimental phases: (1) control, (2) hyperoxia, (3) hypoxia, and (4) recovery. By pulling on the silk ligature, the nerves were then sectioned and the four experimental phases were repeated.

Groups IV and V investigated the inspiratory off-switch excitability before and after the removal of the cerebellum. Before the acquisition of any data, the cat's head was tilted forward in the stereotaxic device and the caudal portion of the occipital bone was cut away with rongeurs. The dura was cut and mineral oil was poured over the exposed cerebellum. The stimulus strengths were then determined and the cerebellum was then removed by vacuum suction. After complete removal of the cerebellum, the floor of the fourth ventricle was covered with Surgicel. Most cats became slightly apneustic for a few minutes subsequent to this procedure. Those animals that continued to breathe apneustically for long periods were sacrificed.

The purpose of experimental Group VIII was to determine the effects of mean carotid sinus pressure on the excitability of the inspiratory off-switch. All cats were prepared with the surgical procedures previously described. In addition, a technique described by Manning (137, 187) was used to vary and control the carotid sinus pressure.

Before the cats were decerebrated, the external carotid arteries and the common carotid arteries were cannulated bilaterally with the aid of a Zeiss stereomicroscope. These four catheters were all advanced toward the region of the carotid sinus. PE 60 polyethylene tubing was secured in the external carotid arteries and PE 100 was used in the common carotid arteries. A fifth catheter was inserted into the right common carotid artery and advanced toward the heart. This catheter was then reconnected to the right common carotid arterial catheter that had been advanced to the carotid sinus, thereby permitting reperfusion of the carotid sinus.

After the decerebrated preparation had stabilized and the intercostal stimulation technique had been set up, the animal was heparinized (about 200 units) and the carotid sinus was isolated as seen in Figure 4. Oxygenated blood was obtained from the right carotid artery. This blood was pumped (Cole-Parmer Instr. Co.; Rollerpump Mod. WZ1R057) at a constant flow rate through both of the external carotid arterial catheters. The small diameter of these catheters and the air-filled damping column interacted to minimize the pressure oscillations developed by the pump. Some blood left the carotid sinus through the many small branches that still remained; however, the bulk of the blood flow passed through the sinus in a retrograde fashion and entered the bilateral peripherally directed common carotid arterial catheters. The blood flow from these cannulas merged and a pressure transducer (Statham P23Db) monitored the pressure in the carotid sinus. A screwclamp enabled alteration of the resistance of this circuit. When resistance was

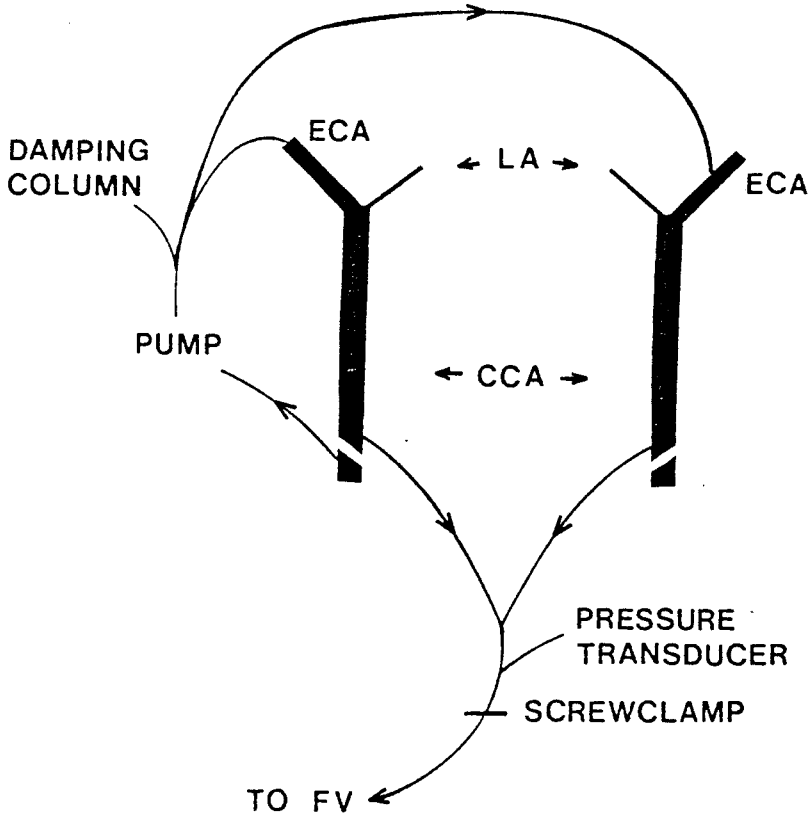

Figure 4. Preparation for the isolation of the carotid sinus. Arterial blood is pumped at a constant flow from the common carotid artery (CCA) into the external carotid arteries (ECA) on both sides of the cat. The carotid sinus is perfused in a retrograde fashion and the effluent blood is collected from the common carotid arteries. Resistance is regulated with the screwclamp and the blood is returned to the femoral vein (FV). The lingual (LA) arteries are ligated.

changed, the carotid sinus pressure could be manipulated from approximately 50 mm Hg to about 175 mm Hg.

Separate stimulus strength versus delay curves were obtained for carotid sinus pressures of 50, 100 and 150 mm Hg. Since each curve would have required about half an hour to complete, a slightly different protocol was needed to avoid the mechanoreceptor adaptation that is known to occur with sustained pressures. The order to delays was once again randomized by the Latin Square. At each delay, the threshold stimulus strength was determined while the carotid sinus pressure was maintained at 100 mm Hg. The carotid sinus pressure was then decreased to 50 mm Hg and the threshold was determined within two minutes. The pressure was immediately elevated to 150 mm Hg and a third stimulus strength was determined before the pressure was returned to 100 mm Hg. The fourth threshold value which then was determined, served as a recovery measurement. This procedure was repeated for all ten delays. Utilization of this technique assured that all stimulus thresholds were obtained under similar conditions; that is, all values were obtained approximately 1½ to 2 minutes after the carotid sinus pressure had been changed. This timing permitted the completion of rapid receptor adaptation, without allowing an appreciable amount of slow adaptation, before the thresholds were determined.

CHAPTER IV

RESULTS

In every decerebrate cat studied during the course of these experiments, a premature phase switch from inspiration to expiration could be produced by stimulation of the left T6 intercostal nerve at any delay greater than 500 milliseconds after the onset of phrenic activity. The inhibition of inspiration elicited by adequate intercostal nerve stimulation followed the stimulation after a relatively short latent period. The inability to elicit phase switches in a few cats at shorter delays was probably due to either poor conduction between the stimulating electrode and the nerve, or damage of the nerve which resulted in a failure of the nerve to conduct. A third possibility exists that even with good neural conduction of the stimuli, the maximal current (5mA) of intercostal nerve stimulation did not provide an adequate excitation of the inspiratory off-switch mechanism. Only those cats which had distinct phase switches at delays of 200 milliseconds after the onset of phrenic activity were used to complete the Latin Square design for each of the eight experimental groups. This criteria excluded only a few cats in which the intercostal nerve may have been damaged during dissection.

The threshold stimulus strengths, which were determined at ten different delays in each cat, were used in the construction of graphs of stimulus strength versus time into inspiration (mA vs. Ti). These

curves indicated the additional excitation required to reach the inspiration off-switch threshold strength decreased as the cats proceeded farther into inspiration, thus demonstrating the time-dependent characteristic of off-switch activation. The mA vs. Ti curves obtained in each cat of Group I with stimulation of the left intercostal nerve are shown in Figure 5. In most of the curves, the threshold stimulus strength decreased as an almost linear function of the time into inspiration. In a few experiments, this curve tended to be hyperbolic in nature with the steepest portion of the curve occurring during early inspiration. The hyperbolic characteristic was lost after averaging the data from all 10 cats as seen in Figure 6. Statistical analysis indicated that stimulation of the right T6 intercostal nerve produces the same off-switch effect as does stimulation of the left T6 intercoastal nerve. However, a significant decrease in threshold was observed at all delays when both nerves were stimulated simultaneously.

The experiments of Group II examined the importance of other somatic afferents on the inspiratory phase switching mechanism. In 10 cats, the left superficial radial nerve and the left sciatic nerve were isolated and mounted on bipolar electrodes. These nerves were stimulated individually at delays of 600 milliseconds after the onset of inspiration. Current strength was increased and stimulus parameters were adjusted as needed in an effort to determine an inspiration off-switch threshold for stimulation of these nerves. The respiratory responses to stimulation of these nerves are displayed in Table I. Stimulation of the superficial radial nerve, a cutaneous nerve, caused an increase in the amplitude of the integrated phrenic signal in most cats, but elicited a phase switch

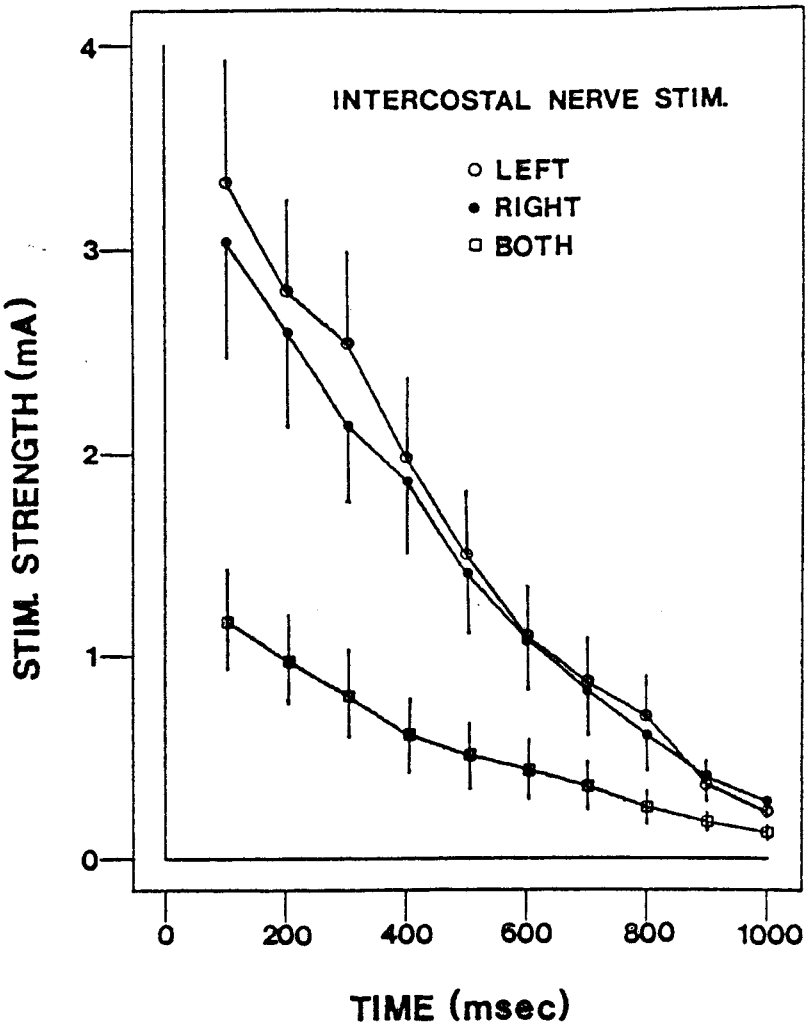

Figure 6. Time-course of off-switch excitability assessed by T6 intercostal nerve stimulation. At each delay, there is no significant difference between the left and the right T6 intercostal nerve stimulation threshold. Simultaneous stimulation of both nerves does significantly lower the stimulus strength required to terminate inspiration. Data from 10 cats (mean $\pm$ S.E.M.).

TABLE I.

Respiratory Response to Somatic Nerve Stimulation

Cat #	Radial Nerve Stimulation	Sciatic Nerve Stimulation
1201	- (PA, f)	- (PA, f)
1208	+ 600 msec	+ 600 msec
1192	- (f)	- (f, Ti)
1180	-	+ train
1211	-	+ 500 msec
1206	- (PA)	- (PA, Ti)
1154	-	+ train
1219	-	-
1218	- (PA)	-
1226	-	+ 500 msec

Abbreviations

-	Stimulation did not prematurely terminate inspiration.
+	Inspiration was prematurely terminated at delays listed.
train	Inspiratory termination was accomplished only by stimulus trains greater than 50 milliseconds (10 pulses, 200 Hz, 5mA).
PA	Stimulation elicited an increased phrenic amplitude.
f	Stimulation elicited an increased respiratory frequency.
Ti	Stimulation lengthened the time of inspiration.

to expiration in only one of the ten animals. Sciatic nerve stimulation proved to be a slightly more reliable means of producing a phase switch from inspiration to expiration. Inspiration was prematurely terminated in one-half of these trials. However, two cats demonstrated prolonged inspiratory durations after sciatic nerve stimulation. For both of these types of somatic nerve stimulation, thresholds could never be found at delays of less than 500 milliseconds. When thresholds were determined, the current strength required to elicit an off-switch response was always greatly elevated from the threshold observed during intercostal nerve stimulation in the same cat. The cerebellum was removed in four cats, two of which exhibited inspiratory termination after sciatic nerve stimulation. Cerebellectomy did not affect the response to stimulation in any of these cats.

Group III was designed to demonstrate the effects of carbon dioxide on the inspiratory off-switching mechanism. In all ten cats, thresholds were determined at each delay with the end-expiratory % CO_2 maintained constant at 4%. The ventilation was then decreased and the % CO_2 rose to approximately 5%. The % O_2 was assumed to fall correspondingly by about 1%. A second mA versus Ti curve was thus determined during hypercapnia. The ventilation was again increased and the end-expiratory % CO_2 returned to 4% before generating the recovery data. These three curves are displayed in Figure 7. As observed in the first series, every animal demonstrated a decrease in the stimulation threshold as the delay increased. When the end-expiratory % CO_2 was increased from 4 to

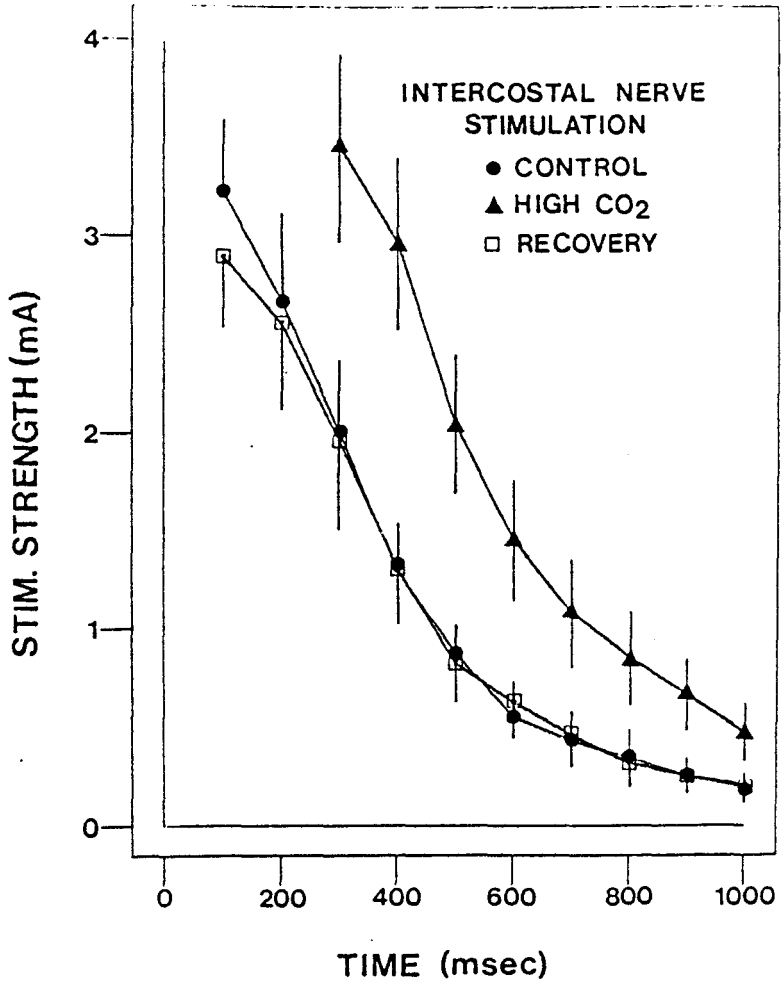

Figure 7. Time-course of off-switch excitability assessed by T6 intercostal nerve stimulation at different carbon dioxide levels. Control and recovery curves are both obtained under conditions of 4% end-expiratory % CO_2. There is no significant difference between these curves. Hypercapnia (5% end-expiratory CO_2) significantly elevates the stimulus strength required to terminate inspiration at all delays. Data from 10 cats (mean $\pm$ S.E.M.).

5%, there was a significant increase at every delay in the amount of stimulation necessary to terminate inspiration. In these cats, the spontaneous respiratory frequency decreased from a mean value (± S.E.M.) of 16.1 (± 2.2) to 11.4 (± 1.1) breaths per minute during hypercapnia. This decreased frequency resulted from the prolongation of both inspiratory and expiratory duration. During hypercapnia, the mean arterial pressure tended to increase (Mean ± S.E.M. - 118.5 ± 6.0 to 126.2 ± 7.4) mm Hg) although the increase was not statistically significant. Thresholds determined during the recovery phase did not differ from those obtained during control ($P \geq 0.25$). This guaranteed that threshold changes resulting from hypercapnia were not attributable to preparation instability or irreversible deterioration.

The experiments of Group IV investigated the inspiratory off-switch thresholds for intercostal nerve stimulation before and after removal of the cerebellum. To complete this study, cerebellectomies were performed in 15 cats. Hemmorhage resulting from the removal of the cerebellum could not be controlled in two of the cats and these animals succumbed to the subsequent depression of systemic arterial pressure. The remaining 13 cats maintained their mean arterial pressure within the range of 85 to 115 mm Hg after cerebellectomy, although there was a slight but insignificant tendency for the pressure to fall from a mean value of 113.0 to 105.6 mm Hg. Most cats became apneustic during the removal of the cerebellum, but this apneustic pattern usually persisted for only a few minutes before reverting to a breathing pattern similar to that observed prior to cerebellectomy. Three cats remained severely

apneustic and these animals were sacrificed. In the ten experiments which contributed data for Group IV, there was no significant difference in the respiratory frequency before (Mean $\pm$ S.E.M. - 13.1 $\pm$ 2.1) and after (14.3 $\pm$ 2.5) cerebellectomy. The mean threshold stimulus strengths for this group are shown in Figure 8. Each cat was ventilated at the same end-expiratory % CO_2 throughout both phases of data acquisition. This graph clearly demonstrates that there is no change in the strength of intercostal nerve stimulation required to terminate inspiration after the removal of the cerebellum.

The results of Group V indicated that the effects of carbon dioxide on respiratory rhythmicity are not mediated through the cerebellum. This experimental series also provided further confirmation of the data obtained from Groups III and IV. Stimulus strength versus time into inspiration curves were acquired under three different experimental phases. These phases (control, high CO_2, recovery) were identical to those utilized in the protocol of Group III. After completion of these three curves seen in Figure 9, the cerebellum was removed and curves were once again determined under the same three phases. The data acquired after cerebellectomy are displayed in Figure 10. Statistical comparison of each point of the pre-cerebellectomy control with the corresponding values for the pre-cerebellectomy recovery (Figure 9), the post-cerebellectomy control and the post-cerebellectomy recovery (Figure 10) indicated that these four curves were not statistically different ($P \geq 0.20$). The stimulus strengths obtained during hypercapnic ventilation were significantly elevated from control values at all delays before cerebellectomy

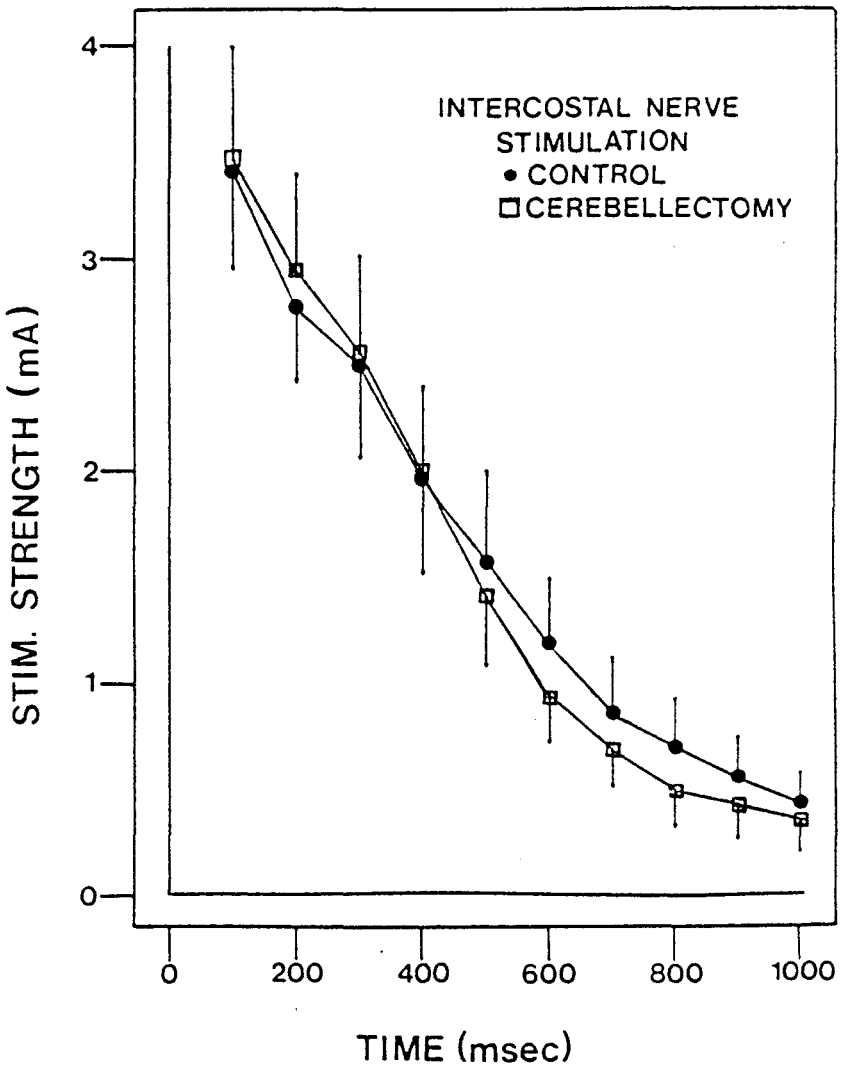

Figure 8. Time-course of off-switch excitability assessed by T6 intercostal nerve stimulation before and after removal of the cerebellum. Cerebellectomy did not significantly change the stimulus strength required to terminate inspiration. Data from 10 cats (mean ± S.E.M.).

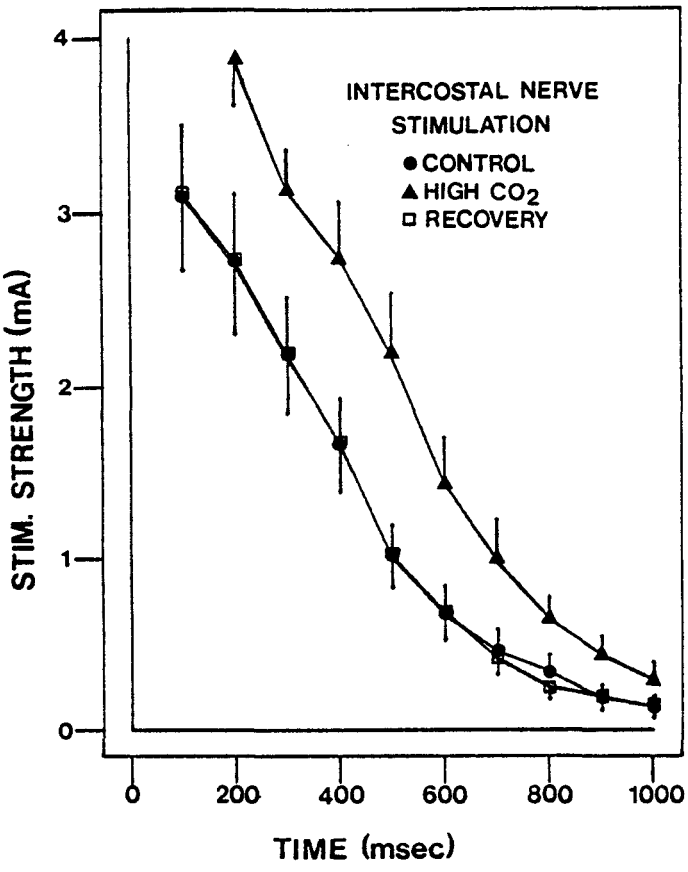

Figure 9. Time-course of off-switch excitability assessed by T6 intercostal nerve stimulation at different carbon dioxide levels before removal of the cerebellum. Control and recovery curves are both obtained under conditions of 4% end-expiratory % CO_2. There is no significant difference between these curves. Hypercapnia (5% end-expiratory CO_2) significantly elevates the stimulus strength required to terminate inspiration at all delays. Although these data are collected in a protocol identical to that used in Figure 7, separate groups of cats were used for the two figures. Data from 10 cats (mean $\pm$ S.E.M.).

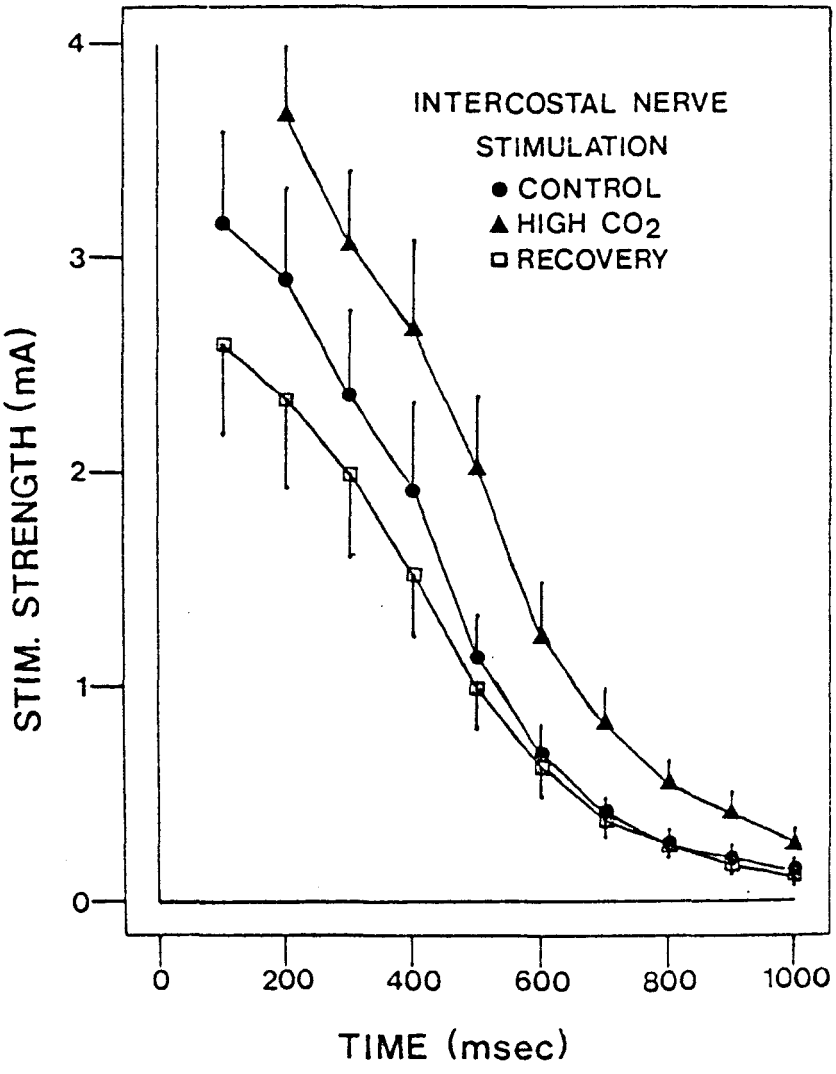

Figure 10. Time-course of off-switch excitability assessed by T6 intercostal nerve stimulation at different carbon dioxide levels after removal of the cerebellum. These curves were determined in the same cats used for Figure 9. Control and recovery curves are both obtained under conditions of 4% end-expiratory % CO_2. Neither of these curves differ significantly at any point from the values shown in the control curve of the previous figure. Hypercapnia (5% end-expiratory CO_2) significantly elevates the stimulus strength required to terminate inspiration at all delays shorter than 800 milliseconds. Data from 10 cats (mean $\pm$ S.E.M.)

and at all delays shorter than 800 milliseconds after cerebellectomy. In these ten cats, the breathing frequency and the mean arterial pressure were not significantly affected by cerebellectomy, although there was a decrease in respiratory frequency from a mean value ± S.E.M. of 12.4 ± 1.11 to 9.9 ± 1.22 breaths per minute. Hypercapnia did not elicit any changes in mean arterial pressure or respiratory frequency in these vagotomized cats either pre- or post-cerebellectomy.

Group VI investigated the effects of oxygen upon the inspiratory off-switching mechanism. While maintaining end-expiratory % CO_2 constant at approximately 4%, the cats were ventilated with room air, a hypoxic gas mixture (17% O_2), a hyperoxic mixture (45% O_2), and finally room air. Spontaneous respiratory frequency was unchanged by the mild hypoxia (Mean ± S.E.M. - 14.8 ± 1.3 bpm), and decreased insignificantly during hyperoxic conditions (14.1 ± 1.4 bpm). The amplitude of the integrated phrenic was measured in relative units from the polygraph records and showed more variability. During the control and recovery ventilation, both with room air, the mean phrenic amplitudes ± the S.E.M. were 17.7 ± 1.9 and 16.9 ± 2.2 respectively. Under hypoxic conditions, the amplitude increased significantly to 23.4 ± 2.2, while during ventilation with the hyperoxic gas mixture, the phrenic amplitude decreased significantly to 10.9 ± 1.5. These changes in phrenic amplitude were accompanied by shifts in the stimulus strength versus the time into inspiration curves as illustrated by Figure 11. Statistical differences from control were demonstrated at the first six delays of the hypoxic curve and at the first five delays plus the 700 millisecond delay of the hyperoxic curve.

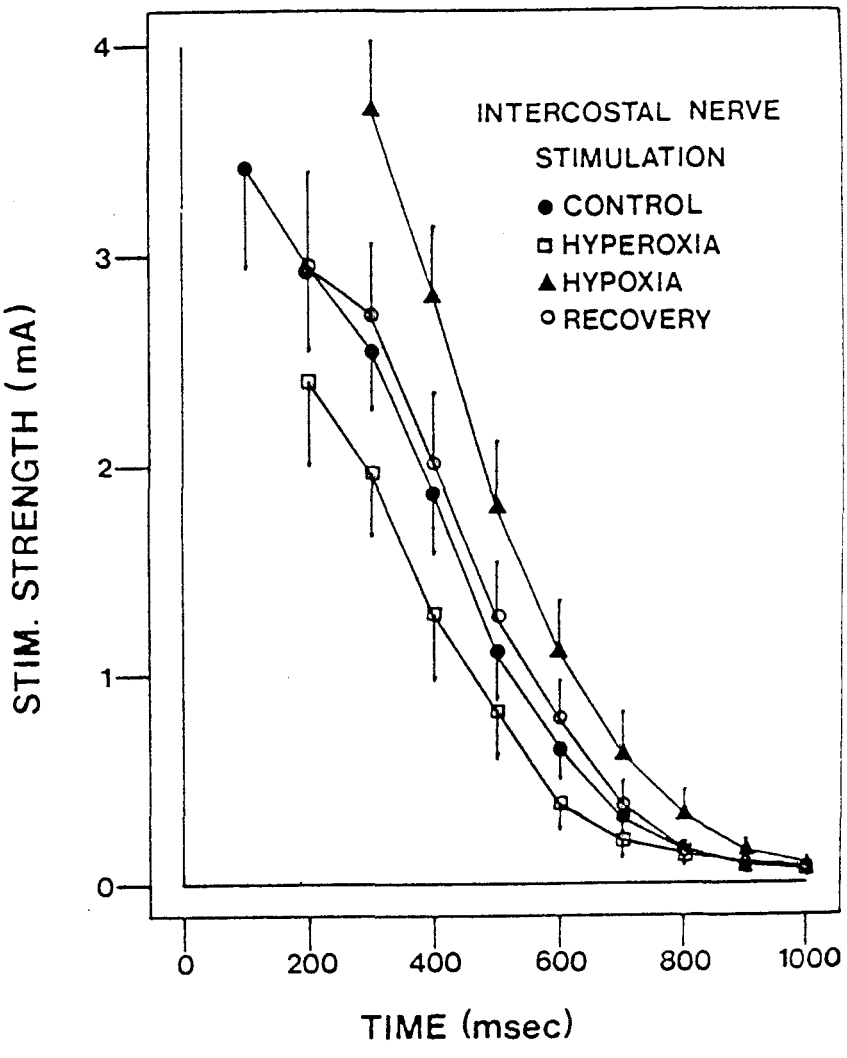

Figure 11. Time-course of off-switch excitability assessed by T6 intercostal nerve stimulation at different oxygen concentrations. All data points were obtained under conditions of 4% end-expiratory % CO_2. During the control and recovery phases the cats were ventilated with room air. These curves are statistically identical. Ventilation with a hyperoxic gas mixture (45% O_2) significantly decreased the stimulus strength required to terminate inspiration at all delays less than 700 milliseconds except the 600 msec delay. Hypoxic ventilation (17% O_2) significantly increased the threshold at the first six delays. Data from 10 cats (mean $\pm$ S.E.M.).

The experiments of Group VII were conducted under eight separate conditions. In each of these cats, the carotid sinus nerves were isolated bilaterally and looped with loose ligatures before the decerebration procedure was performed. After completion of the normal surgical preparations, stimulus strength versus time into inspiration data were collected during ventilation with first room air (control), then a hypercapnic normoxic gas mixture, a hyperoxic gas mixture, and room air (recovery). The carotid sinus nerves were then sectioned bilaterally by tugging on the ligatures. The entire data collection procedure was repeated under the same four experimental phases. Figure 12 demonstrates the cardiovascular and respiratory parameters from one cat that were recorded on the polygraph. During ventilation with 4.0% carbon dioxide the end-expiratory % CO_2 increased to approximately 6.5%. The elevation of alveolar carbon dioxide concentrations significantly increased the amplitude of the integrated phrenic signal from a mean value $\pm$ the S.E.M. of 17.7 $\pm$ 1.7 to 30.1 $\pm$ 3.2 relative units. Although some cats demonstrated changes in respiratory frequency (Figure 12), there was no statistically significant change in the mean respiratory frequency for the group of 10 cats. The respiratory frequency also remained stable during normocapnic hyperoxic ventilation although the phrenic amplitude decreased significantly to 12.3 $\pm$ 1.4 relative units.

After completion of the first four experimental phases, a bilateral carotid artery occlusion was performed. This manipulation decreases the carotid sinus pressure and deactivates the baroreceptors as well as activating the chemoreceptors if it is maintained for more than a few

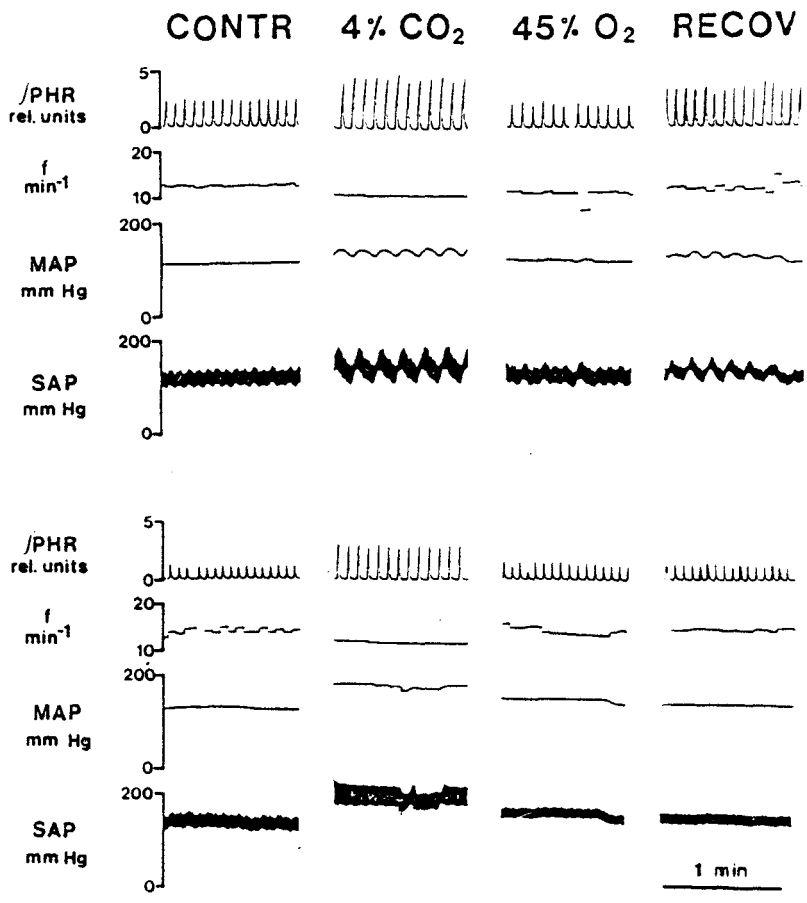

Figure 12. Cardiovascular and respiratory responses during the eight phases of Group VII from a single cat. During control (CONTR) and recovery (RECOV), the cat was ventilated with room air to an end-expiratory % CO_2 of 4%. The cat was also ventilated with 4% CO_2 and 45% O_2. These four phases were examined before (A) and after (B) carotid sinus nerve section. Parameters recorded are top to bottom; integrated phrenic nerve activity, spontaneous respiratory frequency, mean arterial pressure, and systemic arterial pressure.

seconds. Bilateral carotid occlusion produced a substantial increase in the systemic arterial pressure as seen in Figure 13. The carotid sinus nerves were then sectioned. This procedure usually elicited an immediate increase in both respiratory frequency and systemic arterial pressure. Occasionally there was a very transient response to carotid sinus denervation which resulted in a decrease in both respiratory frequency and systemic pressure. Upon completion of the carotid sinus nerve section, a second bilateral carotid artery occlusion was performed. The absence of any change in systemic blood pressure verified that the nerves which had been sectioned were indeed the carotid sinus nerves.

Bilateral section of the carotid sinus nerves resulted in a significant depression of the integrated phrenic signal (from a mean $\pm$ S.E.M. of 17.1 $\pm$ 1.2 to 12.7 $\pm$ 2.1) to a value similar to that obtained during hperoxic ventilation. Accompanying the decreased phrenic amplitude was an increase in respiratory frequency from 12.0 $\pm$ 1.3 to 15.6 $\pm$ 1.4 breaths per minute. Statistical analysis proved this increase in respiratory frequency to be statistically insignificant. In the carotid sinus denervated cats normoxic hypercapnia still caused a significant increase in phrenic amplitude. However, instead of the significant decrease in phrenic amplitude with hyperoxia that occurred in the sinus nerve intact cats, after sinus denervation, there was a slight but insignificant increase in mean phrenic amplitude ($\pm$ S.E.M.) from 12.7 ($\pm$ 2.1) to 14.1 ($\pm$ 3.1) relative units. A complete listing of the mean end-expiratory % CO_2 value, the respiratory frequencies, and the phrenic amplitudes for all eight experimental phases is given in Table II.

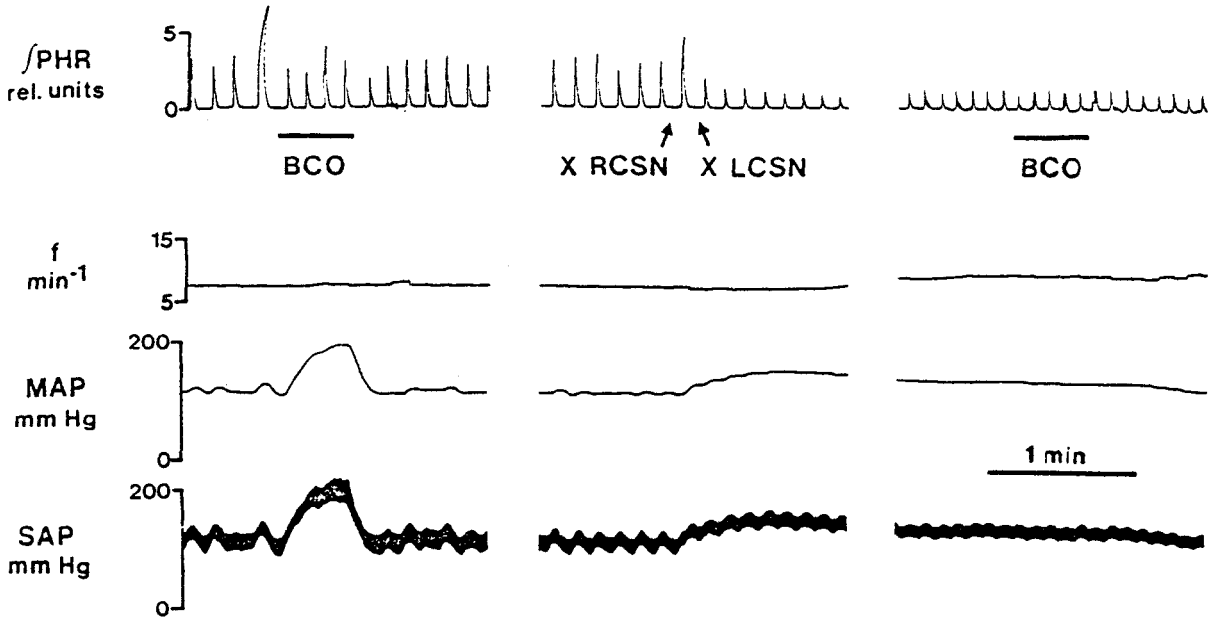

Figure 13. Respiratory and cardiovascular responses to bilateral carotid occlusion (BCO) before and after section of the left (X LCSN) and right carotid sinus nerve (X RCSN). Interruption of the carotid sinus nerves eliminates the response to baroreceptor deactivation. Parameters are listed in Figure 12.

TABLE II.

RESPIRATORY RESPONSES TO THE EIGHT EXPERIMENTAL PHASES OF GROUP VII.

EXPERIMENTAL PROCEDURE	END EXPIRATORY % CO_2	RESPIRATORY FREQUENCY	PHRENIC AMPLITUDE
A. Intact Cat			
Control	4.4 ± 0.13	12.3 ± 1.1	17.7 ± 1.7
Hypercapnia	6.5 ± 0.06*	11.8 ± 1.5	30.1 ± 3.2*
Hyperoxia	4.4 ± 0.13	11.9 ± 1.0	12.3 ± 1.4*
Recovery	4.4 ± 0.13	12.0 ± 1.3	17.1 ± 1.2*
B. Carotid Sinus Denervated			
Control	4.6 ± 0.13	15.6 ± 1.4	12.7 ± 2.1***
Hypercapnia	6.5 ± 0.09**	12.9 ± 1.2	27.6 ± 4.0**
Hyperoxia	4.6 ± 0.13	15.5 ± 1.2	14.1 ± 31.
Recovery	4.6 ± 0.13	16.5 ± 1.1	13.7 ± 2.7

Means ± S.E. for 10 cats
* - Statistical difference from the control value.
** - Statistical difference from the carotid sinus denervated control value.
*** - Statistical difference from the intact recovery value.

In the intact preparation, normoxic hypercapnia significantly increased the stimulus strength required to terminate inspiration at all ten delays (Figure 14). On the other hand, hyperoxia decreased the threshold stimulus strength. Although the differences between the control and hyperoxia curves do not look very large, statistical analysis proved all points except those obtained at delays of 700 and 800 milliseconds to be significantly different from the control value. The recovery curve was identical to the initial control and was not graphed on Figure 14. Since this recovery curve immediately preceded the carotid sinus nerve section, it was used as the control value for comparison with the values obtained after carotid sinus nerve section. Figure 15 demonstrates that the stimulus strengths required to terminate inspiration are decreased significantly, by bilateral carotid sinus denervation at all values except those obtained at delays of 300 and 1,000 milliseconds. Figure 16 displays the stimulus strengths determined in the cats after the carotid sinus nerves were cut. Hypercapnia still elicits a dramatic increase in the threshold stimulus strengths; however, hyperoxia has no effect upon the strength versus time into inspiration curves.

The experiments of Group VIII examined the effects of changes in baroreceptor activity upon the inspiratory off-switch. The special preparation illustrated in Figure 4 was necessary to provide adequate control of the pressure within the carotid sinus. This isolated carotid sinus technique proved to be a very reliable stimulus as indicated in Figure 17. Step changes in carotid sinus pressure were initiated by

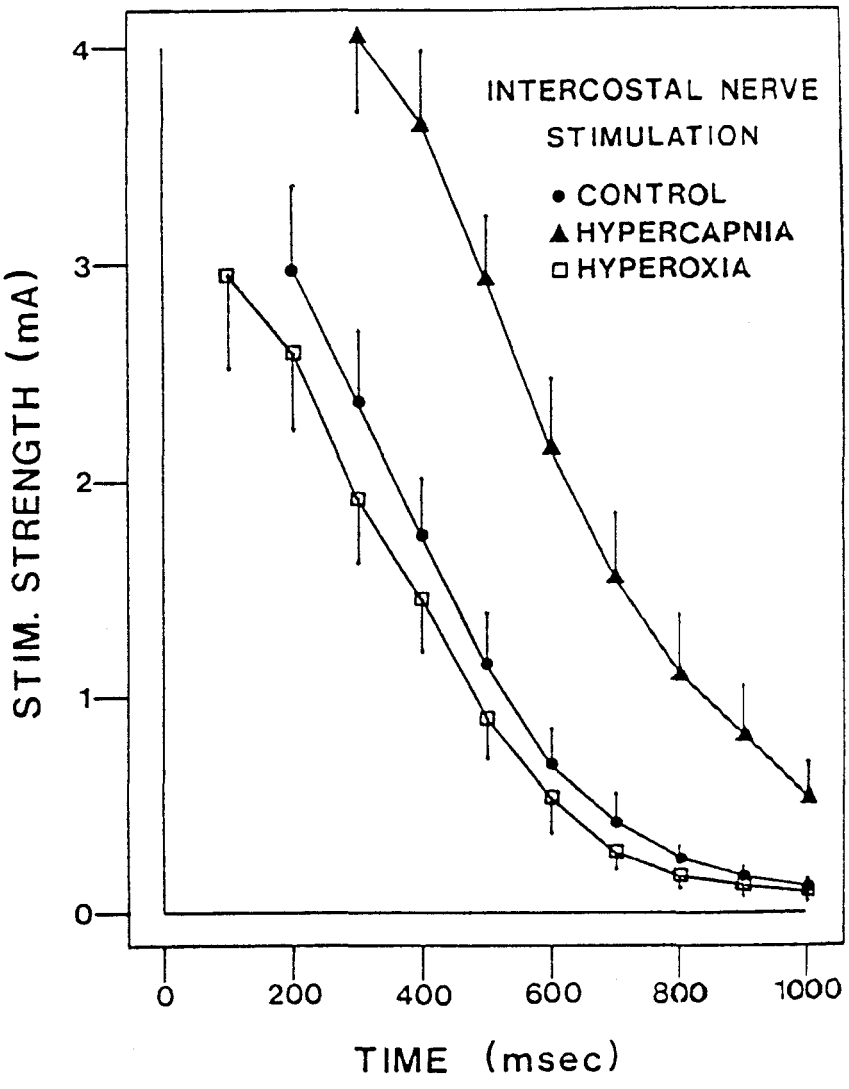

Figure 14. Time-course of off-switch excitability assessed by T6 intercostal nerve stimulation. Ventilation with a gas mxiture containing 4% CO_2 created a situation of normoxic hypercapnia and significantly elevated the stimulus strength required to terminate inspiration at all delays. Hyperoxia (45% O_2) significantly decreased the stimulus strength at all delays except 700 and 800 milliseconds. A recovery curve was identical to the control curve and is not graphed on this figure. Data from 10 cats (mean $\pm$ S.E.M.).

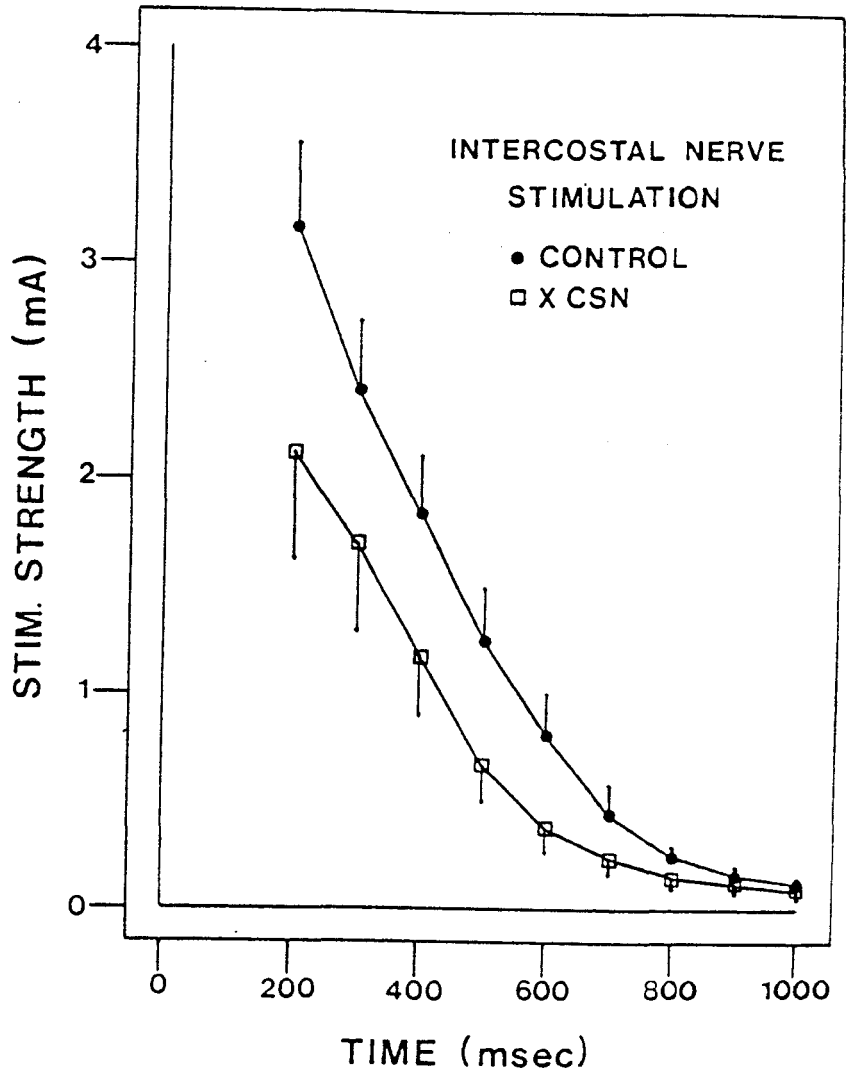

Figure 15. Time-course of off-switch excitability assessed by T6 intercostal nerve stimulation. The control curve was obtained immediately before section of the carotid sinus nerves and is identical to the recovery curve discussed in the previous figure. After bilateral section of the carotid sinus nerves (X CSN), the stimulus strength required to terminate inspiration was significantly decreased at all delays except 300 and 1,000 milliseconds. Data from 10 cats (mean ± S.E.M.).

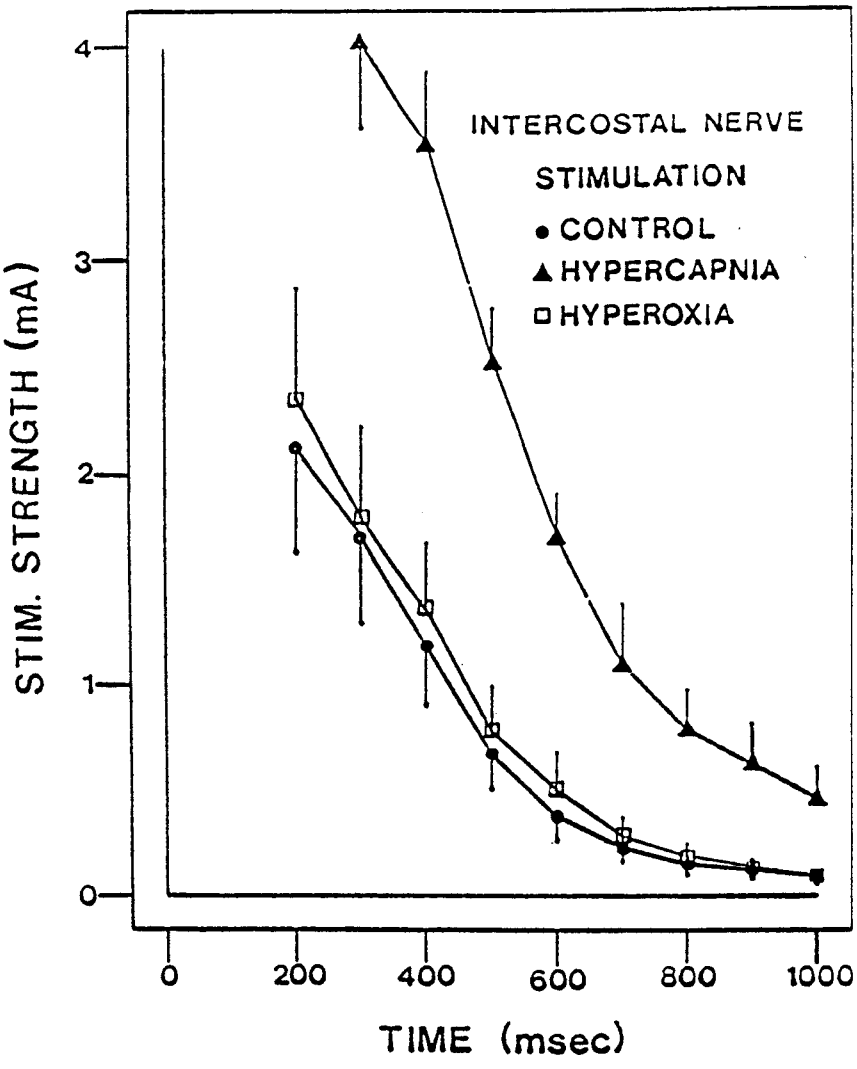

Figure 16. Time-course of off-switch excitability assessed by T6 intercostal nerve stimulation after section of the carotid sinus nerves. These three curves were obtained from the same animals shown in Figures 14 and 15. Conditions were identical to those reported in Figure 14. After elimination of the carotid sinus nerves, hypercapnia still elicits a dramatic increase in stimulus strength at all delays. However, hyperoxia has no effect upon the stimulus strength. Data from 10 cats (mean $\pm$ S.E.M.).

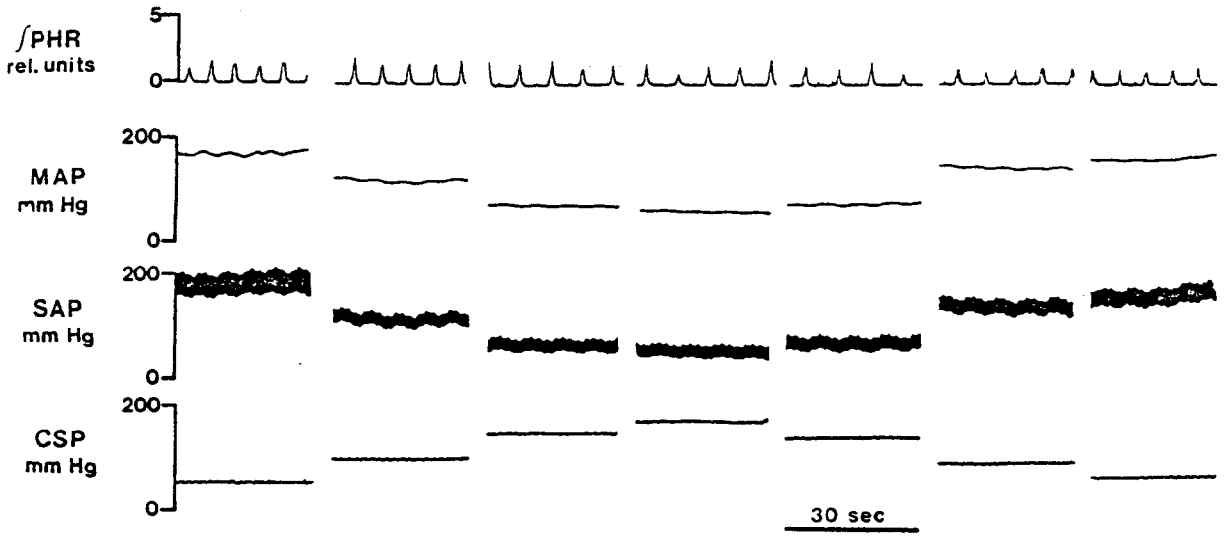

Figure 17. Cardiovascular and respiratory responses to alteration of the carotid sinus pressure. Parameters listed from top to bottom are: integrated phrenic amplitude, mean arterial pressure, systemic arterial pressure and carotid sinus pressure. Note the depression of systemic arterial pressure produced by elevation of the carotid sinus pressure. In this preparation, phrenic amplitude was not influenced by carotid sinus pressure.

adjusting the outflow resistance with a screwclamp. These step changes elicited consistent changes in the systemic arterial pressure. Figure 18 shows the averaged responses to increasing and decreasing carotid sinus pressure in five cats. A distinct hysteresis can be noted. From the curves the baroreceptor "gain" (change systemic arterial pressure/ change in carotid sinus pressure) was calculated and found to be approximately 0.8 to 1.0.

In this study, the procedure for data collection varied slightly from the previous studies. Since static baroreceptor activity is known to adapt during situations of maintained pressure, all stimulus strengths were determined within one to two minutes after changing the carotid sinus pressure. The order of delays was randomized and at each delay, the stimulus strength required to terminate inspiration was determined with the mean carotid sinus pressure maintained at 100 mm Hg, 150 mm Hg, 50 mm Hg, and 100 mm Hg. This proctocol is illustrated in Figure 19. Statistical analysis indicated that in this group of 10 cats there were no significant changes in either mean respiratory frequency or phrenic amplitude with alteration of the carotid sinus pressure. Note that in this particular preparation, phrenic amplitude was decreased during elevated carotid sinus pressures. Four of the 10 cats used in this study responded to changes in carotid sinus pressure with obvious alterations of the phrenic signal. The remaining 6 cats showed no change in the phremic amplitude. This type of response may be observed in Figure 17.

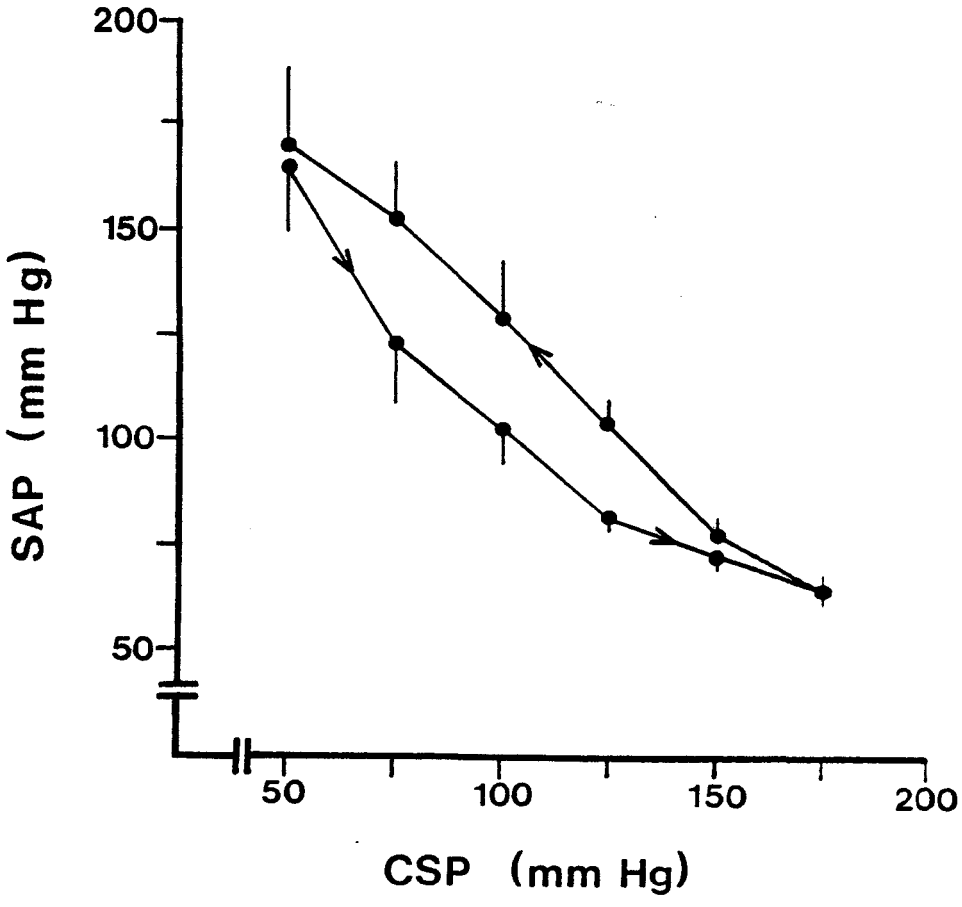

Figure 18. Alteration of mean systemic arterial pressure (SAP) produced by increasing and decreasing carotid sinus pressure (CSP). Data from 5 cats (mean ± S.E.M.).

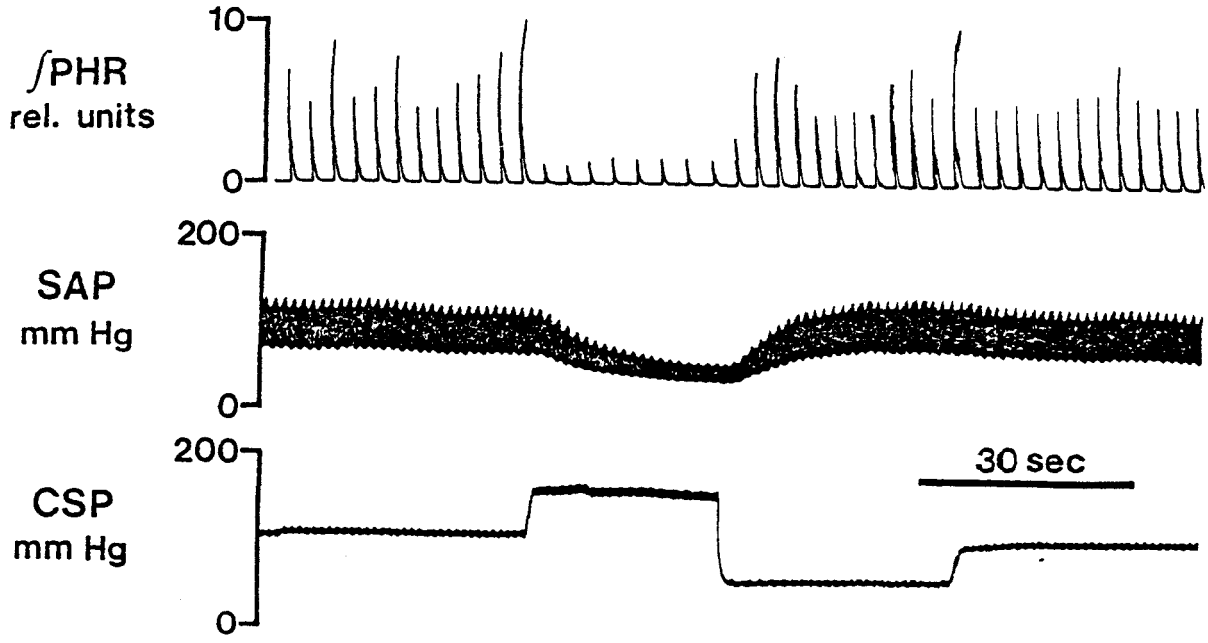

Figure 19. Cardiovascular and respiratory responses to alteration of the carotid sinus pressure. In this particular preparation the integrated phrenic amplitude and systemic arterial pressure were both decreased by elevation of the carotid sinus pressure from 100 mm Hg to 150 mm Hg.

The curves of stimulus strength versus time into inspiration obtained at the three different carotid sinus pressures are displayed in Figure 20. Both the 50 mm Hg curve and the 150 mm Hg curve differ significantly from the 100 mm Hg curve at all ten delays. As an additional control for these experiments, stimulus strengths were determined at a delay of 600 milliseconds in 5 cats both before and after intravenous administration of norepinephrine. The norepinephrine elicited substantial increases in systemic blood pressure without affecting pressure in the isolated carotid sinus. Threshold stimulus strengths were not affected by norepinephrine, therefore suggesting that the alteration of threshold stimuli was due to changes in carotid sinus pressure and not to the accompanying reflex changes in arterial pressure.

Intercostal nerve stimulations were attempted in three cats anesthetized with pentobarbital. Even when stimulating with the maximal current strength of 5 mA, it was impossible to prematurely terminate inspiration within 1,000 milliseconds after the onset of phrenic activity. Therefore, further experiments utilizing pentobarbital anesthetized preparations were discontinued. . .

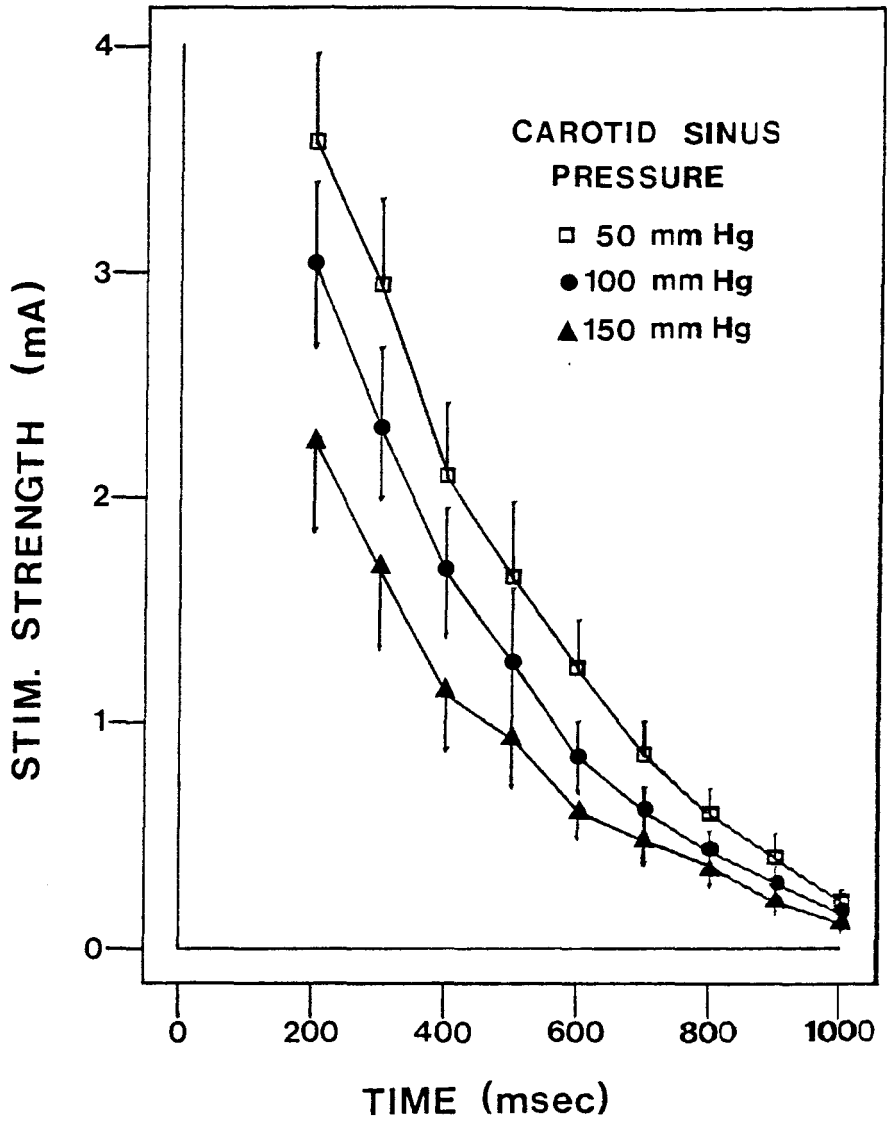

Figure 20. Time-course of off-switch excitability assessed by T6 intercostal nerve stimulation at different carotid sinus pressures. Decreasing the pressure in the isolated carotid sinus caused a significant increase in the stimulus strength required to terminate inspiration at all delays. When the carotid sinus pressure was elevated to 150 mm Hg, the stimulus strength was significantly decreased at all delays. Data from 10 cats (mean ± S.E.M.).

CHAPTER V

DISCUSSION

A. Off-switch Activation by Intercostal Nerve Stimulation

The characertistics of inspiratory off-switch activation have been previously described by studies utilizing the techniques of either pulmonary stretch receptor activation (1, 41, 88, 123) or pneumotaxic center stimulation (22, 47, 82, 83). Both of these manipulations will produce a termination of inspiration if they are of sufficient magnitude. The amplitude of the stimulus strength necessary to achieve inspiratory termination decreases as the delay into inspiration increases (33, 83). This time-dependent characteristic of inspiratory off-switch activation has also been observed with stimulation of the superior laryngeal nerve (19, 135). Since it has been well-documented that intercostal nerve activation may cause a premature termination of inspiration (163, 182), several investigators (83, 101, 165) have suggested that the intercostal muscle afferents may provide an excitatory input to the inspiratory off-switch mechanism. However, no previous study has critically examined the time-dependent nature of intercostal nerve stimulation.

In every decerebrate cat studied during the present series of experiments, the stimulus strength required to produce a phase switch from inspiration to expiration decreased as the delay into inspiration was lengthened (Figures 5, 6, 7, 8, 9, 11, 14, and 20). This supports

the concept that the intercostal muscle stretch receptors may provide an additional input to the inspiratory off-switch. Since phrenic inhibition is not observed during intercostal nerve stimulation in spinal animals (66, 164), it may be concluded that the inhibition of phrenic activity by intercostal stimulation is via inhibition of the medullary neurons which drive the phrenic motorneurons (182). Several investigators have reported a latency between intercostal nerve stimulation and the subsequent inspiratory inhibition of approximately 20 msec (163, 182). Similar latencies were observed in the present studies. This input appears to be processed in a similar fashion to that provided by the superior laryngeal nerve. Adequate stimulation of either the intercostal nerve or the superior laryngeal nerve with a few pulses elicits an immediate inspiratory termination. However, vagal stimulation is not effective unless there is a substantial amount of temporal summation (19, 88, 116). The abrupt phase transition produced by stimulation of either the superior laryngeal or intercostal nerves suggests that these afferents bypass the integrating network involved in the processing of pulmonary stretch receptor input, and instead project directly to the off-switch system.

These results indicate that the assessment of inspiratory off-switch activity may be accomplished through the intercostal stimulation technique. This technique may prove useful for the study of various chemical and mechanical stimuli which contribute to the mechanisms controlling the duration of inspiration. Indeed, it may be argued that this technique has many advantages over those used previously in the

study of off-switching mechanisms. Stimulation of the intercostal afferents is more convenient than lung inflation studies. Also, the afferent systems in the intercostal nerves are not as divergent as the multiplicity of afferents in the vagus nerve. The many types of afferents are all activated to various degrees with vagal stimulation and may therefore initiate several different reflexes in response to stimulation. With the intercostal stimulation model, it is possible to examine the neural control of inspiratory duration without disrupting the normal inputs contributed by the vagus nerves and the pneumotaxic center. This model also avoids the problem of current spread that arises during electrical stimulation of the pneumotaxic center and other brainstem structures.

As is diagramatically presented in Figure 21, the stimulus strength curve profile reflects the difference between the off-switch threshold and the level of excitation of the switching mechanism. In the decerebrate vagotomized cats used in these studies, the main excitatory inputs are derived from the pneumotaxic center and the central inspiratory activity. As the off-switch excitation augments throughout inspiration, the difference between the threshold and the level of excitation decreases. Therefore, less additional excitation is required to bring the off-switch to threshold. This additional excitation is contributed in the present studies by stimulation of the intercostal nerves. One admitted shortcoming of this technique is the unknown nature of the transfer function converting current strength (mA) into the neural input which is delivered to the phase switching system. Shannon (182) has demonstrated that intercostal

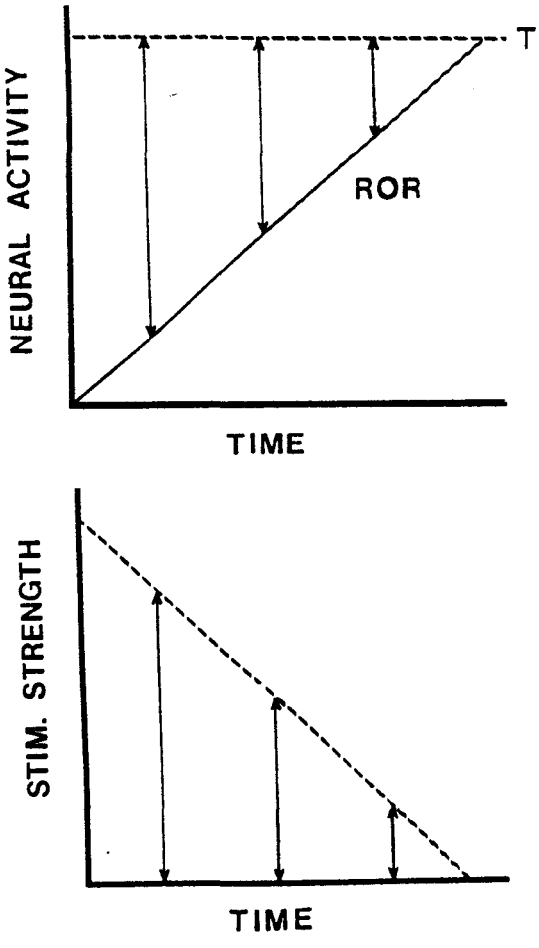

Figure 21. Theoretical diagrams of the function of the inspiratory off-switch mechanism. In the vagotomized cat, the neural activity of the off-switch is derived primarily from the central inspiratory activity which augments with a characteristic rate-of-rise (ROR) as inspiration progresses. When this ROR reaches the level of the threshold (T), inspiration is abruptly terminated. The amount of additional excitation required to terminate inspiration at each instant of inspiration is illustrated by the bottom graph.

nerve stimulation of sufficient strength to activate Group I and Group II afferents inhibits the inspiratory alpha cell discharge whenever phrenic activity is inhibited and augments the inspiratory beta cell (IV cell) activity. This would suggest that both of these types of afferents contribute to the subsequent inspiratory termination. It is assumed that the low current stimulations excite primarily the larger fibers and as current strength is increased, smaller fibers are recruited. The nature of this recruitment may be responsible for the different shapes of the mA versus Ti plots observed in individual experiments (Figure 5). Thus, while it is not presently possible to determine the exact input to the switching mechanism by intercostal nerve stimulation; the model is still very useful in determining qualitative changes in off-switch excitability.

The stimulus strengths determined during stimulation of the left or the right T6 intercostal nerves did not differ significantly (Figure 6). Substantial differences were observed within individual cats, but these differences were not consistent between experiments and they were not apparent after averaging. Since the left and right T6 intercostal nerve stimulation thresholds were not statistically different, it appears that the inspiratory inhibitory response is not distributed preferentially to any one side of the spinal cord. This observation is consistent with the notion that the response is mediated through the medullary neuronal populations which have extensive connections between the two halves of the brainstem. The thresholds observed

during simultaneous stimulation of both nerves were significantly smaller than those seen with a single nerve stimulation (Figure 6).

The fact that inspiratory termination subsequent to intercostal nerve stimulation was easily elicited in all decerebrate cats and absent in the three pentobarbital anesthetized cats supports the idea that barbiturates appear to depress the inspiratory inhibitory chest wall reflex (201). Under anesthesia the reflexes may still be present and operative to some extent; however, much larger stimuli are required to evoke the same response. Indeed, Remmers and Marttila (165) report that under pentobarbital anesthesia, a considerable amount of temporal and spatial summation of intercostal nerve stimuli are required to elicit any effects on respiratory timing. This pentobarbital suppression of supraspinal intercostal-to-phrenic reflexes may account for the observations of Speck and Webber (185) that complete thoracic dorsal rhizotomy in the anesthetized cat does not affect the eupnic breathing pattern.

Several facts suggest that the inhibitory intercostal-to-phrenic reflex may be mediated through the cerebellum. Briefly, the evidence for this is twofold. The intercostal afferents are known to project to the cerebellum (42) and the reflex effects associated with their activation are similar to those elicited by stimulation of the anterior lobe (65, 150). The present experiments of Groups III and IV (Figures 8, 9 and 10) clearly demonstrate that the inhibitory intercostal-to-phrenic reflex is not altered by removal of the cerebellum. Both the cerebellum and the cerebral cortex may have the potential to modify the

reflex, but the reflex is not dependent upon their existence. Pertinent evidence suggests that the reflex is initiated in the intercostal muscle afferents (163, 164), and conducted through the ventrolateral portion of the cervical spinal cord (66, 129) directly to the brainstem (182) where it affects the output of the inspiratory neurons.

The normal functional importance of the proprioceptive information in the neural control of respiration is not known. Since stimulation of only one intercostal nerve inhibits phrenic activity, it seems logical to suggest that these afferents may actively participate in the control of inspiratory duration. Reports in the literature show that reflexes which originate from the intercostal musculature may influence the respiratory pattern during resting breathing (92, 181), chest compression (122, 164, 179) and chest vibration (53). Regardless of their physiological role, stimulation of the intercostal afferents provides a very definite and specific tool for initiating inspiratory termination and assessing the excitability of the inspiratory off-switch.

B. Off-switch Activation by Somatic Nerve Stimulation

The work of Iscoe and Polosa (117) demonstrates that somatic afferents can contribute to the regulation of respiratory rhythmicity. These investigators observed that adequate stimulation of either the hamstring nerve or the superficial radial nerve during inspiration caused an increased rate-of-rise of the integrated phrenic signal as well as a decrease in the duration of inspiration. Stimulation did not elicit an immediate phase transition, but could eventually alter the spontaneous respiratory frequency so that it became synchronized with the repetitive somatic nerve stimulations. Other investigators (127)

have shown that central stimulation of either the femoral nerves or the brachial nerves produces an increase in the breathing frequency and amplitude of the diaphragm EMG.

In this study, the respiratory response to stimulation of either the sciatic nerve or the superficial radial nerve was unchanged by cerebellectomy. Therefore, it may be concluded that the minimal neuronal pathway for these effects does not include the cerebellum. Since Bertrand and Hugelin (22) have shown that neurons in the NPBM of the pneumotaxic center are activated by somatic afferent nerve stimulation, it is possible that the effects obtained after stimulation of the sciatic and superficial radial nerves are mediated by this nucleus.

Results of the prsent study (Group II, Table I) tend to minimize the importance of the sciatic and superficial radial nerves as direct inputs on the inspiratory off-switch mechanism. Neither sciatic nerve nor superficial radial nerve stimulation produced a consistent effect on respiratory timing. When the inspiratory duration was shortened, the phase switch occurred after a much longer latency period than is normally seen with intercostal nerve stimulation. The normal latency between intercostal nerve stimulation and the subsequent inhibition of inspiration is approximately 20 msec (163, 182), while sciatic and radial nerve stimulations exhibited latencies of several hundred milliseconds. This suggests that any effect exerted by the peripheral somatic limb afferents involves an indirect muli-neuronal pathway. It may be possible that the alterations observed with such stimulations may be the result of activation of nociceptive fibers. This is supported by

the study of Heinbecker et. al. (104) who showed that stimulation of fibers which mediate pain sensation from skeletal muscle results in an increase in breathing frequency. The shortening of inspiratory duration elicited by peripheral somatic nerve stimulation is probably mediated indirectly through the increased central inspiratory activity which is due to nociceptive reflexes. The increased central inspiratory activity, as evidenced by the increased rate-of-rise of phrenic activity, brings the off-switching mechanism to its threshold more rapidly. Therefore, inspiratory duration is shortened and breathing frequency is increased.

C. Chemoreceptor Modulation of Off-Switch Excitability

1. RESPONSE TO CARBON DIOXIDE

Various studies have previously demonstrated that the inspiratory off-switch mechanism is sensitive to both chemical and temperature drives (32, 33, 43, 44, 80, 190). Alterations in either body temperature or ventilation would be expected to influence the threshold values determined in the present experiments. Therefore, end-expiratory % CO_2 and rectal temperature were monitored and carefully maintained throughout the data collection periods. End-expiratory % CO_2, which is indicative of alveolar % CO_2, was held constant at a value of approximately 4% unless otherwise noted.

The experiments of Group III provided further confirmation of the effects of carbon dioxide upon the inspiratory off-switch mechanism. When the ventilation was decreased in the present study, there was a concomitant increase in the end-expiratory % CO_2. This increase in

carbon dioxide concentration was accompanied by an increase in the rate-of-rise of the integrated phrenic signal and a significant increase in the threshold current required to elicit an inspiration-to-expiration phase switch (Figure 7). These results support the data of von Euler and Trippenbach (83) who observed that a much greater stimulation intensity, delivered to the nucleus parabrachialis medialis, was required to terminate inspiration as the carbon dioxide level was elevated. The increase in the threshold stimulus strength can be interpreted as an increase in the off-switch threshold (Figure 22). Since the increase in off-switch threshold during hypercapnia is accompanied by an increase in phrenic activity, inspiratory duration may remain relatively constant in the vagotomized cat (33, 125, 188). The slight increase in respiratory frequency in vagotomized cats which is induced by hypercapnia (180) may result from a mismatch between the alterations in off-switch threshold and central inspiratory excitation. An increased frequency would result from a situation where the inspiratory activity is elevated more than the threshold of the off-switch. Grunstein and Milic-Emili (99) have proposed that the central inspiratory activity may always provide a constant proportion of the input to the switching mechanism regardless of the chemical drive.

Removal of the cerebellum did not influence the respiratory response to carbon dioxide (Group V, Figures 9 and 10). Therefore, the basic neural substrate for carbon dioxide modulation of respiration resides within the brainstem, or possibly the spinal cord. The pneumotaxic center has been implicated in the frequency response to hypercapnia (172, 173) and St. John has concluded that the pneumotaxic center is an

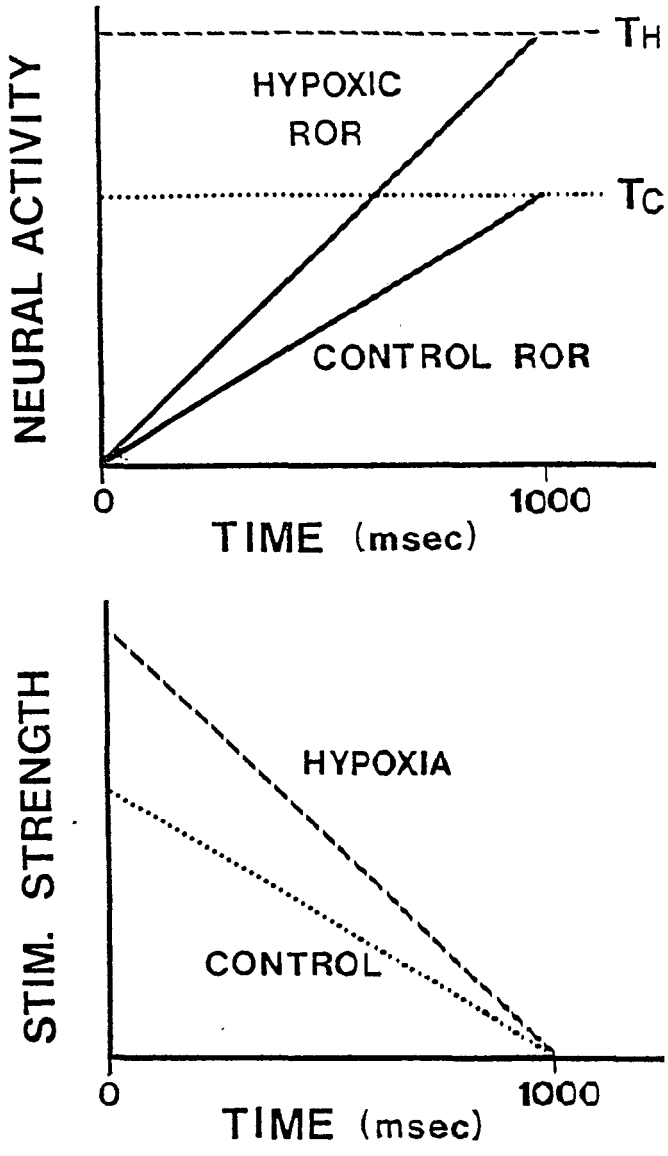

Figure 22. Theoretical diagram illustrating the effects of hypoxia upon the inspiratory off-switch mechanism. During normoxic ventilation (CONTROL), the rate-of-rise (ROR) of inspiratory activity reaches the threshold (T) of the inspiratory off-switch approximately 1,000 msec after the onset of inspiration. The threshold stimulus strength required to terminate inspiration at any instant is displayed on the bottom graph. During hypoxic ventilation, the HYPOXIC ROR is increased, but the inspiratory duration is unchanged. The HYPOXIA threshold stimulus strength curve is also increased substantially at the early portions of the curve, but the CONTROL and HYPOXIA curves converge as inspiration progresses. These two experimental observations are due to the elevation of the off-switch threshold by hypoxia.

integral component of the central chemoreceptor controller system.

As previously mentioned in the methods section, decreasing total ventilation produces a decrease in oxygen concentration as well as an increase in carbon dioxide concentration. Therefore, the results of Groups III and V reflect the response to alteration of both carbon dioxide and oxygen levels. The experiments of Group VII were designed to provide a hypercapnic normoxic ventilation. With this pure hypercapnic stimulation, the off-switch threshold was still significantly elevated (Figures 14 and 16).

The respiratory effects of carbon dioxide are classicically considered to be mediated primarily through the central chemoreceptors, although it is recognized that the peripheral chemoreceptors may contribute to the responses elicited by hypercapnia. Group VII also examined the off-switch excitability before and after section of the carotid sinus nerves. Both phrenic nerve activity and the threshold stimulus strengths were increased by hypercapnia. These responses were unaltered by carotid sinus nerve section. Thus, it may be assumed that the carbon dioxide modulation of the inspiratory activity and the off-switch threshold is mediated primarily by the central chemoreceptors.

2. RESPONSE TO OXYGEN

The ventilatory drive exerted by hypoxia is a function of both the excitatory input contributed by the peripheral chemoreceptors and the degree of hypoxic depression of the inspiratory medullary neurons (94). Although it is well recognized that moderate hypoxia may increase both the depth and the rate of respiration, the central mechanisms

which determine the augmented ventilatory pattern have not been elucidated. It has been suggested that hypoxia exerts an effect upon the threshold of the inspiratory off-switch, since some investigators have observed a hypoxia-induced increase in the depth of respiration without any change in the inspiratory duration (58, 157). A similar result was observed in the vagotomized cats of the present study. The respiratory frequency was unaltered by both mild hypoxic ventilation and hyperoxic ventilation in the cats of Group VI, despite significant increases and decreases in the amplitude of the integrated phrenic signal. Similar changes in tidal volume in response to mild hypoxic and hyperoxic ventilation have been observed in isocapnic vagotomized rabbits (121). However, if the carbon dioxide level is allowed to change, hyperoxia does not cause significant alterations in the tidal volume.

The changes in phrenic amplitude were accompanied by statistically significant shifts in the threshold stimulus strength required to terminate inspiration as illustrated by Figure 11. The interpretation of these data is facilitated by the theoretical diagram displayed in Figure 22. In the vagotomized cat, the main excitatory input to the off-switching mechanism is the central inspiratory excitation (CIE) which is proportional to the phrenic activity. During hypoxia the rate-of-rise of phrenic activity is substantially augmented. If there was no change in the threshold of the inspiratory off-switch, inspiratory duration should be shortened due to the increased rate-of-rise. However, experimental observation has indicated that there is no change in the time of inspiration but hypoxia did increase the threshold stimulus

strength at the early delays. Since both the threshold and the rate-of-rise are increased by hypoxic drives, it may be concluded that hypoxia elevates the threshold level of the inspiratory off-switch and hyperoxia lowers the threshold.

In Figure 11, statistical differences from control were observed at the first six delays of the hypoxic curve and at the first five delays of the hyperoxic curve. An explanation for the absence of significant changes from control during the later delays may be derived from Figure 22. The graph of threshold minus the neural activity indicates the amount of additional excitation required to bring the inspiratory off-switch to its threshold. As inspiration progresses, the hypoxic augmentation of central inspiratory activity begins to compensate for the hypoxic elevation of the threshold level. The net result of these two effects causes the stimulus strength versus the time into inspiration curves to converge.

The experiments of Group VII also responded to hyperoxia with a decreased phrenic amplitude and a decreased threshold stimulus strength curve (Figure 14), thereby confirming the results of experimental Group VI. After section of the carotid sinus nerves the mean phrenic amplitude decreased (Table II) as did the threshold stimulus strength (Figure 15). These alterations are similar to the effects elicited by hyperoxia. Both hyperoxia and carotid sinus denervation decrease the input from the peripheral chemoreceptors. Thus it would appear that during normoxia there is a substantial peripheral chemoreceptor drive which elevates the inspiratory off-switch threshold.

After carotid sinus nerve section hypercapnia still elicited dramatic increases in the mA versus Ti curve, but hyperoxia had no effect (Figure 16). Since there was no change in the threshold stimulus strength curve in response to alteration of the oxygen concentration, it may be concluded that the major effects of oxygen upon the inspiratory off-switch mechanism are exerted through the peripheral chemoreceptors and are not exerted directly upon any brainstem structure. Careful scrutiny of the data indicates that both phrenic activity and the threshold stimulus strength demonstrated a slight tendency to increase duing hyperoxic ventilation of the carotid sinus denervated cat. These findings suggest that there may be a small respiratory depression during normoxic ventilation which can be eliminated by hyperoxic ventilation (94, 145, 168).

D. Baroreceptor Modulation of Off-Switch Excitability

A curvilinear inverse relationship between blood pressure and ventilation has been observed in animals that have intact carotid sinus nerves. After section of the carotid sinus nerves, there remains a small increase in ventilation with severe hypotension (151). This suggests that hypotension may influence ventilation by both central and peripheral mechanisms. The experiments of Group VIII were designed to separate these central and peripheral mechanisms and control them independently of each other. Similar preparations have been utilized in the investigation of cardiovascular baroreceptor reflexes, but the technique has not previously been applied to the careful examination of the baroreceptor reflex modulation of inspiratory off-switch excitability.

One previous study has indicated that baroreceptor activity may influence the off-switch excitability (182), but another investigation demonstrated that the volume-related vagal control of inspiratory duration is unaffected by changes in arterial pressure (98). To clarify the effects of baroreceptor activity upon the inspiratory off-switch mechanism, the threshold stimulus strength of intercostal nerve stimulation required to terminate inspiration was determined at three different mean pressures within the carotid sinus. As carotid sinus pressure increased, there was a significant decrease in the stimulus strength required to terminate inspiration. This suggests that baroreceptor activity may directly lower the threshold of the inspiratory off-switch.

There are, however, several other possible explanations to be considered for the responses to altering carotid sinus pressure. First, it is possible that the threshold alterations were due to the reflex changes in systemic blood pressure. Figure 18 demonstrated that elevation of the carotid sinus pressure to 175 mm Hg resulted in a decrease of the mean arterial pressure to 60 mm Hg. At this low systemic pressure, there may be a decrease in the brain blood flow and the subsequent activation of central chemoreceptors (151). However, based on the experiments of Group III and V, the activation of central chemoreceptors would be expected to elevate the off-switch threshold. Therefore, the threshold alterations seen in the experiments of Group VIII are probably not due to changes in the central chemoreceptor activity. This conclusion is also supported by an additional control experiment which

was performed in five of the same animals. In this control manipulation, norepinephrine was administered intravenously at a dosage sufficient to produce substantial increases in the systemic arterial pressure. Despite increases in mean arterial pressure from 90 to 150 mm Hg, the pressure within the isolated carotid sinus region was unaltered by this procedure. Threshold stimulus strengths were determined before and after norepinephrine administration and were found to be unaltered by this manipulation. The results of this control suggest that changes in systemic pressure were not responsible for the alteration of off-switch thresholds.

It is quite possible that alteration of the carotid sinus pressure could cause changes in the blood flow through the carotid body. Substantial decreases in blood flow would be expected to increase the discharge of the peripheral chemoreceptors. Such augmented chemoreceptor activity would elevate the off-switch threshold as demonstrated by the experiments of Groups VI and VII. Similar elevations of the off-switch threshold were observed in this study during perfusion of the carotid sinus region with a pressure of 50 mm Hg. However, Biscoe et. al, (25) have shown that changes in the arterial pressure between 60 and 160 mm Hg have little effect upon the resting discharge of the carotid chemoreceptors. Likewise, reduction of the carotid body venous blood flow from 60 to 10 microliters/minute did not augment the chemoreceptor discharge (25). In the experiments of Group VIII, to minimize chemoreceptor activation during changes in carotid sinus pressure, oxygenated blood was pumped directly from the common carotid artery into the carotid sinus preparation at a constant flow rate.

Perfusion pressure was manipulated by adjusting the outflow resistance without altering the blood flow rate through the isolated sinus. In light of the data obtained by Biscoe et. al., (25) it is assumed that the threshold responses to alteration of the carotid sinus perfusion pressure were elicited primarily by changing the level of baroreceptor activity.

An interesting problem is posed by the phrenic response to altered carotid sinus pressure. Four cats responded to changes in carotid sinus pressure with obvious alterations in the amplitude of the integrated phrenic activity; when the pressures were elevated, the phrenic activity decreased. The other six cats in the study demonstrated no change in the phrenic amplitude at the different carotid sinus pressures. However, all of the animals showed variations in the threshold stimulus strength which were due to changes in the carotid sinus pressure. It is suggested that the phrenic activity may be considered an output event, while the off-switch excitability may be considered a processing event. The consistency of the processing responses and the variability of the output responses to alteration of the carotid sinus pressure may be reconciled by the proposal that many uncontrolled factors may contribute to the output event. This explanation is, however, unsupported by experimental evidence and exists as nothing more than conjecture in the mind of the investigator.

The results of this study suggest that normal baroreceptor activity depresses the threshold of the inspiratory off-switch. Elimination of the baroreceptor fibers should therefore elevate the threshold.

Section of the carotid sinus nerve does eliminate baroreceptor fibers, but it also disrupts active carotid chemoreceptor afferents. Since both of these afferent fiber types are thought to modulate the activity of the inspiratory neurons and to influence the threshold level of the inspiratory off-switch, carotid sinus nerve section does not abolish any one effect upon the off-switch mechanism. Figure 15 demonstrates a decrease in the threshold stimulus strength after carotid sinus nerve denervation. Such an effect would be expected after elimination of the threshold-elevating chemoreceptor fibers. However, disruption of the baroreceptor afferents should increase the threshold stimulus strength. Therefore, it is concluded that the chemoreceptors play the more important role in the control of inspiratory off-switch excitability during normal ventilation and normotensive situations.

CHAPTER VI

CONCLUSIONS

1. Stimulation of the intercostal nerve with a brief stimulus train of adequate strength elicits an abrupt termination of inspiratory activity.

2. The short latency between intercostal nerve stimulation and the inhibition of inspiration and the distinct time-dependent nature of threshold stimulus strength establish intercostal nerve afferents as direct inputs to an inspiratory off-switch mechanism.

3. Stimulations of the superficial radial nerve and the sciatic nerve do not have a direct, time-dependent effect upon the inspiratory terminating mechanism; therefore the intercostal-to-phrenic inspiratory inhibitory reflex is not part of a generalized reflex which results from peripheral somatic nerve stimulation.

4. Removal of the cerebellum does not alter the integrated phrenic nerve response to stimulation of the intercostal nerve; neither does it alter the response to hypercapnia.

5. Hypercapnia elevates the threshold of the inspiratory off-switch mechanism and augments the central inspiratory excitation. This effect is exerted primarily through the central chemoreceptors.

6. Mild hypoxia increases the inspiratory activity and elevates the off-switch threshold in normocapnic vagotomized cats. Conversely, hyperoxia decreases the inspiratory activity and lowers the threshold of the inspiratory off-switch. The effects of hypoxia and hyperoxia are

mediated primarily through the peripheral chemoreceptors and may be abolished by section of the carotid sinus nerves.

7. Baroreceptor activity lowers the threshold of the inspiratory off-switch.

CHAPTER VII

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APPENDIX A

A. Anatomy and Physiology of the Intercostal Musculature

The midthoracic external intercostal muscles lie beneath the semispinalis, longissimus dorsi, and iliocostalis muscles. The external intercostal muscle fibers are oriented in a rostrocaudal direction perpendicular to the ribs. Upon contraction they pull the rib cage upward and outward. This motion expands the rib cage and therefore facilitates inspiration.

Removal of the external intercostal muscle exposes the external intercostal nerve and the internal intercostal muscle. The external intercostal nerve arises from the smaller of two branches of the ventral ramus (3, 5), penetrates the internal intercostal muscles and passes between the external and internal intercostal muscles in a position immediately posterior to each rib. There are thirteen pairs of ribs and intercostal nerves in the cat. Numerous small branches leave the external intercostal nerve and terminate in the external intercostal muscles exclusively.

The internal intercostal muscles lie between adacent ribs and are oriented at a forty-five degree angle relative to the overlying external intercostal muscles. Contraction of the internal intercostals decreases the intercostal space and facilitates expiration by decreasing thoracic volume.

Removal of the internal intercostal muscle exposes the main intercostal nerve which lies immediately adjacent to the parietal pleura and against the caudal edge of the rib. This nerve lies in close proximity to the intercostal artery and care must be taken when dis-

secting out the main intercostal nerve for stimulation. This nerve is the largest branch of the ventral ramus and gives off many small branches along its entire length. These branches involve numerous projections to the internal intercostal muscles, a lateral branch to the abdominal oblique muscles, a ventral branch to the rectus abdominis muscle, and several cutaneous branches. Each of these branches may innervate musculature from several adjacent intercostal spaces (Figure 1).

During spontaneous respiration the gamma and the alpha motor-neurons innervating both the external and internal intercostal muscles are rhythmically excited during inspiration and expiration, respectively. This rhythmic activity is governed by a central suprasegmental mechanism and is controlled in a similar fashion to phrenic motor activity. Individual intercostal motor units are primarily wholly periodic and are active only during one phase of the respiratory cycle. However, there are some units which have a tonic activity that may or may not be respiratory modulated (5). These patterns of activity may be related to the dual function (respiratory and postural) of the intercostal muscles.

Like most skeletal muscles, the intercostals contain a large number of sensory structures called muscle spindles which measure length. The muscle spindle consists of a bundle of specialized fibers, the intrafusal fibers, which develop negligible tension. These fibers are innervated by gamma motor (or fusimotor) fibers while the main contracting fibers, the extrafusal fibers, are excited by alpha motor neurons. Contraction of the intrafusal fiber exerts a powerful action

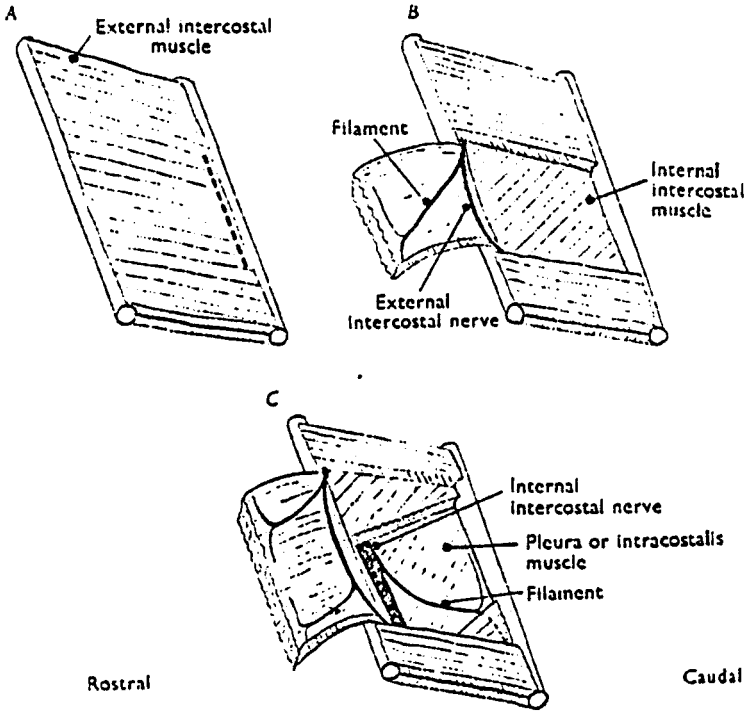

Figure 1. Schematic diagram showing orientation of various intercostal muscles and nerves. From (5).

on the receptor terminals located within the muscle spindle. Since spindles lie in parallel with the extrafusal muscle fibers, contraction of the latter unloads the spindle and the intrafusal bundle becomes deformed. Occasionally the spindle intrafusal fibers are innervated by branches of the same motor axons which supply the extrafusal muscle. These axons are termed Beta axons (2).

Cat spindles contain two or three large intrafusal fibers which are called nuclear bag fibers. In addition there are usually three to five smaller fibers called nuclear chain fibers (2). The total number of axon branches which enter one spindle varies from eight to twenty-five. Each spindle has one primary sensory ending (group Ia) consisting of multiple spiral terminations which wrap around the nuclear region of the intrafusal fibers. Secondary sensory endings (group II) in the cat consist of spiral terminations around each nuclear chain fiber and less extensive spray-like terminations on the nuclear bag fibers. The spiral and spray terminations are connected to the same group II afferent neuron.

Stimulation of the main intercostal nerve may activate afferent fibers originating from many different regions. When stimulated individually each of the many branches will elicit inspiratory termination. Generally it is easier to inhibit phrenic activity with stimulation of one internal intercostal nerve, lateral intercostal nerve or lumbar nerve than with one external intercostal nerve since the former nerves are larger and innervate both skin and muscle (6). In order to elicit inspiratory termination, it is necessary to recruit most of the group I fibers as detected by dorsal root compound action potentials (6). In

some cases, additional recruitment of group II fibers produces more pronounced inhibition. Regardless of the nerves stimulated, additional recruitment of group III pain fibers does not appear to alter the inspiratory inhibitory response (6).

Remmers (4) has suggested that group II afferent fibers carry sensory information from secondary muscle spindle endings in the external intercostal muscles. The receptors from which the group I afferents arise are not known but are thought to be tendon organs since these receptors are known to have a spinal inhibiting action on alpha motoneurons of the muscle in which they are located. Cutaneous afferents in the main intercostal nerve may contribute to inspiratory termination as suggested by the work of Aminoff and Sears (1), these investigators demonstrated segmental inhibition of inspiratory intercostal efferent activity in response to stimulation of afferents in the cutaneous branch of the lateral intercostal nerve.

APPENDIX B

B. Equipment

The basic building block of modern electronic equipment is the integrated circuit operational amplifier. This type of circuit is a direct-coupled device with differential inputs and a single-ended output. Such an amplifier responds only to the voltage difference between the two input terminals. Operational amplifiers may be connected in two basic amplifying circuits, the inverting and the non-inverting configurations. By combining both configurations it is possible to create the differential amplifier. Differential amplifiers can be used to discriminate against undesirable common-mode noise components while amplifying signals that appear differentially.

Operational amplifiers can also be combined with resistors and capacitors to form active filters. These circuits are frequency-selective networks that favor certain frequencies of input signals at the expense of others. Three common types of filters are the low-pass filter, the band-pass filter and the high-pass filter. All of these filters may be placed in series with amplifying circuits.

A brief description of equipment used in these studies follows:

1. CARBON DIOXIDE ANALYZER

The LB-1 Medical Gas Analyzer was used to determine end-expiratory concentrations of carbon dioxide. This device utilizes the non-dispersive infrared analysis technique which is based on the phenomenon that various gas molecules absorb energy from different portions of the infrared spectrum. The analyzer is very specific since the detector is filled with the same gas that is being measured (i.e. CO_2).

When a sample containing CO_2 is drawn through the sample cell by a vacuum pump, the gas in the sample absorbs some of the infrared energy that is directed through the sample beam. This makes the sample beam less effective in heating the CO_2 contained in the top portion of the detector. The CO_2 in the bottom portion of the detector receives more energy and therefore heats more rapidly. The unequal pressure changes resulting from heating causes a thin metal diaphragm in the middle of the detector to expand upward, which causes a change in capacitance. A preamplifier in the pick-up head acts as a charge amplifier to convert the varying capacitance signal into a voltage signal.

Under standard sampling conditions of 500 ml/min., through the standard inlet tube, the response time for a 90% response to a step change in CO_2 is approximately 100 msec. This response time is sufficiently fast to accurately reproduce the contour of the concentration of CO_2 in expired air.

2. PRESSURE TRANDUCER

Changes in resistance within a Wheatstone bridge circuit provide the basis of operation for the Statham pressure transducers used in these experiments. The force exerted by the pressure of the blood is exposed to a thin, stiff metal diaphragm on which a very sensitive resistor is mounted. As the resistor is slightly deformed by the external forces, there is a change in resistance. This unbalances the Wheatstone bridge configuration and the voltage then may be measured across the bridge. Because metallic strain guage elements are very sensitive to temperature changes, the use of a single strain guage element is un-

common. Instead, pairs of strain guage resistors are usually employed within a bridge circuit, with strains in the opposite directions applied to adjacent arms of the bridge. Such a configuration achieves full temperature compensation and minimizes voltage drifts due to temperature.

3. PHRENIC PROCESSOR

The phrenic processor used in this laboratory is a custom-built device modified from the basic circuit provided by Morton I. Cohen. In this circuit a square wave "gating" signal and an integrated signal may be obtained from the amplified activity of any electrical signal. In these experiments the activity recorded from the phrenic nerve was amplified to a level of approximately 2 volts peak to peak. The first stage of the processing circuit produced a full wave rectification of the raw signal. This rectified signal was integrated with a leaky integrating circuit. The time constant of the integrating amplifier was usually set at 200 msec. An inverting amplifier and a non-inverting amplifier then produced two amplified integration signals which had very fast rise times and saturated the amplifiers. These signals were then differentiated and each of the differentiated signals were fed into a Schmidt trigger which was adjusted to trigger a square wave signal whenever the signal rose above a certain level. The output of the two Schmidt triggers marked the onset and the termination of phrenic activity. A final stage of the processor initiated a positive 4 volt signal when the first Schmidt trigger fired and this signal continued until the second trigger occurred. The output of this stage was TTL compatible and was used to trigger the WPI stimulating circuit.

4. STIMULUS ISOLATION

The WPI Series 800 Stimulus Isolator used for these studies utilizes phton coupling to provide isolation of the stimulation circuit from both the signal source and from ground. A stimulus isolation unit delivers a certain amount of current between two electrodes, however, the absolute value of the voltage in the two electrodes is not fixed. If bipolar stimulating electrodes are not isolated from ground, then one of the electrodes is usually at ground, and the other electrode will be at a value that is considerably larger than if the electrodes were isolated.

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APPROVAL SHEET

This dissertation submitted by Dexter Franklin Speck has been read and approved by the following committee:

Charles L. Webber, Jr., Ph.D., Chairman of Committee
Assistant Professor, Physiology
Loyola, Stritch School of Medicine

Robert D. Wurster, Ph.D.
Associate Professor, Physiology
Loyola, Stritch School of Medicine

Walter C. Randall, Ph.D.
Professor, Physiology
Loyola, Stritch School of Medicine

Roger Shannon, Ph.D.
Associate Professor, Physiology
University of South Florida, Tampa

Jack L. Feldman, Ph.D.
Associate Professor, Physiology and Anesthesiology
Northwestern University Medical Center, Chicago

The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the dissertation is now given final approval by the Committee with reference to content and form.

December 9, 1980
Date

Charles L. Webber, Jr.
Director