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The Anterior Hypothalamus: Its Interaction with Respiratory Control

Harold Albert Spurgeon
Loyola University Chicago

THE ANTERIOR HYPOTHALAMUS: ITS INTERACTION WITH RESPIRATORY CONTROL

by

Harold Albert Spurgeon

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BIOGRAPHY

Harold A. Spurgeon, born May 28, 1942, in Macomb, Illinois, was educated in the Bushnell-Prairie City public schools. He received a B.A. from Monmouth College in 1964, then worked two years as Research Associate to Dr. W.A. Himwich, studying cerebral blood flow.

In the Fall of 1966, he entered Loyola University Graduate School, Department of Physiology. A recipient of an NIH Predoctoral Fellowship, from 1967-1970, he served under Dr. C.N. Peiss, studying cardiovascular control and cardio-pulmonary regulation. He married the former Loretta Ulrich in the Summer of 1971, and is currently employed as Senior Research Associate to Dr. Donald Priola at the University of New Mexico, Department of Physiology.

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CHAPTER I

INTRODUCTION

Historically the hypothalamus has been implicated as an area of the central nervous system which can modify respiration. The hypothalamus has been investigated in terms of its cardiovascular, thermoregulatory, behavioral, and to some extent its respiratory interaction. Because it is evidently a site of integration of some importance for vegetative function, and because of the qualitative nature of existing data, the general area of the anterior hypothalamus was explored as a CNS mediator of respiratory response. Clinical implications of patients unable to maintain adequate respiration in the absence of conscious respiratory control indicate the potential importance of an auxillary control site.

Any attempt to simplify the system necessarily removes essential components important in the overall physiological response. The purpose of this study is to clarify and quantitate the role of the anterior hypothalamus in mediation of the respiratory response. Such interaction may be indicative

of a potentially functional pathway for mediation of respiration in such clinical patients.

The present study addresses itself to two problems: 1) Quantification of functional responses to an electrical entraining stimulus; and 2) Development of a data handling technique utilizing modern instrumental and computer techniques capable of reducing the experimentally measured parameters to a manageable status.

Chapter II

LITERATURE REVIEW

A. Historical Overview

The history of respiratory control developed slowly but concurrently with the general body of scientific knowledge. Possibly the earliest recorded evidence that respiration was controlled from the brain, and that this control was not of a simple nature, was provided by Galen (49). He observed, while serving as physician to the gladiators (Ca. 145 A.D.), that some gladiators suffering from neck injuries ceased breathing entirely, while in others diaphragmatic breathing persisted. Prompted by this observation, Galen sectioned the cord of newborn pigs at C2, and showed that complete cessation of respiration ensued. Section at C6 (below the phrenic outflow) abolished thoracic breathing, yet diaphragmatic respiration persisted. Little further work was done in this area until 1760, when Lorry (cited in 88) repeated Galen's experiments, confirming the instant abolition of respiration upon severing the spinal cord. Cruikshank, in 1797, found that breathing could be stopped by making a transection between the medulla and spinal cord (cited in 49).

More precise definitions of the areas essential for respiration really began with the work of Legallois in 1811 (cited in 49,88). His experiments showed that the entire brainstem was not essential for maintenance of respiration. Having removed all the brain rostral to the medulla, Legallois noted that respiration persisted as sections were made at succeeding lower brainstem sites, with little alteration due to a transection at the pontine-medullary border. When the sections were continued posteriorly to include the origin of Galen's pneumogastric nerves (now the vagus), respiration immediately stopped. Both the technique and localization were improved by Flourens in 1842 (cited in 49,88). He described a "vital node," which was a circumscribed area of less than one millimeter at the apex of the calamus scriptorius. Destruction of this area abolished respiratory movements. Flourens characterized this node as extending bilaterally some 2 1/2 millimeters from the midline (cited in 88).

The existence of a narrowly circumscribed area mediating respiratory control proved difficult to duplicate in later experiments. A diffuse system concept began to emerge, whose neurones were scattered throughout the brainstem with a higher concentration in the reticular formation in the caudal half of this structure. Gad and Marinesco ruled out bilateral discrete centers by cauterization of discrete areas of the medulla, and further showed that the reticular substance of the floor of the fourth ventricle was indispensable for the respiratory act

(cited in 49). Bechterew, Kronecker, and Marckwald (cited in 49,99) were also instrumental in providing evidence against the discrete center concept of respiratory control.

Longet (cited in 37) lesioned tissue in the vicinity of the "saisbeau intermediare du bulbe" located at the same level as the vagal nucleus, and observed immediate cessation of respiration. He also reported that destruction of tissue in the vicinity of the vagal nuclei did not interfere with respiration.

The fundamental concept of functional interactions among the components of a respiratory center dates back to the time of Marckwald (cited in 70). In a series of experiments, he determined that stimulation of the central end of the sectioned vagus could induce both expiration and inspiration (49). He was also able to elicit inspiration, and sometimes expiration, by electrical stimulation in the medulla. Marckwald concluded: 1) that the respiratory center consisted of an inspiratory and a less reactive expiratory center, 2) that the respiratory center was located in the medulla below the stria medullaris, and 3) that the respiratory center had an apparent automaticity and rhythmicity which was imparted by way of afferent impulses and projection to the inspiratory center (75,76).

B. The Brainstem

Evidence that the brainstem respiratory neurones control the rhythm of breathing was first offered by Marckwald (75,76).

He observed that transection of the rabbit brain through the pons allowed normal respiration as long as the vagi remained intact. If the vagi were transected, the animal inspired deeply, and remained in the maximally expanded position until death by asphyxia occurred. If the transection was intercollicular, vagotomy merely slowed and deepened the respiration. Marckwald concluded that the medullary respiratory centers had no inherent rhythmicity, but mediated tonic inspiration, or "inspiratory cramp." The vagal mechanism and a second system of inferior collicular origin periodically interrupt the "cramp" mechanism, thereby converting it to a rhythmic respiration. Either mechanism alone could suffice, but the vagal inhibitory system was thought to play the major role.

Respiratory physiologists were to wait over 40 years before refinement of Marckwald's concept of brainstem respiratory control. In 1922, Lumsden (72) reviewed Marckwald's findings and concluded that his concept of periodic inhibition was incorrect. Lumsden pointed out that Aducco (cited in 73) had paralysed inspiration with large doses of chloral, yet expiratory activity continued. Lumsden (73) widely opened the chest of a cat, clipped the edges of the lobes of the lungs, and respirated the animal by a continuous inflow of gas mixture from the trachea. Thus, the asphyxial conditions of earlier investigators were avoided, as were movements of the

thorax. Lumsden coined the term pneumotaxic center and concluded that rhythmic respiration, at least in the cat, was effected by an inspiratory mechanism, the apneustic center, at the level of the stria medullaris. An expiratory center, located just caudally, was also essential. Both centers were controlled by a higher center, the pneumotaxic center, in the upper half of the pons. Furthermore, he maintained that exclusion of the pneumotaxic center abolished rhythmic respiration even with intact vagi (cited in 88). Areas above the corpora quadrigemini were reported to have little respiratory effect when lesioned in the cat (72). Utilizing serial transections of the cat brain beginning rostral to the posterior border of the corpora quadrigemini, and proceeding down the pons to the level of the pyramidal decussation, Lumsden (72) was able to separate out most of the individual components of respiratory control. Yet he maintained that the gasping center, which appears to be analogous to the "vital node" of Flourens, was essential for inspiration.

The first attempt at systematic exploration of the brainstem by electrical recording was made in 1936 by Gesell, Bricker, and Magee (40,41, and cited in 3). They found that the predominant respiratory electrical activity is located in the reticular formation of the caudal medulla. Respiratory potentials were found "in the greatest abundance and greatest certainty" in the region of the obex (40). In twenty experiments exploring the mesencephalon and diencephalon, only five sets

of respiratory potentials were recorded. Potentials also were traced down the cord to T12, with the bulk of the respiratory potentials in the lateral cell columns. Pitts (82) explored the brainstem pons, medulla, and upper cervical cord in 1939. He was first to arrive at a definitive formulation of the function of the inspiratory and expiratory areas.

Gesell, Atkinson, and Brown (38) described three types of electrical response as recorded with small electrodes in the exposed medulla. First, a slowly augmenting type of discharge was reported in which the activity builds up slowly but subsides abruptly. Second, a rapidly augmenting type was seen with rapid buildup and slow decrease in activity. A third type was noted, characterized by a steady discharge throughout its active period, but periodically interrupted. The augmenting discharges occurred almost exclusively during inspiration.

Salmoiraghi and von Baumgarten (101) reviewed three theories proposed for control of respiratory neurones in the brainstem: first, rhythmicity is due to some inherent property of periodicity within the inspiratory neurones; second, rhythmic discharge from vagal stretch receptors in the lung interrupt a tonic activity; and third, rhythmicity results from a reciprocal innervation between two networks of neurones within the brainstem which are activated or facilitated by a negative feedback. They reported some of the first intracellular microelectrode recordings from brainstem respiratory neurones (101). They

found a shift in the resting membrane potential of inspiratory cells toward threshold as activity began in the phrenic. This reduced resting level persisted until the last spike in a series, at which time the membrane returned to previous resting level in 300 msec. Hyperpolarization of expiratory cells was seen during this time. A similar pattern was seen for the expiratory system, with hypopolarization during expiration. The behavior of these systems, facilitating the ongoing activity of the phrenic by hypopolarization, may help explain the reverberation-like response of the phrenic. Burns and Salmoiraghi (18) proposed an alternate mechanism for repetitive firing in the phrenic. Inspiratory and expiratory neural groups with a series of rich interconnections arranged in such a way that excitation could spread from the excitable members of one group to suppress the firing in the reciprocal group was proposed. By some unspecified means, the inhibition could be reversed. However one group could become dominant resulting in a repetitive discharge in the network. Thus the network would reexcite the center.

There is considerable controversy regarding the accuracy and efficacy of work done on the brainstem. Hukuhara et al (55) maintained all previous work done on the brainstem respiratory centers was in error since the presence of respiratory response by stimulation does not mean a center has been found. Discrete potentials in a region without supportive experiments to corroborate the findings is not adequate proof of center

localization.

Cohen pointed out (21) that he had been able to record specific inspiratory and expiratory neurones in the brainstem. There also existed a sizeable population of neurones which appeared to be phase spanning. These fired in a somewhat paradoxical manner, partially in inspiration and partially in expiration.

Nesland et al (80) determined that the frequency response of medullary respiratory neurones to different levels of stimulation was graded and often a non-linear function. The magnitude of effect at a given stimulus level varied from cell to cell. Some medullary neurones discharged with relatively low frequencies at high levels of ventilation, while others discharged with essentially maximum frequency at relatively low levels of ventilation. Nesland et al pointed out, however, that the total impulse output of a group of medullary neurones bears a close direct relationship to the minute volume. Changes in tidal volume may thus be correlated rather well with changes in the discharge frequency of medullary respiratory units.

Some measure of the difficulty of attaining reliable respiratory recordings comes from a comment of Salmoiraghi and von Baumgarten (101). These two highly competent investigators studied, in the course of a year, 51 cats, managing

to attain only 7 successful intracellular penetrations of brainstem respiratory neurones.

Bronk and Ferguson (15) in 1935, studied the prolonged rhythmic afterdischarge in the respiratory center. Using small extracellular electrodes, they found that the action potential of the phrenic nerve was maintained even during curare paralysis. They implied that periodic stimulation from peripheral afferents is unnecessary for the maintenance of quiet breathing.

Many investigators have utilized stimulation techniques in an attempt to define the limits and interactions of the brainstem respiratory mechanism. Borison (12) studied a response he chose to call "spasmodic," which is analogous to sneezing, emesis, or coughing. He localized this response in the dorsolateral region of the mesencephalon including structures corresponding to the descending vestibular tract and nucleus, tractus solitarius and its nucleus, and the vagal and glossopharyngeal rootlets. A connection between the spasmodic response and the mechanism of pneumotaxis was suggested. Brookhart (16) utilized small electrodes and closely controlled stimulus parameters to stimulate precise points in the brainstem. He failed to confirm the existence of discrete, compact inspiratory and expiratory centers in the cat. Based on his data, he concluded that the medullary reticular formation is the site of primary respiratory neurones, with no detectable differentiation of function within that structure.

Cohen and Hugelin (24) pointed out that the response to reticular stimulation is not a simple facilitation of inspiration. Such stimulation resulted in a complex integrated reaction modifying several variables of the respiratory cycle. Included were changes in the duration of the inspiratory and expiratory phase, rate of growth of the phrenic discharge, as well as its decay, and the end-expiratory level of phrenic firing. Cohen and Wang (25) found a possible pathway through the pons. Inspiratory spasmodic and coughing responses were obtained by electrical stimulation in the dorsolateral region in the pons just caudal to the pneumotaxic region and caudal to the level of the inferior colliculus. These responses were elicited more medially in the caudal pons, and some evidence was presented to indicate that the pathway of this tract began to migrate toward the midline at the level of the colliculi.

Cohen and Hugelin (23) showed that increased respiratory rate evoked as a result of medullary stimulation produced relative changes in the duration of the phases of the respiratory cycle; both phases may be reduced. Midbrain reticular stimulation: 1) reduced the duration of the expiratory phase, 2) increased the slope, and 3) increased the peak amplitude of the averaged phrenic signal. Different stimulation thresholds were seen. The shortening of expiratory phase seemed to have a lower threshold than that for increased slope of integrated phrenic activity. Finally, the effect on peak

amplitude of integrated phrenic traffic occurred only at the highest stimulus levels. Cohen and Hugelin used 300 Hz, 0.2 msec pulses, at intensities of 1 to 5 volts. They interpreted the results as follows. The decrease in expiratory duration would lead to increased respiratory frequency. The increased rate of growth of the phrenic discharge would increase instantaneous air flow. The peak (end-inspiratory) phrenic discharge would result in increased tidal volume.

Bach (5) indicated that the facilitatory and inhibitory systems of the brainstem respond to circulatory, respiratory, and somatic reflexes about equally. He compared the response of each of these systems during a series of afferent stimulations for those systems in the brainstem. His conclusion was that each system responded appropriately without regard to the specific nature of the stimulus, with no difference in the sensitivity of any one system.

Katz and Perryman (64) showed that the response of the central respiratory areas to peripheral stimuli may be reversed depending upon the intensity of the stimulus. They stimulated a mixed peripheral nerve and found a critical frequency of about 10 Hz below which an expiratory apnea was produced. Above this frequency, an increase in respiration rate was seen. In addition, the apneic response was voltage dependent, and could be reversed by increasing stimulus voltage. The response did not seem to be attenuated or magnified in any way by vagotomy or bilateral carotid occlusion. The

response was maintained until cessation of the stimulus, i.e. no accommodation was observed.

Transections have been used in the study of brainstem respiratory periodicity. The apneusis so produced may have been a result of tissue damage in the adjacent areas. However, Rijlant (98) has shown in the apneustic animal that a respiratory reflex can still be evoked by stimulation of the appropriate centers in the brainstem. Yet, spontaneous respiratory activity can be destroyed in the cat or rabbit, according to Rijlant, after localized lesions in the posterior extremity of the floor of the fourth ventricle. This is not to be interpreted as a definitive location for destruction of the respiratory center nor of suppression of the inspiratory motor neurone. A brief stimulation, often as low as 5 Hz but sometimes as high as 30 Hz, when delivered to the cervical cord or the phrenic or intercostal motor neurones, produces a homolateral response. Such responses often are characterized as identical with the normal spontaneous respiration. The implication here is that the input from the periphery, in the presence of a discrete lesion as described above, tends to set up some type of reverberating system which reinforces the short duration stimulus input. Such a reverberating circuit may help to explain the mechanism of ongoing phrenic activity following the initiation of inspiration.

Of importance was the finding of Rijlant (98) that

a unilateral lesion placed laterally in the brainstem serves only to suppress respiratory movements on the ipsilateral side. Although the precise location of the lesion was not supplied, it was apparently low enough to block efferent traffic. Stimulation below the lesion resulted in respiratory activity of the sort mentioned above. Due to the ongoing spontaneous, bilateral nature of the respiratory response, it was presumed that an interrupted synaptic connection existed to the nonlesioned side. Some pathways originating in more rostral areas of the brain probably pass through this lateral area of the brainstem. Rijlant proposed that his experiments favored the interpretation of direct phrenic and intercostal control from areas he designated only as "supra-bulbar," with the activity of the bulbar centers being primarily "modulatory."

Hoff and Breckenridge (51) offered additional supportive evidence. They postulated that the inevitable apneusis following low decerebration is not a primary phenomenon, but a secondary occurrence which can be produced or abolished almost at will. They suggest it was not an inherent output of the inspiratory center freed of its inhibitions, but a "superimposed" drive, 'occluding,' in the Sherringtonian sense, the normal output of the medullary center." An exaggerated responsiveness of the inspiratory mechanism following pontine decerebration was suggested. Apneusis in the cat may and often is maintained for the first few minutes, during

which there are no signs of normal respiratory activity. However, in the cat, as well as the dog, apneusis does not entirely replace the obscured but present normal periodic respiration. The presence of these movements, no matter how small, indicate there is still a periodic discharge retained in the preparation. Only at the extreme of apneustic drive is this periodicity lost due to the inspiratory spasm. As the apneustic drive decreases with time, signs of periodic respiration return.

Salmoiraghi and Burns (103) indicated that a relatively small discrete lesion about 4 millimeters long in the midline at the region of the obex was sufficient to totally arrest respiratory movements in the dog. Note that potential lateral descending pathways have been spared. They concluded, in this decerebrate and decerebellate preparation, that there was no evidence for existence of respiratory pacemaker cells capable of spontaneous discharge in the absence or neighboring neural systems.

Many authors have concluded that the residual breathing in an animal subjected to vagotomy and pontine transection may be due to an incomplete transection or to an extension of the pneumotaxic center down to the medulla. Borison (12) suggested it would be equally plausible to have an accessory tract from higher centers remaining in the area, possibly from his "spasmodic center."

Breckenridge and Hoff (13) noted in the vagotomized

animal with a transection below the level of the stria acoustica, that the apneusis produced was considered to be an epiphenomena arising from "supra-medullary centers and not intrinsically associated with the genesis of respiration." Respiration was envisioned as a basic periodicity of the medullary centers regulated secondarily by facilitatory and suppressor areas of the brainstem. Wang and coworkers (118) suggested that normal respiratory rhythm originated in the pontine apneustic center with periodic modulation by inhibitory impulses from pulmonary stretch receptors by way of the vagus, and also from the pneumotaxic center in the rostral pons. They claimed that an uninhibited medullary inspiratory center maintained tonic activity and further that rhythmicity was not a property of the medullary inspiratory center. Several authors, including Stella (113) and Pitts (88), investigated this point in terms of CO_2 response, but found no evidence indicating such an uninhibited inspiratory center. Tang (116) showed that vagotomy abolished the increase in rate but not the increase in depth in response to an increased CO_2 tension. Pneumotaxic center ablation coupled with vagotomy reduced the increased depth response, but exaggerated the rate increase. The Hering Breuer reflex was thus thought to control the respiratory rate in the absence of the pneumotaxic center, and tended to maintain a relatively constant depth of respiration. Tang concluded that the pneumotaxic center normally controls

respiratory depth, and in the absence of the Hering-Breuer reflex as a consequence of vagisection, maintains a constant rate of respiration. In the series of animals reported, all were chronically or acutely decerebrate, many were anesthetized, and most had mid-collicular transections. It is difficult if not impossible to reach valid conclusions about the possible interactions of centers when large portions of the CNS have been totally excluded.

Breckenridge et al (14) pointed out that bilaterally vagotomized preparations with transections rostral to the medulla presented a rate and depth pattern which appeared to be essentially normal. Such evidence was offered in support of the concept that the basic respiratory act was developed within the medulla itself, and that no supramedullary centers (including the pneumotaxic center) were necessary for the development of respiratory periodicity.

Burns and Salmoiraghi (18) suggest that inherent respiratory periodicity in the cat - and probably also in man - is not a highly regular phenomena, a finding they said was not consistent with a respiratory pacemaker site.

In spite of the wealth of information on mechanisms controlling respiratory rate, little information exists regarding depth. Tang (116) implicated the pneumotaxic center for control of both rate and depth. Elimination of the pneumotaxic area greatly reduced brainstem regulation of respiratory

depth, and exaggerated the respiratory rate response to increased inspired CO_2.

Cohen et al (24) showed that several variables in the control of respiratory arousal may be influenced independently; therefore they must represent the activity of several separate subsystems in the respiratory complex. This view focuses attention on the fact that respiratory control involves more than a simplistic model whose chief function is the genesis of an inspiratory or expiratory act. The reticular formation, according to Cohen, acts to facilitate the system triggering the onset of inspiration. A second activity acts to augment this system for the maintenance of inspiratory discharge. This would increase the phrenic firing rate. A third function would be to inhibit the system responsible for the cessation of inspiration. It may readily be seen that such parameters as respiratory rate, depth, tidal volume, and velocity of both inspiratory and expiratory air movement, as well as duration of each phase of respiration could be modified independently if such multifactor control exists.

The possibility, therefore, appears likely that the final integrative process determining discharge to respiratory effectors need not necessarily occur in the medullary respiratory area. There exists a growing body of evidence that this integration may equally be operational at spinal and suprabulbar sites.

Literature dealing with specific effects of anesthetics on respiration is not extensive. Wang and Nims (119) found that chloralose was a more profound respiratory depressant than nembutal, and that the specific ventilatory response to CO_2 was also depressed. Urethane was found to cause no consistent changes in respiration in animals anesthetized with 1 gram per kilogram body weight (119). Florez and Borison (34) indicated that respiratory rate was decreased by small doses of pentobarbital and urethane, but that full anesthetic doses increased the sensitivity to a CO_2 stimulus: chloralose produced a greater depressant effect than either pentobarbital or urethane.

Gesell et al (42) pointed out that the interaction of rate and depth components of respiratory control are exquisitely sensitive to the level and type of anesthesia. For instance, lung inflation results in inhibition of further inspiration. Light anesthesia permitted a much more rapid and sensitive response to lung inflation, whereas with deep levels of anesthesia the lung inflation reflex was totally blocked. These findings appear to confirm the high sensitivity of the reticular formation to many anesthetics.

C. The Vagus

The vagus serves as an important afferent pathway in the control of respiration. Widdicombe (121) has reviewed its function in terms of receptor stimulation. Inflation of the lungs may modify central neuronal activity by some or

all of the following: Hering-Breuer inflation reflex (inflation inhibits diaphragmatic contractions); Head's paradoxical reflex (inflation increases diaphragmatic contraction in the presence of partial vagal blockade); irritation (lower respiratory tract irritation causes an expiratory effort or coughing); pulmonary vascular (pulmonary congestion sensitizes pulmonary stretch receptors, and may induce reflex hypotension and bradycardia). All the above are mediated via vagal fibres, the response being abolished by vagotomy.

Gesell (42) et al described the increase in breathing rate resulting from vagal stimulation during inspiration. Gesell (40) proposed that the vagal reflex may provide the basic mechanism for rate control. Relatively shallow breathing before vagotomy was supplanted by somewhat deeper, slower respiration after vagotomy, presumably by removal of vagal tone. Chemoreceptive reflexes and central stimulation were seen to augment the central discharge and in the absence of the vagal reflex, tended to be inhibitory on respiration. Gesell indicated the final result of an imbalance between chemical and vagal stretch inputs depended upon the relative strength of the two impinging signals.

An excellent treatment of the role of vagal afferents in respiratory control is provided by Liljestrand (71). In this review, the results of numerous investigators are summed up. It would appear that at least three sets of afferent fibres are involved in the vagal respiratory reflex. Fast

fibres of the A-alpha type, with conduction velocities approaching 80 m/sec tend to inhibit inspiration. These were activated only with high frequency stimulation. A second, slower group resembling the A-beta tactile fibres gave a weak inspiratory response and a slight increase in respiratory rate at low rates of stimulation. At higher rates of stimulation, the response became purely expiratory, with marked retardation of respiration rate. The third group of fibres were of the 1B type, resulting in a strong inspiratory reaction with marked acceleration of breathing from either high or low frequency stimulation. Shulgin and Rice (cited in 124) first described the graded response of the respiratory system to vagal stimulation. Wyss (124) built on this concept, and cited the investigations of several authors which support the concept that vagal input to the respiratory system involves at least one internuncial neurone. The evidence seems to support the concept of a dual path interconnection of the vagal and spinal afferents.

Nesland et al (80) found the vagus to be inhibitory to both inspiratory and expiratory neurones in the brainstem. It appeared that the vagus modulated the graded response of the respiratory neurones, acting chiefly by limiting the duration of each burst of impulses. Pitts (74) found that the expiratory facilitation effect of stimulation of the central vagus may be completely overriden by stimulation of the brainstem with a moderate stimulus strength. He

concluded that the concept of the vagus as a modulator of respiratory period generation is not correct. Cohen (19) postulated that the respiratory system contained two subsystems for vagal afferent input; a predominantly medullary system closely linked to vagal input, and a second system, predominantly pontile, and only remotely related to vagal input temporally. Wyss (125) had shown that the frequency of stimulation of the vagal afferents of monkey produced two different respiratory responses. Low frequency stimulation (30 Hz) produced an acceleration in respiratory rate and a shift toward the inspiratory position. Higher frequencies (150 Hz) gave rise to an opposite reaction, with slowing of respiration and a shift toward expiration. He also demonstrated that a graded respiratory response could be obtained by varying the frequency of stimulation at the central end of the transected vagus. These he related to the inflation and deflation reflex (124,132).

Pitts (86) showed that respiratory inhibition as a result of vagal stimulation was of a more complex nature than limited synaptic connections might provide. A burst of stimuli delivered to the vagus for approximately 0.8 seconds produced an inhibition of phrenic firing which lasted 1.4 to 3.0 seconds, followed by return to spontaneous activity. Pitts assumed a resetting of a central mechanism resulting from the applied vagal burst.

Woolcock et al (123) studied pulmonary dynamics as

a result of vagal stimulation in dogs. Narrowing of the peripheral airways resulted, with increased lung compliance and elastic recoil of the lung resulting in a considerable decrease in total lung volume as a result of the stimulations. Extensive neural control of the central and peripheral portions of the bronchial tree was indicated. Katz (64) reported that stimulation of peripheral afferents resulted in a 13 to 92% decrease in minute volume. However, high voltage, high frequency stimulation usually caused an increase of 8 to 14% in minute volume. Inspection of the figures of the paper indicate that in some cases the respiratory rate was inhibited, while in other cases the respiratory rates were more than doubled concurrent with an increase in depth.

Gesell et al (38) postulated that the vagal signals which are at a maximum during peak inspiration, may in fact switch their drive from an inspiratory to an expiratory center. Thus the tapering off of inspiration which merges into expiration might be explained with the vagus acting as the mediator in the switch from inspiration to expiration.

Destructive lesions have been used to study the interrelations of the brainstem respiratory system and the vagal-receptor system. Wyss (124) localized the primary reflex areas for vagal input. The nucleus of the tractus solitarius and its adjacent areas seem to be the primary point of entrance for vagal respiratory fibres. Connection with the expiratory center seems to occur at the cranial end of

the tractus solitarius while the more caudal extent of this nucleus appears to convey traffic to the inspiratory center. He proposed the net effect of the vagal input would depend upon the level of vagal firing. The vagal input, although projected to both inspiratory and expiratory centers, seems to have its predominant effect on the inspiratory center. This indicates that the inspiratory center is more sensitive to vagal afferents. Only at higher vagal firing rates would control be switched from a facilitatory effect to one of inhibition of the inspiratory center, with resulting facilitation of the expiratory center.

In 1947, Wyss (126) showed that selective elimination of either the inspiratory or expiratory effect of vagal stimulation could be brought about by small lesions in the region of the tractus solitarius and the dorsal portion of the immediately adjacent lateral reticular formation. The critical lesion for the expiratory component is in the cranial portion of the tractus solitarius just caudal to the point of entrance of the vagal fibres. The inspiratory component can be blocked by lesions in the caudal 2 to 3 mm of the tractus solitarius system.

Stella (113) studied the effect of thoracic compression as a means of terminating the apnea normally associated with midpontine section. He found that even moderate tension was often sufficient to restore regular respiration which continued only as long as the tension was maintained.

Even animals in which elevated CO_2 or chemical stimulation had been unsuccessful regained normal respiration as a result of this procedure. He implicated a vagal reflex involving a decrease in the thoracic volume. As proof, however, he performed a median sternotomy and manipulated the costo-vertebral joints. The apnea was interrupted only when the transection was made in the lower pons. The response to middle pontine section was small or absent.

Liljestrand (71) described lesions in the tractus solitarius and adjacent lateral reticular formation which abolish the effect of central vagal stimulation on respiration. He suggested a "vagal expiratory center" closely approximated to the site of the lesion, and further stated that vagotomy often destroys this center. The "vagal inspiratory center" was located about 2 mm caudally in the tractus solitarius and the lateral reticular formation at the caudal portion of the calamus scriptorius. It extended 2-3 mm caudally. Discrete lesions in either center produced no apparent alteration in the other center.

D. Upper Brainstem and Forebrain

Many investigators, for example Spencer in 1894 (111), Sachs in 1911 (111), and others have shown that electrical stimulation of the anterior forebrain may cause changes in the rhythmicity of respiration. Bucy and Case (17) pointed out that various investigators as early as 1885 were able to augment respiratory rate and amplitude in rabbits by stimulation

of a small area in the lateral wall of the third ventricle, just anterior to the cephalic end of the aqueduct of Silvius. Papez (83) described three reticulo-spinal tracts, each of which has later been shown to possess respiratory-related potentials when explored with microelectrodes (40). Kabat (61) carried out a comprehensive study of hypothalamic influences on respiratory patterns in cats lightly anesthetized with nembutal. Stimulations were performed from the level of the olfactory bulb to a point just posterior to the inferior colliculi. There is difficulty in interpretation of his data, however, because he grouped all responses into only four types. It is thus difficult to determine the specific nature of the stimulus response. For example, one of the classifications was that of increased amplitude of respiration only, yet Kabat states there was usually an increase in rate as well. The second type dealt with increased respiratory rate. Type three referred to decreases in rate or in amplitude. Finally, significant increases in amplitude with decreases in rate were reported.

In 1933, Ranson and Magoun (93) found a marked acceleration in respiratory rate (as high as 240 per minute) by stimulation of the lateral hypothalamus and the perifornical region. Control rates in these cats were 20-30 per minute. With the onset of stimulation, an immediate increase in respiratory rate to an average of 90-100 resulted. It was also reported that the cessation of stimulation was not

followed by a period of apnea, and the animal returned rapidly to control rate. In some animals, repeated electrode penetrations in this area permanently increased the rate. In one instance, a control rate of over 90 was reported, which the animal maintained until sacrificed. At autopsy, hemorrhage was observed in the area of the hypothalamus. They found respiratory reactions were minimal in the preoptic area rostral to the level of the anterior hypothalamic nuclei. The first responses were seen about 0.5 mm behind the optic chiasm at a level just rostral to the rostral pole of the ventromedial hypothalamic nucleus. More caudal levels through the posterior hypothalamus and mammillary body showed many responses.

In 1934, Leiter and Grinker (69) produced apnea by the stimulation of the anterior hypothalamus. The apneic period lasted 20-30 seconds, with no indication whether the animal spontaneously broke out of the expiratory arrest. Stimulation in the same area but more lateral showed only a slowing of respiration. These authors postulated, on the basis of nerve degeneration studies, that a single neurone may project in the reticular structure from the hypothalamus to the lumbar cord. Part of the data was based upon the degeneration studies of others. Leiter and Grinker (69) characterized the respiratory response to hypothalamic stimulation under light anesthesia as an initial slowing followed a few breaths later by increases in both rate and

depth. Stimuli were delivered for 30 seconds from an inductorium. "Stronger" stimulation brought about a "tetanicapnea" which resulted in increased amplitude and rate of respiration on cessation of the stimulation. In a few of the animals studied, a definite stimulation of respiration occurred simultaneously with motor movements.

In 1935, Ranson, Kabat, and Magoun (92) described more definitively the respiratory response to hypothalamic stimulation. Specific stereotaxic coordinates were given for the series of stimulation in pentobarbital anesthetized cats. Stimuli were begun at Horsley-Clark coordinates AP 12, L 6, H+3 and repeated down to H-5. The series was then repeated with the electrode moved successively one millimeter closer to the midline. The authors qualitatively stated that stimulation of the hypothalamus in these areas resulted in an increased rate and depth of respiration, as well as a rise in blood pressure. Ranson et al also showed that stimulation of points rostral to the optic chiasm caused respiration to become slow and shallow, while the same stimulation delivered caudal to the optic chiasm increased both rate and depth.

Ranson et al (92) implanted chronic electrodes in the hypothalamus at unspecified coordinates, then stimulated the animal while alert. The respiratory result was an increase in both rate and depth of respiration. In discussing this procedure, Ranson pointed out that the

respiratory responses observed were almost invariably associated with what he interpreted as emotional excitement of the awake animal.

Kabat, Magoun, and Ranson (62) mapped points in the midbrain and forebrain from which cardiovascular responses could be elicited. They did not detail the respiratory responses other than to note that in many instances during hypothalamic stimulation the respiratory response was found to occur independent of that found for blood pressure (94).

In 1936, Gesell, Bricker, and Magee (40) indicated that they considered the reticular formation to be the site of a major integrative area. Their conclusions were based primarily on a report by Finley (33) who had reported two cases of acute respiratory failure which revealed post-mortem congestion in the upper cervical cord and destruction of anterior horn cells bilaterally. In addition, there was evidence of bilateral destruction in the reticular formation. Gesell et al, provide a comprehensive list of authors who have attempted removal of the midbrain without "seriously affecting the mode of breathing," as well as a list of authors dating back to the time of Legallois in 1812, whose work indicates that the thalamus had no effect on respiration. The magnitude of disagreement on this point can be seen, however, when one examines a second list, running some two pages and covering the period 1812 to 1936. This list details papers in which changes in respiration

were produced by stimulation in the hypothalamus.

The studies of Gesell et al (40) on stimulation in the thalamus, hypothalamus, and in the mesencephalon, even though they resulted in respiratory changes, did not convince them that the hypothalamus should be included as a necessary part of the respiratory apparatus. They pointed out that the scarcity of respiratory potentials may indicate that the upper centers exert very little influence on breathing. They also indicated however, that some unknown step in the procedure may have eliminated these higher respiratory potentials or restricted their pathways in such a way that they had not been detected. Even though Gesell et al attempted small-electrode recordings from the upper end of the thalamus to a point 2 centimeters caudal to the thalamus, in only five out of an unspecified number of penetrations were cells showing respiratory rhythm found. Due to the nature of the equipment available in 1936, the absence of any respiratory potentials does not preclude their existence. However, the authors concluded that any change in respiration evoked as a result of stimulation in the hypothalamus must then be of a reflex nature.

Smith reported in 1938 (108) that stimulation of the cerebral cortex during the inspiratory phase can result in early termination of the inspiratory phase and rapid return to the expiratory position. He also outlined an area in the cortex which was facilitatory in terms of its respiratory

response. Both the inhibitory and the facilitatory areas were effective when stimulated if either the spinal cord below the phrenic outflow was transected or if the phrenics themselves were sectioned. Thus Smith concluded that mediation of the response is over both phrenic and intercostal nerves. Some areas of the cortex responded paradoxically depending upon the level of anesthesia employed, being facilitatory under light anesthesia, but inhibitory under deeper narcosis. Speakman and Babkin (110) showed that a shift in respiratory contractions from thoracic to diaphragmatic occurred when the lateral end of the anterior sigmoid gyrus was stimulated. Light anesthesia was essential for this purpose. They also demonstrated areas of inhibition and facilitation of respiration in the cortex.

Studies of Pitts et al (89,91) indicate an involvement of the hypothalamus in respiration, but further reference is not made to the exact anatomic location of this respiratory response nor of its magnitude other than to state that the response does not appear as profound as that evoked by brainstem stimulation. Hugelin and Cohen (53) studied the effects of stimulation of the posterior hypothalamus and the subthalamus. Stimulation in these areas yielded an immediate termination of the expiratory act followed by the rapid onset of inspiration. A more rapid increase in the phrenic discharge frequency and a greater amplitude of the integrated phrenic signal resulted, primarily

due to an increased respiratory rate secondary to shortening of the expiratory period. Expiratory time was always shortened, but the effect on inspiratory time was variable. Polypneic animals showed a decrease in respiratory rate mediated primarily by an increase in inspiratory duration. It was suggested that reticular stimulation acts on multiple components in the respiratory centers.

Fink, Katz, Reinhold, and Schoolman (32) noted that supracollicular transection produced a marked increase in respiratory frequency, presumably by unmasking a caudal facilitatory influence through removal of some forebrain inhibition. Redgate and Gellhorn (96) showed that lesions in the lateral hypothalamus as a result of RF current caused an immediate decrease in rate and/or depth of respiration in the lightly anesthetized cat. They concluded that impulses from the lateral hypothalamus exert tonic facilitatory action on the respiratory center. Some lesions were seen to produce only a reduction in the respiratory rate without changing amplitude. It was also shown that a significant decrease in respiratory activity results from a well circumscribed lateral hypothalamic lesion. Redgate (95) reported that microinjection of thiopental into the hypothalamus decreased the duration of inspiratory apnea elicited by a medullary stimulation. The same dose given systemically produced no respiratory effects. Redgate concluded there is a tonic hypothalamic discharge exciting inspiratory activity. Segundo

et al (107), reported that bilateral lesions of the anterior thalamic nuclei in cats produced a period of marked respiratory acceleration. They also showed that discrete lesions in these same nuclei can produce an inhibition of respiration.

Gellhorn and Ballin (36) have described the effects of afferent input on hypothalamic potentials. They found that the increase in respiration correlated with an increase in evoked hypothalamic discharge. The actual characteristics of the respiratory responses were not reported. Bach (4) studied interaction of the somatic, respiratory, and cardiovascular responses to reticular stimulation, and suggested that the concept of the reticular formation as a generalized facilitative or inhibitory center is probably invalid. The magnitude of each response was compared before and during stimulation of a specific bulbar site. Zilov (133) recorded extracellular activity from mesencephalic reticular units, and concluded that only 20% of these cells responded with facilitation or inhibition to a wide variety of stimulation procedures. There is no data offered regarding the types of cells studied nor of the physiological significance of the individual cell populations. He indicated, however, that others have reported figures as high as 80% in similar studies. In 1936 Le Gros Clark (68) produced an excellent anatomical description of ascending tracts to the hypothalamus in Macaque monkeys. These tracts, defined by means of fibre degeneration studies, were from a wide variety of areas

primarily associated with peripheral afferents.

Few similar studies have been done in man. White (120) stimulated the lateral wall of the third ventricle of man, using 3-4 volts and 60 Hz. Cardioacceleration without elevation in blood pressure was observed. No alterations in respiration were seen. Stimulation of the anterior thalamic nuclei in man and cat were compared by Baird, Guidetti, Reyes, Wycis, and Spiegel (6). In anesthetized cats, stimulation of the anterior nucleus resulted in a slowing of respiratory rate and a reduction of amplitude, often with complete arrest of respiration for as long as one minute. Respiration after cessation of stimulation was characterized by a deep inspiratory movement. Stimulation of comparable sites in man yielded similar results. A period of apnea of 2 1/2 minutes was recorded in one patient.

Kaada and Jasper (60) studied respiratory responses to stimulation of the temporal pole, insula, hippocampus and the limbic gyrus in man. They found inhibition of respiratory movements in expiratory position resulting from stimulation in the anterior end of the hippocampal gyrus, the ventral and medial surfaces of the temporal pole, anterior portion of the insula, and anterior limbic gyrus. Segundo, Arana, Migliaro, Villar, Garcia Guelfi, and Carcia Austt (107) studied respiratory responses in man from the fornix and wall of the third ventricle. They reported a depression of respiration or expiratory apnea when the

fornix was stimulated. However, stimulation of the wall of the third ventricle caused a marked acceleration in respiration with an increase in the expiratory position of the thorax. Baird et al (6) indicated that efferent inhibitory impulses from the cingulate gyrus reach respiratory centers by a dual pathway, part of which courses around the anterior thalamic nuclei. The other part synapses in this structure. Bilateral lesions in the anterior thalamic nuclei in cats produced a period of reduced respiratory reactivity. The authors also demonstrated that anterior thalamic stimuli can inhibit respiration in cats.

Spencer (111) described cortical centripetal fibres in monkeys which were inhibitory to respiration. These fibres project from the cortex through the lenticular nucleus to form part of the olfactory limb or the anterior commissure. This bundle passes caudally to the inner side of the anterior cornu of the lateral ventricle in the midline to a point where it decussates to form the anterior fibres of the anterior commissure. These fibres then pass downward and outward from the midline close to the infundibulum above the optic commissure, thence above the inner end of the optic tract. Spencer described these fibers as running above and just internal to the level of the aqueduct and then passing through the red nucleus.

One of the physiological responses to elevated temperature is panting. Smith (108) demonstrated in the dog

and cat that an area in the anterior composite gyrus of the cerebral cortex is a powerful inhibitor of respiration in both normal and panting animals. He also demonstrated panting-like responses from stimulation of the cortical respiratory acceleration areas. Smith also cites Pinkston, Bard, and Rioch, who demonstrated that the removal of the cerebral cortex results in the absence of true polypneic panting. Smith postulated that each of these responses are mediated through a mesencephalic pathway. The result of unilateral cortical stimulation was shown to be bilaterally distributed. Its inhibitory effect was distributed between both intercostal and phrenic pathways. Fink, Katz, Reinhold, and Schoolman (32) studied the blood gas composition resulting from supracollicular transection, and noted the possibility for a facilitatory influence from the forebrain area. Supracollicular transection produced a marked increase in respiratory frequency, presumably by unmasking a caudal facilitatory influence. After intracollicular transection, there was a sudden fall in respiratory rate. Supracollicular preparations sometimes exhibited respiratory patterns similar to polypnic panting. Magoun, and Lilienthal and Otenasek (cited in 32) believed this respiration pattern originated in the hypothalamic temperature regulating mechanism. Foster and Ferguson (cited in 32) observed panting at rates above 200/minute resulting from hypothalamic heating. Pitts, Magoun, and Ranson (91) studied the effect of

decerebration on respiration. They found that if the major part of the hypothalamus were spared, spontaneous panting began and continued for an hour or longer. They suggested that this decorticate panting was mediated through the pneumotaxic center and its descending pathways. They speculated in a footnote, however, that the possibility exists for a descending pathway which is merely coexistive with those from the pneumotaxic center. After a unilateral destruction of the hypothalamus, Keller (65) reported the persistence of a bilateral functional response to heat stress.

Pitts (88) reviewed the role of hypothalamic mediation of thermoregulation as it pertains to respiration. He suggested that thermal panting depends upon impulses descending from the anterior hypothalamus to the pneumotaxic center, causing it to become the dominant factor in respiration (88,90). Conscious control of respiration in man, who does not normally pant, is capable of elevating minute volume well beyond 150 L/minute presumably by similar mechanisms (88).

E. Efferent Pathways

Although there is ample evidence which confirms reciprocal connections of the respiratory neurones, their projections to the final common pathway in the cord appear to be uncrossed. Rijlant (97) studied cats following a 3 mm midsaggital section caudally from a point 2 mm rostral to the caudal end of the fourth ventricle. As a result of this

relatively discrete lesion, Rijlant reported the respiratory effectors on both sides of the body acted independently. He concluded that the coordinating mechanism lies in the midline at the posterior margin of the fourth ventricle. A more extensive midline transection resulted in a transient inhibition of respiration followed two to three minutes later by very slow respiratory cycles. Salmoiraghi and Burns (102) reported bilateral responses both in terms of respiratory movements and extracellular recordings of respiratory units following a complete midline transection in the brainstem from the obex to a point 2 mm rostral to that point. They concluded that in the area of the medullary pyramids and slightly rostral to it, there is a convergent crossover of respiratory neurones.

Based on both transection and electrogoagulation data, Hukuhara, Sumi, and Okada (57) proposed that the pathways of primary respiratory fibres descend through the lateral reticular formation uncrossed but converge toward the obex. They concluded therefore that the primary descending pathways may be uncrossed.

Gill and Kuno (44) pointed out that a possibility exists for integration at the cord level in the phrenic neurone. They presented evidence showing that impulses descend through the cervical cord which produce both excitatory and inhibitory action on the phrenic motor neurones. The relative effect of these two descending pathways depends

upon the portion of the respiratory cycle studied.

Cohen and Hugelin (24) described the response of phrenic neurones to stimulation in the reticular formation, the mesencephalon, dorsal border of the red nucleus, lateral part of the hypothalamus caudal to the mammillothalamic tract in the fields of Forel, and the zona incerta. The result of these stimulations was a complex integrated reaction modifying serveral variables, including duration of expiration, rate of growth of phrenic discharge, and end-expiratory level of phrenic firing. The magnitude and relative contributions of each variable depended on the control status of the cat; however, typical effects were those which would lead to increased ventilation in the normally respiring animal.

Further evidence for a more diffuse potential pathway was reported by Batsel (7). He pointed out that the limits of the respiratory areas as defined by truncation, electrical activation, or chemical stimuli are only in approximate agreement. Respiratory areas were found to exist more laterally when defined in terms of potential recordings rather than response to stimulation, thus indicating that the tonically active system may be more lateral than previously thought. The result of stimulation may be to enhance the firing of otherwise silent backup centers. Even the origin of the efferent system therefore seems in doubt.

Allen (cited in 10) concluded that descending

respiratory motor tracts were in the anterior columns and in the ventral part of the lateral columns, suggesting that the ventral and lateral reticular spinal tracts serve respiratory function. Pitts (85), however, stated that in cats the pathways were in the anterior column and in the anterior part of the lateral column of the cord. They appeared for the most part to be uncrossed.

Smith (108) stimulated the isolated cortex, and found many small points bilaterally which were inhibitory to respiration. Other areas facilitated inspiration. Simultaneous bilateral stimulation of points with comparable topographic representation in both hemispheres was more effective in evoking respiratory responses. When the phrenic and vagosympathetic trunks were sectioned, the response also was found with intact phrenics and cord section below the point of exit of the phrenic. Thus the phrenics and intercostals have a dual control over respiration, one sufficing in the absence of the other.

Hukuhara et al (55) recorded respiratory action potentials from the spinal cord. Although no precise localization was given, from a photomicrograph in the paper it appears that the respiratory cells in the cervical cord are localized in the lateral column.

Cross connections were thought to exist in the respiratory system only in the region of the medulla between the respiratory neurones of one side and the effector

motor nuclei of the other. Hitchcock (45) reported that the respiratory tract with maximal effect on respiration is found in close approximation to the spinothalamic tract in the cervical cord.

In man, there seems little doubt that the reticulospinal tract itself is concerned with respiration. Hitchcock (50) reported that patients with high cervical cordotomy often function quite well when awake, yet have been known to die in their sleep. He stated "it is not uncommon to see a patient with spinal injury, or with disease, patients with no difficulty in voluntary respiration, but in the complete absence of rhythmic automatic respiration." In this context Belmusto et al (10) were of the opinion that the spinothalamic and respiratory tracts were intimately intermixed. Hitchcock and Leece (50) proposed that voluntary control of respiration may exist through a corticospinal path, with the anterior horn cells serving as the final common pathway for both corticospinal and reticulo-spinal tracts. Conclusions were based on data describing respiratory modifications in a group of 14 patients operated for intractable pain. Sears (105) studied respiratory reflex connections in motor neurones of the cat spinal cord and presented evidence for a direct spinal reflex in the control of respiration. This reflex did not involve the brainstem per se. His primary evidence was based on stimulation and concurrent intracellular recording distal to a complete cord transection.

Liljestrand (71) concluded that insofar as the efferent pathways were concerned, the descending respiratory motor tracts were located in the ventral columns and ventral parts of the lateral columns. Based on an extensive study of these pathways utilizing intracellular techniques, he concluded that these pathways descend primarily uncrossed. He reported ipsilateral paralysis of the respiratory muscles after hemisection of the cord at the C1 level. Cross connections, however, exist on both sides of the thorax and at the diaphragm; if an animal is allowed to recover from a unilateral hemicordotomy, bilateral respiration results, indicating that potential pathways in the cord do in fact exist and can become operational. Sumi (114) studied the function of interneurones in the respiratory complex at the level of the spinal cord, and suggested that these interneurones could play an integrative role in the regulation of intercostal respiratory unit discharge. Evidence for a possible integrative site in the cord separate from the respiratory center was presented by Eklund, von Euler, and Rutkowski (31), by Critchlow and von Euler (27), and by Sears (104). All demonstrated gamma efferents in the intercostal muscles and described effects of chemical and evoked respiratory cycle stimuli on the gamma efferent system.

Sears (106) described the results of intracellular recordings from respiratory motor neurones in the thoracic spinal cord of cats. Utilizing antidromic stimulation of

the internal and external intercostal nerves to determine the nature (expiratory or inspiratory) of the impaled cell, he concluded that a rhythmic depolarization toward threshold occurred during the active period of the thoracic motor neurones. Hyperpolarization of the antagonist cells also was observed. Sears concluded the source of these facilitating and inhibiting potentials was of central origin, but may be modified by interneurones with short axons. He emphasized that the source of these drive potentials was probably separate from the classical respiratory centers, yet concluded that the respiratory motor neurone itself is the supreme determinant of integration of the modulating drives responsible for phasic activity.

Wyss (124) revealed the possibility for inhibition of inspiratory motorneurones as a result of stimulation of final descending pathways. The work tends to be rather complex in terms of stimulus parameters and sites of activation, which makes interpretation difficult. He also pointed out that several investigators have speculated about direct inhibition of motor neurones in a two neurone spinal reflex system. Bronk and Ferguson (15) demonstrated that the internal intercostal muscles function during expiration and the external intercostals during inspiration in the cat. However, in the quietly breathing decerebrate cat, both internal and external intercostal nerve traffic was often absent, returning only after some degree of

airway obstruction was introduced. Interestingly, the external intercostal traffic returned at lower obstruction levels. At times the external and internal intercostal nerves were found to fire in synchrony with inspiration. Gradation was found to occur in the intercostal firing which correlated with the depth of inspiration. Adrian and Bronk (2) did not find evidence of gradation in single fibres of the phrenic at different levels of respiration.

Evidence involving the portion of the cord responsible for respiration in man has been outlined by Belmusto (10) based on results of high cervical cordotomy or partial cordotomy. It was concluded that the anterior quadrants bilaterally from C1 to C3 from the lateral margin of the cord to a point 3 to 5 1/2 mm medial to the lateral border contain the entire respiratory projection.

Jones, Beargie, and Pauly (58) showed that in bipeds an additional postural function must be attributed to the intercostals. Using surface electromyographic techniques, they indicated that the first rib is elevated actively during inspiration in man, but that the balance of the ribs apparently follow passively with no noticeable increase in intercostal firing. They were unable to separate the external and internal intercostal traffic with their technique, so were unable to confirm Bronk and Ferguson (15) regarding simultaneous contraction of the internal and external intercostals. Other evidence cited by Jones

et al (58) indicates that the role of the intercostals in all but maximal forced respiration may be mainly to supply a splinting tension to the ribs while the main force of respiration proceeds from below. In a follow-up study, Jones and Pauly (59) elaborated on their previous findings. They showed, during forced inspiration, that the scaleni, sternomastoid, and anterior edge of the trapezius as well as the intercostals play important roles in development of inspiratory movement.

A possible ascending pathway has been described by a number of authors, including Decema, von Euler, and Thoden (28). They felt that the pathway had bilateral representation in the ventral part of the lateral funiculi, as bilateral section in this area was necessary to abolish the reflex believed to be due to intercostal receptors.

F. The Phrenic Neurone

The classical final common pathway for respiration, the phrenic motorneurone, has been the subject of much study. Much of this is based upon the technique of Adrian and Bronk (2) who first described a method of teasing apart the fibres of the phrenic nerve until small branches were obtained. The discharge characteristics of the fibres could then be studied. They reported about 150 fibres in each nerve trunk in the cat. By careful teasing, Adrian and Bronk obtained what they felt were 5 to 6 remaining intact fibres. Any attempt to reduce the number of fibres

still further resulted in destruction of the nerve and obviously no recorded potential remained.

Pitts (87) found that the phrenic neurone of the cat fired at peak discharge frequencies well below 50 Hz, with the majority of the population showing discharge rates in the range of 10 to 30 Hz at the peak of inspiration. Pitts further pointed out (85) that the degree of respiratory activity during inspiration is a function both of the number of neurones firing and the degree of activity in those neurones. Wyss (129) showed that the number of active fibres in the cat during spontaneous respiration varies between 0 and 40. The number of units found were distributed almost equally between the two phrenics, and were found primarily in the C-5 and C-6 branches. A periodicity in phrenic firing was found by Cohen (21), described as a series of alternating bursts and silent periods. He described an oscillation in phrenic impulse traffic which varied from cat to cat. It ranged from 70 to 100 Hz, and seemed to be superimposed upon the basic phrenic traffic, resulting in a modulation of phrenic discharge.

Pitts characterized the phrenic discharge more fully in 1946 (88) as beginning at a fairly slow rate which slowly augmented toward the peak of inspiration, then decreased steadily at the beginning of expiration. He also indicated that a certain unspecified number of units were active throughout the inspiratory cycle, while a few others were

brought into play at or near the peak of inspiration.

Cohen (21) pointed out a direct relation between phrenic nerve discharge and tidal volume. An approximately proportional relationship exists between integrated phrenic or diaphragmatic discharge and the tidal volume.

Contrary to the results of Adrian and Bronk, Pitts (88) found that the phrenic was capable of recruitment. Because of the recruitment, as well as the different times of activation of the individual fibres in the inspiratory cycle, Pitts postulated that there might be several possible pathways, all of which might converge and result in the firing of the phrenic cells. Thus, stimulation in discrete areas of the inspiratory center of even moderate intensity or of changing stimulation frequency might lead to recruitment and an increase in the discharge. Pitts (87) utilized a two-shock technique to show this facilitation. The degree of facilitation resulting from stimulation in the inspiratory center was, however, dependent upon the portion of the respiratory cycle in which the test stimuli were delivered. When the conditioning shock and the test stimuli were delivered during expiration, a greater facilitation was seen. Facilitation was, in effect, greater when the inspiratory center was in a normally quiescent state.

Cohen (21) showed that single shock stimulation of the pneumotaxic center produced a resultant reduction in phrenic discharge followed by a rebound burst with a

latency of about 5 msec, indicating that very low levels of input were sufficient to reduce the integrated output of the phrenic.

Kahn and Wang (63) measured electrical activity in the brainstem and phrenic nerve. After bilateral vagotomy and pontine lesions, the central neurones and the phrenic fired continuously at high rates for 20 to 60 seconds. The onset and cessation of firing in both central and peripheral sites was synchronous. The phenomenon was independent of rate and inflation pressure.

Controversy still exists about whether or not the control mechanism responsible for phrenic outflow and that controlling intercostal outflow might be separate. Smith showed that, at least for stimulation of the inhibitory areas of the brainstem, the outflow to the phrenic nerve and intercostals are influenced in the same qualitative manner. Pitts, Magoun, and Ranson (91) and Rijlant (97) have shown that the outflow from the brainstem to the phrenic is apparently uncrossed.

Studying the diaphragm, Gesell (39) found that the gradation and intensity of respiratory movements was apparently the result of an increase in the firing frequency of the phrenic, and not due primarily to the recruitment of additional fibres. This relationship held throughout the lower intermediate range of respiration. Gesell was stimulating the primary respiratory center. Under these

conditions there is recruitment of the intercostals, but, as noted, not of the phrenic.

G. Data Reduction

Because of the nature of this study and the instrumental methods employed in evaluation of the data, a brief review of similar data reduction techniques is presented.

Methods of automatically handling phrenic discharge information in relation to other respiratory signals have been described by a number of authors. Each computer based technique has its own merit, and all suffer the same inherent inadequacy: primarily that of recognizing the onset of a physiological signal and discerning that signal in the presence of noise or other false triggers. No method, including the approach developed in this work, has been able to completely avoid these problems.

Steen and Crane (112) have developed on-line data processing for measurement of ventilatory volume. Cohen (20) has made extensive use of computer techniques as they apply to the phrenic neurone. In his earlier work, Cohen utilized a spike determining network, signal squaring network, and finally a pulse counter. Each respiratory signal was hand-divided into intervals for cycle-by-cycle analysis. Later he developed a computer, apparently of his own design, to derive histograms of phrenic discharge.

The problem of automatically determining the onset and amplitude of a bioelectrically generated spike potential

is one of considerable difficulty in spite of the relatively uncomplicated appearance of the signal. Digital computer separation of extracellular bioelectric signals has been covered in detail by Mishelevich (77), who provides a comparison of his techniques and that of other authors. The principles he employs closely parallel the assumptions made in the computer reduction employed in the present study. However, the type of data and output format are different.

Any computer based recognition program must do three essential things: first, it must recognize the presence of a spike as opposed to a noise pulse; second, it must determine signal amplitude. Third, the signal sequence with respect to time must be determined. Mishelevich (77) used a window technique for both signal amplitude and pulse width to derive a standard pulse for each signal which fit the predetermined window criteria.

Chapter III

METHODS

Experimental animals were 2-7 Kg mongrel cats of either sex anesthetized with intravenous thiopental (sodium pentothal). Initial doses were 20 mg/Kg, and supplemental injections were given as required during the course of the experiment. All cats were prepared with PE60 polyethylene catheters in the femoral vein for supplementing anesthesia and for administration of isotonic saline. A PE90 catheter was inserted into the aorta via the femoral artery for continuous monitoring of arterial pressure, using a Statham P23Gb pressure transducer connected to a Honeywell Model 1300 Carrier Amplifier. The amplified signal was recorded continuously on a Precision Instruments Model 6200 Analog Tape Recorder in the FM mode, and was simultaneously displayed on the face of a Rycom wide-screen oscilloscope for direct visualization. The amplified arterial pressure signals were also led into two special circuits which provided direct digital display of mean blood pressure and heart rate using Digitec model 200 Digital Voltmeters

A ventral midline incision was made in the neck, and both vagus nerves were dissected free and loosely ligatured. A metal "T" cannula was inserted into the trachea, the lateral arm of which was connected to a one foot length of 3/8" tygon tubing arranged below the axis of the cannula in such a way as to create a trap for tracheal secretions. Coupled to the arm of the "T" was a Fleisch pneumotachograph. This pneumotachograph consists essentially of a metal tube containing a honeycomb screen, which induces a small pressure drop in the air flowing across it. The ports on either side of the screen were connected to a Grass PT5A differential pressure transducer, the output of which was led to a second Honeywell Carrier Amplifier. The differential pressure signal was proportional to air flow velocity over the range encountered in the animals used in these experiments. The resulting curves of air flow velocity were displayed on the Rycom oscilloscope and simultaneously recorded on tape in the FM mode. Device linearity was tested and found acceptable.

The animal was then mounted in a Kopf Model 1600 stereotaxic frame. A midline incision was made with electrocautery from a line at the superior orbital ridge caudally to a spinal level of C8. The temporalis muscle and periosteum were reflected to expose the skull. By means of a dental drill and fine round dental bur, a bilateral skull plate was made from approximately A16 to A8, and extending approximately 5 mm to either side of the midline (101).

The plate was lifted carefully and the midsagittal sinus was dissected free. Any resulting bleeding was controlled by application of small moist Gelfoam pledgets (Upjohn) using gentle pressure. The entire cortex thus exposed was bathed in warmed mineral oil to prevent surface evaporation and subsequent drying. The dura was then lifted on the left side at anterior 12 and an incision made extending 4 mm lateral from the midsagittal sinus. Alternatively, two small holes were eroded through the dura at 1 and 3 mm lateral to the midline utilizing the RF cautery at very low current. This technique usually yielded a dry field and provided a stable preparation.

An incision was then made through the muscle of the neck and the phrenic nerve was carefully isolated at the C6-C7 spinal levels. The nerve was not sectioned, but a small bundle was isolated and draped over bipolar stainless electrodes mounted on a stereotaxic carrier. Umbilical tapes were passed through small stab incisions in the loose skin and were used to secure the skin flaps to the frame of the stereotaxic instrument in such a way as to provide a pocket. Mineral oil was dripped through a heat exchanger in series with a blanket type water heater and K-pad, which was being used to maintain the animal's body temperature. The warmed mineral oil was continuously dripped into the pocket and allowed to bathe the phrenic nerve. By carefully isolating the nerve with minimal

disruption of the fascia, oil could be introduced at a rate of about 100 cc/hour, which was sufficient to maintain a constant pool. If more radical dissection was carried out, it was sometimes necessary to line the pocket with gelatin or agar to reduce the rate of oil leakage.

The potentials from the phrenic nerve branch were amplified with a Grass P9B amplifier and were simultaneously displayed on a Tektronic Model 561 Oscilloscope and recorded on an FM tape channel.

All stimulations reported here are confined to the A12 stereotaxic plane. Preliminary experiments indicated this area to be the most reactive. Within this plane, it was found that the maximum activation of respiratory rate and amplitude was obtained from a relatively restricted region. Figure 1, a cross-section through the cat brain at plane A12 shows anatomical landmarks on the left and locations of the electrode tip in all animals reported. Electrodes were positioned into specific stereotaxic coordinates according to the following terminology:

A12: L1: H-4 Site M4

A12: L3: H-4 Site L4

A12: L1: H-5 Site M5

A12: L3: H-5 Site L5

The electrodes were initially positioned and the optimal respiratory rate response obtained, beginning at the H-2 coordinate in the A12 plane. All points were subsequently

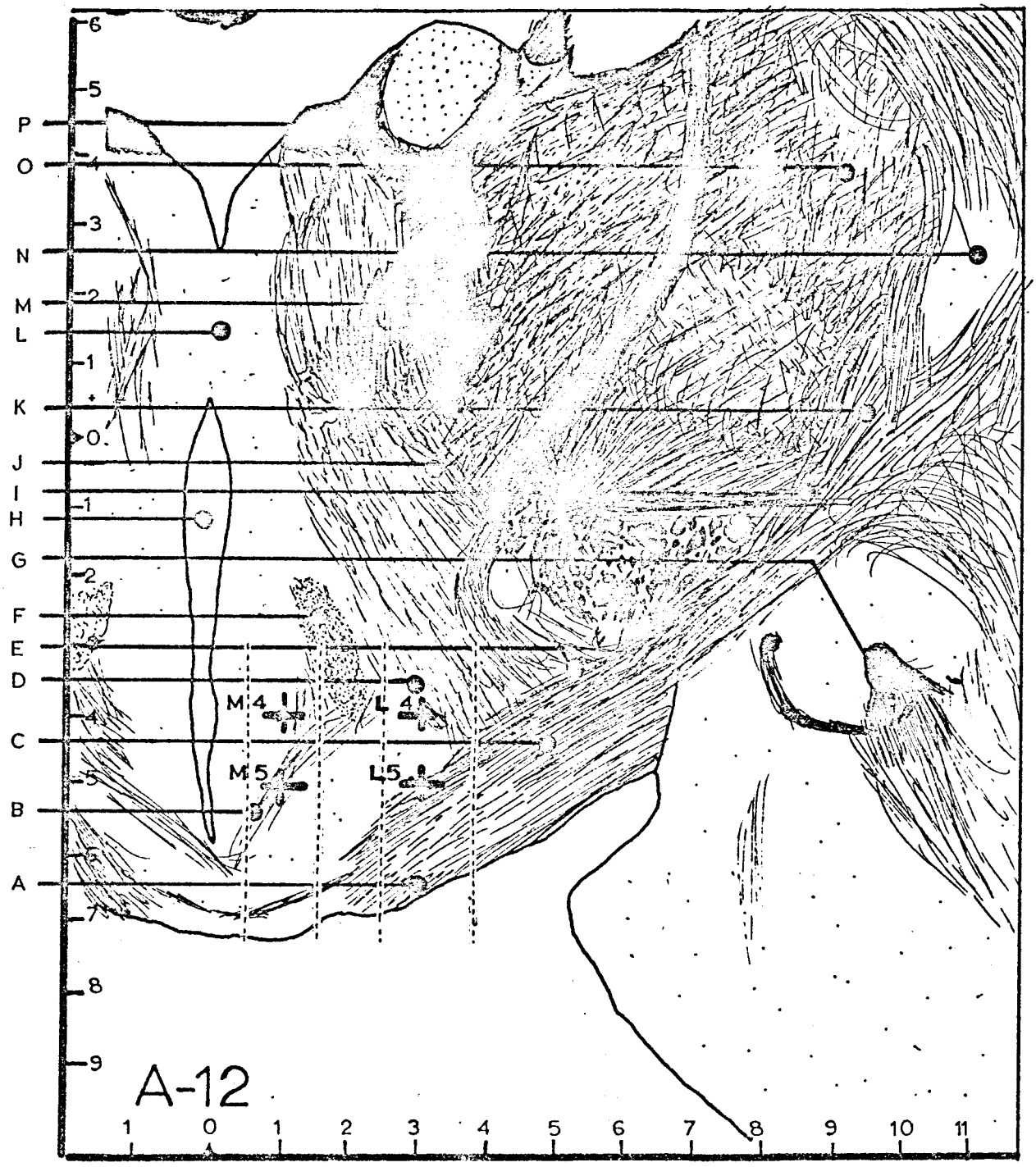


Figure 1.

Cross section through diencephalon showing stimulation sites. See following page for details.

Figure 1: Site Designations

Major structures in the cat brain surrounding the area found most reactive in this study. The large crosses denote the anatomical sites most reactive in this the A12 plane. Letters and numbers nearby indicate the shorthand notation used to designate each site. Major structures, according to the designation of Snider and Niemer (109) are:

A	Tractus opticus
B	Commissurae supraopticae
C	Tractus opticus
D	Fasciculus prosencephali medialis
E	Ansa lenticularis
F	Columna fornicis
G	Fasciculus longitudinalis associationis
H	Ventriculus tertius
I	Fasciculus thalamicus
J	Fasciculus mamillothalamicus
K	Globus pallidus
L	Massa intermedia
M	Lamina medullaris medialis
N	Putamen
O	Capsula interna
P	Stria medullatis thalami

Average electrode positions, as computed from measurements taken from the histology, were lateral 1.1 for the medial track, and 3.05 for the lateral track. Extremes found for each track were .5 to 1.5 mm in the medial track, and 2.5 to 3.8 in the lateral track. Of the thirteen cats, three showed electrode tracks with no detectable error, and an additional three were 0.2 mm or less from desired track. The mean value and range of electrode tracks is shown schematically by broken lines in Figure 1.

stimulated as indicated above in all animals. Following the initial series of stimulations, recording was initiated at the sites indicated above in random order.

Electrodes used were of the bipolar concentric type, consisting of 22 gauge stainless steel shells insulated with Eccobond epoxy, and an inner core of 27 gauge nichrome insulated with Formvar. The inner core extended 1.5 mm past the shell, with an uninsulated tip 1.0 mm long.

Preliminary experiments indicated optimal respiratory responses were obtained by using an approximately logarithmic stimulus train. The following system was used. Rectangular, monophasic stimulation pulses were delivered from a Grass Model S-5D stimulator with an isolated output. Pulse duration and voltage parameters were controlled directly from the S-5D. Frequency, train duration and inter-stimulus interval were derived from external circuits. Stimuli were delivered in crescendo trains of constantly increasing frequency according to an approximately logarithmic function determined by the discharge of an RC network. This network fed its voltage output to a high impedance Voltage Controlled Generator (Wavetek Model 134) whose output, as short duration pulses, was fed as a gating signal to the S-5D stimulator. The V-C-G voltage source was charged during the off period between stimulation trains, and the duration of each train as well as the total number of trains were controlled by a Grass Model S-4 stimulator

modified to provide pulse widths as long as 1 second. Frequency of the S-4 thus controlled the rate at which each subsequent train occurred. The S-4 pulse duration controlled the length of the train and, due to the V-C-G arrangement, the upper frequency of stimulation and total number of stimuli. A synchronizing signal was also derived from the stimulator and recorded on a separate tape channel to facilitate later data analysis.

Electronic data processing techniques were utilized in reducing the raw data. Analog signals recorded in the FM mode during the course of the experiment were converted to coded digital signals in real time and stored in digital form on magnetic computer tape. Raw nerve traffic was converted by means of discriminative height analysis and was also stored in digital form. The details of these procedures are given below in the chapter on data analysis.

The plane of anesthesia required for the success of the stimulation was critical. Light pentothal narcosis, maintained just below the threshold of spontaneous movement by titration, proved most dependable. Reasonably stable levels could be obtained after 3 to 4 hours, which persisted fairly well. As further precaution, however, the stimulus sites were randomized to minimize any effect of varying anesthesia level. Although no control for basal metabolic rate was included, 2 to 3 minutes control was included between each stimulation, during which the

animal almost invariably returned to the prestimulation rate and depth. Although the possibility exists that the animal was not in a basal state during the control periods, it was at least at comparable levels of activity before each stimulation regardless of site stimulated.

In the analysis of data, several relationships have been plotted using the number of stimuli as one variable. In a logarithmic stimulus train, it is equally possible that the significant variable is the interspike interval. Analysis of this factor requires another complex computer program, and this analysis has been deferred for subsequent work.

Chapter IV

DATA ANALYSIS

The physiological signals recorded on FM magnetic tape were played back in real time to a PDP-12 Computer programmed to analyze a series of variables in the recorded data. Because of the nature of the computer program it has been felt advisable to provide the reader with a narrative account of the type of computer programming employed and an over-view of the individual steps and assumptions in the program. The computer program itself consisted of an executive program to determine the type of operation involved, a series of sub-programs to perform those operations and finally a series of executive control programs to determine the output of the computer. The complete program as entered into the PDP-12 computer is provided for reference and is included in this dissertation as Appendix I. By way of explanation the first column on that print-out is the line number. This line number is listed in octal as a consequence of the print-out available from the computer and will be used as a reference for discussion of individual operations. The second number

column is the computer address being used and is a machine reference. The third column is the binary machine operation code. The balance of the programming columns represent the sub-routine name where applicable; the operation column, describing in alpha-numeric code the kind of operation provided; and finally a comment line indicating in very abbreviated form the type of operation being performed. Both "LINC" and "PDP-8" modes of programming were used.

The PDP-12 computer is capable of being operated in a number of modes. Two modes were used simultaneously in this program, that of 8-mode or strictly digital computer and that of a Linc mode computer utilizing a U.S. Government developed program which allows some degree of user interaction with the computer while computation takes place. The computer was controlled by a basic one kilohertz analysis time. However, insofar as the phrenic nerve was concerned, a maximum interval of 120 microseconds occurred between samples. Because of the nature of the phrenic nerve spike detection program used, the phrenic input channel could be sampled in as little as 30 microseconds. The special function register of the computer was enabled (line 10) allowing the computer to write on tape simultaneously with the gathering of information. This particular step was accomplished by allowing the computer to store incoming signals in two alternate memory fields, storing in one while interrogating the second and recording that data on the magnetic

tape for a later second pass analysis. Lines 27 through 50 programmed the internal crystal-controlled clock of the computer and were utilized only once in the routine. A calibration sequence was provided allowing input levels to be sampled and established as baselines. These levels were then added to or subtracted from the incoming signals when the computer was in the data gathering mode. The fast sample option was enabled at line 66 after the calibration procedure and the set-up procedure to enable the computer to gain 18 microseconds per sample, in effect, reading the last sample and beginning another sample while continuing computation. In the normal mode the computer would lose 18 microseconds in acquiring the current sample. For this reason, in reading the program the reader may find some difficulty in determining just what channel is being sampled. Wherever possible the comment for the actual channel sample has been included in the extreme right column.

Line 67 begins the executive routine for the read-in of data gathering mode of the program. At line 67 the computer interrogates the internal 1 kilohertz clock and if 10 milliseconds have elapsed, the computer initiates a routine designed to sample the slow analog signals of respiration and blood pressure. Simultaneously, the computer continues to gather neural signals, and if the memory bank assigned is filled, initiates a transfer to digital tape. The computer routine interrogates the blood pressure channel

and the respiratory channel to determine the digital equivalence of the analog values of these signals at that time. In addition, for the respiratory channel the maximum change per 10 milliseconds time period in the positive going signal is computed to derive the air flow velocity slope and if an inspiration is in progress a value is computed for a relative tidal volume. These values are recorded every 1.25 seconds. Tidal volume values are not corrected for cycle length at this time, but are converted during the alternate readout program employed. The rationale here is that time is saved in making this computation. A second readout path enables the value to be converted to a true tidal volume. If 10 milliseconds has not elapsed the computer is directed to sample the phrenic nerve input channel and determine the signal amplitude of that channel. The maximum sample rate of the PDP-12 is 18 microseconds. During this 18 microseconds, however, the computer may interrogate other portions of its memory or perform calculations not directly connected with the sampling operation. For this purpose several updating steps and some computation steps have been interwoven into the actual sampling routine.

Provided no 10 milliseconds flag has been raised, the computer is directed to go to line 320 for the routine labelled Nsamp and at this point the computer interrogates two input channels. One channel contains the marker signal or synchronizing signal from the stimulator and if a stimulus

sync is present the computer is directed to ignore the phrenic input channel and return to test the clock again to avoid the presence of a stimulus artifact. In effect this gates the phrenic channel during the presence of an electrical stimulus. Note that the computer is actually directed to ignore any neural input during the time that stimulus current is being delivered. Although other methods are available to eliminate the stimulus artifact, they are basically amplitude dependent. It was felt that by actually correcting the neural channel values to a lower value (by omitting the possibility of stimulus artifact) a more rigorous test would have to be met to show differences in firing during a stimulation series. The computer is also directed to determine if the signal indicating the onset of stimulus represents the beginning of a stimulus pulse. This is important since several samples may be taken during the pulse duration.

If the computer determines, by comparison with the last sample taken of the stimulus mark channel, that the signal represents the onset of a stimulus, the time value is stored in a temporary memory location labelled WORD2 indicating the presence of a stimulus. For every stimulus recorded during a 10 milliseconds epoch the computer will store one count in this word. The current value determined for the stimulus artifact channel is then put in a buffer for comparison with the next value as a method of determining whether or not the next value will be the first of a

series of values corresponding to one stimulus artifact. Providing no stimulus channel information is present, the computer is then directed to sample the phrenic channel, and in sampling the phrenic channel the computer will determine whether or not a gate has been set for the presence of stimulus artifact. If that gate has been set it indicates that the computer has not determined the current stimulus artifact channel to be the first of a series of pulses in the stimulus artifact but that a stimulus is present. The computer then exits to the executive routine to check the clock and to see if it should return to sampling the stimulus channel and the nerve channel, or to return to sampling slower analog channels.

Providing neither gate is set the computer then scales down the phrenic nerve input signal by a factor of 4 (line 362) and checks its polarity and level. Scaling the input rounds off the low order bits, thus minimizing the effect of small amplitude changes in the input signal. This reduces the possibility of detecting a false peak in the later peak determination program. Such a scaling also aids in the establishment of a stable baseline, since values oscillating closely about zero volts input will be rounded off toward lower values, and if negative, results in their being dropped as detailed below. Operating baselines are thus easier to establish, and drift problems in the input are minimized.

If the signal is negative the value is discarded and the computer again returns to the executive routine. The rationale here is to clip the negative halves of all peaks, since phrenic nerve traffic has been recorded as an AC signal. If, however, the signal is positive, a series of routines is instituted to determine the level of the signal and also whether it is coincident with the last sample level in a routine designed to determine the peak of a current sample. If the signal is falling, the second to last sample is tested to determine if it were also less than the next to last sample. If the middle sample of three serial samples is higher than either of the other two, that middle sample defines a peak and its amplitude is stored as the peak level of the spike. If neither of these criteria have been met and if the signal is greater than either the next-to-last or the second-to-last signal it is determined that the signal is rising and each of the previous storage values are updated so that the second-to-last signal is discarded and is replaced by the next-to-last signal, which in turn is replaced by the current signal. The computer then returns to the executive routine.

If a peak is detected, a gate is set so that the computer is not allowed to record another peak until a negative peak or a trough is determined by a similar routine. When this trough has been determined the gate is cleared, allowing the computer to again record a positive peak should it occur.

This prevents ragged edges or noise in the phrenic nerve signal from triggering the computer, because signals must now be three samples apart in order to describe a peak. The frequency response for triangular waves with a sharp point then is limited to about 800 cycles (by test) in this kind of a routine and on the low end is limited to about 100 cycles because a broad peak or a flat top will not be discriminated as spikes by the computer. Both criteria therefore help to eliminate noise. Input amplifiers with selected filters were used to condition this signal so that the rise time would be rapid enough for triggering the computer. When a spike is detected the computer then determines the amplitude of that spike and stores it in one of seven bins in a routine labelled LEVEL (line 403). This routine breaks the signal up into approximately 10 millivolt units of actual signal. It should be noted that the actual signal can be adjusted by means of an input amplifier so that absolute calibrations in terms of microvolts or raw signal amplitude are not reported here.

Because of the AC nature of the complex signal such a calibration was not considered to be of particular advantage in the current work. Because the physiological signal was recorded at approximately a 1 volt peak level (which has been scaled down to a fourth of that value) the computer signal for a normal physiological signal will be about 250 millivolts. The first 50 millivolts of this signal are discarded since this represents an approximate background noise

level. If the signal is less than 7 counts above noise level (10 millivolts) the computer again discards the signal. The computer then increments by 14 digital counts (15 millivolts) and determines the BIN in which the signal will fall. Signals greater than 250 millivolts are discarded as being noise or movement artifacts at the high end of the signal amplitude spectrum. If a peak is determined, one digital count is stored in that bin in a coded sequence at temporary storage sites WORD1 and WORD2. Because of time restrictions of the on-line signal processing routine and because of memory restrictions in terms of the number of pieces of data to be handled, a maximum of seven spike counts per amplitude level is allowed per 10 millisecond interval.

A maximum of 39 counts may be stored during any 10 milliseconds under ideal conditions. At the end of the 10 millisecond epoch when blood pressure and respiration are sampled, the values of WORD1 and WORD2 are stored in a buffer zone for transfer to computer digital tape. At the end of 1.25 seconds, when the buffer memory is full, the computer is directed to store the contents of that buffer memory, shift to the alternate buffer site, and resume calculations while the computer executes a storage function. When the computer determines that a clock signal has occurred in the executive routine, i.e. 10 milliseconds, it is directed first of all to store WORD1 and WORD2 buffers, and reset WORD1 and WORD2 to zero. During the analog sampling

routine the computer is periodically directed to go back and check the phrenic nerve routine.

In spite of the apparent length of the heart rate and respiratory channel calculations, a maximum of 12 microseconds elapse without a checking of the phrenic sample. First the computer compares the blood pressure input signal to the previous 10 millisecond sample and determines if a minimum has occurred. This minimum is calculated for 1.25 seconds and has been part of the problem in this routine. However, the primary value of this routine has been in the analysis of phrenic nerve activity and in spite of the problems in calculating blood pressure on a 1.25 second epic, useful data can be obtained. The computer then determines if the blood pressure is a new maximum for the 1.25 second interval. If either of these criteria are met the temporary buffer is filled with a value for a new minimum and a new maximum which are transferred to the tape at the end of 1.25 seconds. When a new minimum occurs the computer is directed to check a time tally to determine the time from the last minimum. This value is then used to determine the heart rate. A series of gates is included in the routine to prevent the computer from triggering on adjacent minima should the computer began its 1.25 second sampling period on the falling limb of a blood pressure tracing. At the conclusion of the blood pressure and heart rate routine, the computer is directed to sample the respiratory

channel. If the respiratory input is negative the computer is directed to return to the executive routine and the negative half of the airflow curve is discarded. If the signal is positive, in other words inspiratory, the computer is directed to store a value of 4 in bits 0 to 2 to WORD2 as an indication of a positive respiratory signal. Then the computer will determine the time of onset of inspiration, determine the maximum change from the last respiratory value, and determine the amplitude of the respiratory channel at that time. This value is added to memory for computation of relative tidal volume, which represents inspiratory volume per 1.25 seconds. Later programs then convert this value to tidal volume by computing cycle lengths and adjusting relative tidal volume accordingly. When the tape has been filled, the computer is directed to halt the sampling operation and to return to a display mode which can only be activated by the operation of the sense switches. The computer will remain in the halt mode until restarted or until playback is initiated. During playback, several options were available to the operator which could be activated by the operation of computer console switches in combination with specific characters from the teletype console. Displayed on the computer screen are 1.25 seconds of data as retreived from the computer tape. These data can be displayed as time histograms with one point representing 10 milliseconds and each signal amplitude displayed separately. Seven lines of data in addition to a line

indicating the presence of a stimulus pulse and the status of the respiration (inspiration or expiration) are thus displayed on the left half of the screen. On the right half, digital data are presented for tidal volume, heart rate, blood pressure, and current tape address.

Alternatively, the operator can combine the seven levels of data into a composite histogram which is displayed across the entire screen along with the stimulus mark and respiratory information. The computer can access either the multichannel or the composite histogram non-destructively.

In addition several destructive modes of operation are available. These enable the operator to acquire the original data from tape, manipulate it, or restore it. However, if it is desired to move backward in the manipulation scheme, the operator must reacquire the original data from computer tape.

Because the 1.25 second sampling periods are random with respect to the respiratory cycle, the onset of respiration may not correspond with the beginning of a storage frame. Therefore the operator can update the material being stored to display the histogram for any 1.25 second period by setting zero time to any of the following: a) onset of inspiration; b) 50 milliseconds before the onset of inspiration, or any other delay the operator may choose; c) the onset of the first stimulus in a train of stimuli; d) the onset of any integral number of stimuli after the onset of a stimulus train; e) any specific time point defined by a

cursor displayed on the screen of the computer, and controlled by the operator. In each of these modes of operation, the computer shifts all data points the required number of spaces in the display, then completes the 1.25 second sample period by accessing the next succeeding block of digital tape and using the appropriate data points to update the balance of the display.

In any of the modes listed above, as well as the "as sampled" mode, the computer may be directed to add the display as shown on the screen (perhaps after the above manipulation) to an averaging memory. This feature can be used to compile an average histogram whose initial time is any of those listed above. Digital printouts of the values listed at each histogram point are available in all modes of operation listed above including the averaged histogram modes.

All of the above mentioned routines are available within the body of the program and may be called immediately. A satellite program may also be loaded to perform other calculations on the data. This program has many of the display features of the parent program, but does not allow the gathering of data directly from the analog input, nor the calculation of averages. This program enables the operator to assign an identifying code to each 1.25 second tape block on the ten minute tape after previewing the data in any of the display modes of the parent program.

After the precoding is completed, the computer is initiated in an automatic mode directing it to compute a series of average values and print them with corresponding identifying symbols. Fifteen categories are available enabling the data to be averaged into one of fifteen groups. Data may be added at random to these values, so that a given stimulus condition may be sampled initially, the computer directed to store succeeding values in other categories, and then return to the initial category some time later for an identical stimulus. When the operator runs out of categories, a special symbol is inserted, and the computer is directed to print out the results of its calculations and restore memory to zero in preparation for another computation run on an additional fifteen groups.

Values computed in this mode of operation are as follows: number of cycles sampled, tidal volume (corrected in the routine for sample length), number of firings occurring at each amplitude level in phrenic recording (separated into inspiratory and expiratory components), air flow slope, inspiratory and expiratory cycle times, total cycle time, number of stimuli delivered in each phase of the cycle, total nerve firings. From these values, respiratory rate, minute volume and expiratory to inspiratory stimuli ratio were calculated.

The criteria for data processing depended upon the choice of a complex phrenic recording rather than a single

unit recording. As reviewed in the literature, the phrenic consists of approximately 150 fibres with a peak firing frequency of about 30 Hz. Utilizing this figure, and considering that the portion of the phrenic used for recording represented roughly 1/10 of the total nerve trunk, one would not ordinarily expect more than 7 impulses of a given amplitude in a 10 millisecond block of time.

To separate out the phrenic complex action potential into its component parts, the following assumptions were made: First, the action potential occurring as the result of activation of a single fibre would present a constant, or nearly constant amplitude. This assumption is accepted as essential criteria in the recording of extracellular single cell traffic, and would therefore appear valid here. Second, it was assumed that occlusion would occur in which synchronous firing of two cells would result in the production of a spike appearing as a third cell of greater amplitude. High speed samplings minimize this factor, although it could not be eliminated entirely. It was also assumed that the occlusion effect would remain nearly constant for a given set of recording conditions. Occlusion of peaks would have to be nearly synchronous, as each peak need only be separated by 100 microseconds to be discriminated by the window technique used.

Samples, and subsequent firing level assignments, were made at a maximum of 200 microseconds apart. This long sample

loop was necessitated every 1.25 seconds as the routine to store data on tape from computer memory was initiated. The majority of the samples were taken every 100 microseconds or less, with some test loops occurring every 18 microseconds. In any case, resolution was limited to 4000 peaks per second, or expressed somewhat differently at 5 peaks per millisecond. Any specific population firing at a rate faster than this would be truncated. Each occurrence of a peak was assigned a value of 1 for that level, and added to a register representing total firings for that level. Every 10 milliseconds, the value of that register was transferred to a temporary buffer memory representing the point in time of the preceding 10 millisecond sample, and the register reset to zero. The bins formed were therefore indicative of the number of pulses occurring at each amplitude level for the 10 millisecond epoch. Time was coded by serial address location. Because of memory and storage cycle restrictions, all firing level values were coded into three binary bits and packed four per computer word. Thus the restriction of seven or fewer firings per level per 10 millisecond epoch is introduced. It should be noted that a possible, systematic error can exist here as more than seven firings at a given level would result in overflow and a corresponding insertion of one count in the next higher firing level for each seven firings at the lower level. No check was included for this contingency, as the processor time increase was felt to

be more detrimental than the possibility of occasional overflow. Within these limits, it is felt that the program employed accurately represents the physiological findings of this study.

Chapter V

RESULTS

A. General Observations

Based upon the results described in this dissertation, there would seem to be little doubt that the hypothalamus has the potential for exerting control over the respiratory system above and beyond that of simply modulating rate and depth of respiration.

Although in the course of preparation of this dissertation over 90 animals were studied, the results here represent the last 13 animals in the series. Determinations were made on a total of 240 stimulations representing a total of 8674 respiratory cycles. Over 600 different relationships were calculated and plotted for comparison. In addition, over 900 computer printouts of digitized data, and accompanying computer generated graphics were obtained, the latter on 35 mm film using the electronic shutter provision of the program. Examples of these are presented in Figures 42-47. *(Relationships characterized by low correlation coefficients have been excluded from all Figures.)* The results to

follow then, represent a sampling of the overall experiments, but are thought to accurately portray the physiology of the respiratory interaction of hypothalamic stimulation and respiratory patterns.

In the graphs to follow, the lines represent the calculated least squares regressions. The ends of the line signify maximum and minimum abscissa values obtained in all thirteen cats reported. Numberical values for averages are shown in Table 1, along with the standard error of the mean. Points on each line are weighted averages representing collectively 240 stimulation sessions in thirteen animals, for a total of 9366 respiratory cycles. An equivalent number of controls are also included.

All points were stimulated in all animals. For each general relationship presented, e.g. tidal volume versus inspiratory time, the correlation coefficient was calculated for each experimental site both before and after vagotomy. All relationships in which the correlation coefficient of the regression line was not found significant at 0.1 level or higher have not been plotted. Thus, in a given graph showing for example only one regression line, the lines have been calculated for stimulation in the other sites in the hypothalamus, but are not plotted, since they were not significant.

Because of varying control levels, a ratiometric approach has been used in describing the data. Broken lines

indicate a ratio of 1.0, which signifies no change in the measured parameter from control. For convenience, the ratio of control to stimulated parameter is designated the S/C ratio. An S/C ratio of less than 1.0 signifies inhibition, while an S/C ratio greater than 1.0 indicates facilitation of the response, with the magnitude of the S/C ratio providing a measure of the extent of increase or decrease from control. Representative analog data is shown in Figures 48-51.

Stimulations are separated into two phases. The phrase "stimulations in expiration" indicates the number of stimuli delivered per respiratory cycle between the time air flow assumed a negative value (expiration) and the next positive crossing, which marked the onset of inspiration. A similar reasoning defines "inspiration." Because no attempt was made to synchronize the beginning of the stimulus train with any phase of respiration, the animal was free to react to each component of the stimulus train. Because of this freedom, each experiment generated many pieces of information regarding the response of the respiratory system to stimulation at designated sites in the hypothalamus. Vagotomy was performed in all animals, and the stimulus sequence repeated. Vagotomy reduced the control respiratory rate in all animals, however the control employed for vagotomized stimulations is that after vagotomy, and does not reflect this change.

The specific effects of hypothalamic stimulation on the highest amplitude sample of phrenic discharge (level 7)

are shown in Figures 2-5. In Figure 2, it is evident that stimulation at two different hypothalamic sites during the inspiratory phase of respiration evoked markedly different effects on the degree of phrenic discharge during the inspiratory phase. The broken line indicates a ratio of one between the phrenic discharge immediately prior to stimulation and the discharge during stimulation. Stimulation at the L4 site had only a slight effect on this ratio. Stimulation at the M5 site, however, evoked an average of 8.5 times increase in the discharge of the phrenic using the same range of stimuli as those delivered at the L4 site. To attain the control value, signified by a ratio of 1.0, site M5 would require 7 stimuli, while site L4 would require 13. Both sites exhibited a positive slope between stimulations and discharge. One might presume that the bulk of the increase in discharge was due to the stimuli occurring before the onset of inspiration. Figure 3 describes this relationship. For the electrode site M5, there was a significant difference between the regression slope of the curve describing inspiratory and expiratory stimuli on the magnitude of discharge during inspiration. The L4 site showed an increased gain in response to stimulation in expiration. Fewer stimulations in expiration were required to produce the same degree of enhanced phrenic firing. Note also in Figure 3 that vagotomy effectively reduced the phrenic inspiratory firing response to M5 stimulation site to an average of 1.08 times the control,

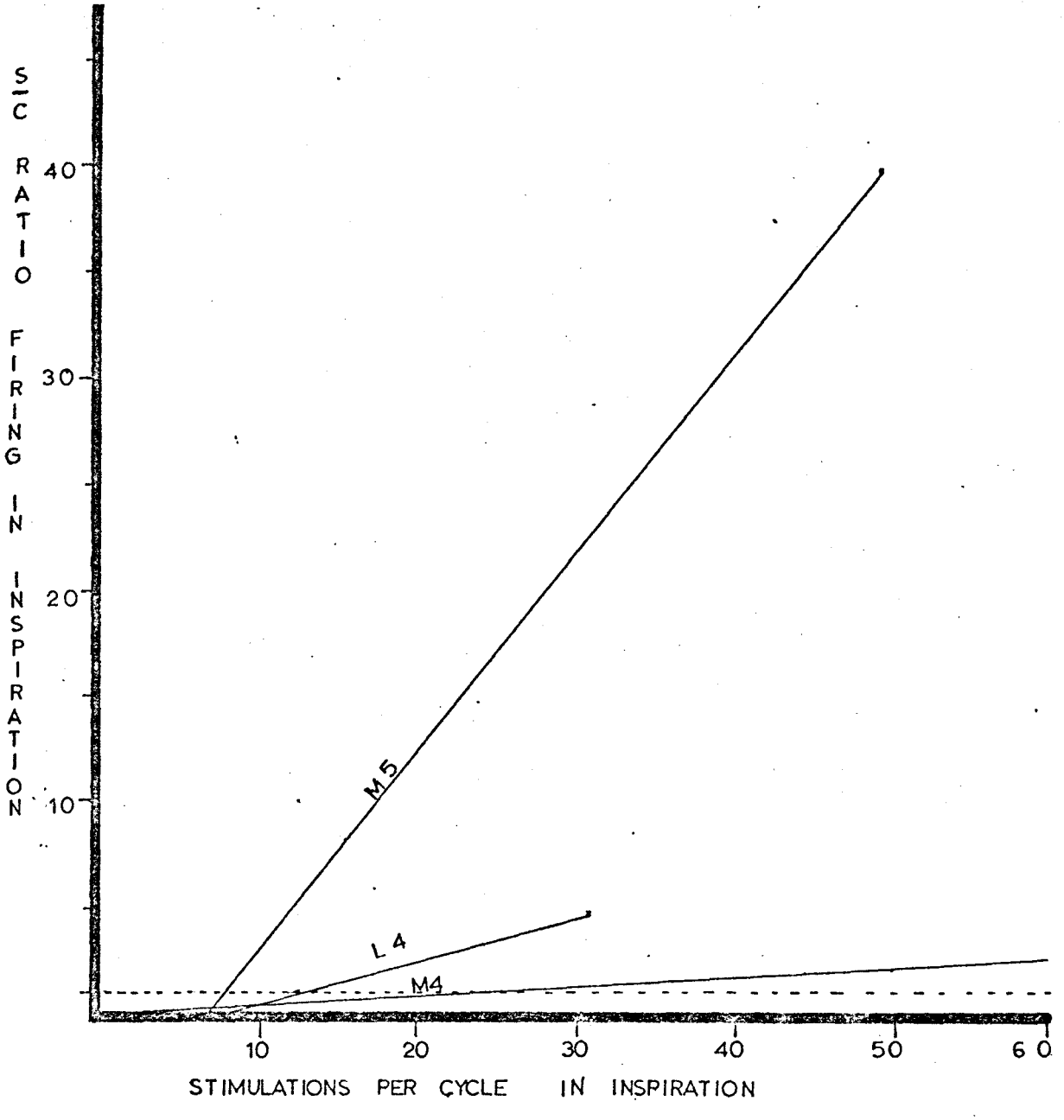


Figure 2.

Solid lines show the relationship between the number of stimulations in inspiration for each respiratory cycle and the resulting change in phrenic nerve discharge during the inspiratory phase. These changes are plotted as the ratio of stimulated cycle discharge to the control proceeding the stimulation. The broken line depicts the ratio of 1.0 point, indicating no change from control. In all subsequent Figures, ratios will be designated as S/C.

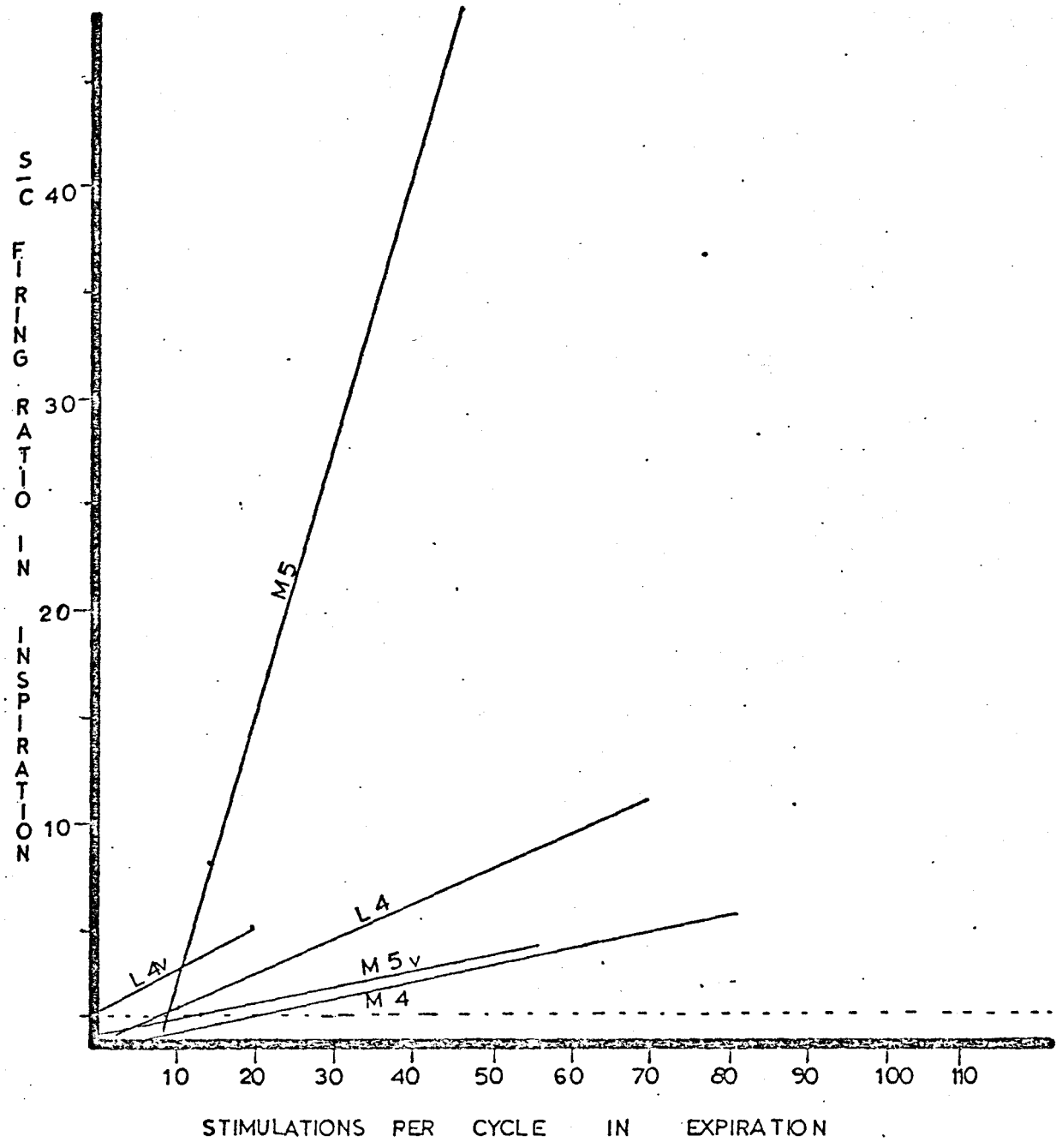


Figure 3.

Relationship between the total number of stimuli delivered per cycle in expiration and the S/C discharge in expiration. Only the significant regression slopes are plotted here, and only the level 7 firing is shown. At a ratio of 1.0 (broken line), no change would be noted from control. In this and subsequent Figures, a "v" following the designated stimulation site represents the response after bilateral vagotomy.

i.e. only a slightly enhanced discharge. The M4 site was not significant before vagotomy, but after vagotomy a significant regression slope was obtained. For the L4 site, a significant regression was found before vagotomy but the functional relationship which existed before vagotomy was eliminated as a result of vagisection. The L4 site appeared more effective than the M4 site in increasing the phrenic discharge after vagotomy. Stimulations at L5 produced no significant change in phrenic discharge when stimulated either in inspiration or expiration. Comparing points where inspiratory discharge during stimulation exceeds control (ratio of 1.0), both L4 and M5 crossed at only 8 stimuli, and M4 crossed at 26 stimuli for pre-vagotomy values. After vagotomy, M5 required 11 stimuli.

To determine whether stimuli delivered in inspiration may have a significant effect on discharge in expiration, these parameters were compared. Figure 4 demonstrates the effect of inspiratory phase stimuli on expiratory phrenic discharges. The term expiratory here denotes a portion of the respiratory cycle in which air was not being inspired, and necessarily includes phrenic firing preceeding the onset of air movement inward. A pronounced effect was observed when the M5 site was stimulated. An average of seven times increase in phrenic discharge over control was exhibited for firing level 7. No other sites tested produced any significant change in phrenic discharge of level 7.

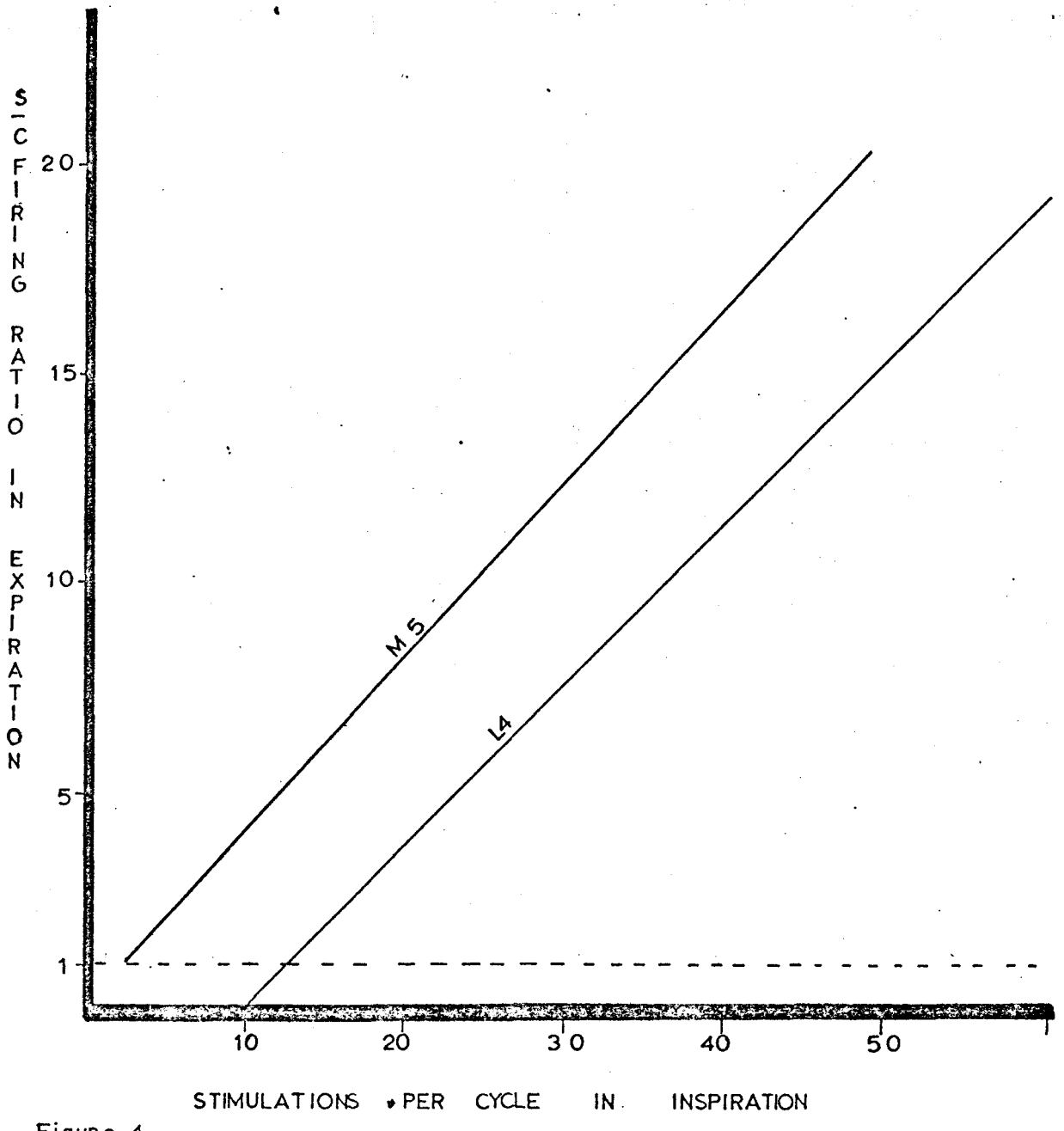


Figure 4.

Relationship between the number of stimuli delivered during the inspiratory phase of each respiratory cycle and the change in phrenic discharge during expiration as a result of the stimulation. This change is expressed as a ratio comparing the stimulated cycles to the control cycles immediately preceding the stimulation. Only site M5 is portrayed, as this relationship failed to yield a significant correlation for the other sites tested.

Discharge patterns for Figures 5 and 6 describe essentially the same patterns except for site M4. Figure 5 demonstrates the effect of expiratory stimulations on the discharge ratio in expiration, while Figure 6 describes the same ratio changes as a function of total stimuli delivered per respiratory cycle. Compared in terms of total stimulations per cycle, the M4 site after vagotomy had a large significant effect on phrenic discharge. An average increase of 17 times in the phrenic discharge was measured, while with comparable numbers of stimuli, the M4 site prior to vagotomy produced only a three-fold increase. Before vagotomy, the M4 and M5 sites were seen to have comparable regression slopes both in terms of total stimuli and of stimuli in expiration. In all cases, 5 to 8 stimuli were required before any effect was seen in the discharge frequency. Vagotomy decreased the effect of expiratory stimulations on expiratory firing at site M5, while it increased the effect at site M4, located only 1.0 mm dorsal. The significance of the L4 slope was lost altogether after vagotomy. Comparing Figures 4 and 5, it would seem that the predominant effect of stimulation at site M4, at least in terms of potentiating the phrenic discharge, was evoked during the period preceeding inspiration. This was manifested as a large increase in phrenic discharge before onset of active inspiration, with little effect on the phrenic discharge once active inspiration was initiated (cf. Figure 3).

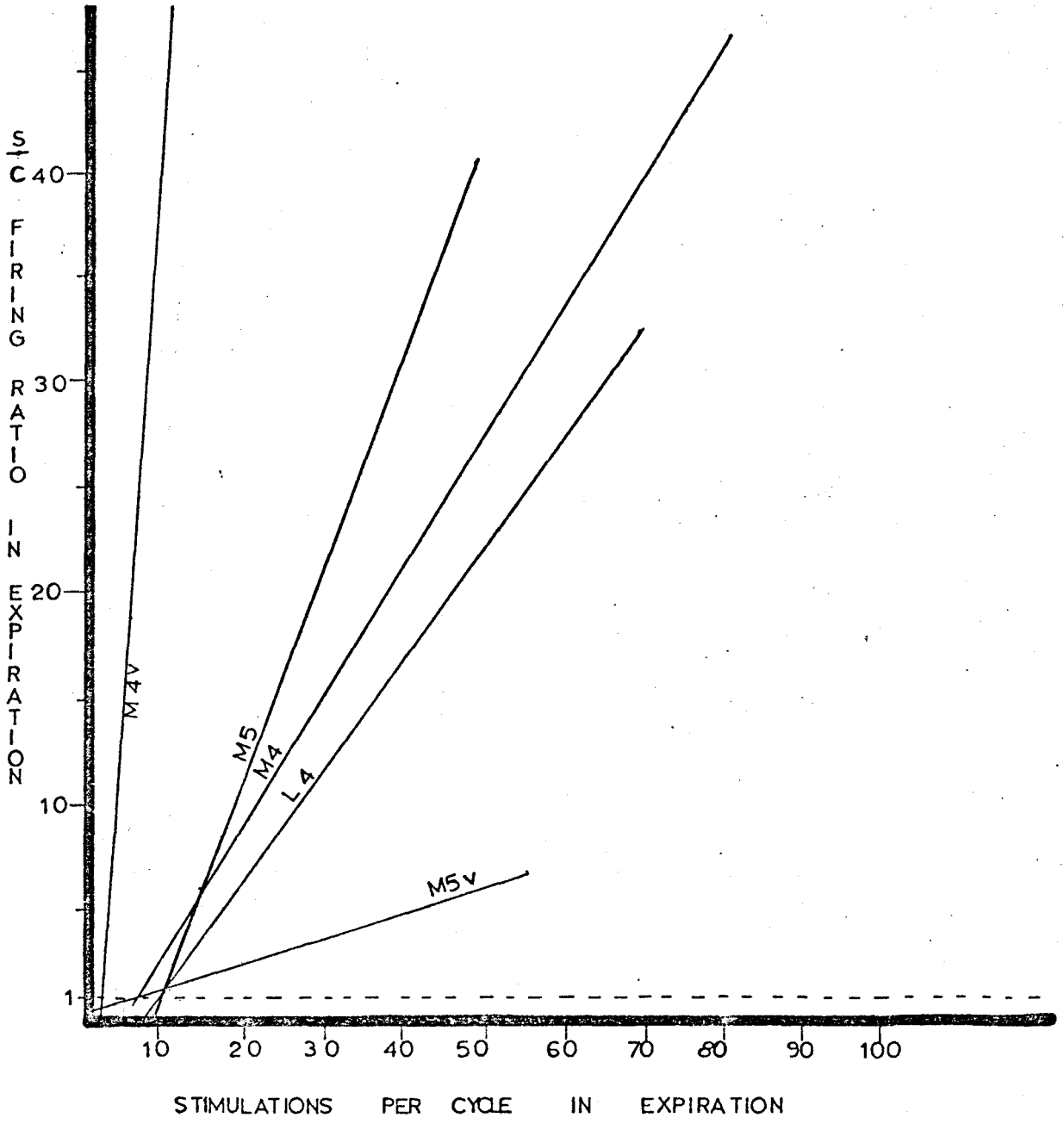


Figure 5.

The relationship between the number of stimuli delivered per respiratory cycle during the expiratory phase as compared to the S/C change in phrenic nerve discharge during expiration.

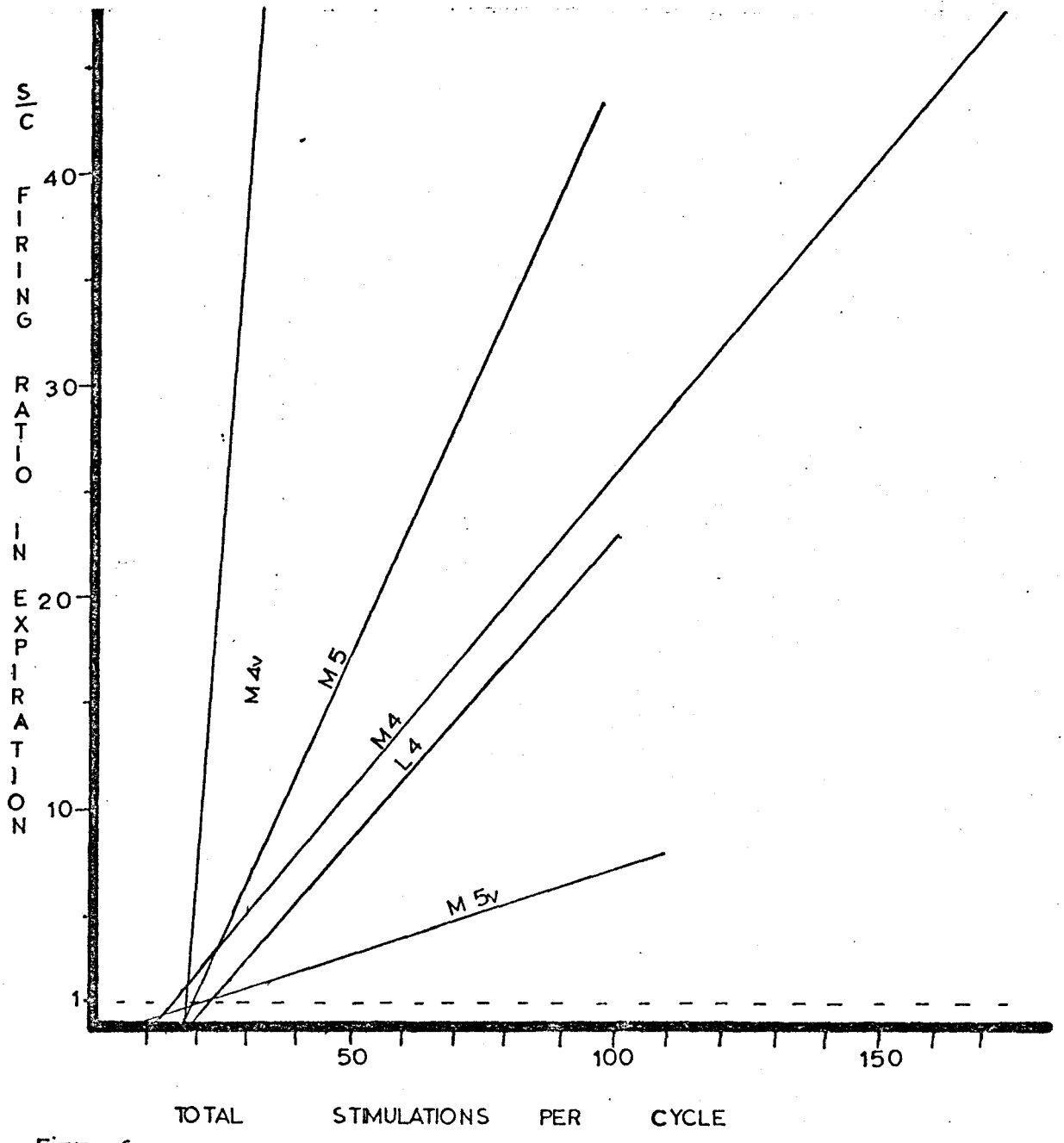


Figure 6.

The relationship between the total number of stimuli delivered per respiratory cycle and the S/C phrenic discharge of amplitude level 7.

B. Effect on Respiratory Time

Figure 7 describes the shifts seen in the relative times of inspiration to expiration as a function of the ratio of stimuli delivered in each phase of the respiratory cycle. Results of this analysis indicate that longer expiratory times were produced as the number of stimuli delivered in expiration approached that of inspiration. However, respiratory rate was almost invariably increased (cf. Table I). The result of stimulation can be interpreted as primarily a shortening of inspiratory time, with a consequent increase in the expiratory:inspiratory time ratio. After vagotomy, this effect was not seen to be significant at the M4 site. However, both the L5 and M5 sites demonstrated significant slopes after vagotomy. Since the average expiratory:inspiratory ratio was always less than 1.0, and since the system was free to seek its own ratio of expiratory to inspiratory stimuli per cycle, fewer total stimuli were more effective in producing larger changes.

Tidal volume might reasonably be expected to vary as a function of respiratory time. Figure 8 demonstrates the relationship observed between the ratio of respiratory time (expressed as the ratio of stimulated to control time) and a similar ratio of tidal volume. From this graph, it appears that two distinct populations are represented. Stimulation at either the M5 or L5 coordinate produced tidal volumes of 1.4 and 1.6 times, respectively, above that of control. The

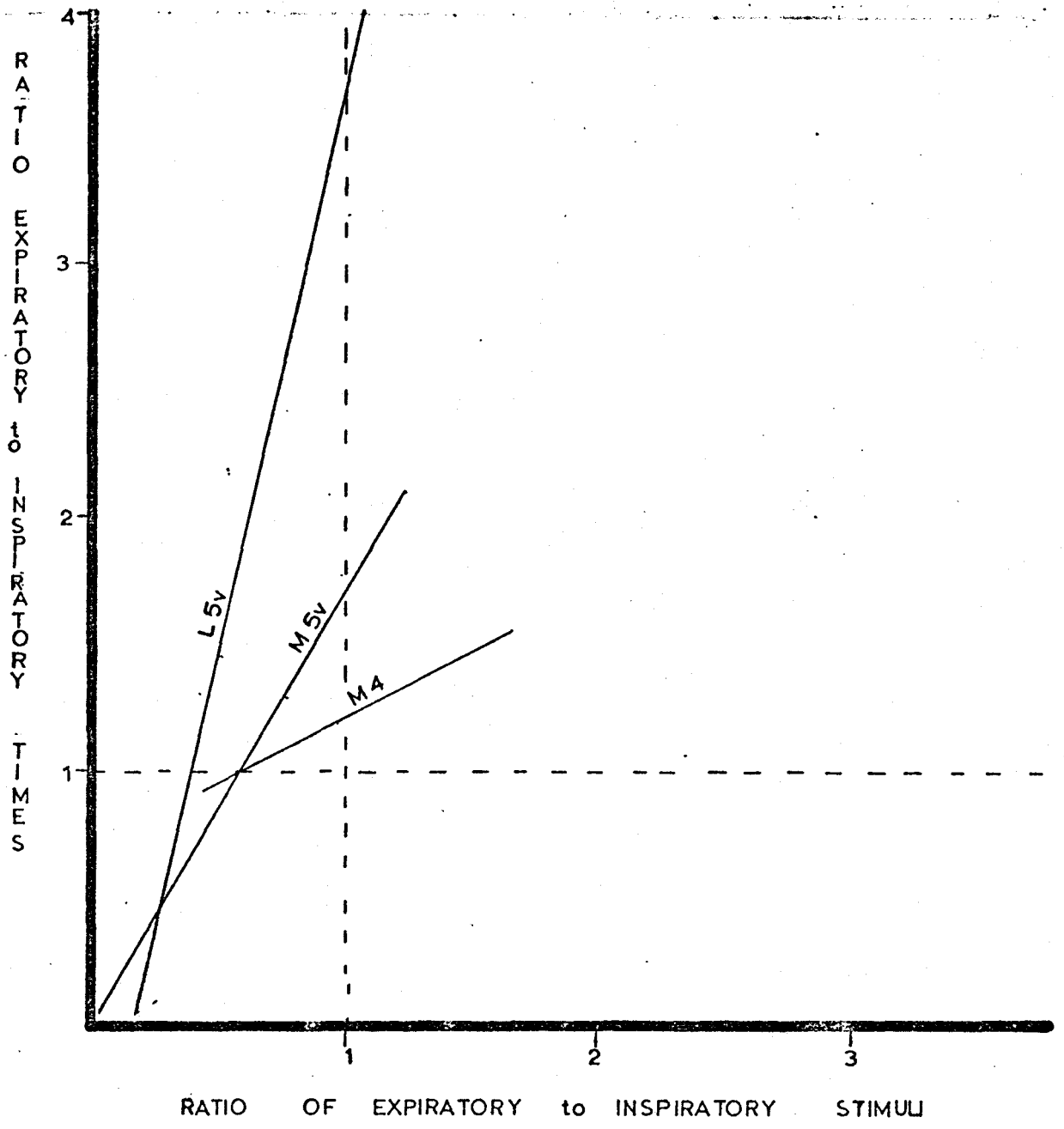


Figure 7.

The relationship between the ratio of stimuli in expiration to those in inspiration is plotted against the S/C ratio of expiratory to inspiratory cycle times.

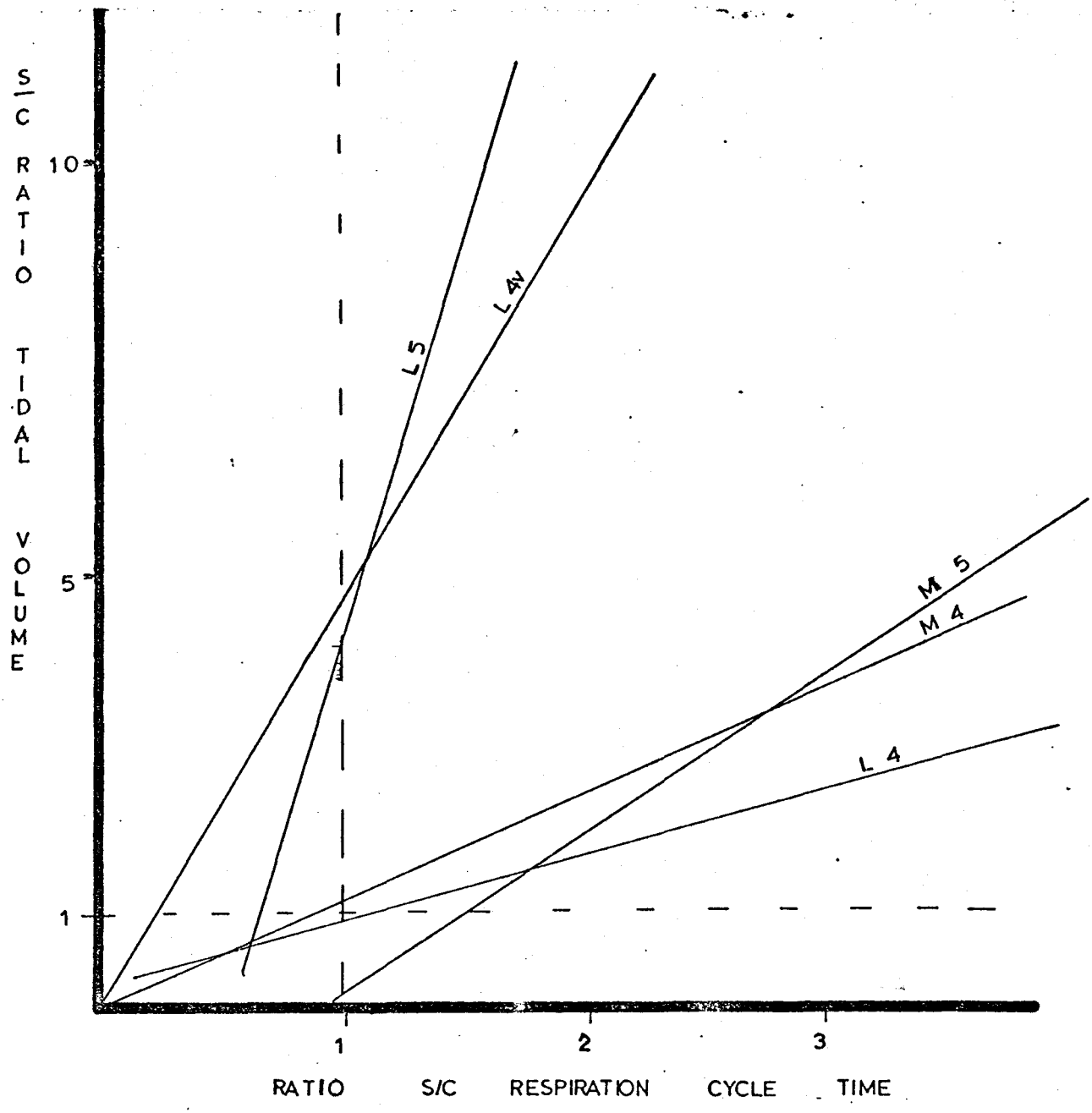


Figure 8.

The S/C ratio of tidal volume plotted as a function of the respiratory cycle time.

M5 site reduced the average respiratory frequency to 0.83. The L4 and M4 populations, however, showed reduction in tidal volume by about 50% with doubling of the average respiratory rate. Comparing ratio of 1.0 intercepts, the tidal volume would be equal to control at the following rate ratios: L4, 1.0; M4, 0.9; L5, 0.7; M5, 1.5. Interpreted another way, the rate would be unchanged at the following tidal volume ratios: L4, 1.0; M4, 1.2; L5, 4.0; M5, 0.2. The slope of the L4 line after vagotomy would yield a tidal volume of 5.0 at control rate. Control tidal volume would be attained at 0.25 rate.

Figure 9 portrays the relationship between the ratio of stimulated and control expiratory times and the ratio of tidal volumes. Site M5, the only one found significant for this relationship, evoked an increase in tidal volume as a result of stimulation for virtually zero expiratory time. As tidal volume is increased, the time necessary for expiration relative to inspiration increased, indicating that tidal volume may be compromised as a function of expiratory time under the conditions of the experiment. However, since the control tidal volume was attained at a very low expiratory time ratio, a resetting of the mechanism controlling tidal volume must have occurred as a result of the stimulation. Tidal volume, extrapolated to an expiratory time ratio of 1.0 would be approximately 2.2 times control.

Figure 10 describes the same tidal volume relationship

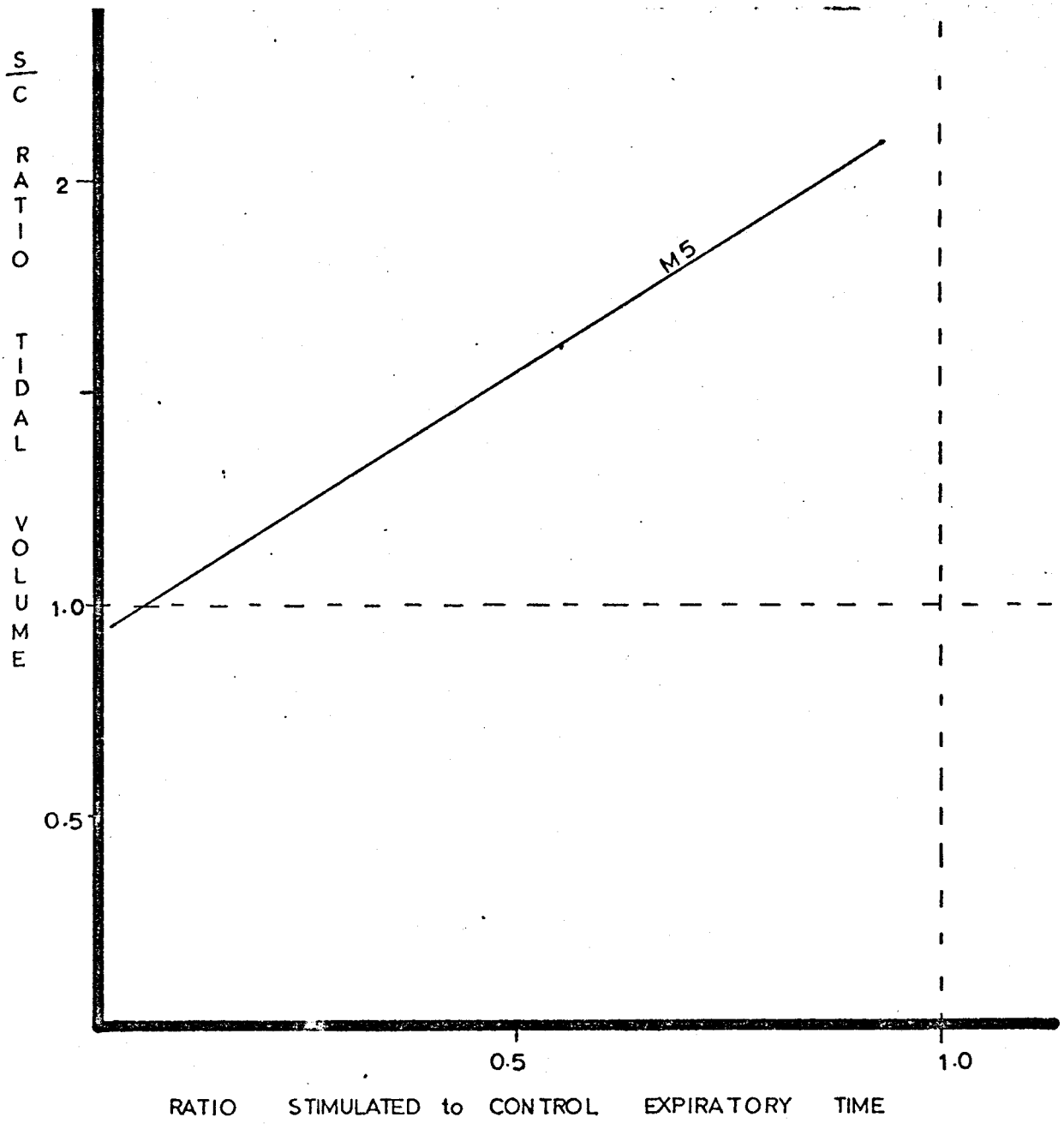


Figure 9.

Effect of stimulation at M5 on the S/C ratio of tidal volume as a function of S/C ratio of expiratory time. The 1.0 ratios are indicated by the broken lines.

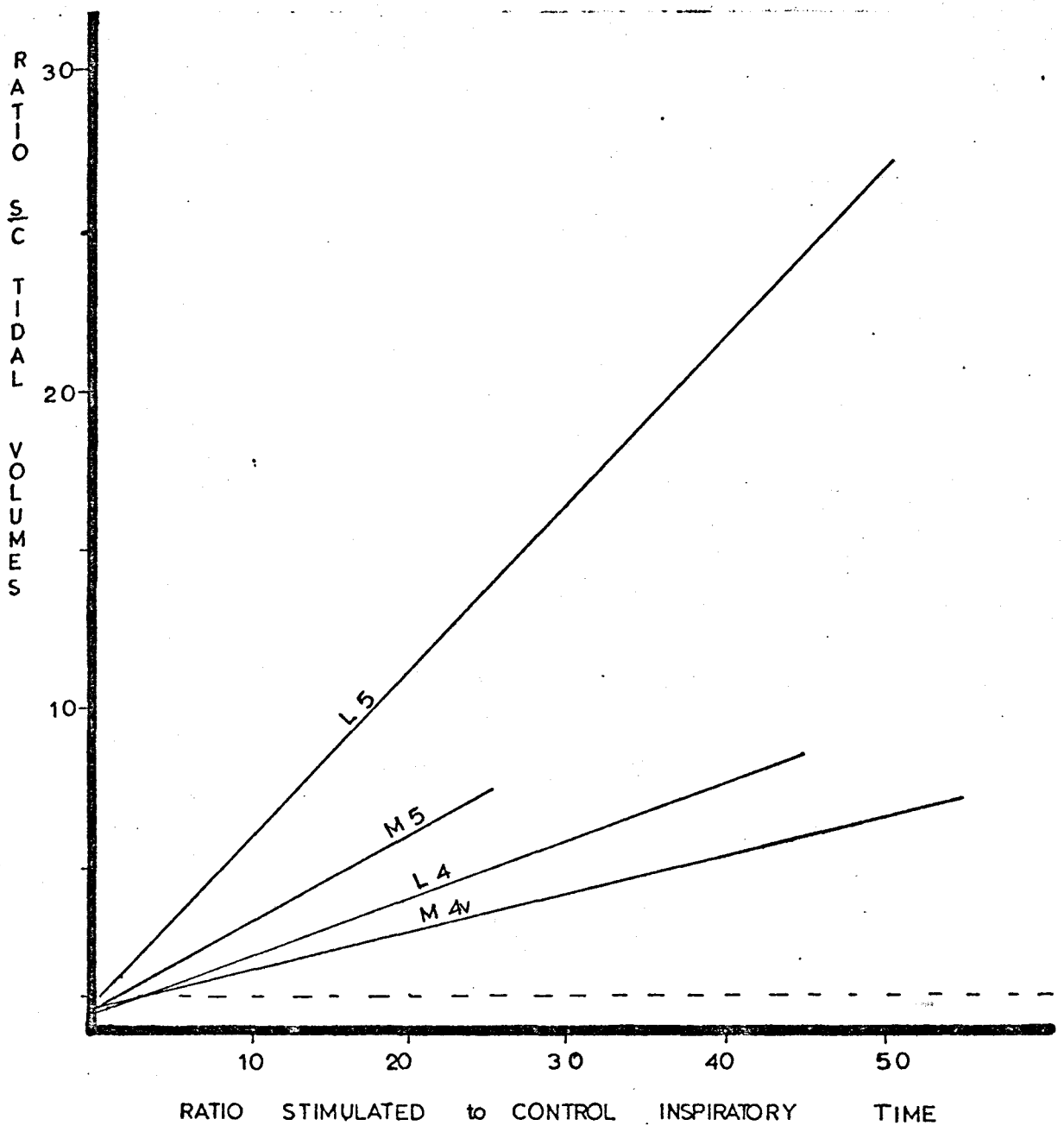


Figure 10.

Relationship between S/C ratio of control inspiratory time and S/C ratio of tidal volume.

as a function of inspiratory times. Here again, two separate populations seem to be represented. The L5 site (average value) lengthened inspiratory time above control by about 5%, but increased tidal volume by about 40% above control. The M5 site increased inspiratory time about 300%, with only a 45% increase in tidal volume. Inspection of Table 1, which shows only the average values obtained for each parameter and does not reflect the regression slope determinations, shows that the H-4 sites shortened inspiratory time before vagotomy, while the H-5 sites lengthened this time. After vagotomy, a shortening of 1/3 to 1/2 the control was seen at each site. At the point where the control stimulated time equals stimulated inspiratory time, the L5 and M5 populations have about equal effects on tidal volume, showing an increase of 1.3 times control. The slopes relating this relationship are different however. Site L4 has a ratio of about 0.6 for tidal volume. To achieve a tidal volume equal to control, the inspiratory time for site L4 stimulations would have had to increase to 3.5 times control. An approximately doubled inspiratory time would be required for site M4 after vagotomy.

Tidal volume may also be expressed in terms of its relationship to the hypothalamic stimuli. Figure 11 expresses the ratio of expiratory to inspiratory stimuli and the tidal volume ratio. Again it appears that two populations

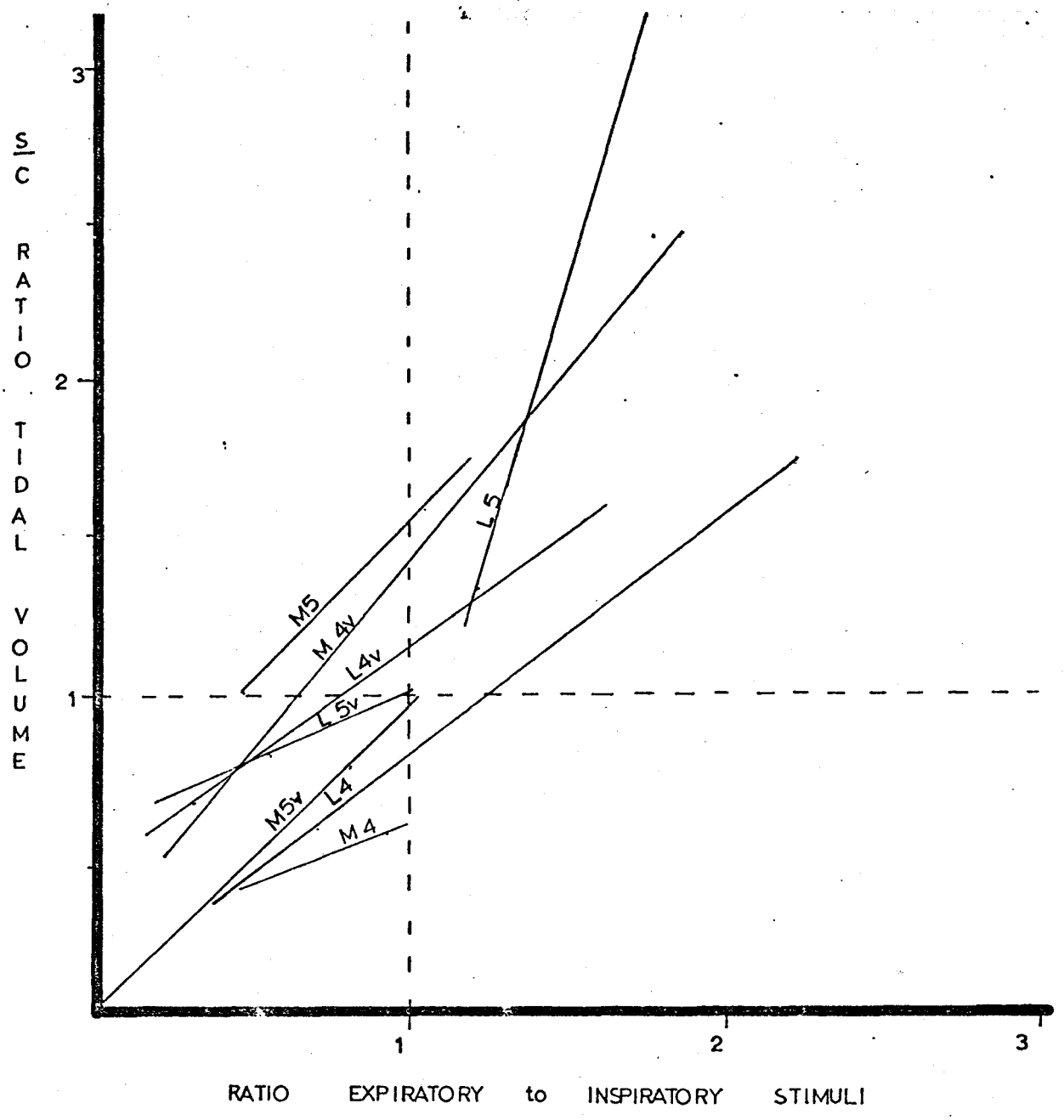


Figure 11.

The Figure relates the ratio of stimuli delivered in expiration to those during inspiration to the ratio of tidal volume during stimulation compared to tidal volume during the preceding control period. Relationship between the S/C ratio of stimuli delivered in expiratory and inspiratory periods and the S/C ratio of tidal volume.

can be discerned. The increase in tidal volume evoked as a result of hypothalamic stimulation seems to have a higher gain regarding the ratio of stimuli applied during expiration at the H-5 sites. The H-4 sites exhibited both decreased gain and lower than control tidal volume. Note that the L5 site appears to behave like the H-4 sites after vagotomy. Because no direct control was exerted over the number of stimuli occurring in each phase of the cycle, this ratio might reflect either the number of stimuli necessary to "prime" the system, or may have been an indirect consequence of the length of the stimulus trains. It would appear, however, that the system seeks some ratio of stimuli depending upon the site being stimulated. The apparent order of sensitivity for increased tidal volume is M4, L4, M5, L5. Following vagotomy, the M5 site remained the dominant one for tidal volume, all others clustering about a decreased tidal volume point. Before vagotomy, the tidal volume would be equal to control for the following values of stimuli ratio: Site L4, 1.2; M4, 2.2; L5, 0.9; and M5, 0.5. After vagotomy, both L4 and L5 intersect at 1.0, M4 at 0.7, and M5 at 0.45. It should be noted that as the number of expiratory stimuli exceeds those in inspiration, i.e. the ratio exceeds 1.0, the tidal volume ratio exceeds 1.0 for the H-5 sites both before and after vagotomy, and for the L-4 sites after vagotomy. The L4 site before vagotomy exceeded the tidal volume equality line only slightly

to the left of the expiratory:inspiratory equality line. Only M4 prior to vagotomy required a large number of stimuli delivered in expiration relative to inspiration to produce tidal volumes above control.

Stimuli delivered in inspiration were ineffective in causing increased tidal volume. Stimuli delivered in the expiratory phase, however, had marked effects on tidal volume. Figure 12 depicts some of these relationships. From this graph, it appears that sites M5 and L5 have similar tidal volume gain to expiratory stimuli. However, the markedly different responses seen after vagotomy suggest that two different sites of integration may be involved. Once again, the H-4 sites appeared to have a low threshold to the number of stimuli presented per respiratory cycle, yet depression of tidal volume was evident, which may have been due to the greater effect on respiratory rate evoked by stimulation at these sites (cf. Table I). The L4 site would require a large number of stimuli to overcome its depressant effect on tidal volume, and under the conditions of the experiment, expiration would be complete before a sufficient number had been delivered. For no change in tidal volume, site L4 would have required 27 stimuli in expiration, while both L5 and M5 produce this change with only 11.9 stimuli. After vagotomy, site M4 required 11 stimuli, L4 required 17, and L5 a total of 26 stimuli during expiration.

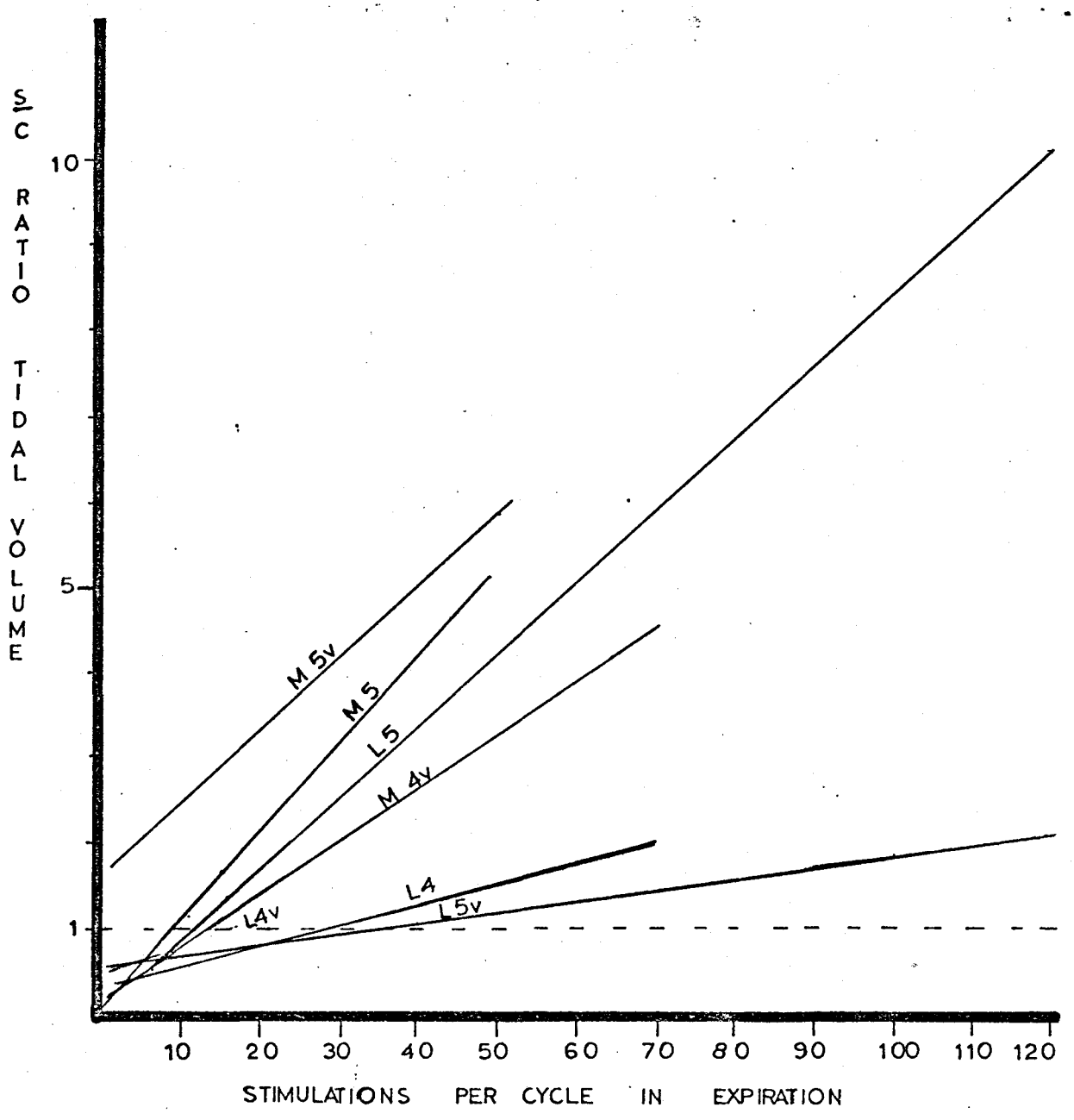


Figure 12.

The number of stimuli delivered per cycle during the expiratory phase is plotted on the abscissa. On the ordinate is plotted the S/C ratio of tidal volume. Two distinct populations can be seen.

C. Changes in Maximum Air Flow Velocity

The greatest relative changes during hypothalamic stimulation were those involving the maximum rate of air flow movement during the initial phase of inspiration (MAF). The averages obtained for each site are summarized in Table I. Graphical representation of the regression line, for each case in which the regression was significant, is shown in Figures 13, 14, and 15. Figure 13 compares the ratio of MAF and ratio of respiratory time. Site M5 produced an increase in MAF with increasing rate. An average of 4.9 times control was seen. After vagotomy, an ever steeper rise was seen in the slope relating these two parameters. Average increase in MAF was 6.1 times control. After vagotomy, the gain of site M5 increased while L5, with nonsignificant slope after vagotomy, produced a slope intermediate between the M5 pair. From the data presented in Table I, it may be seen that only at the L5 site was MAF during stimulation reduced following vagotomy. Even then the average value was above control. At a rate comparable to control, MAF would be approximately 8 times control. The same site before vagotomy had a value of 4.5 at control site. Site M5 after vagotomy would have a MAF of approximately 9 times control at rates equal to control.

Figure 14 presents the regression slopes relating the MAF to the ratio of inspiratory times. It will be noted that site L4, before vagotomy, appeared relatively

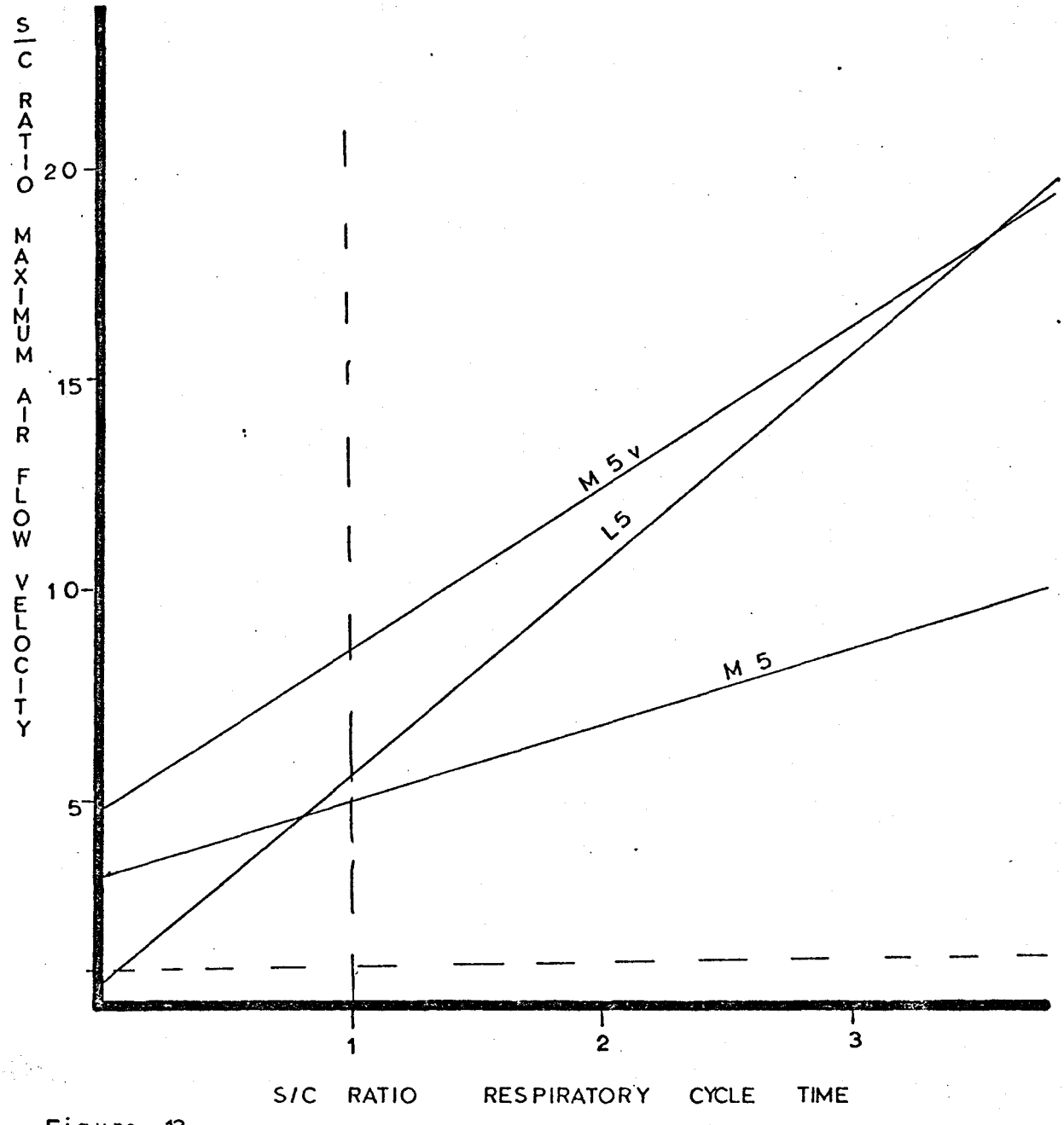


Figure 13.

Effect of changes in S/C ratio of respiratory time on S/C ratio of maximum inspiratory air flow velocity.

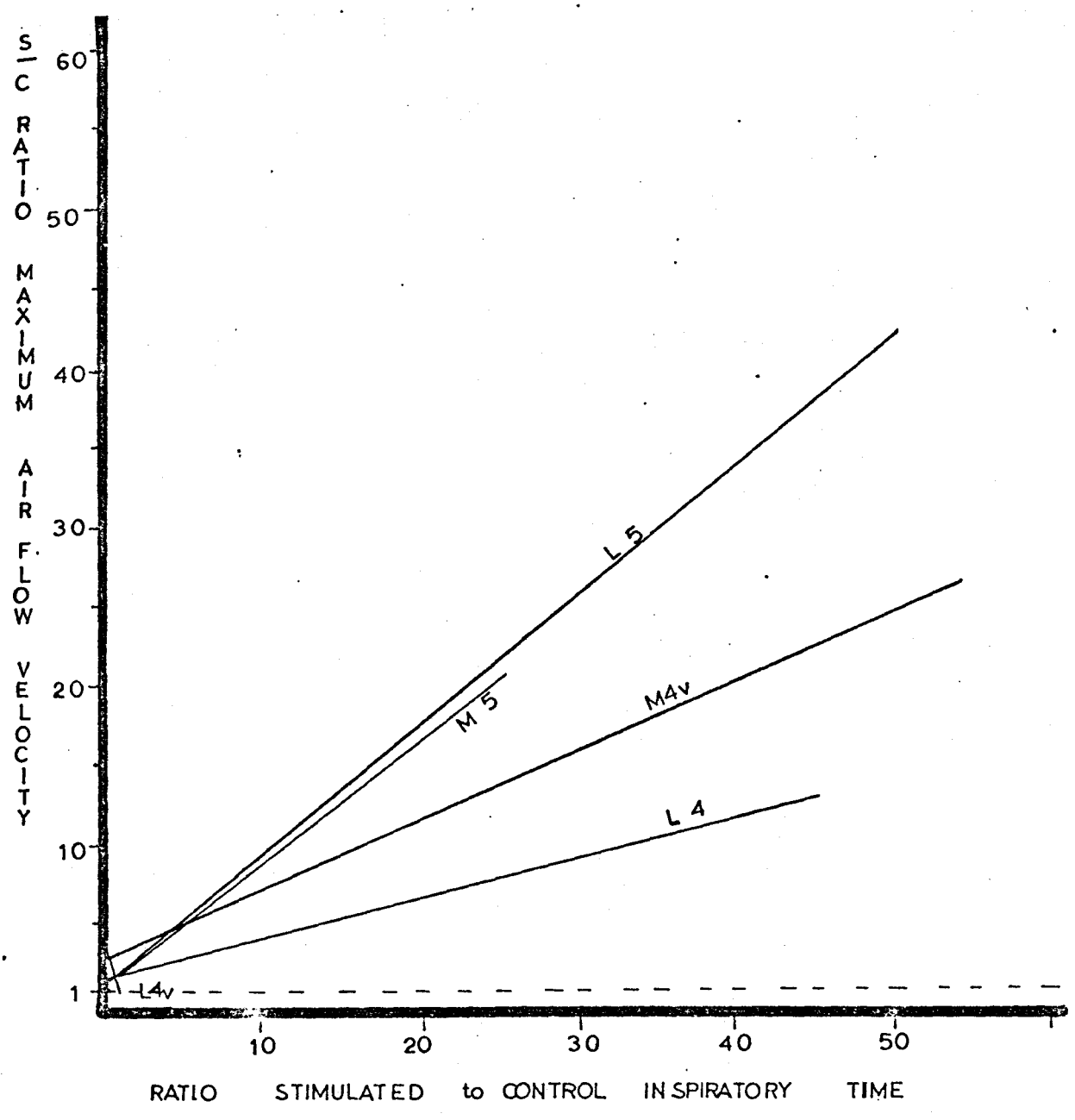


Figure 14.

The significant relationships between the ratio of stimulated to control maximum inspiratory air flow velocity and the ratio of stimulated to control inspiratory times are plotted.

independent of this variable, doubling the MAF but with little change compared to the ratio of inspiratory times. L5 showed a somewhat more sensitive relationship. Its sensitivity slope closely paralleled that of M5, but the initial effect on MAF was less. The slope of the M4 site after vagotomy approximated that of the M5 site before vagotomy, although the magnitude of response was less. Site L4 showed an inverse relation between inspiratory time and MAF after vagotomy. Comparing the points for an inspiratory ratio of 1.0, L4 would have an increased MAF about 2.2 times control, L5 about 2.8, and M5 about 3.5 times control. After vagotomy, M4 intersects at 3.3, while L4, with a negative slope, would have an intersection at 2.0. If inspiratory time were doubled, L4 stimulation would produce an MAF equal to control.

Figure 15, comparing expiratory times to MAF, shows that only site M5 produced a significant regression slope. The slope of the line here is approximately twice that of the inspiratory relationship, and again shows a positive relationship between increasing expiratory time and increasing MAF. No other points were significant when compared in this way. Extrapolated to control expiratory time, a six-fold increase in MAF would result.

D. Vagotomy

Some effects of vagotomy on the evoked changes in respiratory parameters resulting from hypothalamic stimulation are presented in Figures 16-25. In each case P refers

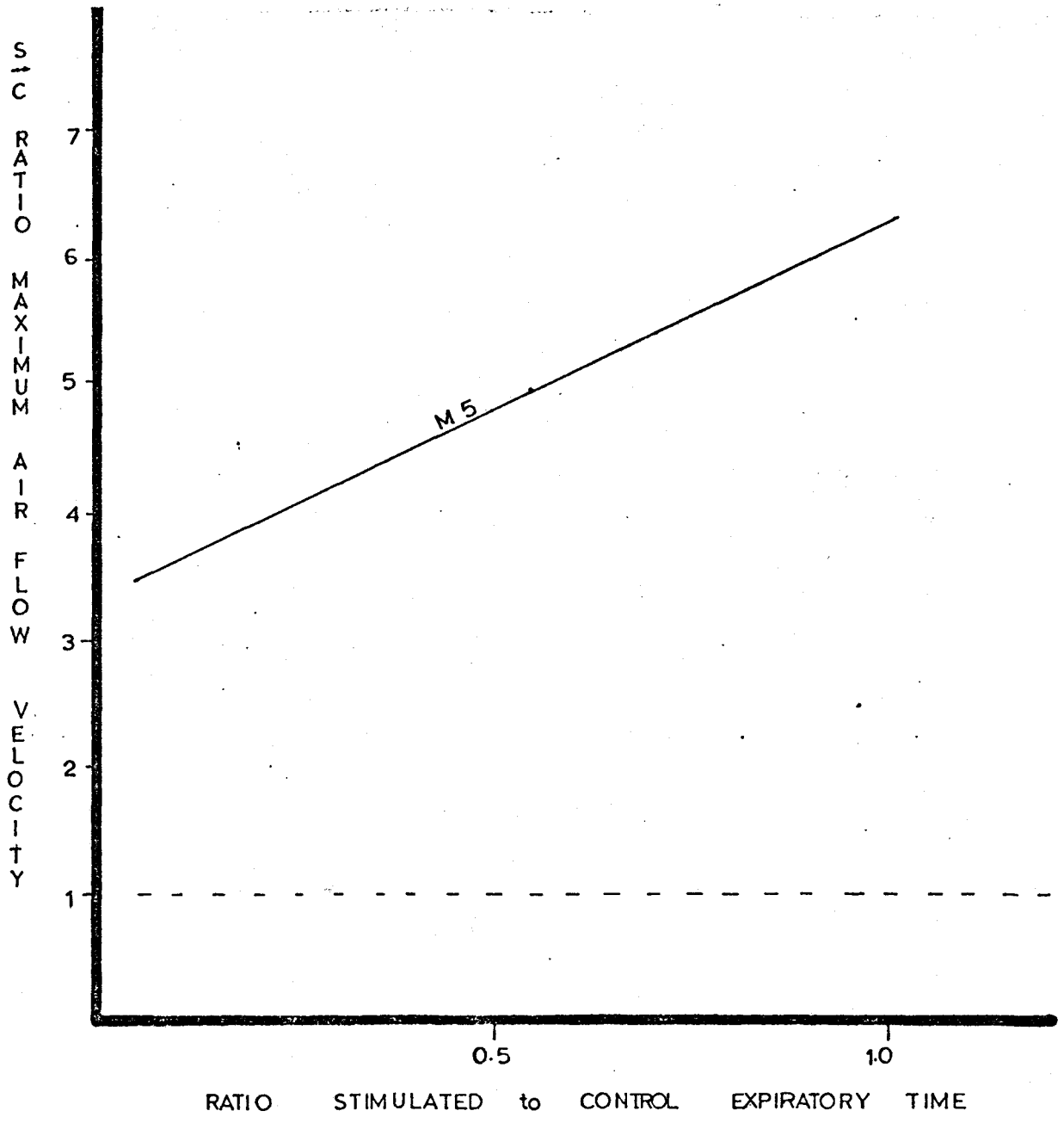


Figure 15.

Relationship between S/C ratio of expiratory time and S/C ratio of maximum inspiratory air flow velocity.

to the confidence level for difference of regression slopes for the pair.

Figure 16 shows the relation between the total number of stimuli delivered per respiratory cycle and the ratio of rates comparing the animal during stimulation to his own control immediately prior to stimulation. In this Figure, respiratory rate decreased to approximately 0.8 of its control value under the influence of stimulations at site M5. There was a positive gain relationship between the two variables, with increasing stimuli resulting in a slightly slower rate ratio. However, after vagotomy, stimulation in the same site showed a reduced gain indicating an increased sensitivity to total stimuli for the change in respiratory rate. Increasing the number of stimuli decreased the respiratory rate obtained by stimulation. About 70 stimuli would be required to reduce the increased rate to a ratio of 1.0 as compared with control. Only 10 were required before vagotomy.

Figure 17 shows a similar relationship for the M5 site as a function of the number of stimuli delivered in expiration. Here the vagotomy line was displaced downward, indicating that for a given number of stimuli, a relatively larger increase in respiratory rate is seen. Stimulations in expiration may have two effects. Either the system may be primed in some way to increase the rate of respiration, or the expiratory phase of the cycle may be shortened. Although no significant relationship could be found for shortening

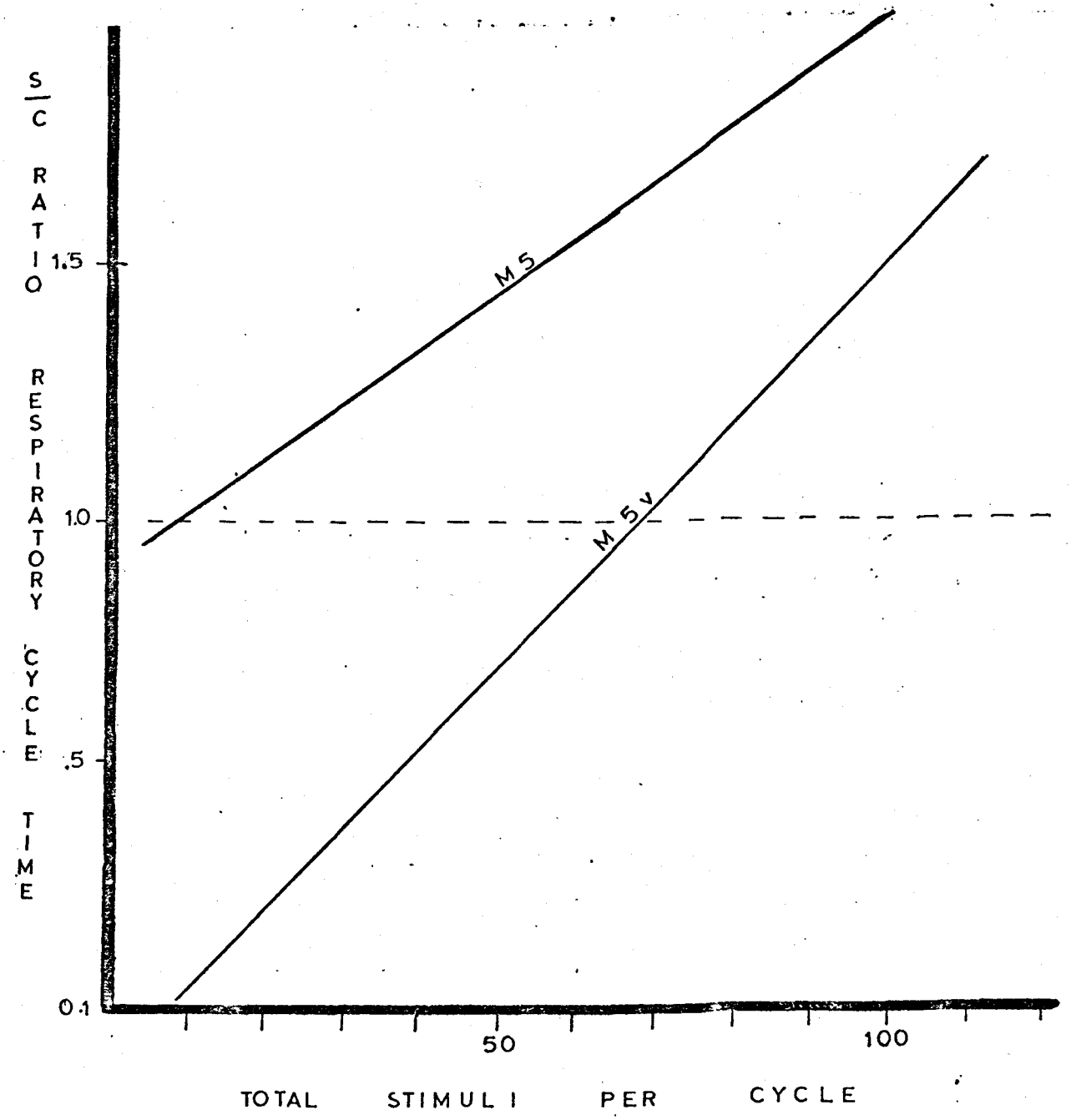


Figure 16.

Effect of total number of stimuli delivered per respiratory cycle on respiratory time before and after vagotomy. The P value indicates the significance in the difference between the 2 slopes.

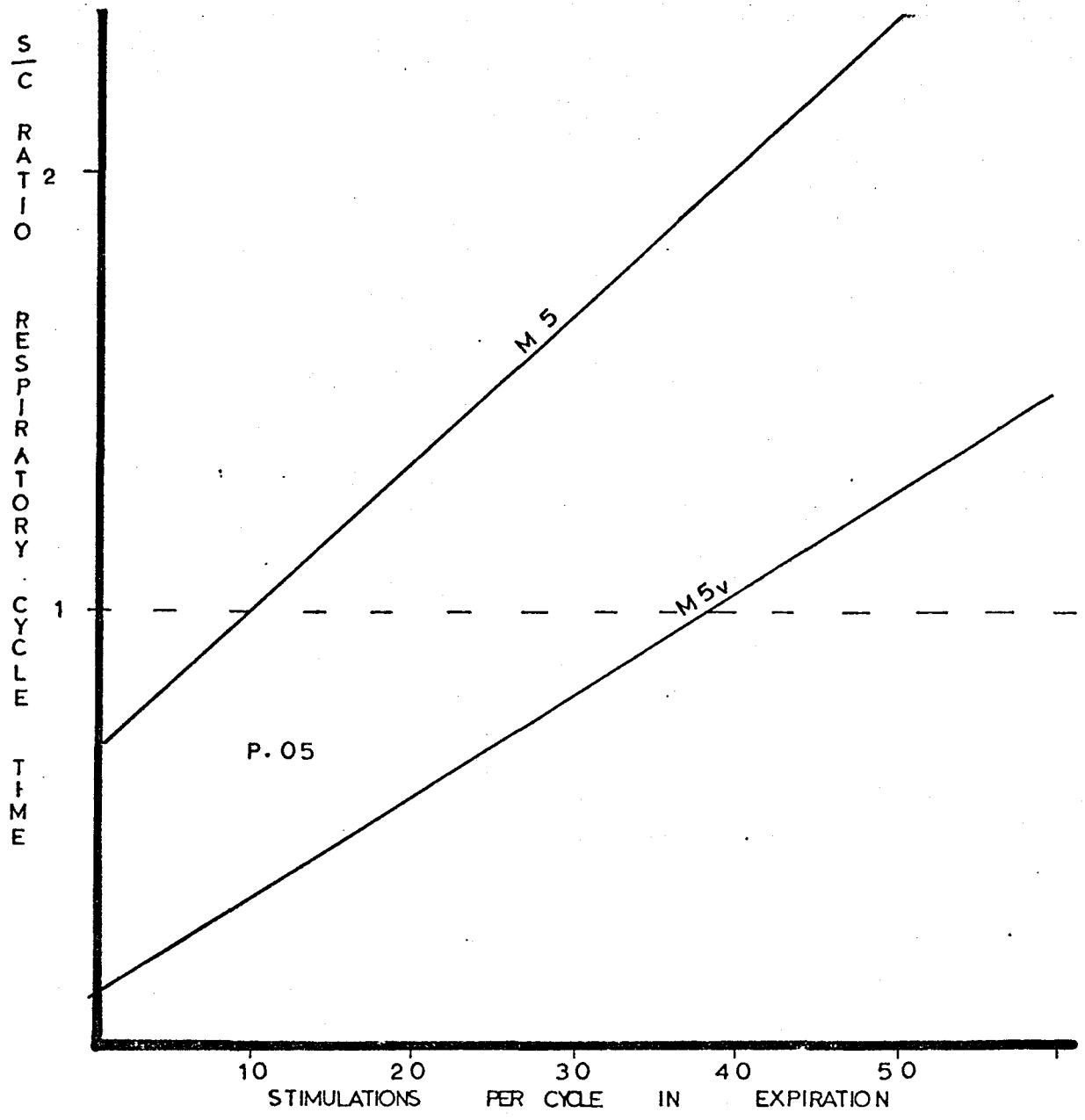


Figure 17.

Effect of total number of stimuli delivered in expiratory cycle on respiratory time before and after vagotomy. The P value indicates the significance in the difference between the 2 slopes.

the expiratory phase, the average expiratory time for this site (see Table I), was a ratio of .994, indicating little change from control. In fact, compared to the change during prevagotomy state, the average expiratory time lengthened. The major change seemed to be in inspiratory time as depicted in Figure 20. A significant difference in the slope relating inspiratory stimuli and rate changes is shown in Figure 18. Only the M4 site was found to have significant slopes both before and after vagotomy, with a P of .01. Here more stimuli decreased the respiratory rate toward that of control. Following vagotomy, the system was more sensitive to the number of stimuli delivered, with a small change in the number of stimuli resulting in a large decrease in the effective rate change from control. Slopes of both lines actually were quite similar. The major difference noted here was not in the gain but in the threshold, as vagotomy changed the average response point from .58 to .29 of control value.

Figure 19 depicts the effect of vagotomy on the inspiratory time as a function of the total number of stimuli delivered. Site M5, the only one significant for both non-vagotomized and vagotomized preparations, showed two prominent differences. Prior to vagotomy, the effect of increasing numbers of stimuli per cycle was to lengthen the inspiratory time relative to control. Following vagotomy, the sensitivity of the system changed. The gain of the M5 site

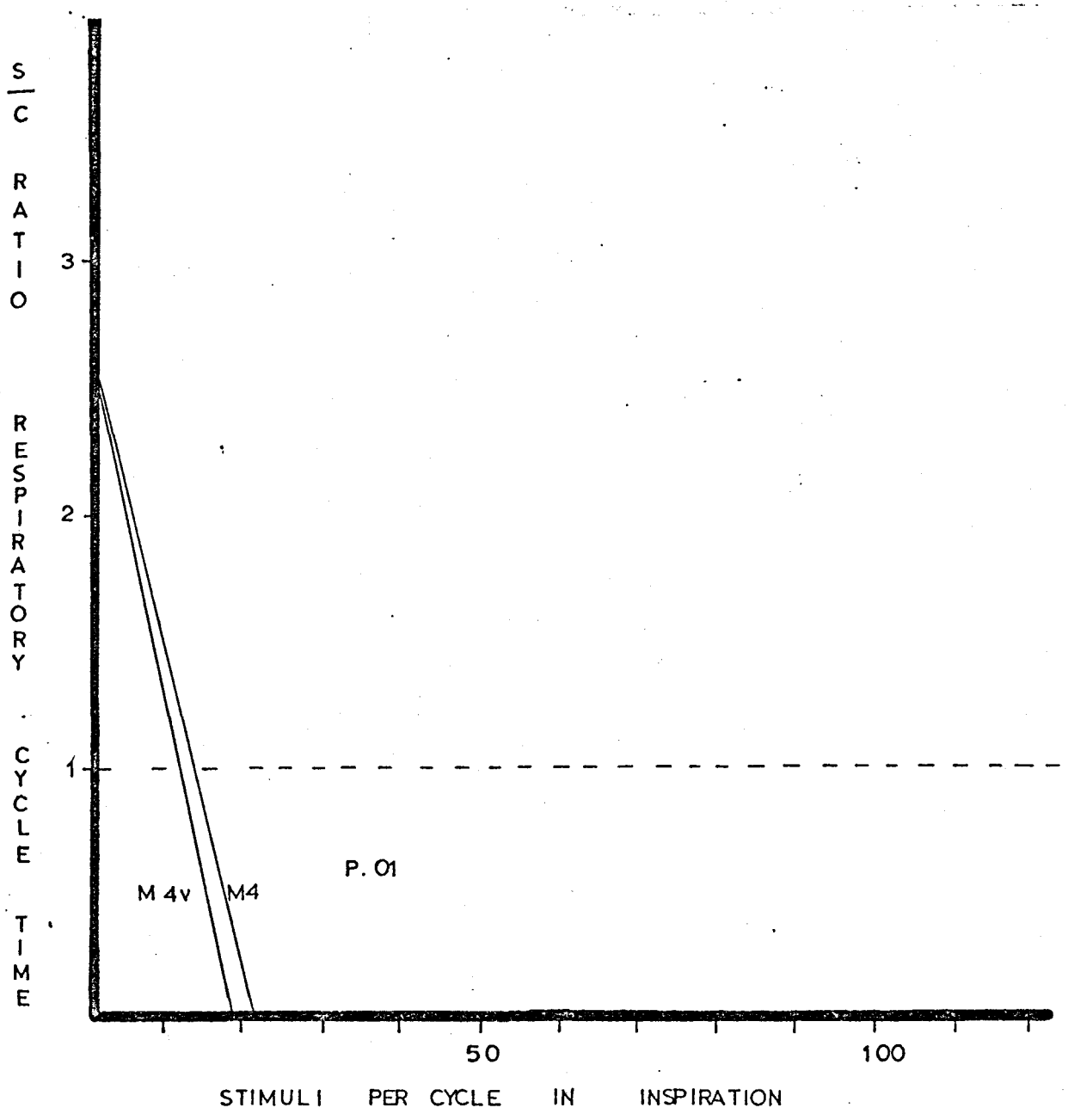


Figure 18.

Effect of total number of stimuli delivered in inspiratory cycle on respiratory time before and after vagotomy. The P value indicates the significance in the difference between the 2 slopes.

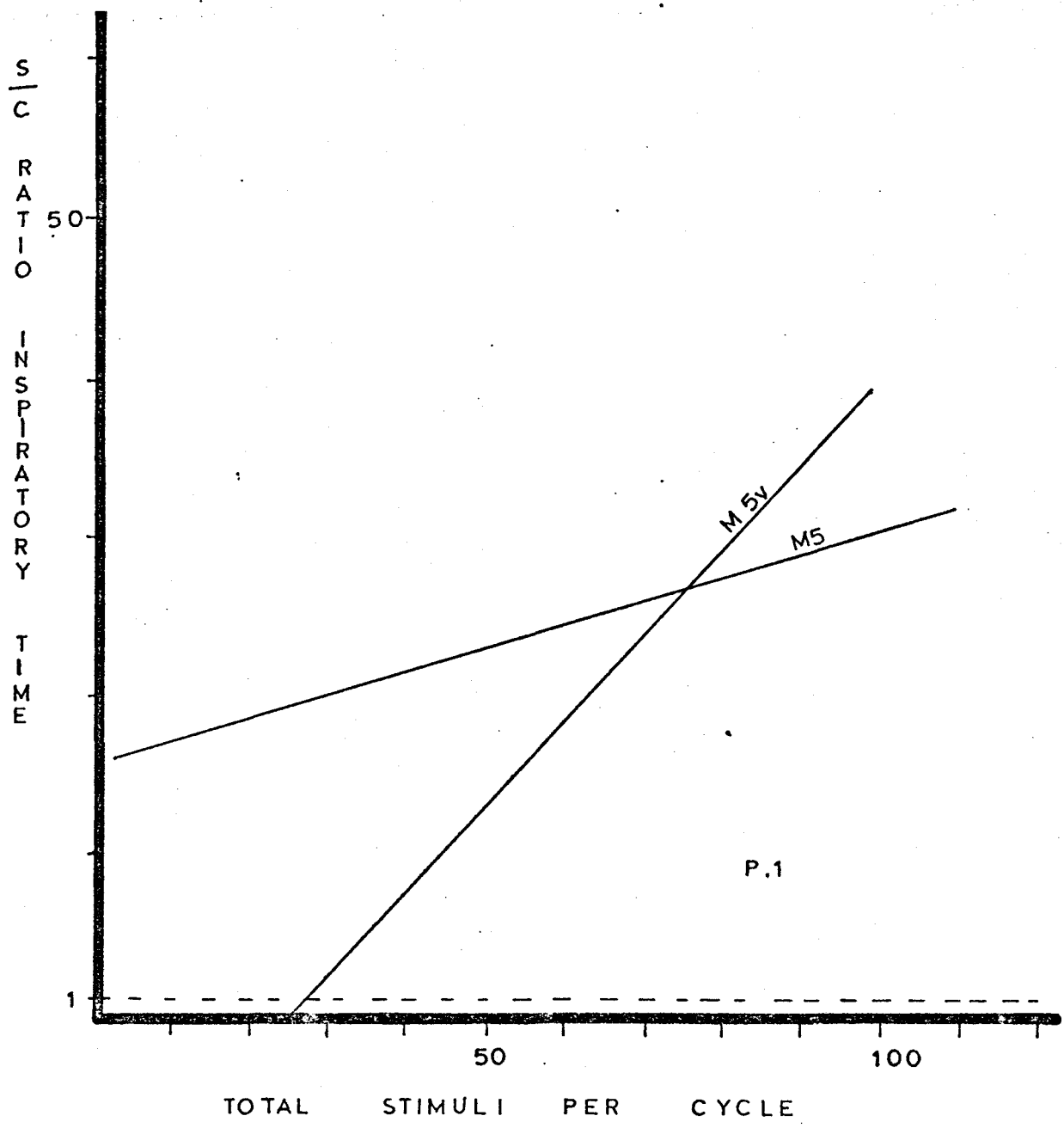


Figure 19.

Effect of total number of stimuli delivered per cycle on the S/C ratio of inspiratory time.

following vagotomy was increased, as was the threshold for changes in inspiratory time. Average response values here seem to indicate a change in the mode of the response as well, as inspiratory time before vagotomy was 3.1 times control, and only 0.42 after vagotomy.

In Figure 20, the relationship between the M4 and M5 sites are plotted. The lengthening of inspiratory time by M5 stimulation to about 3.1 times control was not seen in the M4 site. Here the inspiratory time was essentially unchanged as a function of the stimulation. After vagotomy, however, the M5 site and the M4 site produced comparable changes in the threshold, but the gain of the M4 site was definitely greater than that of the M5 site when compared after vagotomy. Before vagotomy, stimulation at site M4 produced little change in inspiratory time, while after vagotomy the gain was greatly increased, approaching that of site M5 before vagotomy. The number of stimuli necessary to produce an inspiratory time ratio of 1.0 were the following: Site M4 before vagotomy 16, after vagotomy 17; Site M5 before vagotomy 15.5, and 20 after vagotomy. Stimuli delivered in inspiration had little significant effect on expiratory time except at site M4 as shown in Figure 21. Here the slopes of regression are also changed as a result of vagotomy. Initially stimulation resulted in a change in expiratory time due to increasing numbers of stimuli, but after vagotomy there was no relation between stimuli

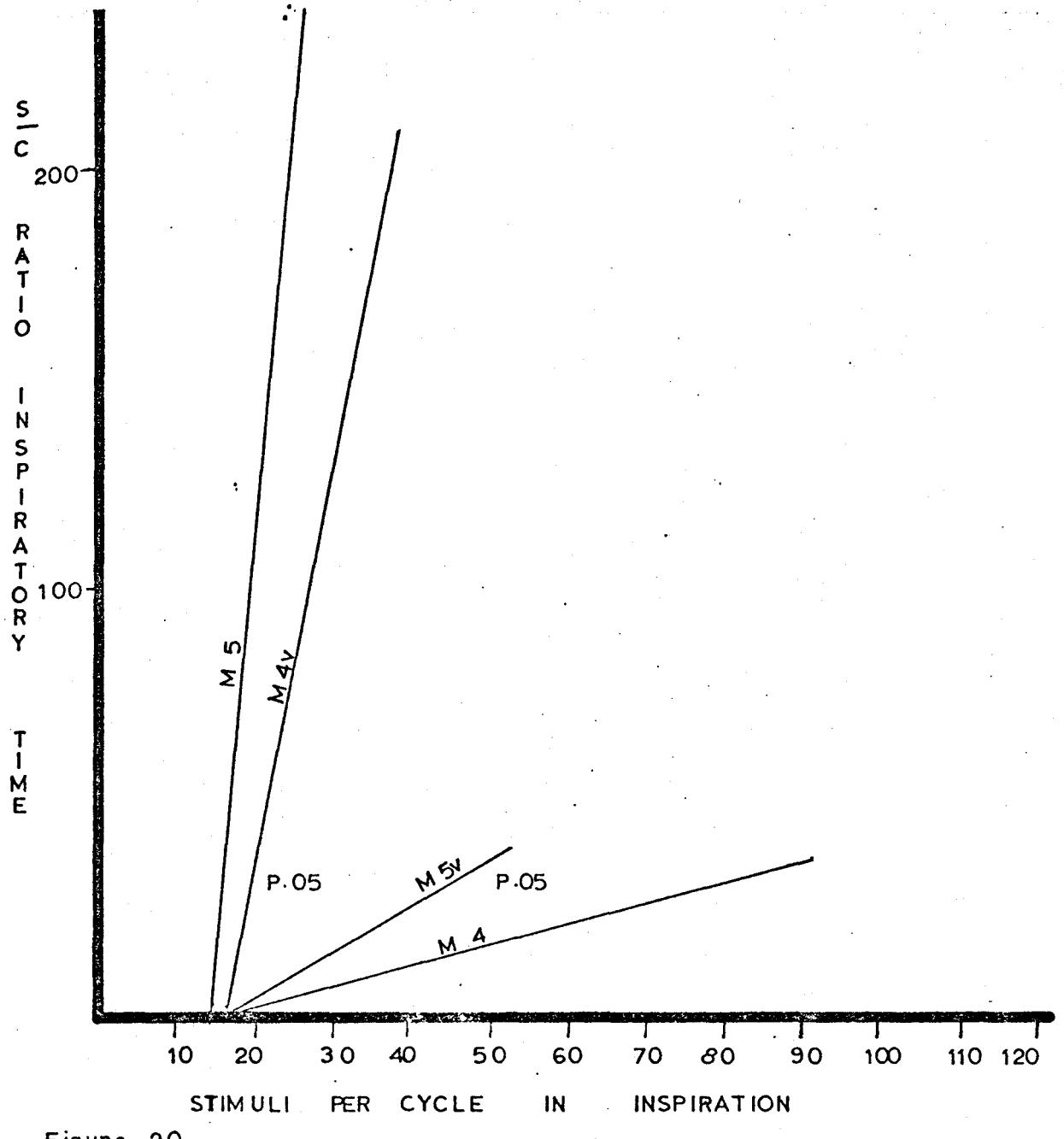


Figure 20.

Effect of number of stimuli delivered in inspiration on the S/C ratio of inspiratory time.

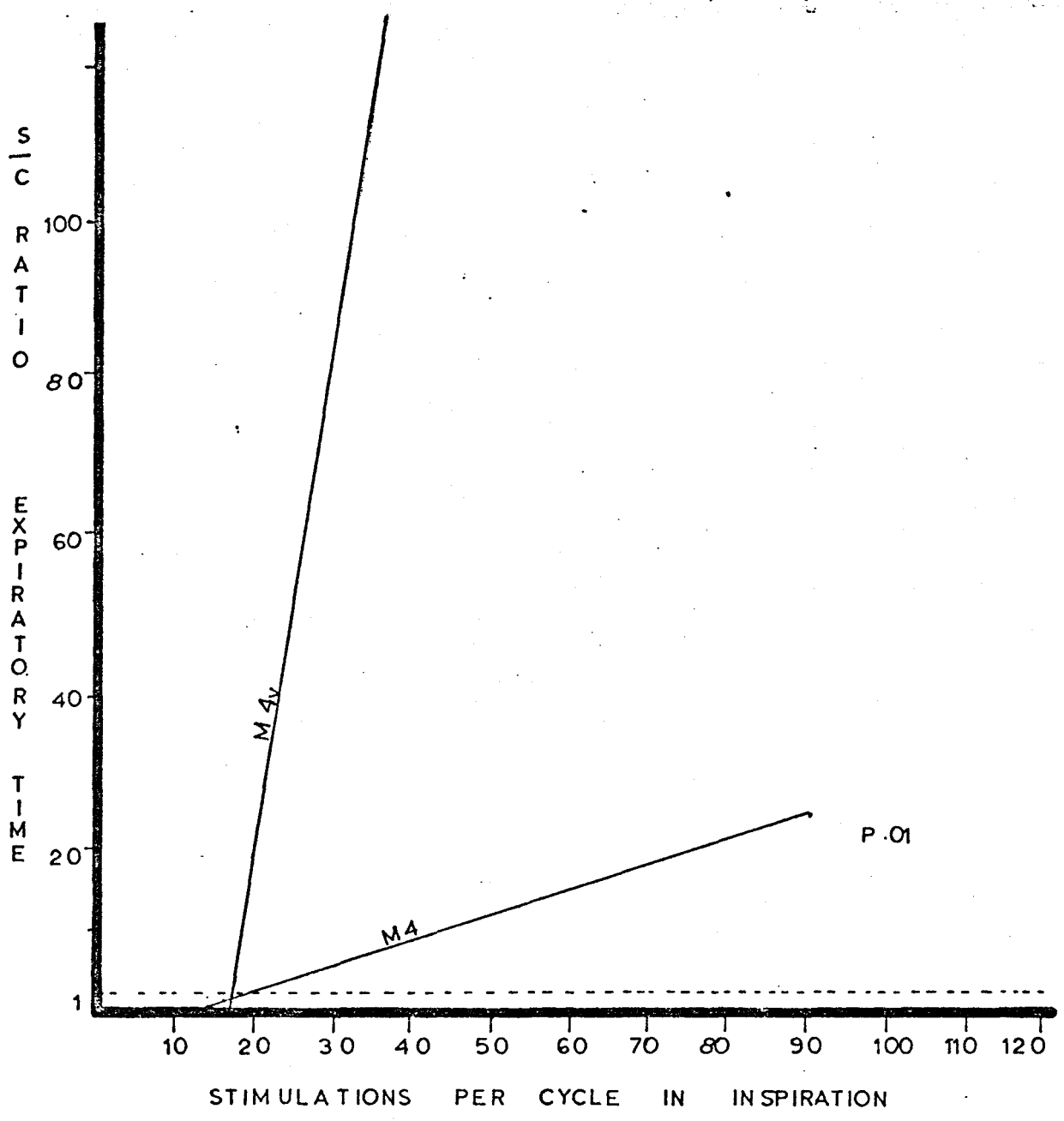


Figure 21.

Effect of number of stimuli delivered in inspiration on the S/C ratio of expiratory time.

and change in expiratory times, as signified by zero slope. The regression lines intersect at a ratio of 1.0, requiring 17 stimuli.

Interestingly enough, stimulation at site L5 failed to produce significant changes in phrenic nerve discharge. However, the relationship plotted in Figure 22 suggests this site may have an influence on tidal volume. An increase in tidal volume was seen to correlate with increased numbers of stimuli delivered in expiration. The critical number of stimuli for crossover from inhibition to facilitation was 11 per cycle. After vagotomy, the slope decreased significantly, although the directional relationship remained. The crossover point for a tidal volume ratio of 1.0 would be at approximately 27 stimuli per cycle.

Figure 23 depicts the relationship between pre- and post-vagotomy stimulation of site L4. This graph indicates a small positive relationship between number of stimuli per cycle and increasing tidal volume, although for the number of stimuli employed here, it yielded a decreased tidal volume from control. Vagotomy decreased the amount of reduction in tidal volume at lower numbers of stimuli. The intercept of this line with the tidal volume ratio of 1.0 was increased from about 50 to over 98 stimuli per cycle. Part of this increase may be explained by Figure 24. Before vagotomy, MAF was increased about 2.5 times control, with little direct relationship between the number of stimuli in

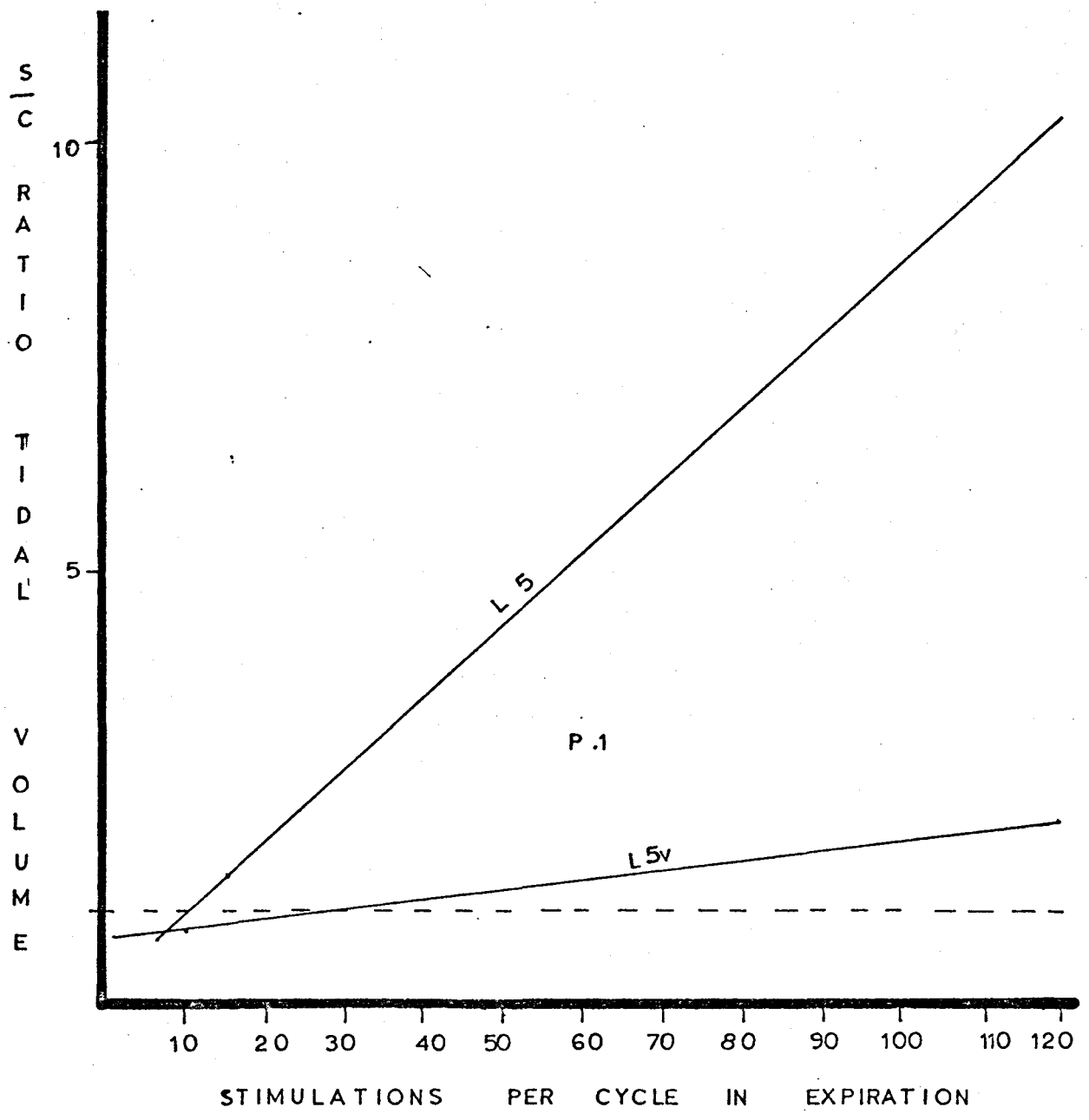


Figure 22.

The effect of vagotomy on the relationship between the number of stimuli delivered per cycle in expiration and the S/C ratio of tidal volume.

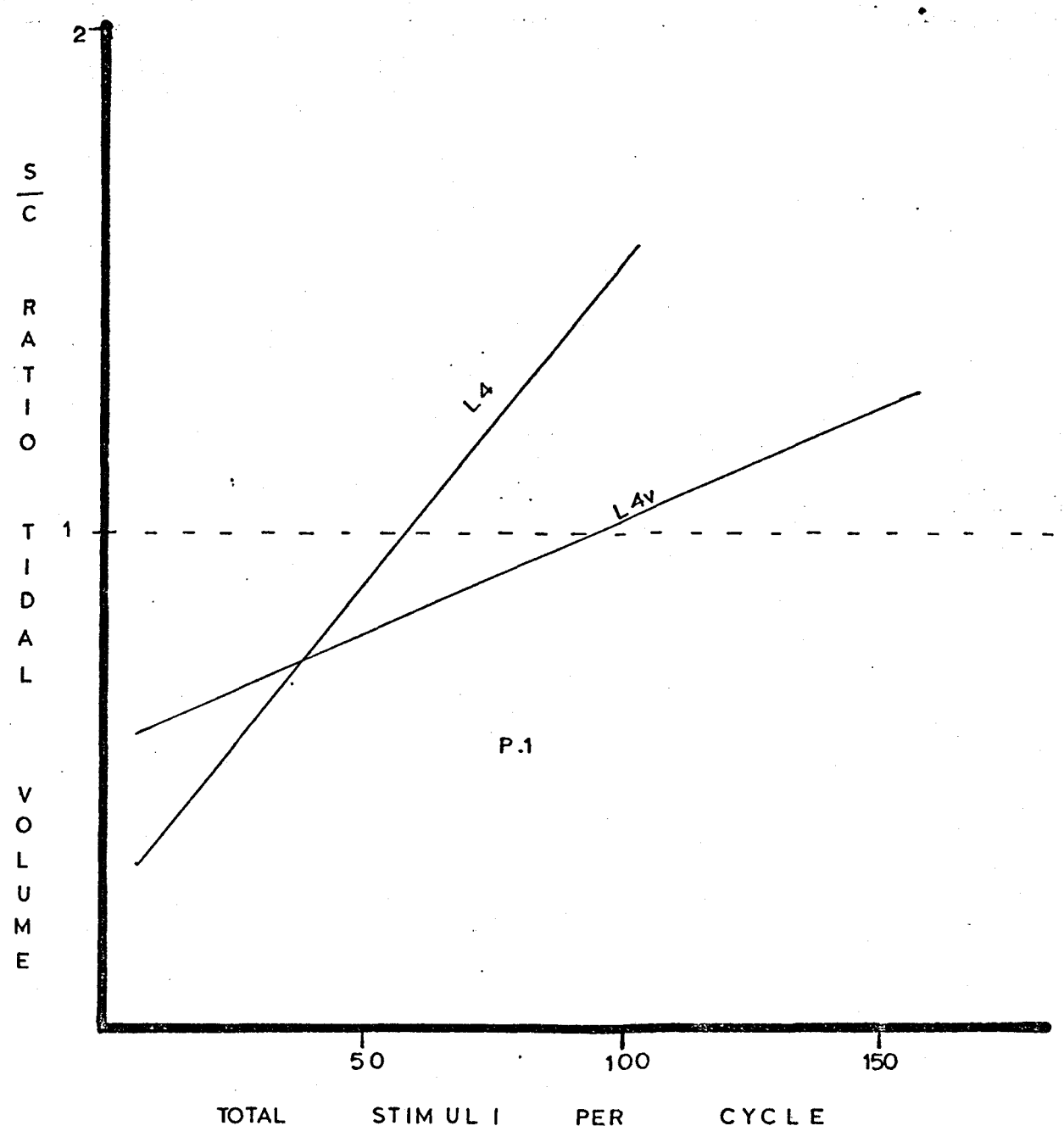


Figure 23.

The effect of vagotomy on the relationship between the total number of stimuli delivered per respiratory cycle and S/C ratio of tidal volume.

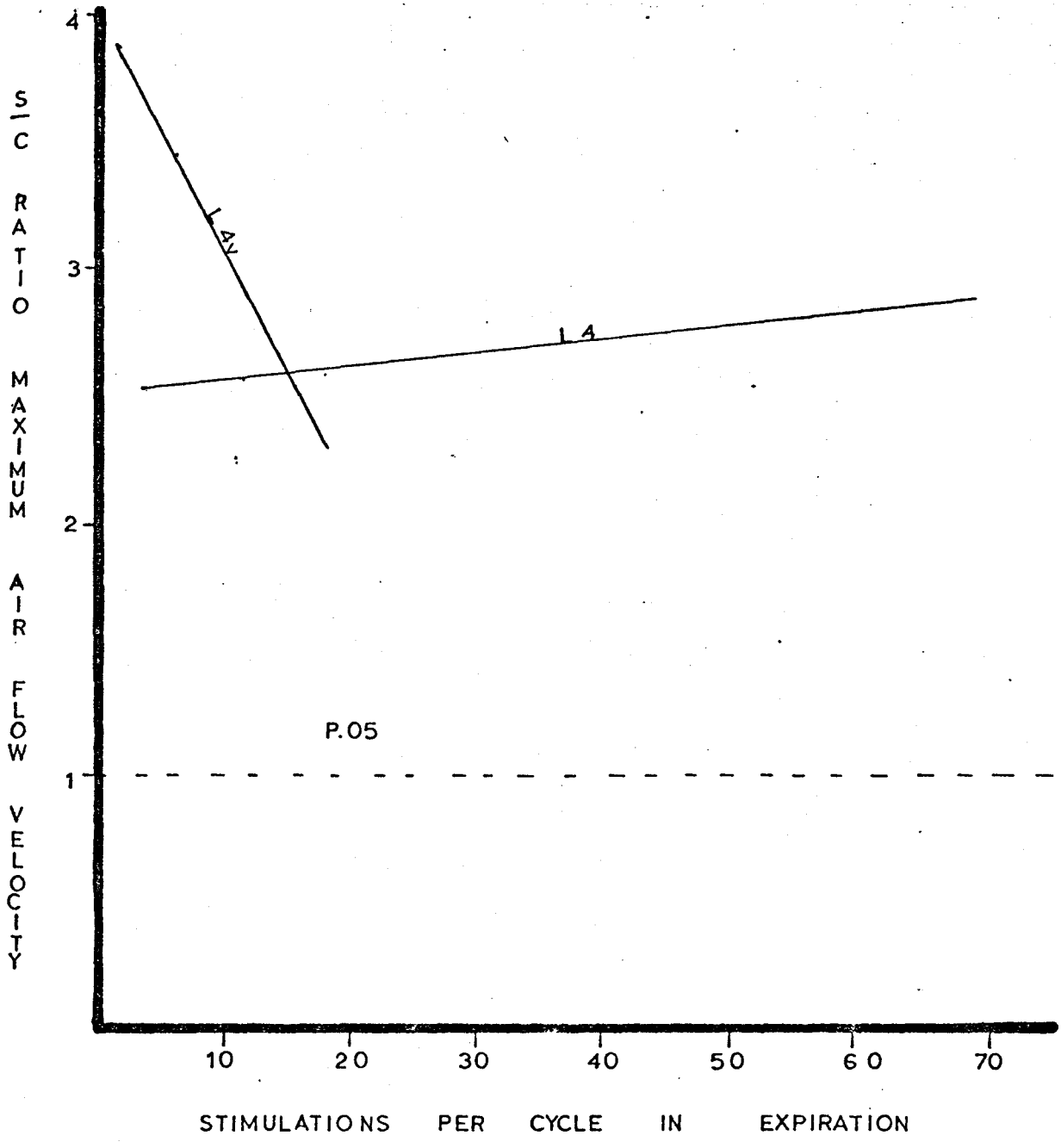


Figure 24.

The effect of vagotomy on the relationship between the total number of stimuli delivered per cycle in expiration and the S/C ratio of maximum air flow velocity in inspiration.

expiration and the resulting increase in MAF. After vagotomy, site L4 showed a reduction in the magnitude of the increase in MAF with increasing stimuli in expiration, with peak increase in MAF coming at the low end of the stimulus number spectrum.

E. Changes in Phrenic Discharge

Figures 25-30 detail the responses evoked in phrenic discharge patterns from stimulation at site M4. Numbers indicate responses obtained before vagotomy, and the "v" those after acute bilateral vagotomy. Only those slopes whose correlation coefficient was significant at P values of 0.1 or less were plotted.

Figure 25 indicates responses of the S/C phrenic discharge in expiration driven by total stimulations per cycle. Note that the five lower levels were depressed below control, but the average values for levels 6 and 7 were increased. Vagotomy increased the slope of the level 7 discharge and greatly reduced level 6 average value. Comparing pre- and post-vagotomy data, the slopes for level 4, 5, and 7 were significantly different at respective P values of 0.1, 0.01, and 0.001. All control regressions were significant at P=.005 level. The P values given here refer to the confidence limits for points fitting the calculated regression line. It is in a sense a "goodness of fit" for the experimental data points, and represents the correlation coefficient for data fit. The significance value for

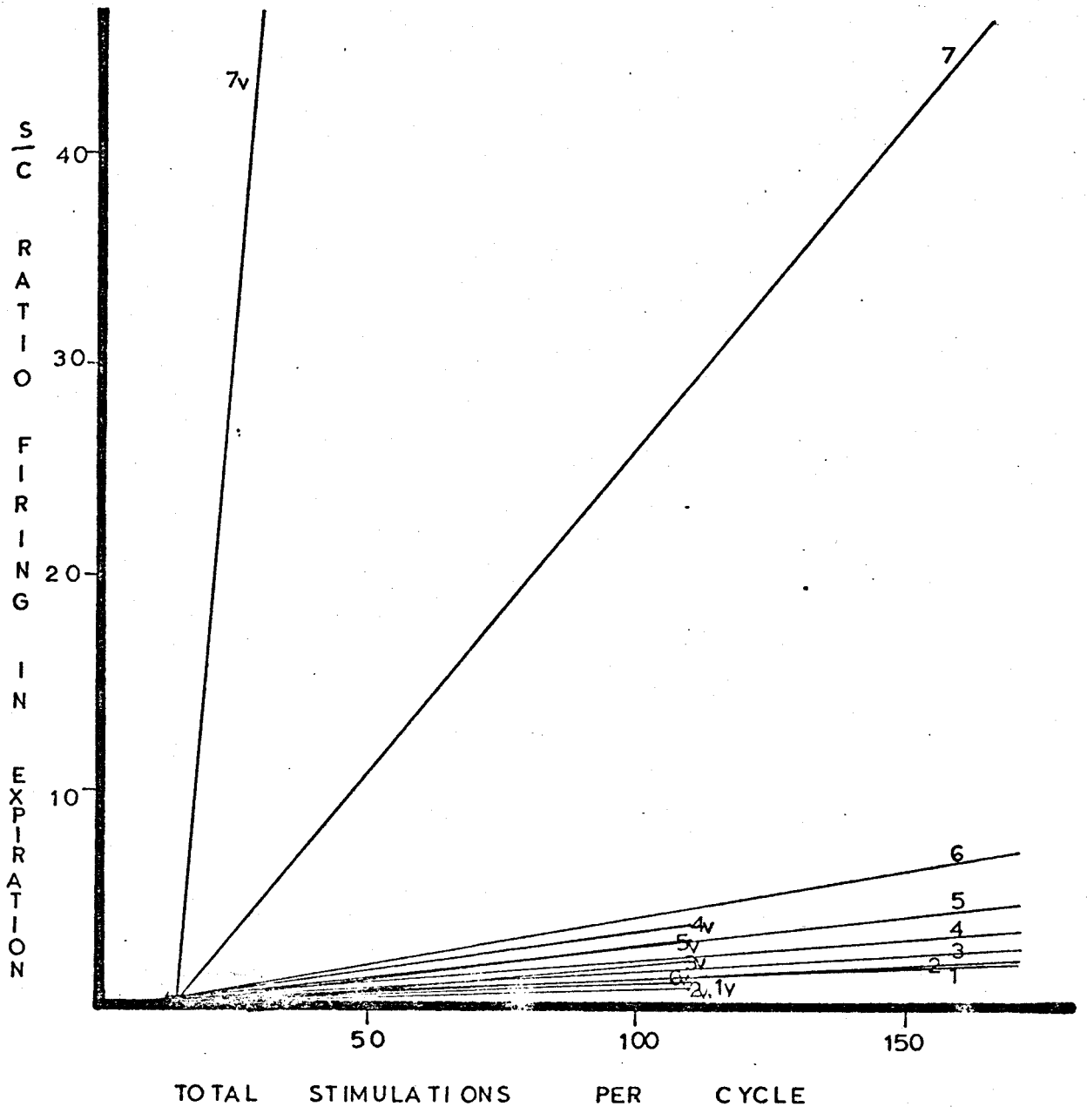


Figure 25.

Phrenic nerve discharge regression slopes are shown for site M4 in each case where the line was significant. Total stimuli delivered per cycle is correlated with the S/C ratio of discharges per cycle. Numbers 1 through 7 designate amplitude level (see text).

difference of two slopes, used elsewhere in this dissertation, refers to the confidence level for the assumption that two regression slopes represent two unique populations. Thus a low P value for the correlation coefficient indicates a great deal of scatter, while a low P value for regression slopes indicates the two proposed populations really are one and the same population. Because of these relations, two populations may have low correlation coefficients, yet represent two distinct populations.

Figure 26 indicates the relative lack of changes in the inspiratory phase of phrenic discharge as compared to total stimulations. Prior to vagotomy, the relatively wide degree of scatter produced the curves shown with relatively low correlation coefficients. Level 6, for example had a P=.2. After vagotomy, level 7 P=.2, level 1 P=.1, and all others were significant to .05 or above, with levels 5 and 6 at the .005 level. Significant differences between slopes after vagotomy as compared to the pre-vagotomy state were: level 2 P=.1, level 3 P=.05, and level 5 P=.02 with all others .2 or above.

In Figure 27 the changes in firing ratio observed due to stimulations in site M4 during expiration are detailed. It will be noted, by comparison with Figure 25, that the major response in increased phrenic discharge was due to an increased firing in the expiratory phase of respiration, i.e. before significant amounts of air are moved.

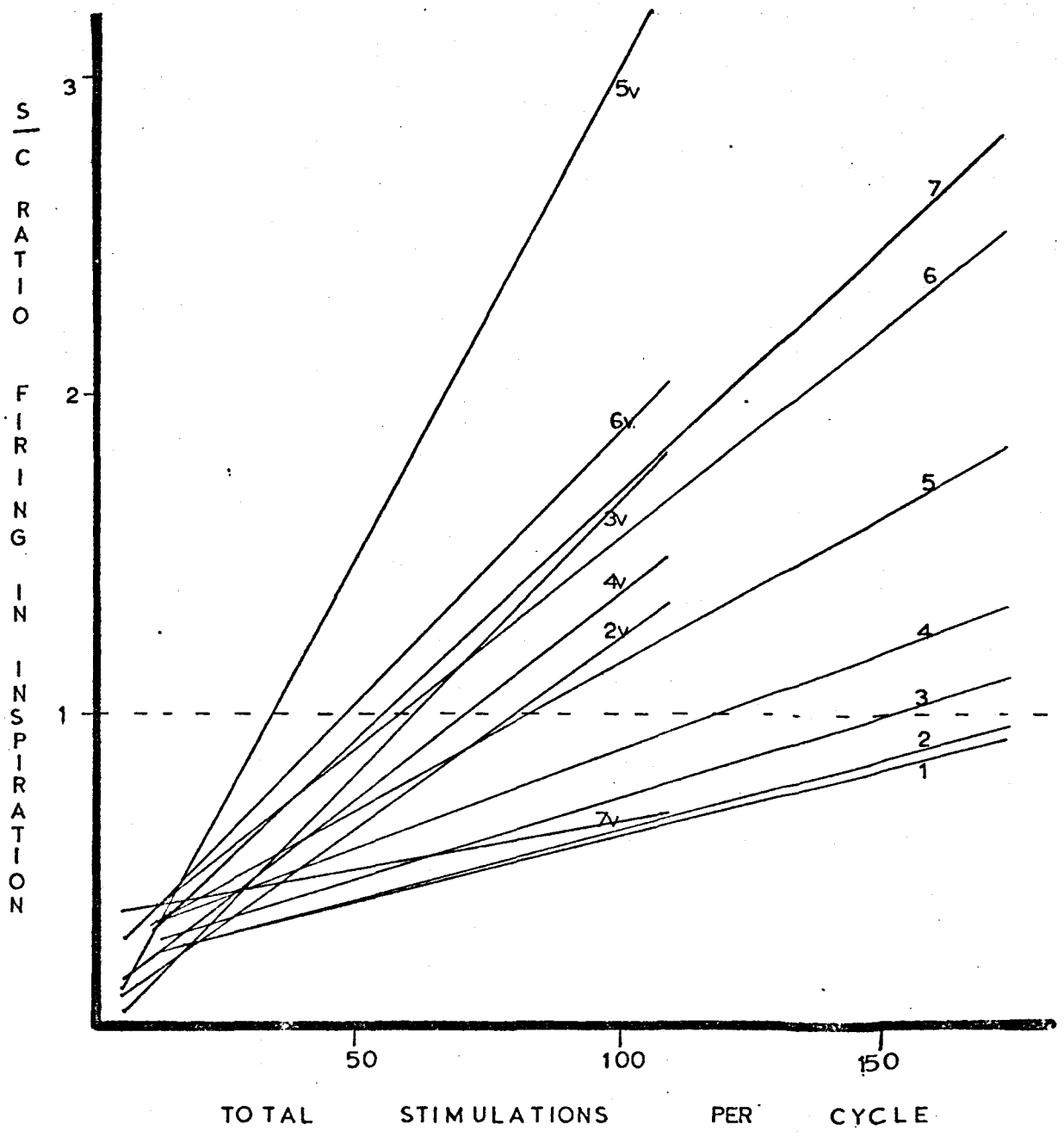


Figure 26.

Effect of total stimulations per cycle on S/C ratio of phrenic nerve discharge in inspiration for site M4. Phrenic discharge is indicated for each level before and after vagotomy at which regressions with significant correlation coefficients were found.

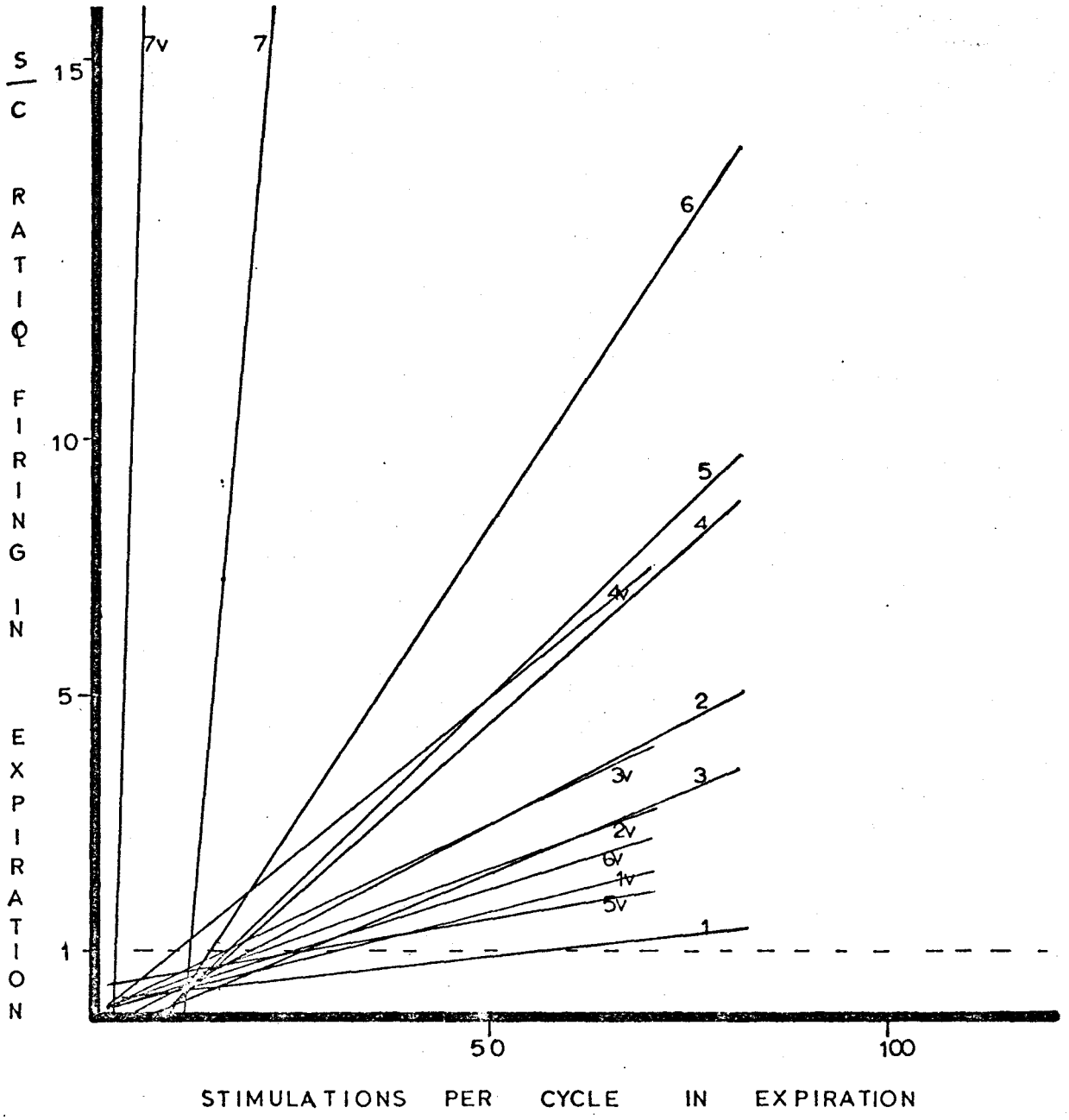


Figure 27.

The significant regression lines relating stimuli in expiration and the S/C ratio of expiratory discharge at site M4.

The relative discharge in inspiration was actually decreased (cf. Figure 26). A greater reduction in the lower levels of discharge (1-4) was noted in inspiration as compared to expiration. After vagotomy, the respective levels are well-represented by points located on the same slope lines. In expiration, however, this relationship was not necessarily true, as the upper levels of discharge (4-7) appeared to have considerably different slopes.

The effect of stimulations in expiration upon inspiratory discharge is shown in Figure 28. Prior to vagotomy, the lower levels were similar. Striking differences were noted in the slopes of levels 5-7 after vagotomy. The effect of expiratory stimuli on inspiration discharge seems to be two-fold. The sensitivity to level 7 discharge seems to be decreased as a result of vagisection, while the lower level slopes were enhanced. This was not true for levels 6, 5, or 4.

Stimulations in inspiration as they pertain to changes in the ratio of discharge are detailed in Figures 29 and 30. Here it may be seen that a uniform positive relationship exists for discharge in both inspiration and expiration for the lower level discharges. After acute vagisection, however, no significant relationship was found. Higher level discharges were not found to be significant in either case.

Figures 31-36 present the results of stimulation at site L4 correlated with the ratio of changes from control

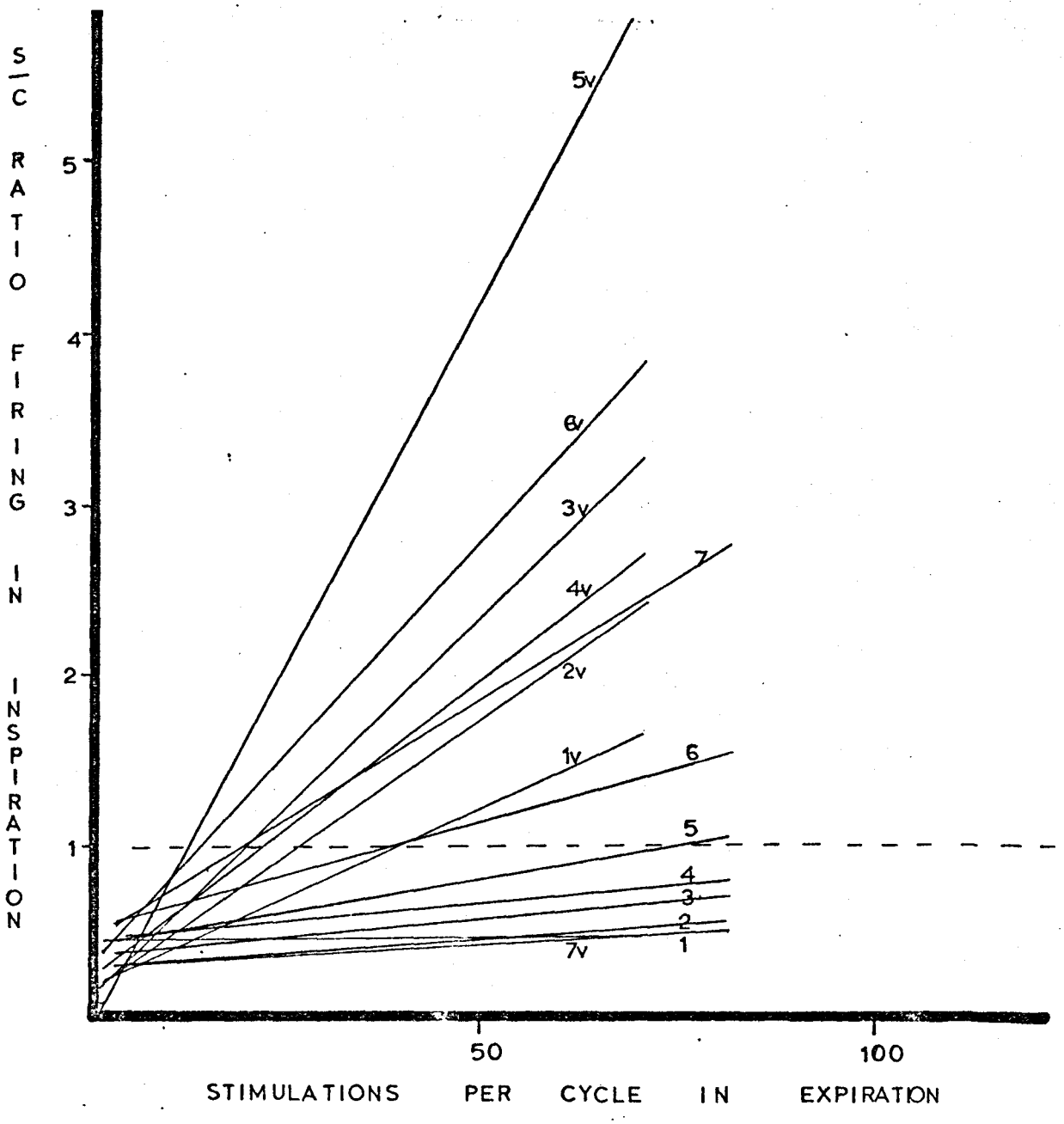


Figure 28.

Effect of number of stimuli in expiration on the S/C ratio of phrenic discharge in inspiration for site M4.

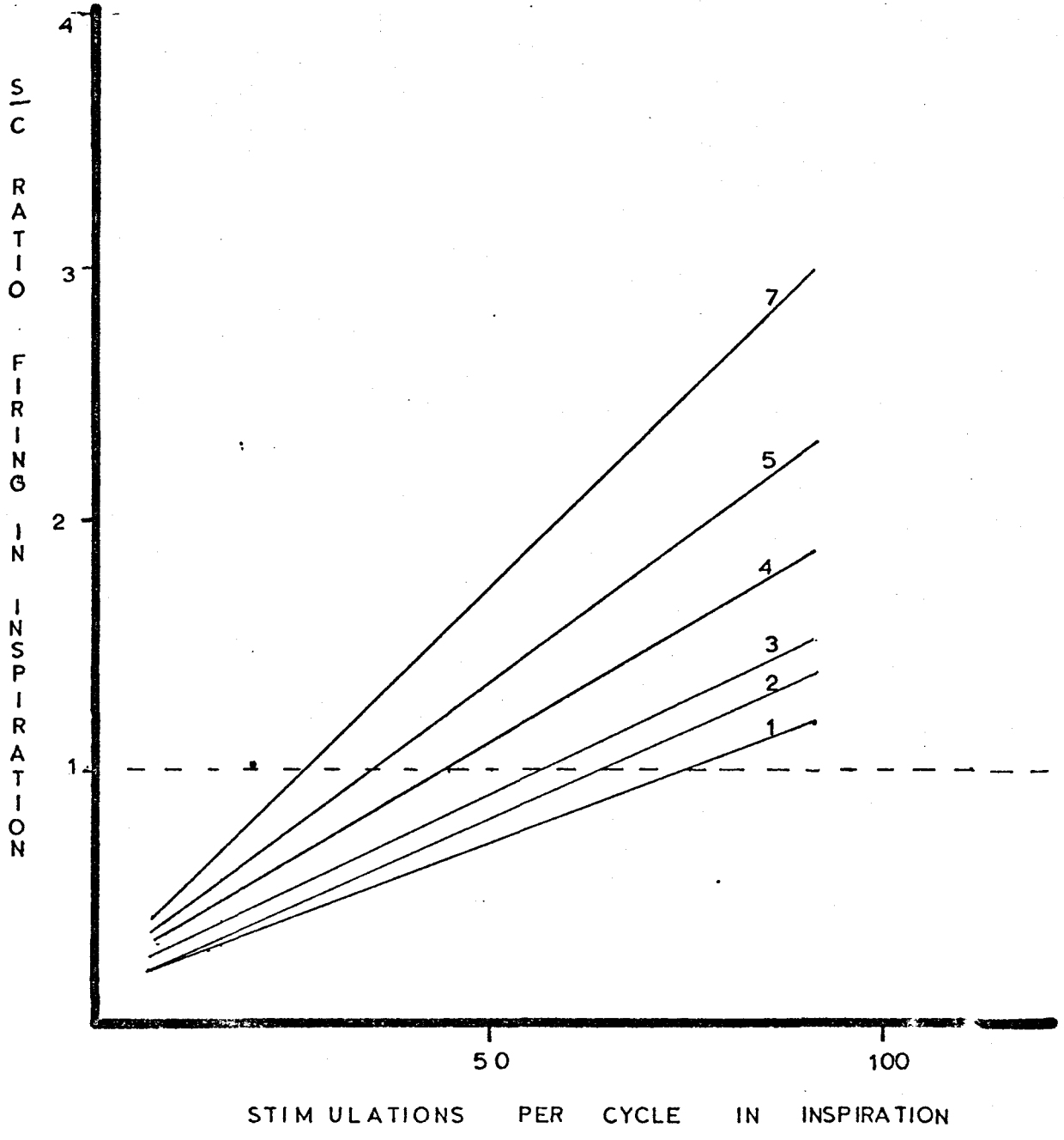


Figure 29.

Effect of number of stimuli in inspiration on the S/C ratio of phrenic discharge in inspiration.

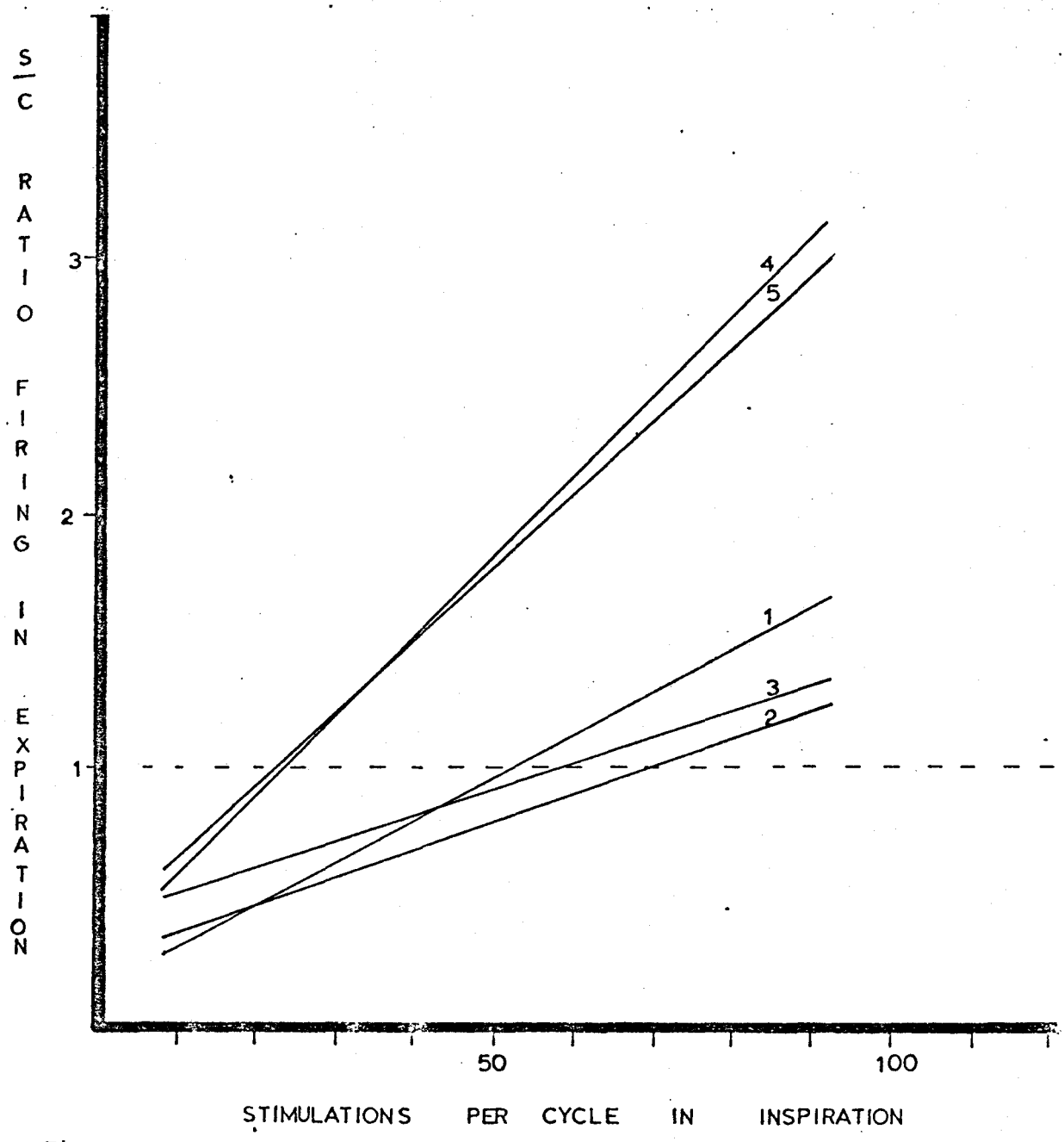


Figure 30.

Effect of number of stimuli in inspiration on the S/C ratio of phrenic discharge in expiration for the 5 amplitude levels in which regressions had significant correlations coefficients. After vagotomy, none of the levels had significant regressions.

discharge. The primary difference between this site and M4 was the pronounced increase in discharge of phrenic level 7 in both inspiration and expiration. No statistically significant slopes were found for the relation between total stimulations per cycle and expiratory discharge after vagotomy. Prior to vagotomy, it may be seen from Figures 31 and 32 that the response of level 7, both in terms of inspiratory and expiratory discharge changes, was markedly different from that of all other levels. Vagotomy produced no significant relationship between total stimuli and expiratory discharge, but significant relations were found for the lower levels as portrayed in the Figure relating inspiration and total stimulations, Figure 31. Stimuli delivered in inspiration appeared to have a strongly facilitatory effect on discharge in expiration, as shown in Figure 33. Again, decreases in the discharge frequency of the lower amplitude signals was noted, with an enhancement of the discharge of the two higher levels. Vagotomy eliminated the relationship, as no significant slopes were found following this procedure.

Stimuli delivered to site L4 in expiration also produced an increase in the discharge of level 7, and a decrease in all other levels (Figure 34). Vagotomy significantly affected the average discharge of each level as well as the slope of the regression line. The sensitivity to stimuli was increased, resulting in greater augmentation

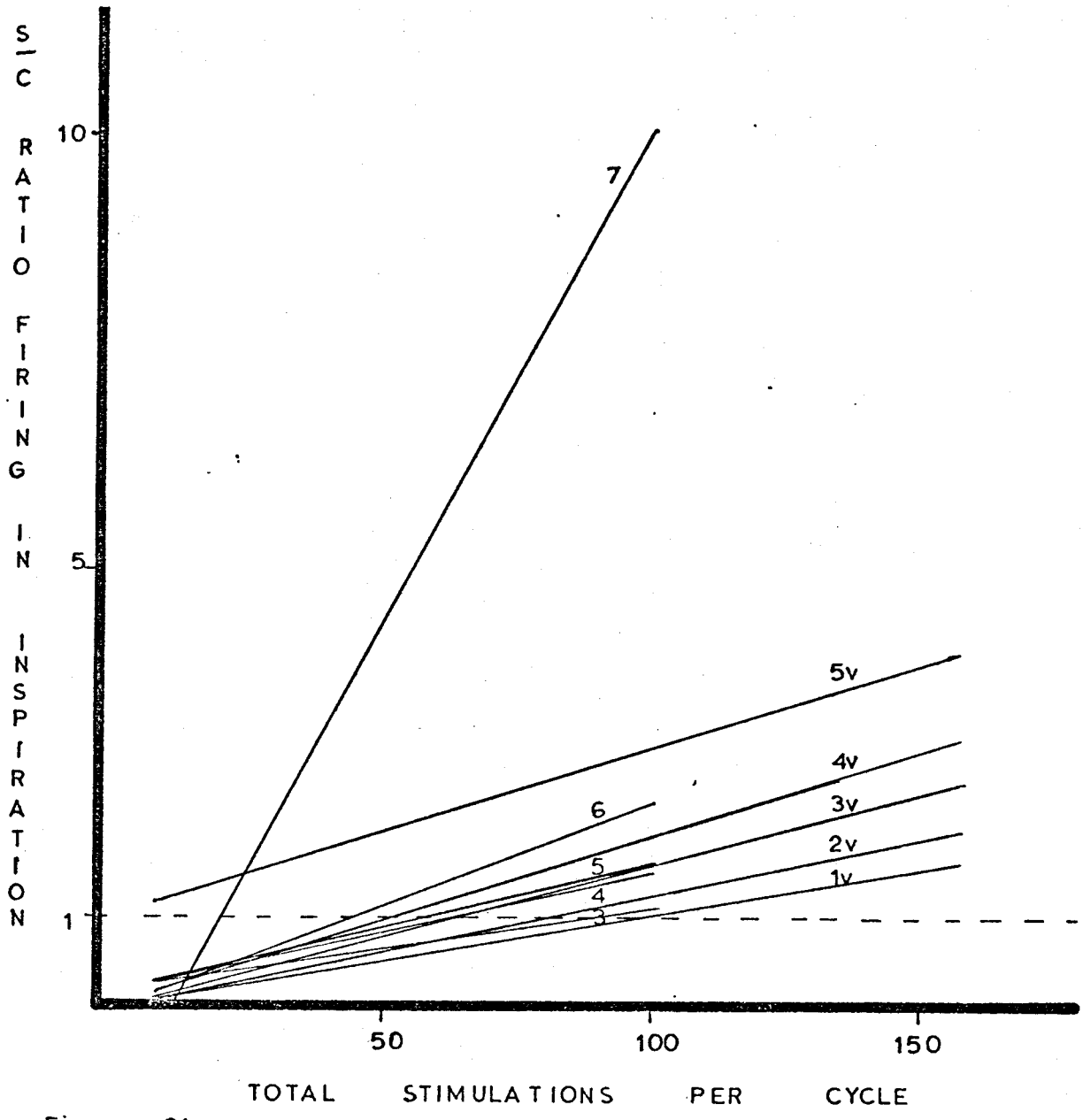


Figure 31.

Site L4: Effect of total number of stimuli per cycle on S/C ratio of phrenic discharge in inspiration.

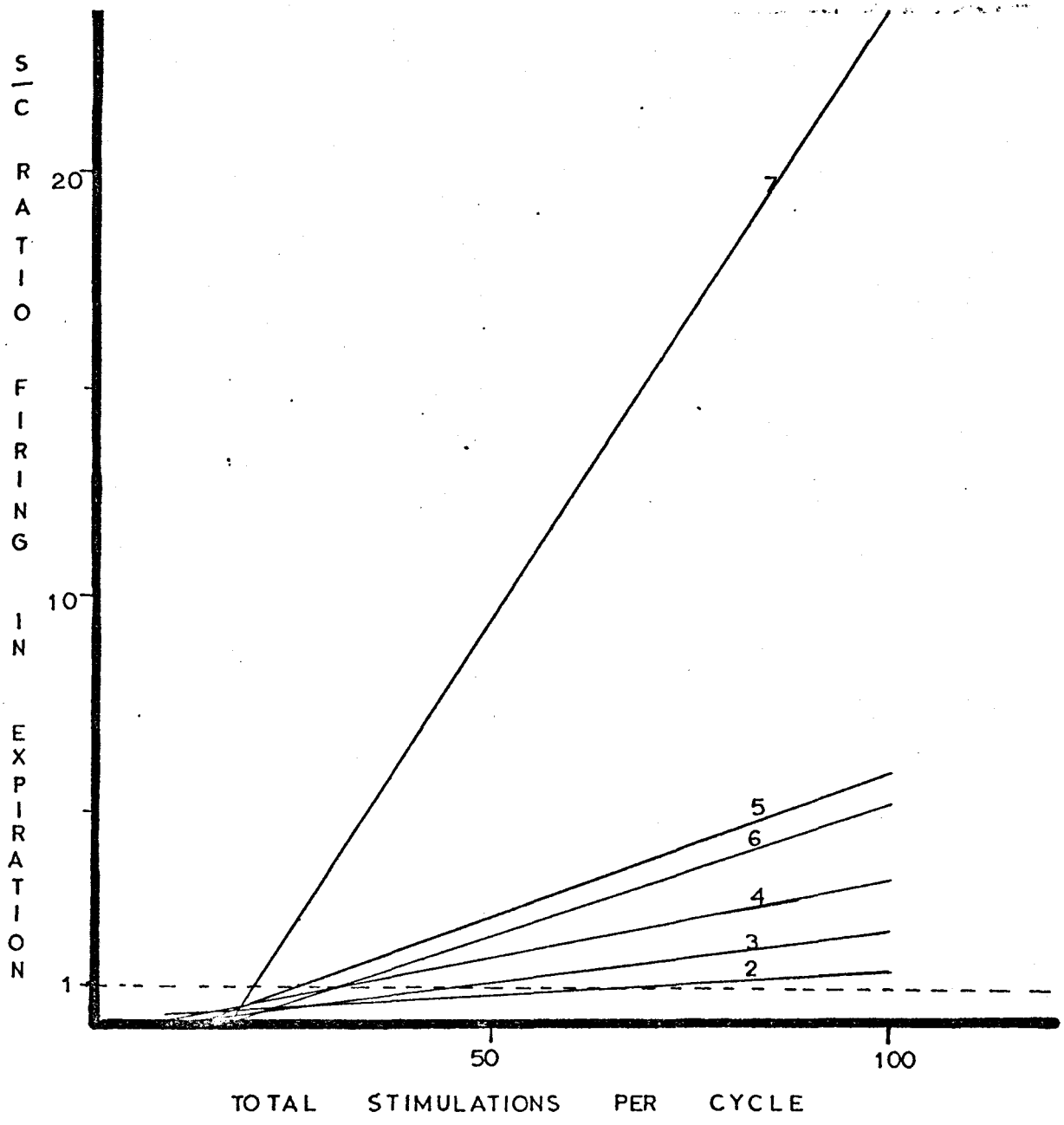


Figure 32.

Site L4: Effect of total number of stimuli per cycle on S/C ratio of phrenic discharge in expiration. All levels showed non-significant correlation coefficients after vagotomy.

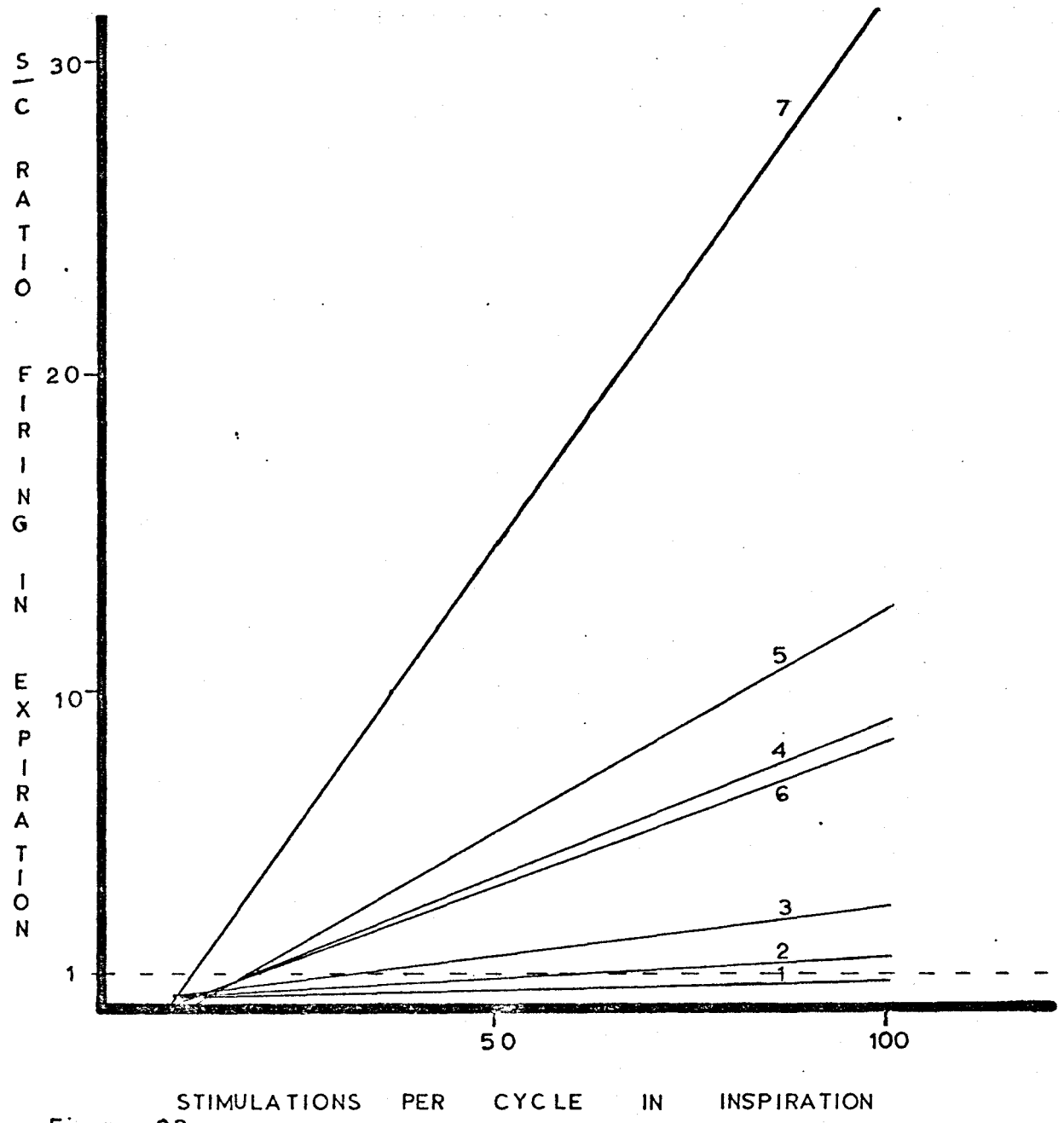


Figure 33.

Site L4: Effect of number of stimulations delivered in inspiratory phase on S/C ratio of phrenic discharge in expiration. After vagotomy, all phrenic levels showed non-significant correlation coefficients.

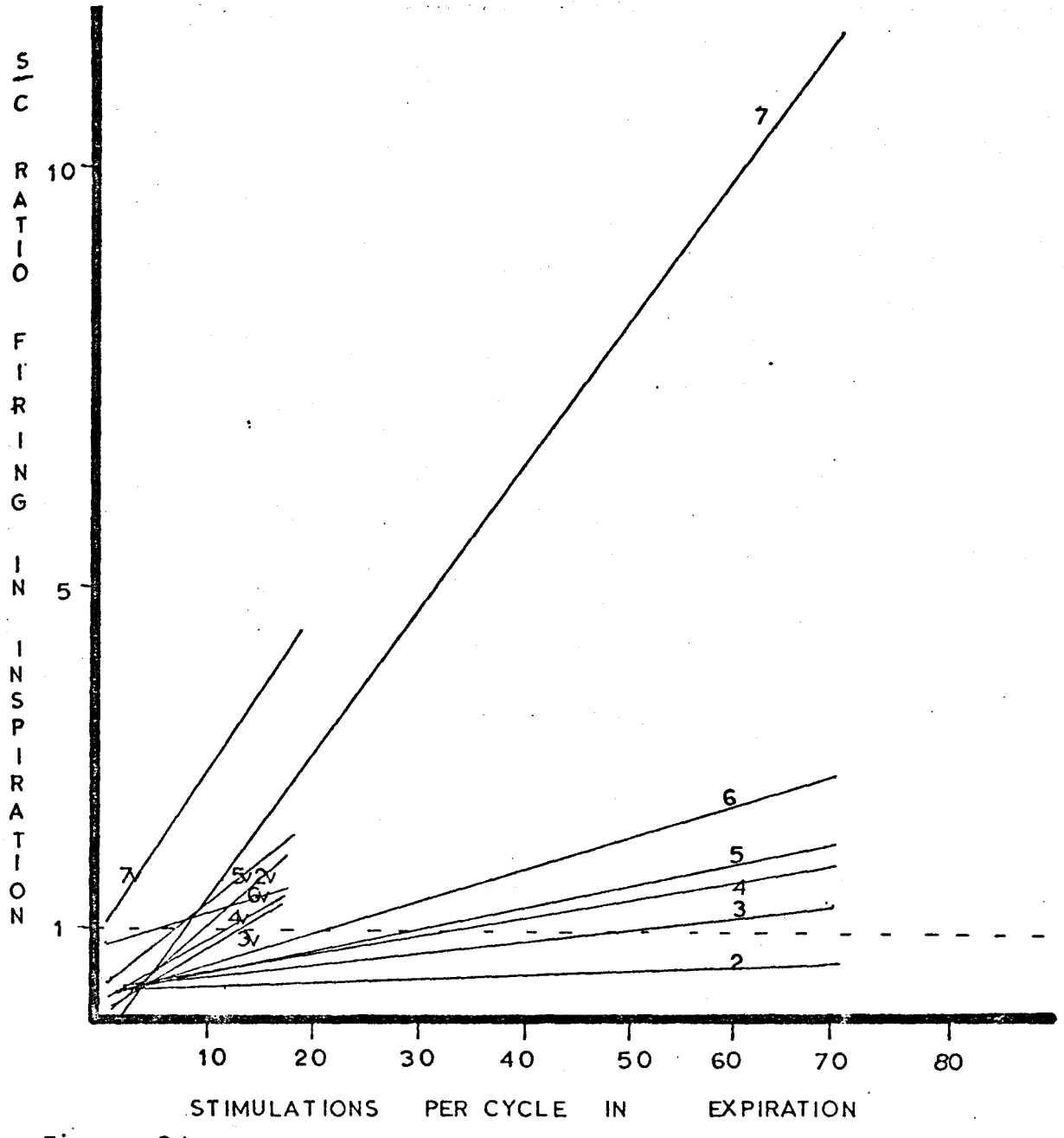


Figure 34.

Site L4: Effect of number of stimuli delivered in expiratory phase on S/C ratio of phrenic discharge in inspiration.

of the phrenic discharge with fewer stimuli. This result suggests that stimuli in expiration at this site have a facilitatory effect on phrenic discharge.

Stimuli delivered in expiration also had positive effects on discharge in expiration, as shown in Figure 35. Here, the positive gain of firing of levels 6 and 7 was converted into a negative gain following vagotomy, with the effect requiring fewer stimuli. Initially, increasing numbers of stimuli resulted in increased firing, but after vagotomy reduced firing resulted.

Finally, the results of inspiratory stimuli on inspiratory discharge are presented for site L4 (Figure 36). Here again a positive relationship between stimuli and level 7 was noted, which was no longer significant after vagotomy. Behavior of all other levels was essentially uneffected.

Compared to the results seen for sites L4, M4, and L5, the most pronounced difference in site M5 discharge was the more general increase in discharge above control resulting from hypothalamic stimulation. Figures 37-39 show this behavior for the cases in which regression slopes were significant. Vagotomy reduced the discharge slopes, but at this site, unlike all others studied, all slopes remained positive and at least one population, level 7, exhibited a ratio greater than one. Strong effects of total number of stimuli were noted on expiratory

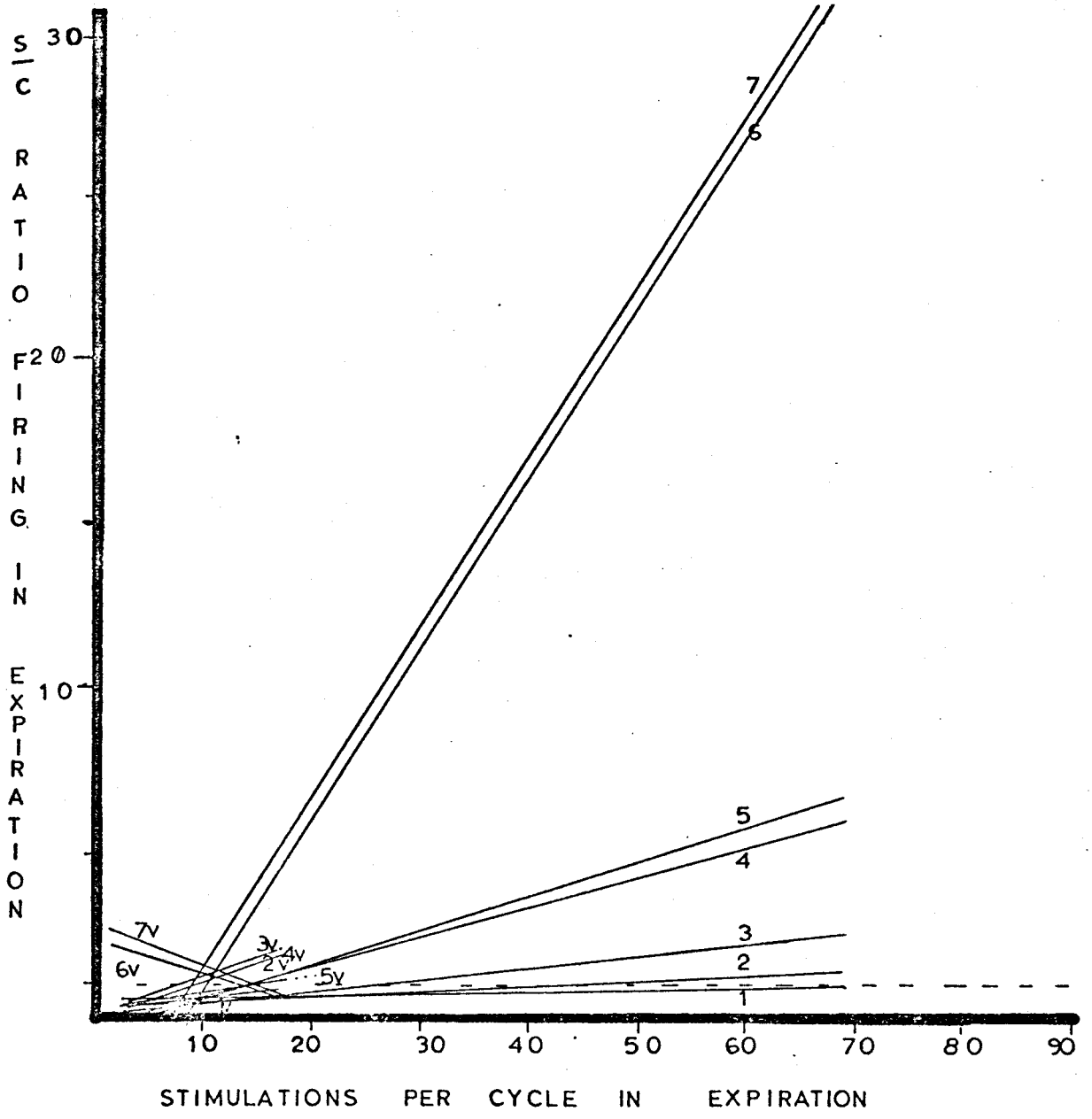


Figure 35.

Site L4: Effect of number of stimuli delivered in expiratory phase on S/C ratio of phrenic discharge in expiration.

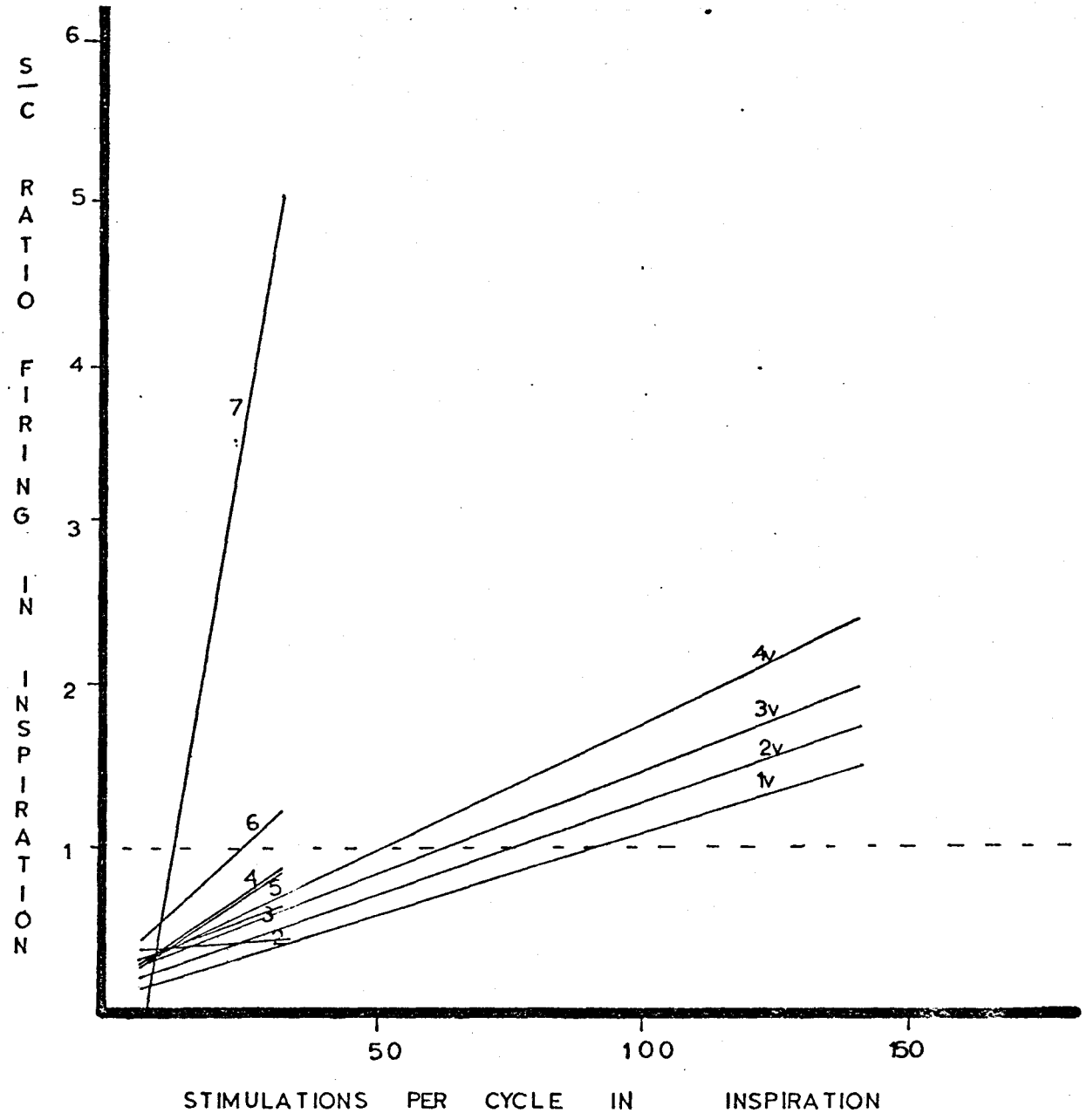


Figure 36.

Site L4: Effect of number of stimuli delivered in inspiratory phase on S/C ratio of phrenic discharge in inspiration.

discharge (Figure 37) and on inspiratory discharge (Figure 38). Expiratory stimuli yielded positive relationships for discharge slopes of inspiratory phase firing as shown in Figure 39. No significant effects were seen in the expiratory population to either inspiratory or expiratory stimuli alone.

Figure 40 shows the effects of stimulation at M5 and L4 sites on MAF. There was little change in either system gain or threshold following vagotomy at M5. Increasing stimulation produces increased MAF. However, for the L4 site after vagotomy, a low threshold for increased MAF was seen, with a negative gain. Increasing numbers of stimuli delivered in expiration decreased the MAF below maximal values, although for the range studied, the MAF was always elevated above control.

Figure 41 shows a similar relationship for MAF as a function of the total number of stimulations delivered per cycle. The same general relationships hold here, however there was a slightly increased gain for site M5 after vagotomy. MAF decreased below control only at very large values for total stimuli at L4.

F. Representative Histograms

Figures 42-47 are composites of actual computer plots photographed from the screen of a Tektronix type 561 oscilloscope modified to monitor the main computer screen. Because of resolution problems in the pictures depicting multi-level phrenic data, it has been elected to present here

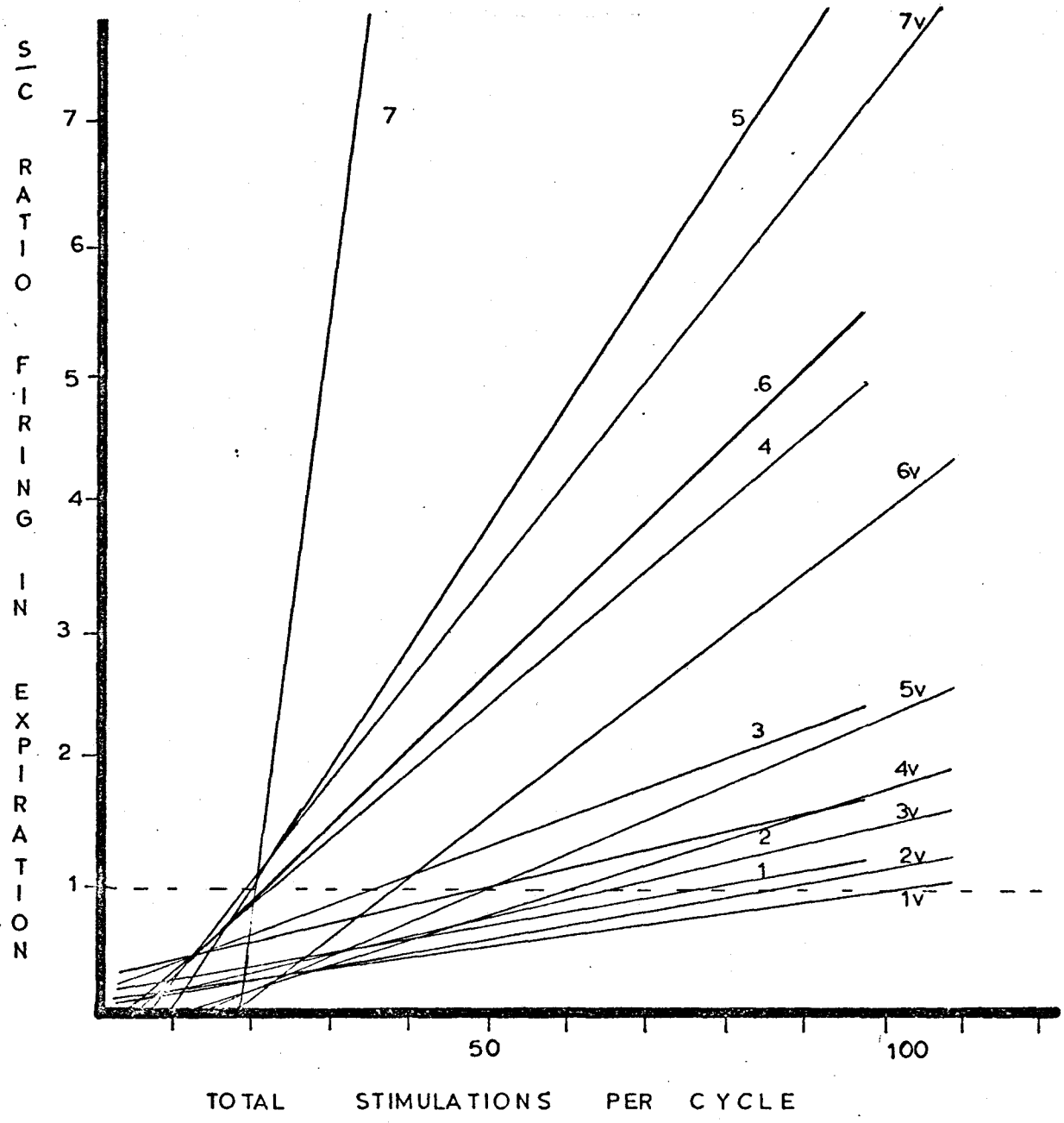


Figure 37.

Site M5: Effect of total number of stimuli delivered per cycle on S/C ratio of phrenic discharge in expiration.

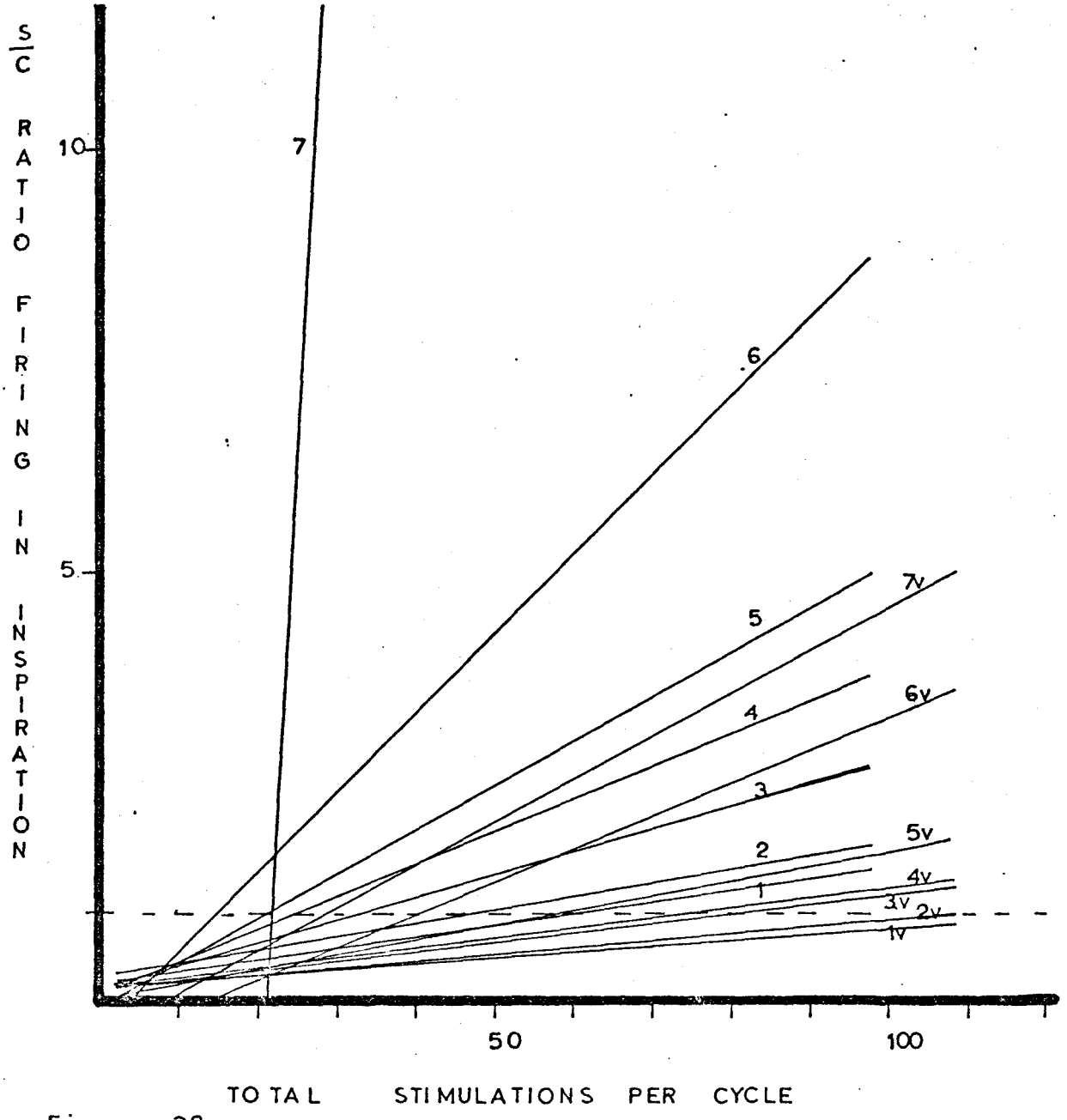


Figure 38.

Site M5: Effect of total number of stimuli delivered per cycle on S/C ratio of phrenic discharge in inspiration.

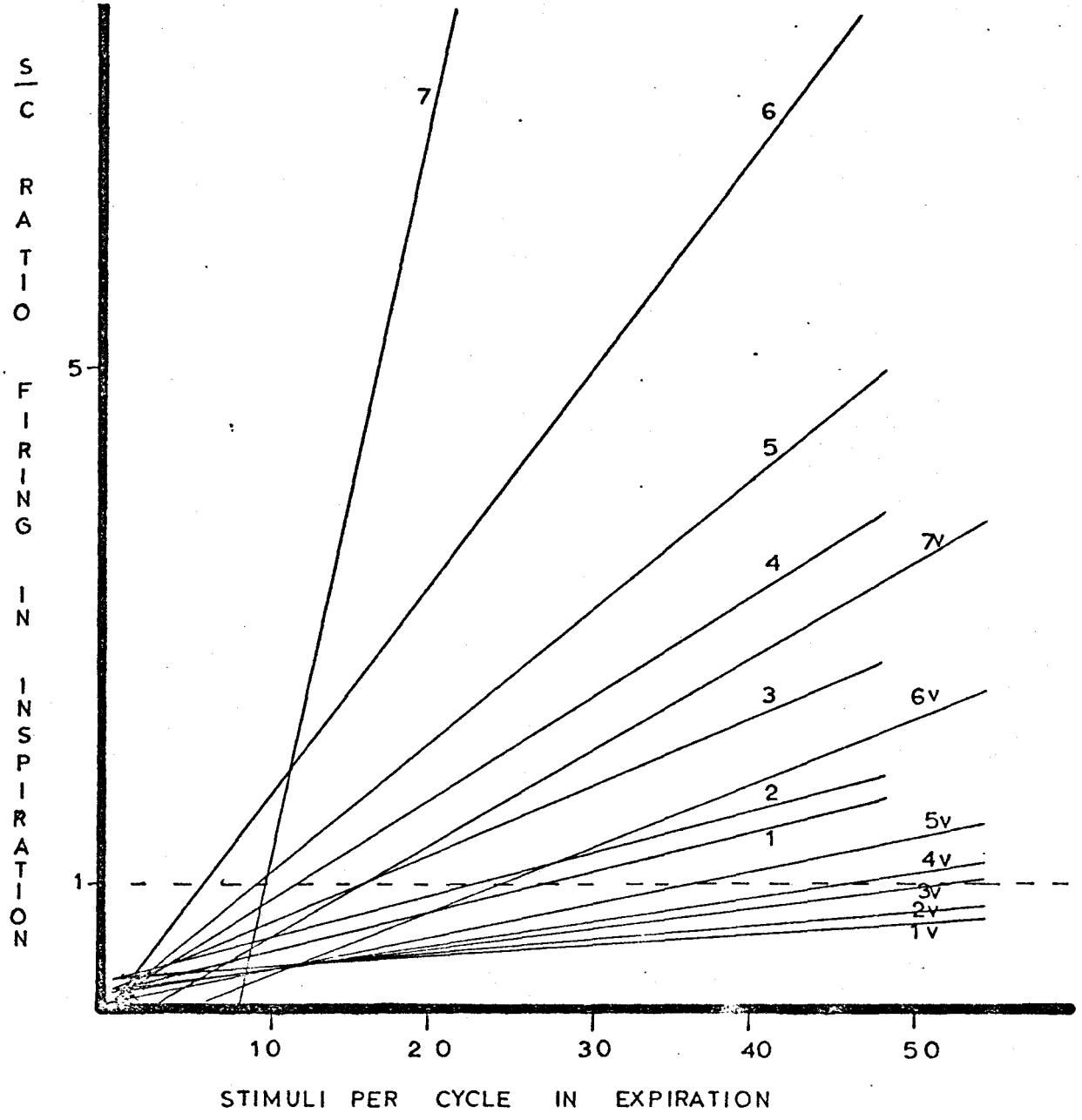


Figure 39.

Site M5: Effect of number of stimuli in expiratory phase on S/C ratio of phrenic discharge in inspiration.

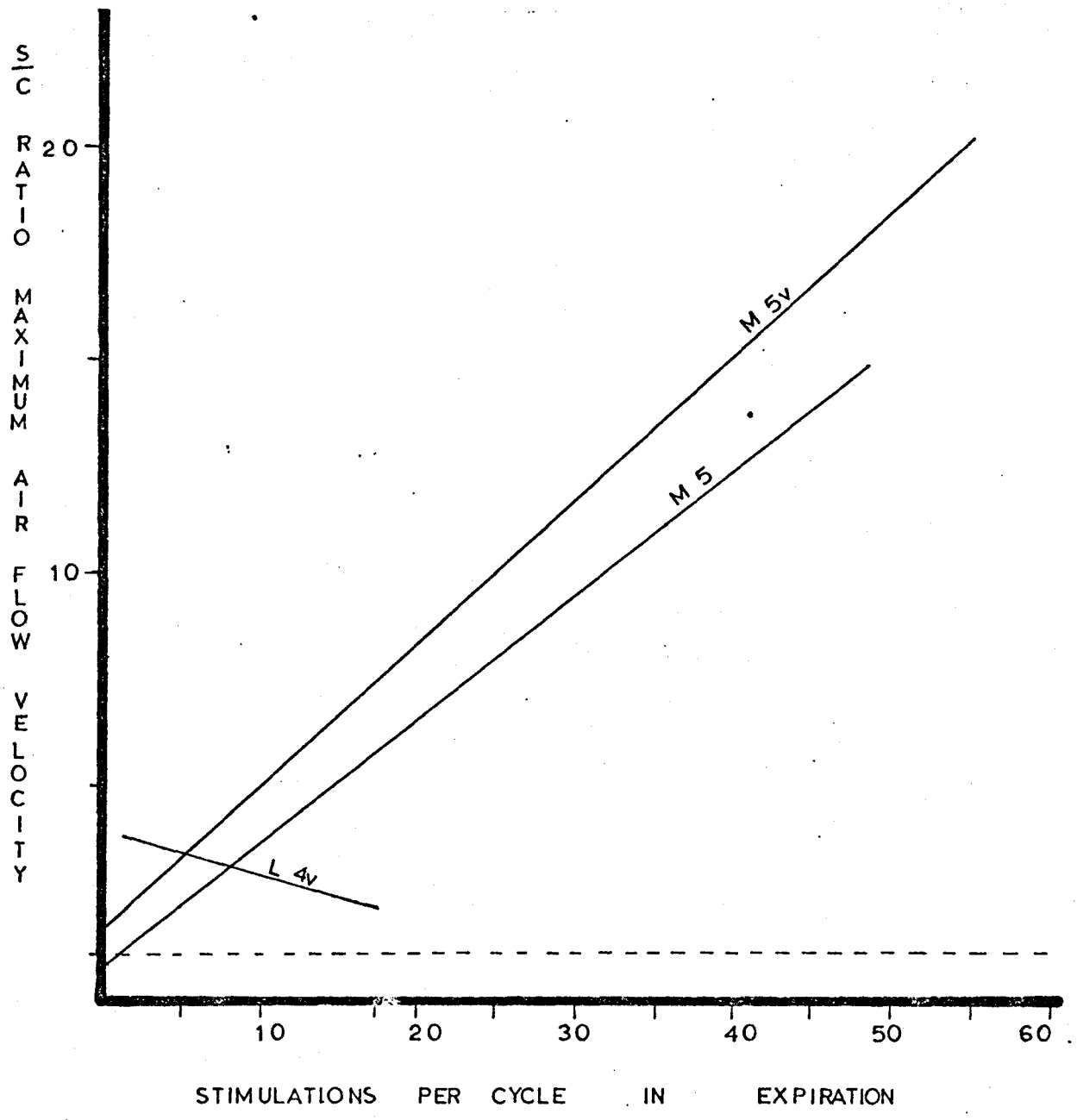


Figure 40.

Effect of number of stimuli delivered in expiratory phase on S/C ratio of maximum air flow velocity.

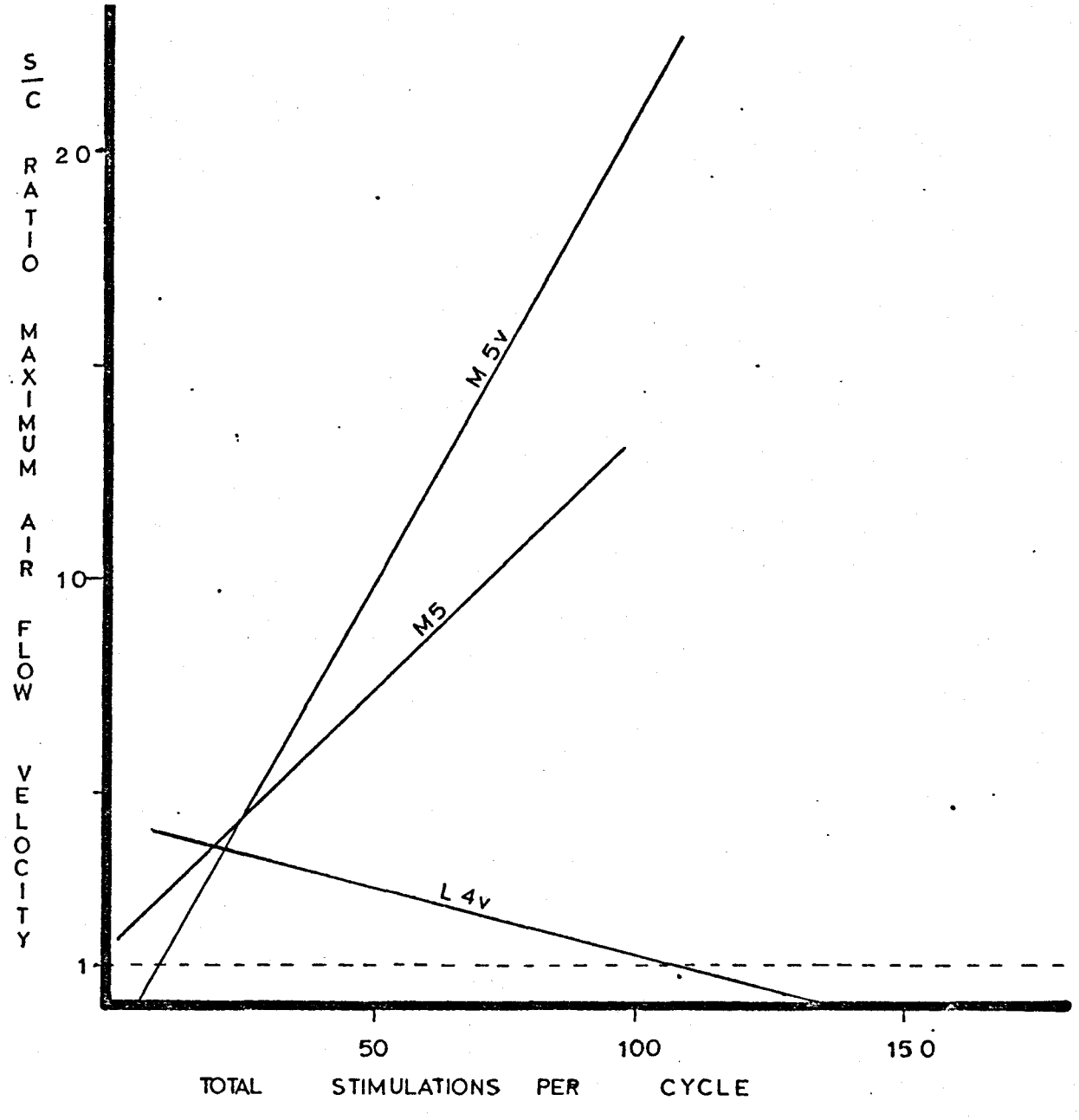


Figure 41.

Effect of total number of stimuli delivered per cycle on S/C ratio of maximum air flow velocity.

only the summated display mode for comparison. Each dot horizontally represents 10.0 milliseconds elapsed time. Each vertical dot level represents a count of 1 in that time bin. Below the horizontal axis is shown the trace representing stimuli and inspiration. Each small deflection indicates one stimulus pulse. The large deflection, which is evident for example in Figure 42, part E, indicates inspiration, which continues as long as the trace remains high. To facilitate data acquisition and storage, no particular synchronization was used with respect to the onset of inspiration. Each storage block immediately follows the preceding 1.25 second block. Where averages for several cycles are displayed, the method of synchronization as outlined in the Methods is specified.

In panels A and B of Figure 42, the effect of increasing the rate of rise in frequency of stimulation on site M4 is presented. Each is the average of four traces, with zero time at the application of the first stimulus. Panel B, with increased stimulus slope, shows an increased phrenic discharge early in inspiration and a greater total discharge. Panel C is the average of 10 successive traces with no synchronization, and is presented to display the random noise reduction effect of averaging.

Panel D shows the average discharge pattern for site M4, with zero time as the first applied stimulus in a train. The response may be compared with panel E for stimulation in

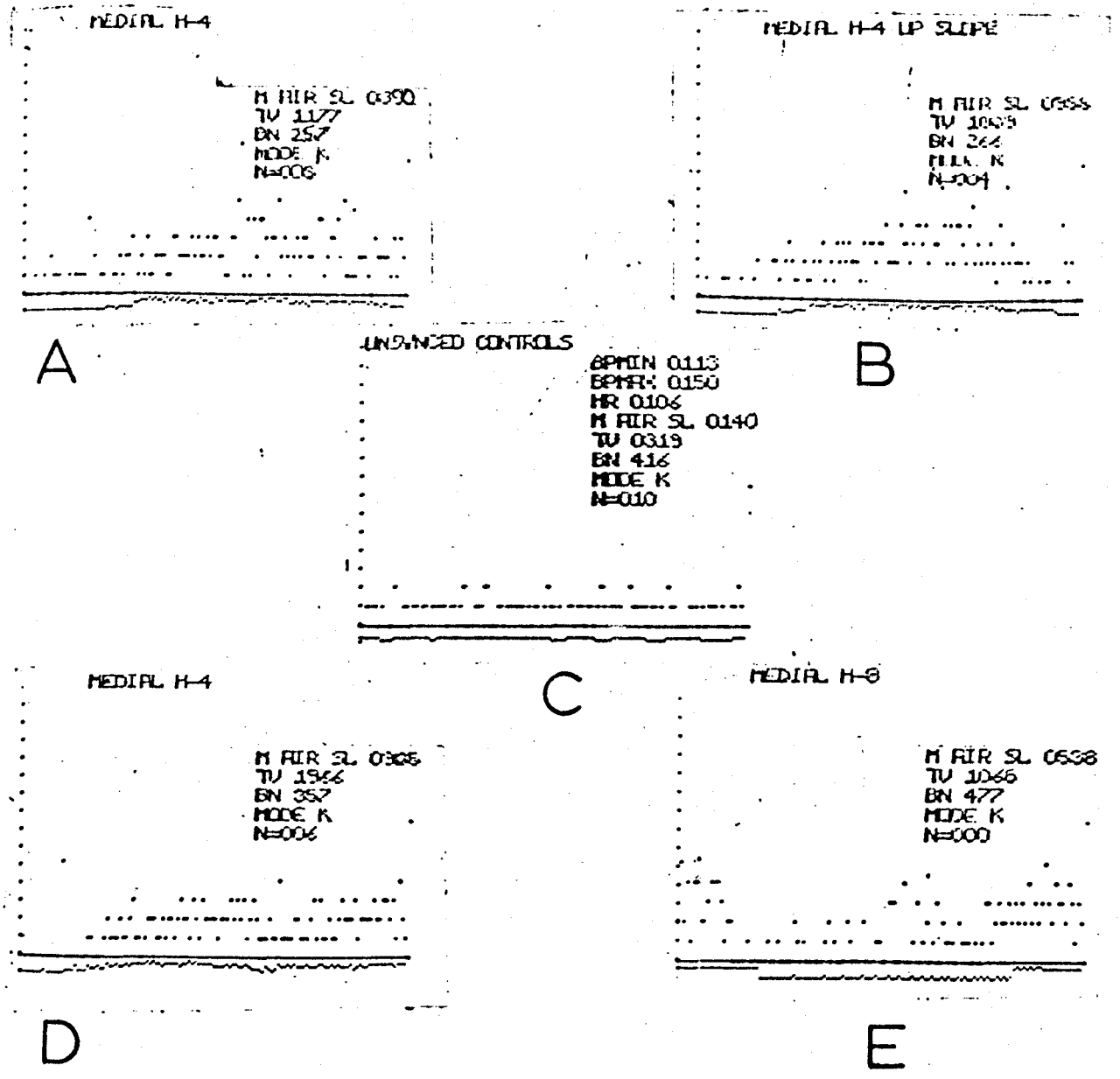


Figure 42.

Representative computer graphic displays. Panels A & B are averages of 4 and 6 cycles respectively, showing the effect of increased stimulus frequency slope on M4 site discharge. Panel C shows the results of 10 cycles averaged without synchrony, showing the degree of random noise rejection. Vertical dots represent a count of 1 in 10 msec period. See text for details.

site M8, for which no particular respiratory effect was found.

Figure 43 shows the effect of the slope of increasing stimuli on evoked respiratory cycles. The stimulation site is M5. In panel A, a control cycle is shown. Panel B shows the average response from 7 cycles at an intermediate stimulus frequency rise rate. Panel C is the average of 3 cycles at a high rate of rise, while panel D is the average of 6 cycles with a slow rate of rise. All panels except control were adjusted to zero time at the onset of the first stimulus in the train. Note that the evoked discharge was higher earlier in inspiration and attained a higher peak discharge for the fast rise in frequency. The onset of inspiration also followed the time of first stimulus more closely.

Figure 44 shows, for another animal, a control (panel A) and single cycles from a series of stimulations at the M5 and L5 sites. Little significant change in the phrenic discharge was shown in the regression slope analysis for site L5. Note that a large increase in phrenic discharge occurred for site M5 (panel B). Little change in the early phases of the L5 site discharge (panel C) was noted.

Figure 45 shows some single cycles selected from responses to stimulation at site M5. A control cycle is shown in panel A. Panel B, with zero time corresponding to the first stimulus delivered, illustrates decreased cycle time. In panel C, stimulation began during expiration, and

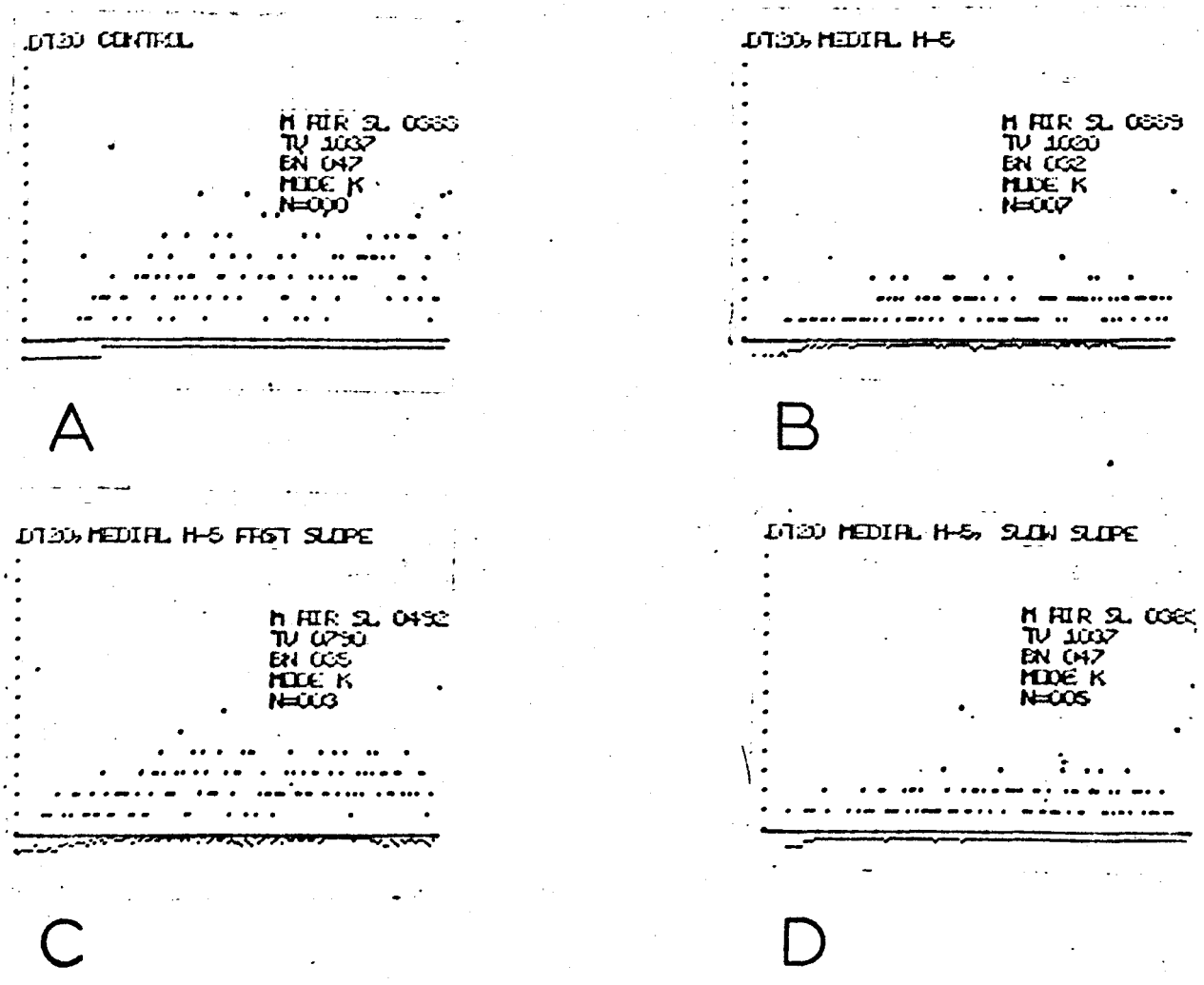


Figure 43.

Comparison of averages of several cycles taken at different rates of frequency increase (slope) for the M5 site. Panel A: control. Vertical dots indicate 1 event occurring in 10 msec. See text. Panel B: average of 7 cycles, at moderate slope. Panel C: average of 3 cycles, rapidly rising frequency slope. Panel D: slowly rising, average of 5 cycles. Except for control, time begins in each panel with the application of the first stimulus of a train.

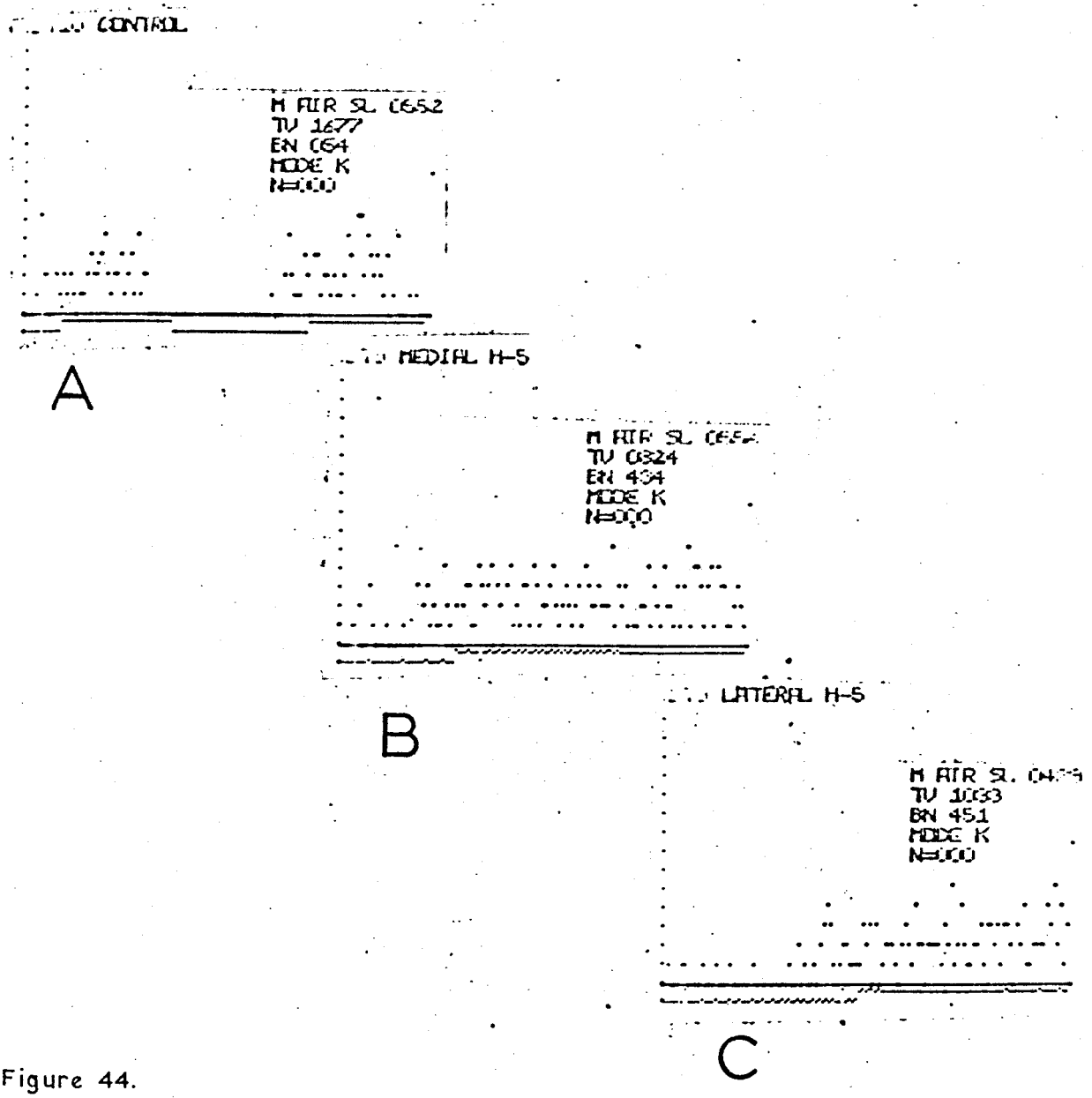


Figure 44.

Single cycles, time = 0 at start of trace indicating the first of a stimulus train (except control Panel A) for the medial (B) and lateral (C) sites. Time scale, 10 millisec per dot horizontal, 1 event per bin vertical. Sum of all firing levels indicated.

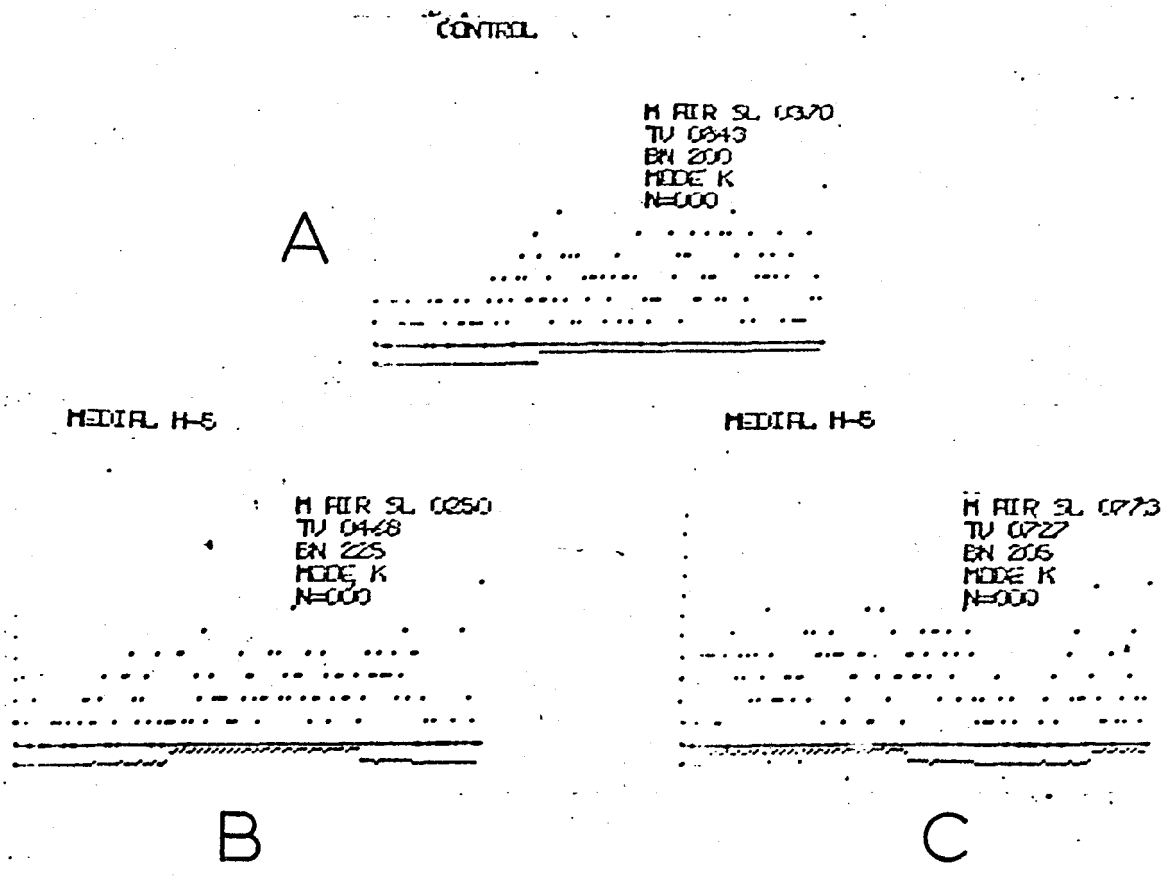
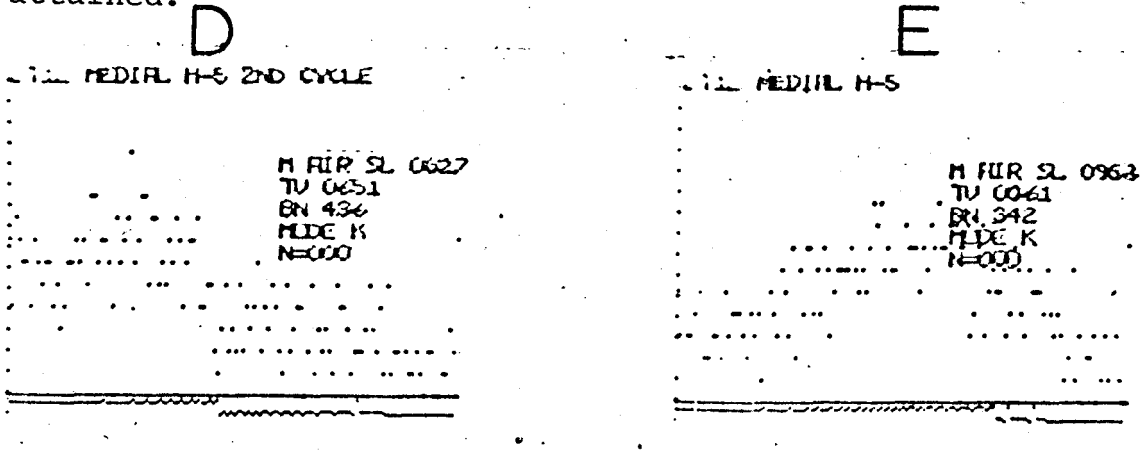


Figure 45.

Panels above indicate control and two representative cycles where synchrony has not occurred. Note the difference in discharge patterns depending on the portion of the cycle involved. Below are the second and a subsequent cycle showing how synchrony is attained.



continued through the end of inspiration into the next expiration. With little delay, the next cycle of stimuli was initiated, resulting in another inspiration. Note the very short period of relative silence in phrenic discharge. In panel E, stimulation began in inspiration, and resulted in an immediate increase in phrenic discharge, which was sustained above control levels until the end of the train of stimuli. In panel D, the same relation is shown for another cycle from another stimulation series in the same animal. Note the slow tapering off of discharge after stimulation ceased.

Figure 46 shows some responses for site M4 in terms of the early cycles after initiation of a series of trains. Panel A is the control for panels B and C. The first cycle resulting from stimulation is shown in panel B, and the third in C. Note the augmentation already occurring in B, and more pronounced in C. Panel C represents the control for another series of stimulations at the same site. Stimulation was initiated in inspiration, and resulted in a prolonged inspiration with an increased inspiratory discharge in the first cycle. The animal began respiration synchronous with the train repetition frequency on the next cycle.

Figure 47 presents a control cycle, panel A, and a series of stimulations at site L5. A variety of responses can be seen depending upon the phase of respiration in which

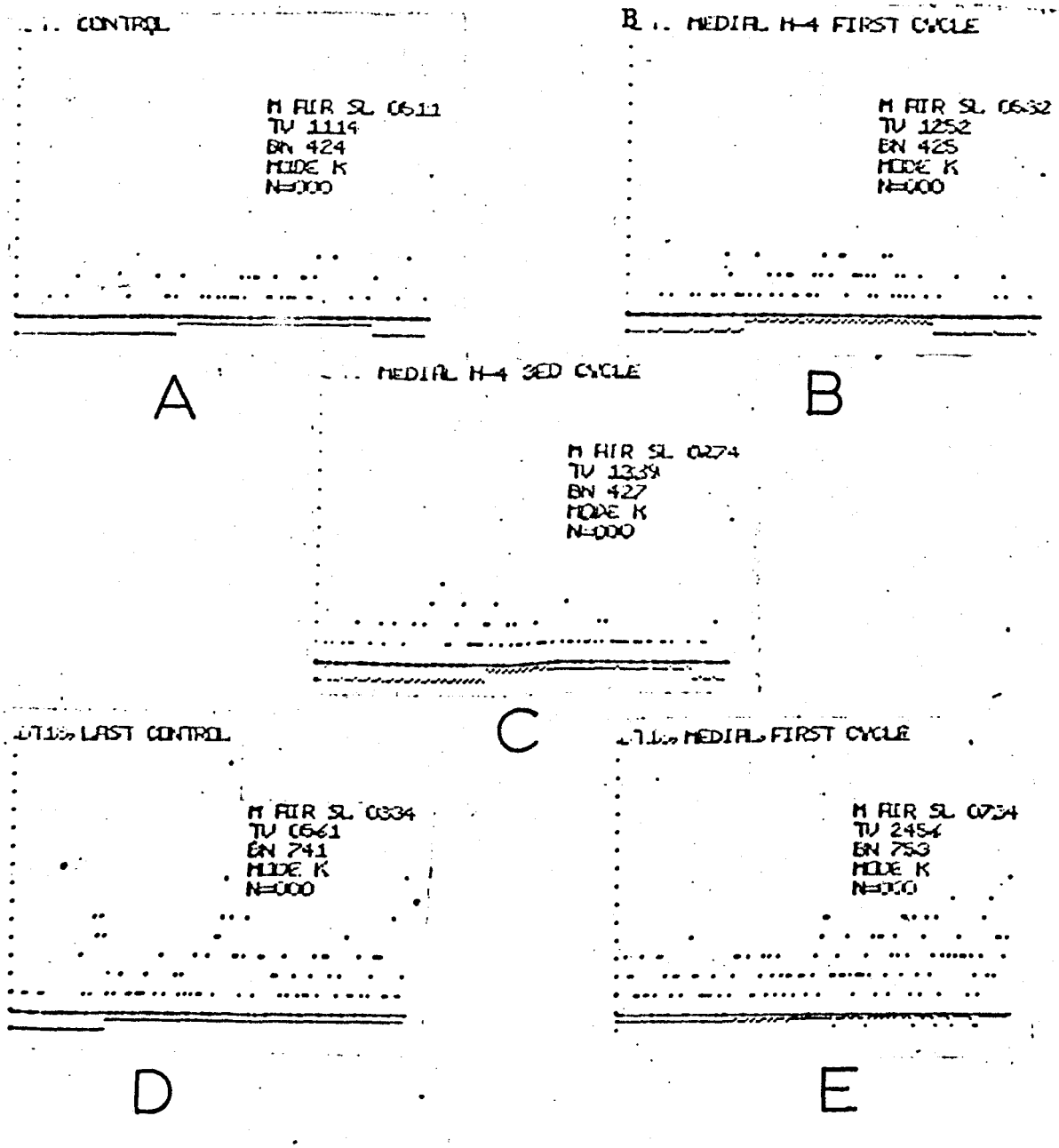


Figure 46.

Two examples of rapidly attained synchronization at respiratory response and stimulus train from M4 site. Time and sensitivity as in previous Figures.

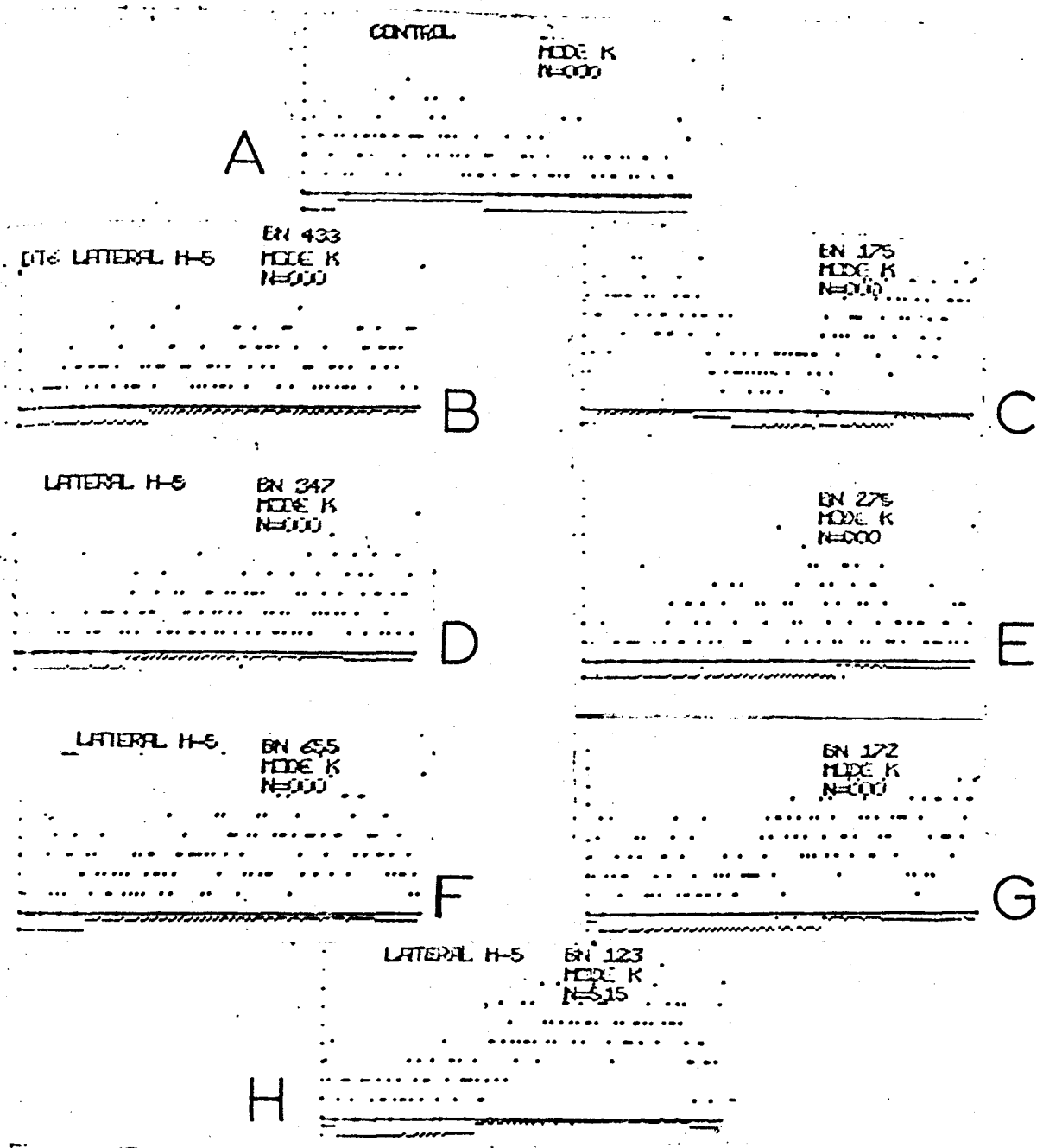


Figure 47.

Some randomly selected representative samples of single respiratory cycles resulting from stimulation of site L5. Time zero represented by first stimulus of the train, with bin width 10 msec. Vertical scale 1 count per division per bin total.

the train began. The wide range here, as well as the apparent inability of the system to lock onto and track the train repetition frequency may account for the lack of significant regression noted for this site.

G. Some Special Observations on Raw Data

As might be expected, computerizing the data runs the risk of removing some of the essential "flavor" of the experiment. In this section, some interesting points extracted from the raw data are presented. These observations do not necessarily reflect the quantitative relationships presented in earlier parts of this chapter, but reflect in a qualitative manner the subjective impressions gathered during the course of the study.

Figure 48 shows perhaps the most exciting finding in the entire study. The animal had undergone the usual set of stimulations, followed by a bilateral vagotomy, and a second set of stimulations. In preparation for lesioning the primary respiratory area at the termination of the experiment, a 23 gauge needle electrode was positioned in the primary respiratory area. Spontaneous respiration ceased immediately, and the animal began to deteriorate. However, stimulation in the L4 site produced functional paced respiration over a period of about 10 minutes. Removing the stimulus resulted in immediate cessation of respiration and again deterioration. Tactile, auditory, and pain stimuli were ineffective. After repeating this sequence

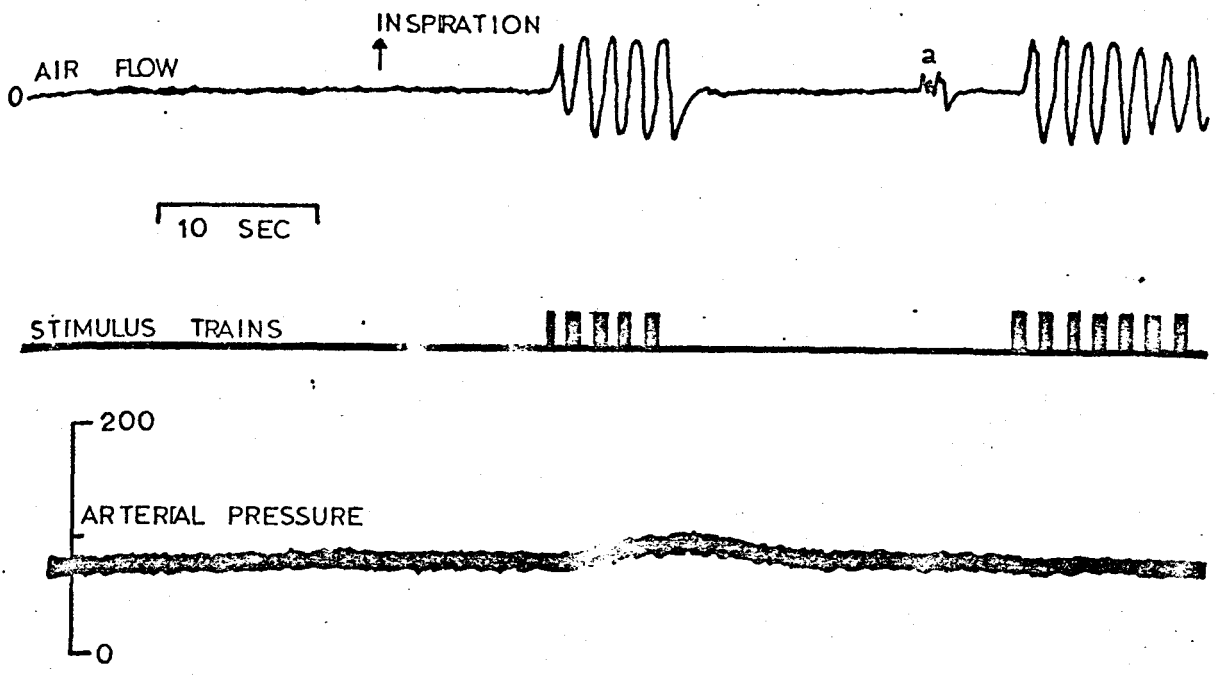


Figure 48.

Reproduction of a high speed oscillographic tracing obtained from an animal in which insertion of the brainstem lesioning electrode produced complete cessation of respiration. Stimuli, represented by the stimulus mark deflections in the "S" trace, were delivered to the hypothalamus at coordinates A-12, L1, H-4. The few stimuli delivered when the train generator was first turned on resulted in a small breath. The four succeeding cycles of stimulus trains resulted in 4 respiratory cycles. The stimulus was turned off, 25 seconds elapsed, and the stimulus repeated. The animal again followed. The small "glitch" in the right portion of the Figure(a), before the second set of paced respirations is not of respiratory origin, but represents the excited investigator bumping into the table. Note also the small rise in aortic pressure. After several repetitions of stimulus application, the stimulus was turned off, and the animal tested to see if spontaneous respiration was merely suppressed, and might restart. The animal expired in less than 5 minutes.

several times, the record shown in Figure 48 was obtained. Because the possibility existed that only a transient apnea had occurred, the animal was finally allowed to expire, indicating that only the hypothalamic pathway remained active, but presumably was suppressed by the anesthesia. Respiration was functional, the amplitude of the air flow velocity tracing approximating that of control before cessation of spontaneous respiration. Respiratory rate definitely followed the rate or train delivery. Note for instance (Figure 47) where only part of the first stimulus cycle was delivered, resulting in a smaller respiratory amplitude.

In Figure 49, the effect of varying pulse duration on the evoked response is shown. Longer duration pulses result in a greater amplitude of the air flow velocity trace. When the duration is decreased by a factor of 10, the animal remains paced above inherent rate, but the peak amplitude is reduced. Note the rapid return to control after stimulation. Also note that the change in aortic blood pressure does not preceed the change in respiratory pattern.

In Figure 50, the effect of train repetition rate on respiratory rate is illustrated. Here, the animal was breathing spontaneously at 38 per minute. Stimulation began at the mark at 42 per minute, and the animal tracked the stimulus trains. At the arrow stimulus rate was increased to 60 per minute, but the animal did not track, shifting instead to a rate of 30 per minute. Such results were seen often when

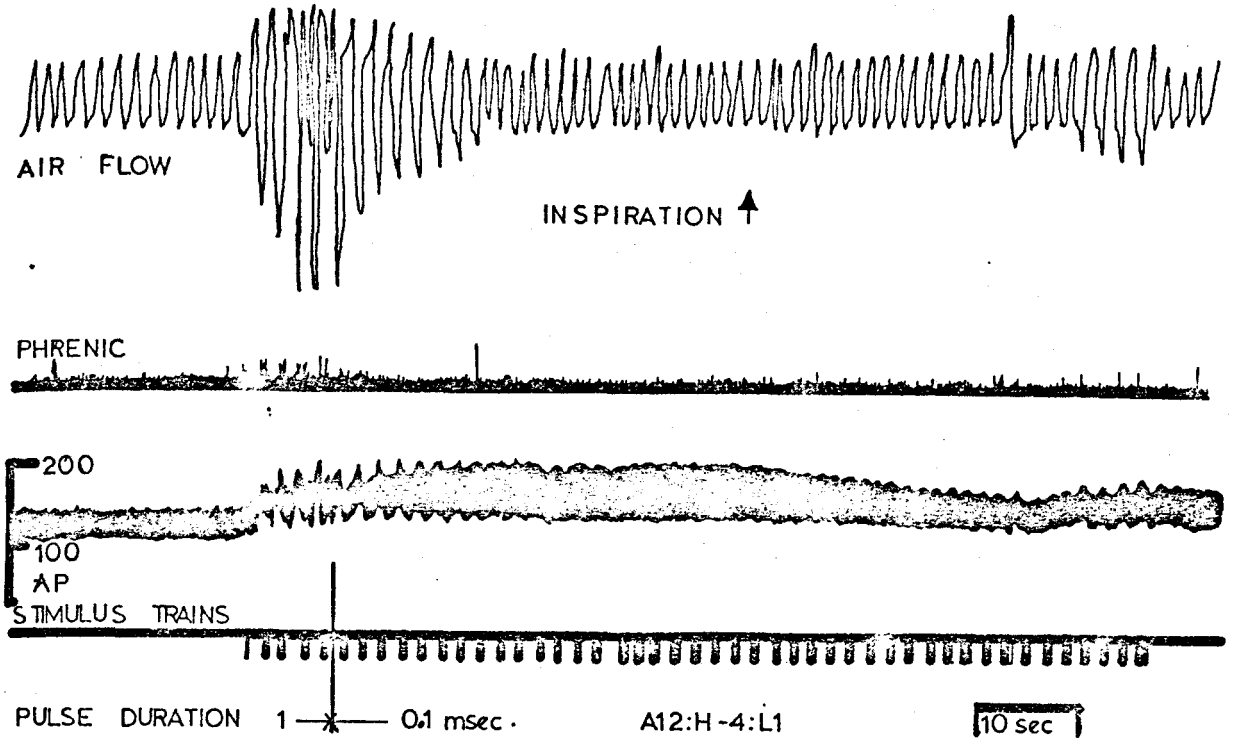


Figure 49.

Effects of two different durations on inherent respiratory rate evoked by stimulation in the L4 site. Four trains are delivered with 1 msec duration per pulse, followed by a series of pulses at 0.1 msec. A black bar denotes the change from 1 to 0.1 msec duration. Note amplitude follows closely. Note also the lack of a definite "off" response on the left of the slide, when the stimulus is turned off. The animal returns rapidly to control rate and depth. Phrenic discharge also follows. Also note the lack of correlation between aortic blood pressure and the respiratory air flow trace.

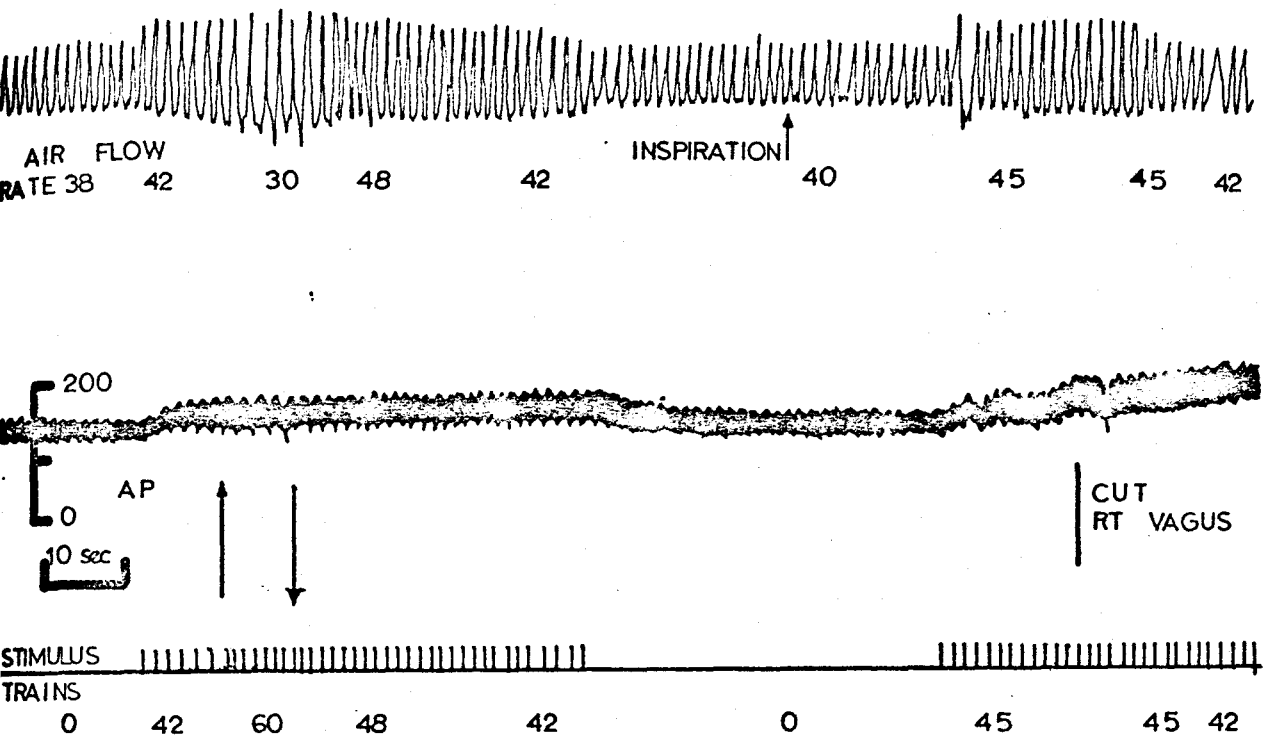


Figure 50.

Effect of stimulus frequency on respiratory rate. In this Figure, the animal is breathing spontaneously at a frequency of 38 per minute. Stimuli are instituted at the mark, with a train rate of 42 per minute, which the animal follows. At the first arrow, the train frequency is increased from 42 to 60 per minute. The animal fails to follow, and decreases his respiratory frequency to 30 per minute, i.e. 2:1. At the second arrow, the frequency is reduced to 48 per minute, which the animal follows. A control period follows, with respiration returning to 40 per minute. Stimuli are again reinstituted, and the right vagus transected at the black bar. Peak air flow velocity decreases, but no change in rate is noted. The phrenic activity also follows hypothalamic stimulation.

too high a pacing rate was attempted. Reducing the frequency to 48 per minute at the second arrow resulted in tracking again at 48 per minute. Upon cessation of the stimulation, the animal returned rapidly to a rate of 40. In the far right portion of the Figure, stimulation was again instituted, and the right vagus transected at the black bar. Note that rate did not change, but the amplitude decreased.

Some single cycles and the effect of change in stimulus parameters are shown in Figure 51. The effects of train duration, rate of rise of the stimulus frequency within the train, and the number of stimuli in each train are detailed. Comparative maximum air slopes measured in the more conventional manner by casting a line on a fast trace are shown in this Figure also.

In an attempt to localize the pathways involved, sections were taken following electrolytic lesions in the lower brainstem. Although massive RF lesions (30-50 milliamperes for 15 seconds, repeated as many as five times) were applied to the brainstem at posterior coordinate P10, no lesion was fully effective in blocking the respiratory response. Figure 52 is a composite tracing of the lesions and the obvious areas of destruction from each experiment. These areas were defined as those with totally disrupted tissue, bordered with darkly staining areas (Kluver and Barrera stain). Questionable areas have been omitted. In all cases, respiratory responses could be obtained from hypothalamic stimulation.

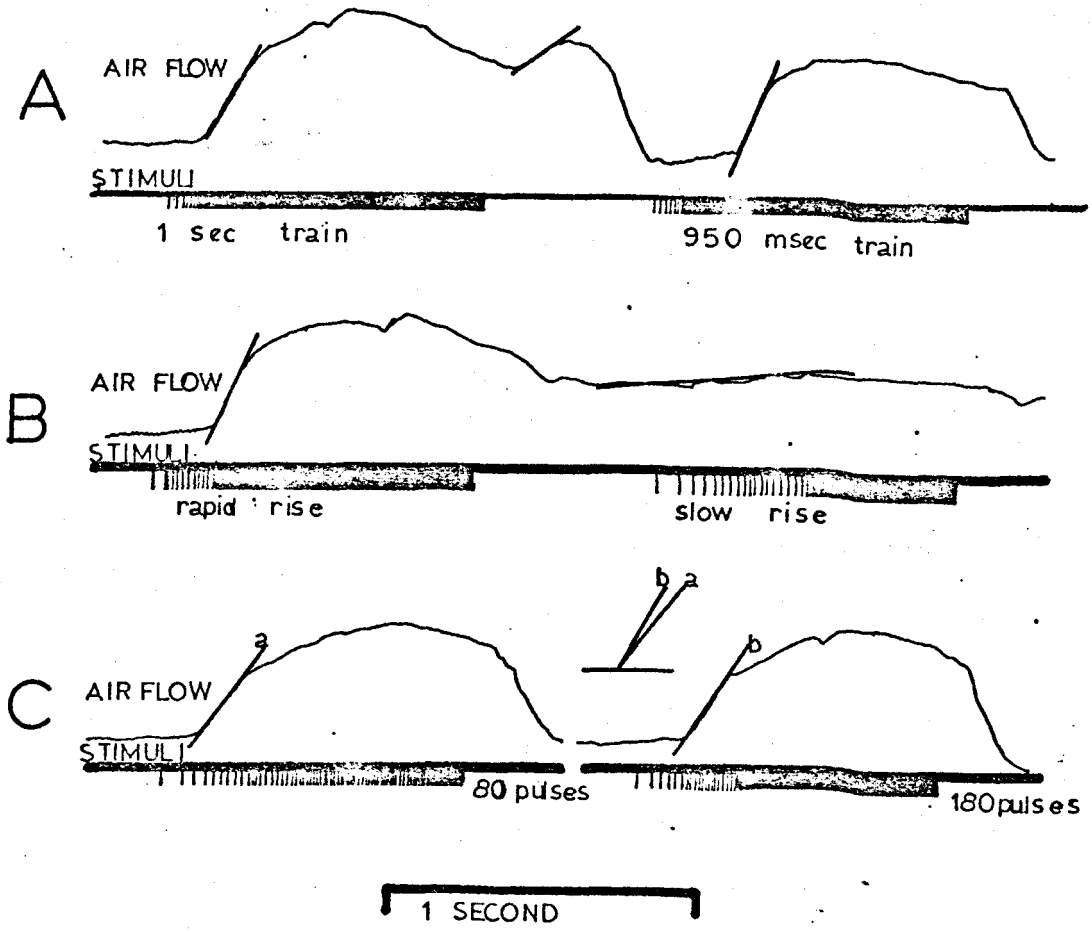


Figure 51.

In this Figure, the effects of number of stimuli delivered per cycle are shown. Air flow patterns are shown for a series of respiratory cycles evoked from a stimulus train delivered to the hypothalamus. In panel A, the left cycle was obtained from a train 1 sec long, while that on the right was from the same signal truncated to 950 msec. Note in the 1 per second stimulation, expiration was interrupted and a new inspiration evoked before expiration was complete. In panel B, a rapidly increasing stimulus frequency within the train is shown on the left, while on the right a very slow rising stimulus frequency was used. Note the profound differences in air flow velocity. In panel C, the rate of stimulus presentation is varied with voltage, pulse duration, and total train duration held constant. On the left, an intermediate log rate of frequency increase is used resulting in a total of 80 pulses delivered during the train. On the right, a faster rate of frequency rise is used, resulting in 180 pulses in the same length of train. One respiratory cycle has been deleted between the right and left portions of the Figure. Comparative maximum initial slopes are shown in the center of panel C.

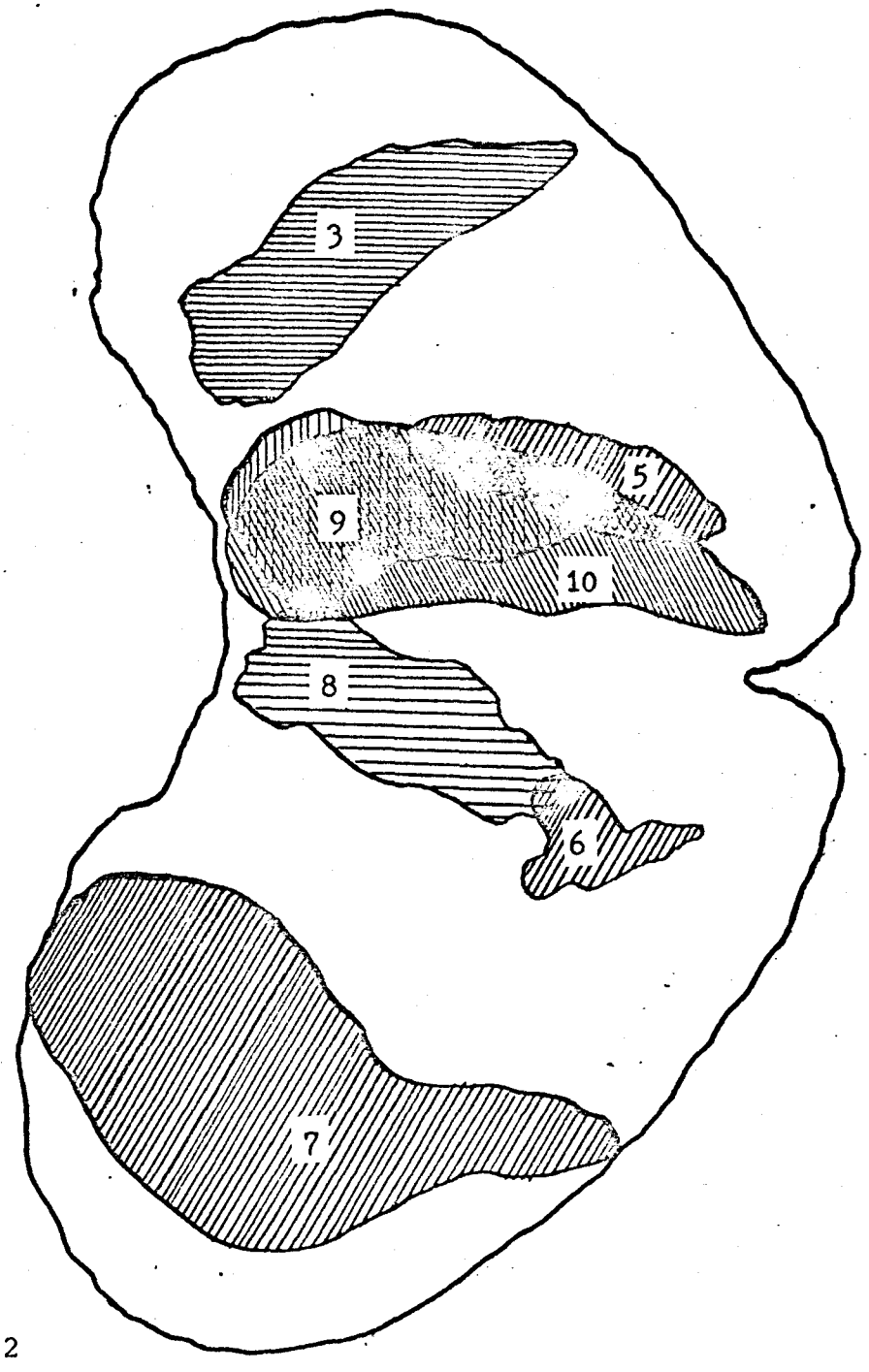


Figure 52

Composite histological reconstruction of areas disrupted by RF lesion which failed to interrupt the hypothalamic evoked response. This is a section through the caudal medulla, 5 mm rostral to the obex.

These experiments only begin to delineate where the pathways mediating these respiratory responses might lie. Further experiments are planned to investigate this promising lead.

Because some controversy exists regarding the relationship between tidal volume and phrenic nerve activity, an estimate was made which would the approximate "integrated phrenic activity." This estimate was obtained by weighting each average level discharge by its amplitude factor. For example, level 1 was multiplied by 12, which is 14 octal, the value used in the original computer program, and added to level 2 multiplied by 24, etc. The average values thus obtained were plotted against the average tidal volume in Figure 53. It can be seen that the proposed linear relationship between tidal volume and integrated discharge did not hold under the stimulus conditions of this experiment. Both L4 and M4 showed decreases in phrenic activity below control values at .5 to .7 of the control tidal volume. Vagotomy further reduced this correspondence at these sites. Site L5 showed an increase of about three times in phrenic activity at a time when the tidal volume was only increased by about 1.4. Vagotomy reduced the average response to one of little change from control in either the tidal volume or phrenic discharge. Site M5 showed an increased tidal volume at decreased phrenic discharge levels, and actually showed an increase in total discharge after vagotomy at approximately the same tidal volume.

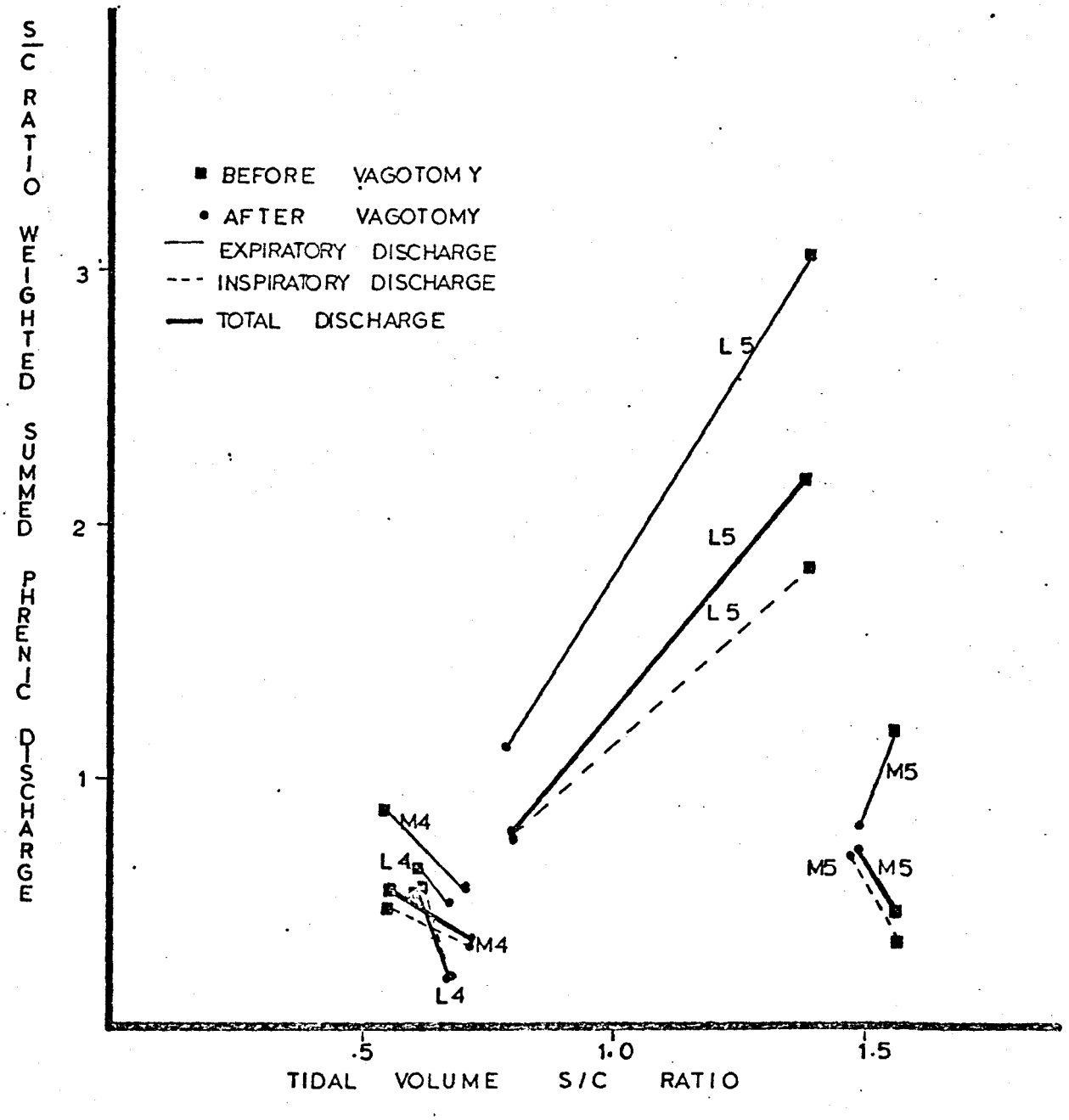


Figure 53.

Comparison of the S/C ratio of tidal volume and the weighted summed phrenic discharge activity. Squares denote mean values before vagotomy, dots after. See text for details.

There appear to be three populations represented on this basis. Site M5 showed reduced phrenic discharge at enhanced tidal volumes. Vagotomy lessened the reduction in phrenic discharge with little effect on tidal volume. Site L5 showed moderate increases in average tidal volume with larger increases in phrenic activity. Both changes were substantially eliminated by vagotomy. Finally, both H-4 sites show, on the basis of stimulations, a reduction in tidal volume and phrenic activity, with less inhibition of tidal volume and greater inhibition of phrenic activity following vagotomy.

Chapter VI

DISCUSSION

It is obvious that hypothalamic modulation of pontomedullary respiratory mechanisms is not mediated through a simple system. No "centers" were found for modulation of any one parameter or group of parameters related to respiratory control. Rather a graded control of specific segments of the overall response was noted in moving from one stimulation area to another. The assumption is made that each of these areas contains a higher concentration of the cell bodies and/or fibres of a descending pathway specialized for a specific portion of the respiratory response. This is based on the observation that movement in any plane away from the sites studied resulted in a decreased respiratory response.

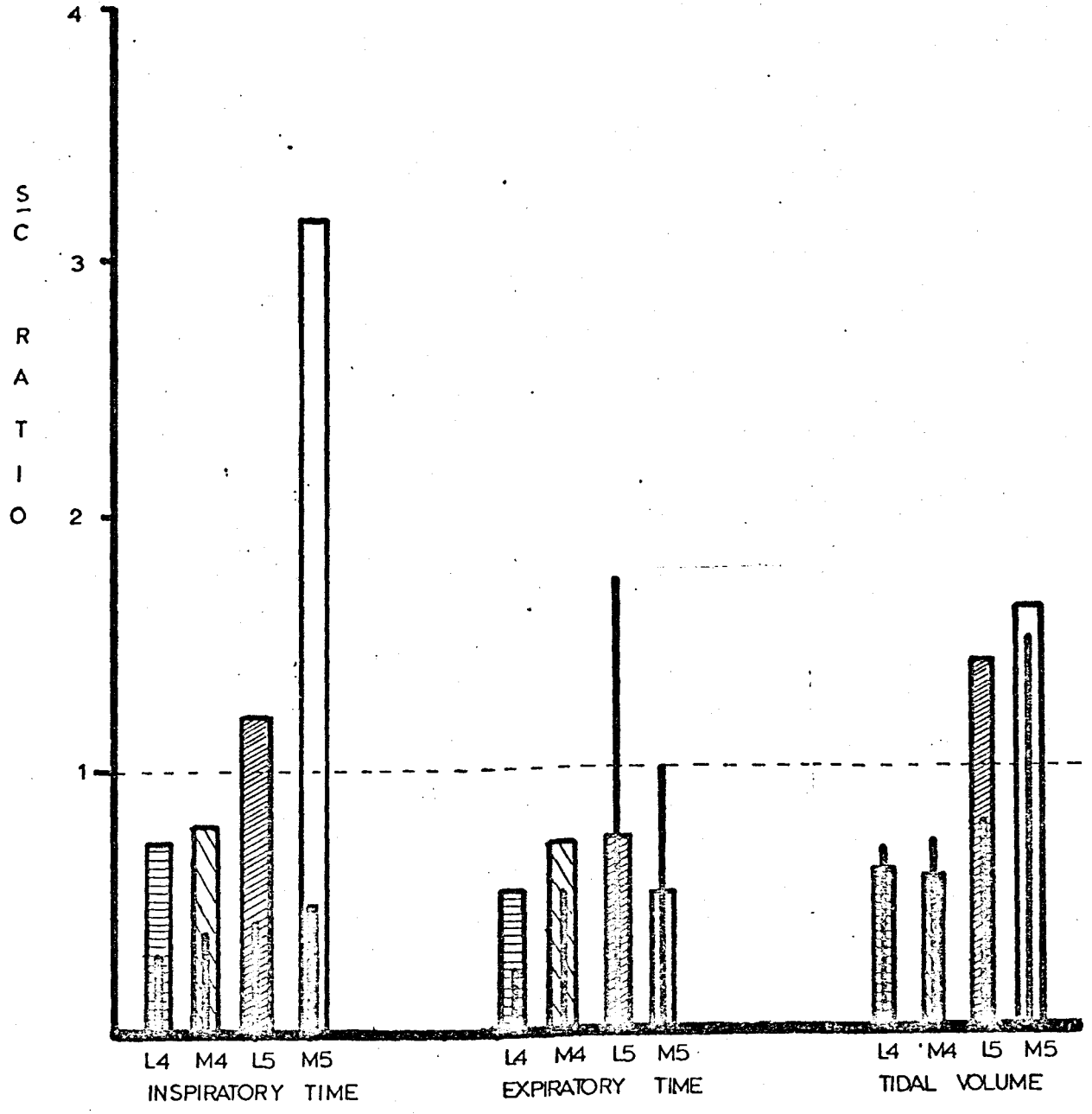
Each site studied produced different respiratory rate responses. Inspection of Table I, and Summary Graph 1 will provide some measure of the responses seen. The increase in respiratory rate evoked by stimulation in each of the designated areas of the hypothalamus was inhibited by the presence of the vagus nerves. All sites studied except M5

TABLE I

COMPARISON OF S/C RATIO OF SOME RESPIRATORY PARAMETERS DURING HYPOTHALAMIC STIMULATION

S/C RATIO vagotomy	SITE L4 before	SITE L4 after	SITE M4 before	SITE M4 after	SITE L5 before	SITE L5 after	SITE M5 before	SITE M5 after
INSP TIME	.708±.013	.302±.013	.797±.042	.384±.056	1.197±.243	.411±.033	3.152±.149	.465±.036
EXP TIME	.566±.031	.216±.020	.687±.027	.554±.033	.734±.061	1.733±.133	.534±.027	.994±.044
TIDAL VOL	.609±.020	.650±.009 **	.551±.021	.686±.021	1.410±.159	.804±.019	1.592±.076	1.485±.117##
MAF	2.464±.033	3.264±.033	2.681±.037	3.105±.040	3.114±.329	2.577±.227#	5.064±.185	6.155±.245 ***
PHRENIC NERVE DISCHARGE RATIOS								
N1 EXPIR	.313±.008	.364±.007	.410±.007	.273±.008	.685±.024	.568±.018	.503±.012	.352±.008
N1 INSPIR	.347±.008	1.173±.034	.305±.006	.248±.006	.649±.023	.570±.016 *	.602±.017	.358±.007
N2 EXPIR	.337±.010	.268±.007	.479±.008	.335±.011	.835±.031	.638±.026	.641±.015	.375±.010
N2 INSPIR	.374±.007	1.207±.033	.331±.006	.292±.008	.699±.026	.617±.014 *	.721±.020	.408±.008
N3 EXPIR	.420±.015	.302±.012	.545±.008	.383±.015	.937±.042	.756±.037	.823±.023	.468±.015
N3 INSPIR	.430±.010	1.025±.025	.385±.006	.342±.011	.780±.030	.673±.020	.965±.031	.443±.010
N4 EXPIR	.586±.035	.310±.018	.713±.014	.458±.026	1.447±.071	.815±.031	1.404±.052	.392±.014
N4 INSPIR	.498±.012	.931±.020	.431±.007	.359±.010	.790±.031	.653±.026	1.270±.044	.478±.013
N5 EXPIR	.604±.041	.205±.014	.840±.018	.343±.010	1.789±.087	.796±.038	2.041±.088	.455±.018
N5 INSPIR	.547±.014	.819±.023	.513±.008	.478±.021 **	.984±.042	.798±.038	1.557±.061	.524±.018
N6 EXPIR	1.666±.045	1.464±.089	1.093±.032	.283±.011	3.279±.239	1.207±.077	1.501±.063	.654±.028
N6 INSPIR	.588±.018	1.059±.034	.646±.013	.559±.015	1.098±.048	.898±.039	2.511±.111	.720±.028
N7 EXPIR	2.229±.226	1.674±.070***	5.682±.263	1.191±.039	12.76±.859	2.214±.111	6.783±.370	1.924±.064
N7 INSPIR	1.588±.077	2.202±.168	.625±.018	.877±.027	1.842±.134	1.403±.066	8.422±.573	1.171±.064
Ratio STIMULATION IN INSPIRATION TO EXPIRATION								
	.679±.019	.299±.006	.923±.022	.376±.007	1.196±.040	.516±.024	1.045±.026	.765±.023

NOTE: Mean values±S.E.M.. "P" values comparing before/after vagotomy≥.001 except *.005;**.05;***.01;#.2;##NS.



Solid bars after vagotomy ; wide, open bars before .

AVERAGE VALUES OF S/C RATIO FOR EACH SITE.

Summary graph 1
Figure 55

produced an acceleration in respiratory rate when stimulated. A small decrease in the average rate was noted at site M5. Vagotomy increased the magnitude of the acceleration in respiratory rate from all sites, and also rendered site M5 acceleratory. The magnitude of rate increase after vagotomy at each site was approximately 3 times that observed before vagotomy. It appears that the influence of the vagus on hypothalamic evoked respiratory rate increase was to attenuate the response by approximately a factor of three. However, it should be noted that the effect normally ascribed to vagotomy is to reduce the control respiratory rate.

The increased S/C ratio of respiratory rate after vagotomy appeared in most cases to be a function of both shortened inspiratory and expiratory times. Larger reductions in inspiratory time than in expiratory time were noted after vagotomy. The entire reduction in cycle time at site M5 can be accounted for by the shortened inspiratory time. It appears that the rate change effect of hypothalamic stimulation can be divided into two populations. Both H-4 sites were seen to increase respiration rate markedly above control, and also above that elicited from the H-5 sites. Both H-5 sites appeared to have the same relative effect on respiratory rate changes after vagotomy, although site M5 exhibited a reversal in effect when compared before and after vagotomy. The slight inhibition in rate noted before vagotomy was converted into a facilitation after vagotomy.

Inspection of Table I shows that some relationships have rather large differences in their S/C ratio dependent on the site of stimulation. For example, the average inspiratory time ratio before vagotomy shows that stimulation resulted in a decrease of 30% (L4) and 20% (M4) respectively, while at the H-5 sites an increase in inspiratory time of 20% (L5) and 215% (M5) resulted. Thus both the direction and magnitude of response were changed by moving only 1 mm in the vertical plane. After vagotomy, a uniform reduction in the inspiratory time was noted, ranging from a reduction of 54% (M5) to 70% (L4).

Expiratory time changes also show some rather wide variations depending on the site stimulated. The range of changes in expiratory time prior to vagotomy, all decreased from control, spanned 47 to 27%. After vagotomy, site M5 was essentially unchanged by stimulation, and site M4 decreased by 45%. Comparison of L4 and L5 presents a striking picture after removal of vagal modulation. L4 stimulation resulted in a reduction of 79% from control, while stimulation of the L5 site, 1 mm away, resulted in an increase of 73%. The corresponding respiratory rates following vagotomy were increased about 150% for both H-5 sites, up 236% for M4 and up 450% for L4. Prior to vagotomy, only M5 showed a decrease in rate (17%). The H-4 sites gave increases of 73-96%, the L5 site 57%.

Tidal volume ratios seem to be split into two distinct

populations prior to vagotomy. Both H-4 sites showed reductions of about 45%, while the H-5 sites showed increases in tidal volume of 40% (L5) and 58% (M5). After vagotomy, slightly smaller reductions were seen in the H-4 sites. The big change occurred at site L5, where the previous 40% increase in tidal volume was converted into a decrease of 21%. The increase at M5 was only slightly reduced to 48%.

Maximal air flow velocity (MAF) provides a measure of the peak inspiratory rate of shortening of the respiratory muscles. The average value of MAF was increased in every situation studied, with a minimum increase of 153%. The greatest increase over control was at site M5, where a 395% increase was seen before vagotomy. The effect of M5 stimulation was even more profound after vagotomy with an increase of 513%. The magnitude of the average response at other sites was much less. L4 for instance, gave a 131% increase before vagotomy and 221% after. M4 showed 165% increase before and 191% after vagotomy. Of all the sites studied, only L5 produced an attenuation of the MAF response after vagotomy (201% increase before, 153% after).

Table II lists some of the actual average values obtained in this study. Control respiratory rate averaged 6 per minute $\pm$ 3 cycles including both the before and after vagotomy measurements. The average respiratory rate during stimulation of site M4 prior to vagotomy was 77 per minute, and 91 per minute after. At site L4, 101 before and 125

TABLE II

WEIGHTED PARAMETER AVERAGES FOR SELECTED RESPIRATORY DETERMINANTS DURING HYPOTHALAMIC STIMULATION

PARAMETER (see notes)	M-4 Control	Vagotomy	L-4 Control	Vagotomy	M-5 Control	Vagotomy	L-5 Control	Vagotomy
N	40	23	20	25	26	65	14	27
Cycles	1744	986	868	1350	800	2112	887	619
TV, control	967	922	947	594	720	811	1220	816
TV, stimulated	423	469	947	341	370	365	638	546
MAF, cont	261	242	270	205	232	216	409	268
MAF, stim	637	682	626	550	662	578	514	478
Expir. time,c	2370	2490	4780	4260	2190	1430	759	980
Insp. time,c	7400	4820	8440	4750	8120	4670	1312	6150
Cycle time,c	9770	7310	13220	9010	10310	6100	2071	7130
RRate,control	6.2	8.2	4.5	6.6	5.8	9.8	3.0	8.4
Expir. time,s	369	301	287	196	459	444	661	558
Insp. time,s	415	362	304	285	741	616	435	658
Cycle time,s	783	663	591	481	1200	1060	1096	1216
RRate,stimulated	77	91	101	125	50	57	55	49
Expir stims	15	6	11	6	15	13	13	10
Inspir stims	16	17	15	22	16	19	12	22
Total stims	31	23	26	28	31	32	25	32

NOTES:

Column heading Control signifies "before vagotomy" while control or c in the parameter list denotes average value prior to stimulation. Stimulated, or s, denotes average value during stimulation session.

N denotes number of experimental sessions, Cycles signifies the total cycles studied in each instance.

TV (tidal volume) in relative units.

MAF (maximal inspiratory air flow velocity) in relative units.

All cycle times in milliseconds, respiratory rates in cycles per minute.

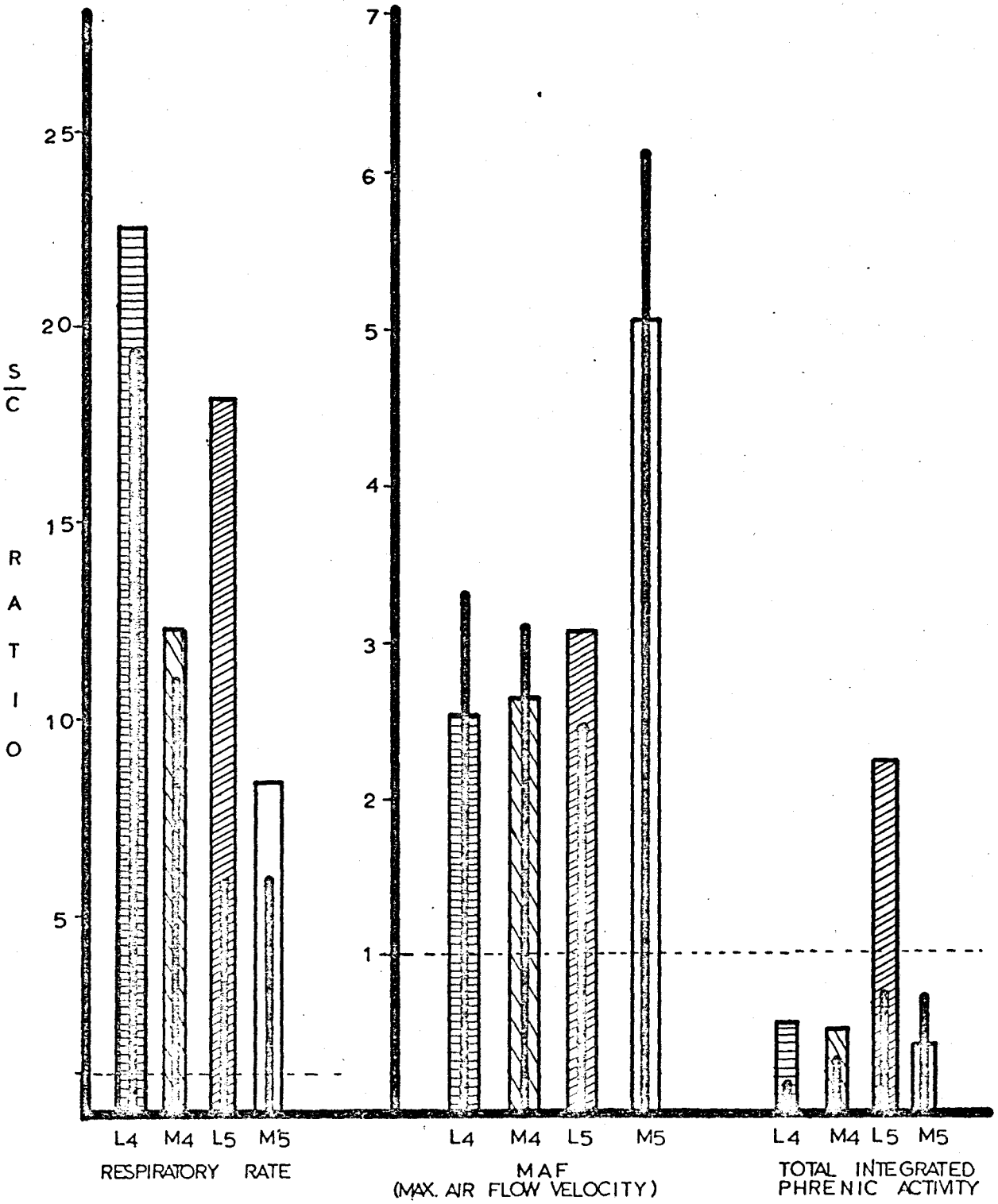
Last three parameters, above, show average number of stimulation pulses delivered per cycle in each instance, which was determined primarily by the animal response and was not predetermined.

after; M5, 50 before vagotomy, 57 after; and L5, 55 before, 49 after. These calculations are based upon the average total cycle duration for each site studied. It may readily be seen from these data that the stimulus trains delivered in the anterior hypothalamus resulted in marked increases in respiration rate. It should also be noted that the average rate obtained in each instance is weighted downward somewhat, since an attempt was made in each animal studied to maximize the stimulus train rate only as high as the animal could be paced. In addition, each experiment and therefore the average values reported in Table II, reflect points taken at below the optimal rates which were used to construct the regressions presented earlier. Maximal respiration rates were sometimes noted with train repetition rates of 250 milliseconds, which would correspond to 240 respiratory cycles per minute. Using the usual definition of panting, these animals were clearly respiring at rates well above panting threshold. Yet the response could hardly be attributed to thermal heating, as the animals returned to approximately control respiratory rate within one or two cycles. That the response was functional is shown by experiments in which the pacing was continued for as long as 1/2 hour with no apparent diminution of response, and again the animal returned quickly to control rate and depth.

Some of the data in this dissertation indicate a lack of correlation between phrenic nerve activity and tidal

volume. Total integrated phrenic nerve activity and tidal volume are well correlated in the quietly breathing cat, but Eldridge (Am. J. Physiol. 221: 535-543, 1971) has recently shown that the relationship is not consistent over a wide range of tidal volumes. Our experiments indicate that the relationship between these two parameters does not hold during hypothalamic stimulation. This raises the possibility that alterations in tidal volume and maximal air flow velocity might be mediated by pathways other than the phrenic nerve. Presumably these pathways involve the intercostal and accessory respiratory muscles. The nature of these pathways remains an open question.

Table I and Summary Graph 2 depict the changes noted for tidal volume. Again, two populations are readily noted. The H-4 stimulations reduced tidal volume below control, even though rate was increased. Vagotomy appeared to lessen the reduction in tidal volume at the H-4 sites, suggesting that the area upon which these cells impinge is inhibited both by the hypothalamus and the vagus. The H-5 populations, on the other hand, showed reduced increases in tidal volume after vagotomy. The implication here is that the absence of vagally mediated reflexes reduced the increase in tidal volume. Part of the difference noted between the two populations is undoubtedly a consequence of the markedly different rate changes associated with each site. Large increases in rate might be expected to reduce



Solid bars after vagotomy ; wide, open bars before . AVERAGE RATIOS.

Summary graph 2.
Figure 56

the tidal volume. Those sites with the largest increases in rate (H-4) also exhibited decreased tidal volume as a result of stimulation. The differences in direction of the tidal volume change after vagotomy cannot be explained on this basis, however. All sites exhibited proportionally greater increases in rate following vagotomy. The decreased tidal volume at the H-5 sites must therefore be a result of different activation pathways from those involved in the H-4 response.

Maximum air flow velocity in inspiration (MAF) showed striking differences. Of all sites tested, only the L4 area was inhibitory to MAF, an effect which was reversed after vagotomy. Both medial sites were facilitated after vagotomy, while the magnitude of response after vagotomy was reduced at L5 and facilitated as well as reversed at site L4. There seemed to be little relation between the MAF and changes in tidal volume, except that the largest increase in tidal volume occurred at sites which also showed the largest increase in MAF. The M5 population seemed to exhibit responses of a different nature insofar as MAF is concerned. The pre-vagotomy S/C ratio was almost twice as large as that at all other sites, and increased further after vagotomy. It might seem tempting at this point to attribute changes in MAF primarily to site M5, changes in tidal volume to both M5 and L5, and changes in rate primarily to sites L4 and M4. Since each of these factors are obviously interrelated, any

such interpretation must remain highly speculative.

The bulk of the MAF response appeared to be related to the number of stimuli delivered per cycle during expiration (cf. Figure 40). An increased sensitivity at site M5 was seen after vagotomy, although the slope remained the same. A negative regression slope was seen for the vagotomized preparation stimulated at site L4, indicating that increasing the number of stimuli had an inhibitory effect on the increase in MAF. Comparison of Figures 40 and 41 shows that the response was primarily related to total stimulations, specifically to the number delivered in expiration.

Some insight into the operation of the system can be gained from relating the changes which would take place with a given number of stimuli delivered to each site. From Figure 12, it may been seen that if 5 stimuli are delivered to each site during expiration, approximately 0.5 of the control tidal volume will result at all except M5 after vagotomy. If 10 stimuli per cycle are delivered, site L4 will attain about 0.6 of control tidal volume, while L5 and M5 will exhibit tidal volumes approximately equal to control. Increased number of stimuli will result in tidal volumes above control. However, something must have acted to terminate the ongoing response to the stimuli, since site L4 on the average only accepted 11 stimuli.

The MAF data can be interpreted using the same

rationale. Site M5 after vagotomy showed not only an increased gain but an elevated threshold to stimulation (Figure 13). Regardless of the change in cycle time evoked, the tidal volume was always increased 5 times above control. Both the threshold and gain of site M5 before vagotomy were reduced. Site L5 showed a very low threshold and a high gain. Comparison of these two sites at a point equivalent to control cycle time shows the minimum elevation in air flow velocity was approximately 4 times, and can go as high as 7.5 for the M5 site after bilateral vagotomy. Greater MAF changes were noted (Figure 14) as stimulated inspiratory time exceeded control, with decreased MAF a consequence of shortened inspiratory time. Finally, a direct relationship relating increasing numbers of stimuli to increasing changes in MAF above control was seen (Figure 41). It was also seen that shortened expiratory time leads, for site M5, to a decreased MAF (Figure 15).

If tidal volume were some function of the relative lengths of inspiration and expiration, one could expect to arrive at a regression equation expressing this relationship. Figure 9 shows the only case in which a significant regression line could be found. Here tidal volume decreased as the expiratory time shortens from control. Yet, the minimum theoretical tidal volume which could be obtained is only 0.9 when expiratory time equals zero. Thus, the relationship must be more complex. Figure 10, which describes

the tidal volume dependence on inspiratory time, also shows a positive relationship between inspiratory time and tidal volume, with similar thresholds but widely varying gains. Lengthened inspiratory time always resulted in increased tidal volume. The relationship must obviously involve other factors. L5 and M5 in this Figure showed two different sensitivities between inspiratory time and tidal volume. If a simple relationship existed relating tidal volume and inspiratory time, one might expect different intercepts consequent on different sensitivities, but the slopes of the lines should be parallel. This was not the case. L4 and M4 after vagotomy appeared initially to reduce the tidal volume, but still exhibited positive slopes relating time and tidal volume. The ratio between inspiratory and expiratory stimuli may well be the critical parameter for determination of tidal volume. Figure 11 indicates the sensitivities of each site. Longer tidal volumes seem to be favored by the delivery of relatively more stimuli in expiration. Comparison of this data with that of Figure 12 shows that the absolute number of stimuli delivered in expiration was apparently the determining factor for tidal volume. Because no significant relationships for the number of stimuli in inspiration could be found, it is concluded that the number of stimuli in inspiration had little direct effect, and was only important as it entered into the ratio of stimuli delivered in each phase of the respiratory cycle.

It is accepted that the hypothalamus has interconnections

with the medulla. The location of pathways modulating respiratory controller output from medullar areas is less well understood. Figure 52 shows where they are not, as these lesions were not sufficient to block the response, although some decrease in the magnitude of the response was noted. This decrease, however, was attributed to the general deterioration of the animal resulting from the massive size of some lesions. It was felt that no lesion could be considered as being in the descending pathway unless the response could be totally blocked.

A series of stimulation were carried out in one cat tracing the reactive areas posterior from the hypothalamus. Responses could be elicited from successively more ventrolateral locations, although with reduced effectiveness, as the stimulus site moved caudally. In this cat, the pathway was traced posterior to APO, then lost. However, Cohen (23) has recently reported the results of stimulation in the rostral pons in areas which would closely approximate the point where the central stimulation effect was lost in the present study. The behavior of the phrenic discharge in his system closely approximates that reported in the present study. He found that short trains of 20-30 stimuli delivered in late expiration produced early termination of expiration and switching to inspiration. A single stimulus produced a short latency reduction of phrenic discharge, followed by a wave of increased

activity. Cohen's explanation implied that the respiratory system undergoes an increase in excitability throughout each phase which primes the system for the next phase. However, the close correspondence of the results of this study and that of Cohen suggests the possibility that these two systems might be common, and that the hypothalamus might be the ultimate source of this increased excitability. In Cohen's experiments, the animals all had midcollicular transections, which removed any tonic influence of the higher brain centers, thereby enabling him to switch the respiratory phases at will. The animals also had neuromuscular block, and were vagotomized.

Gesell, Bricker, and Magee (40) have recorded respiratory potentials from the lateral, medial, and ventral reticulospinal tracts. The anterior projections arising either directly or indirectly from these reticular pathways might be logical choices for descending neurones from the hypothalamus. Kabat (61) stimulated similar areas in the hypothalamus in 1936, and found increased respiratory rate of unspecified magnitude in the same area as represented by site M4 in the present study. Kabat showed that the loci from which he was able to obtain respiratory responses moved progressively more ventral as more caudal levels were stimulated. The tracks became rather diffuse at the level of the colliculus, which may have accounted for the difficulty in tracing them more posteriorly in the

present study. No quantitative data was provided, but it would appear from the qualitative description that the results Kabat obtained were similar to those of the present study.

Ranson and Magoun (94), in a re-examination of the original data of Kabat described above, found that although increases in rate and amplitude of respiration resulting from hypothalamic stimulation were often combined, occasionally these responses appeared separately. Occasionally an increase in rate with a decrease in amplitude was seen. At the level of a plane passing just caudal to the optic chiasma, numerous points yielding vigorous respiratory responses were reported.

They found, however, that at more caudal levels the reactive field spread medially from the lateral portion of the hypothalamus with increases in amplitude or in rate appearing separately. These pathways persited throughout the mammillothalamic tract and lateral parts of the supramammillary nucleus and the posterior border of the mammillary body. Further caudally, inhibitory responses predominated. Such a finding would be consistent with cell bodies located more centrally, with only a diffuse pathway proceeding caudally, making their stimulation relatively more difficult to obtain. They also pointed out that other investigators have found panting responses from the lateral hypothalamus near the mammillothalamic

border.

Ranson and Magoun (94) also applied local heating to the regions used in the present study using 1 Mhz current. They reported increases in respiratory frequency to 225 per minute, comparable to the magnitude of response noted in the present study. However, the short duration and relatively low frequency of the stimulations used in the present study would preclude any significant heating. Ranson and Magoun indicated that 30 to 90 seconds of heating were required to obtain maximal respiratory responses. In the study reported here, the first application of stimulus trains induced an increase in respiratory frequency. Lesions in this area, also reported by Ranson and Magoun (94), reduced but did not abolish the temperature regulation ability of the animal. Thus the possibility of interaction with the temperature regulation mechanism is suggested but not proven. It might be noted in passing, however, that in one cat not included in the detailed analysis reported here, the temperature regulating system failed, raising the rectal temperature to 43°C. In this cat, the response to hypothalamic stimulation was greatly reduced, both in terms of rate increase and increased MAF. Following reduction of the rectal temperature to 38°C, the animal exhibited respiratory responses comparable to those reported here. Whether this decreased reactivity resulted from the low initial values due to the high control respiratory rate,

or was a consequence of reduced reactivity is not known. No changes in the state of piloerection were noted in the present experiments during stimulation.

It might be contended that hypothalamic stimulation overrides chemoreceptor control. Yet in some animals the respiratory pacing was continued for 30 minutes with apparently no deleterious effects. The animal returned promptly to control rate and depth upon cessation of the stimulation. However, small decreases in tidal volume were noted during the course of the prolonged stimulation, indicating some degree of regulation of minute volume in response to exogenous stimulation. From this standpoint, respiratory rate would appear to be the major parameter modified by the stimulation.

Whether the final common pathway for respiratory changes evoked from hypothalamic stimulation involves only the phrenic nerves is open to debate. The reduced reactivity of phrenic discharge from stimulation at site L5, which nonetheless resulted in increased tidal volume and MAF, suggests spinal outflow to intercostal muscles. In addition, the involvement of accessory muscles of respiration observed during the stimulation suggests that the outflow must involve more than just the phrenic nerves. Liljestrand (71) and Sears (104,105) have demonstrated respiratory tracts in the spinal cord. Liljestrand reported that these pathways are in the ventral columns and ventral

parts of the lateral columns, and are chiefly uncrossed. Sears postulated a two neurone spinal reflex with excitatory and inhibitory influences from higher centers. Both of these mechanisms could potentially be modulated by direct hypothalamic projection. The lesion experiments of this study indicate that the pathway must at least pass through the medial or ventromedial portions of the cord. As Sears pointed out (106), the respiratory motorneuron itself must be considered the final level of integration of respiratory activity. How these descending pathways pass to the motorneuron is unknown. Pitts et al (89) found that these pathways exist in the anterior columns and in the ventral part of the lateral columns.

Interaction between hypothalamic drive and the primary respiratory areas is an alternate possibility to direct spinal interaction. Pitts et al (89) concluded that polypneic panting which results from thermal stimulation in the hypothalamus is mediated through facilitation of the pneumotaxic center, resulting in increased respiratory rate. The present study indicates that the reaction is more complex, as shown in the experiment in which a lesion in the primary respiratory area resulted in complete cessation of respiration. The hypothalamic respiration pattern could still be evoked, even though the animal expired after the stimulation was stopped. Thus the apnea was apparently not transient, and the lesions must have spared the proposed

auxilliary pathways.

The potential exists for hypothalamic sites to function as relay stations from higher centers. Volitional respiration may be mediated through this pathway. Smith (108) has detailed some of the respiratory responses resulting from cortical stimulation, and correlated them with alterations in blood pressure and heart rate. Respiratory inhibition is frequently accompanied by increased pulse and mean pressure, with little change in heart rate. In the present study the cardiovascular responses usually lagged behind the respiratory response, and were generally pressor and accelerator in nature, a combination which would be expected to reflexly inhibit the respiratory response. No such direct inhibition was observed.

The hypothalamic influence could also be involved in mediating the ongoing hypo-polarization noted by Salmoiraghi and von Baumgarten (101). It is also possible that phase-spanning neurones (21) may be activated not by the primary respiratory center, but by descending influences from the hypothalamus.

An additional line of evidence for hypothalamic involvement in the respiratory act comes from three experiments not reported in detail in this thesis. In these cats, respiratory periodic potentials were recorded from the region of M5. These potentials could not be found after moving the 7 micron tungsten electrode 1/2 millimeter in

any direction. Although there was considerable background activity, it was obvious that these potentials were periodic with respiration, and appeared to precede the onset of respiratory movements. Kastella and Weiss (personal communication) have succeeded in recording a limited number of these potentials in the same area, and it appears that their relatively slow discharge pattern is augmented by increased baroreceptor discharge resulting from increased pressure in the carotid sinus.

Because of the interaction of the primary respiratory center and the hypothalamic stimulation sites, a precise identification of each of the elements of interaction is not possible. However, it may prove profitable for the reader to investigate the following model for hypothalamic modulation of the respiratory system. Such a model must be regarded as highly tentative. The black box model presented here is segmented along functional rather than anatomical lines. It should be remembered that the surrounding areas of the hypothalamus studied, while in some cases reactive to the stimulus in terms of respiratory response, did not produce as profound or as consistent a response as the four sites discussed in detail earlier. Thus, each anatomical site designated in the model might best be thought of as representing a portion of the total block of tissue, and definitely should not be considered as a center regulating the named function (see Figure 54).

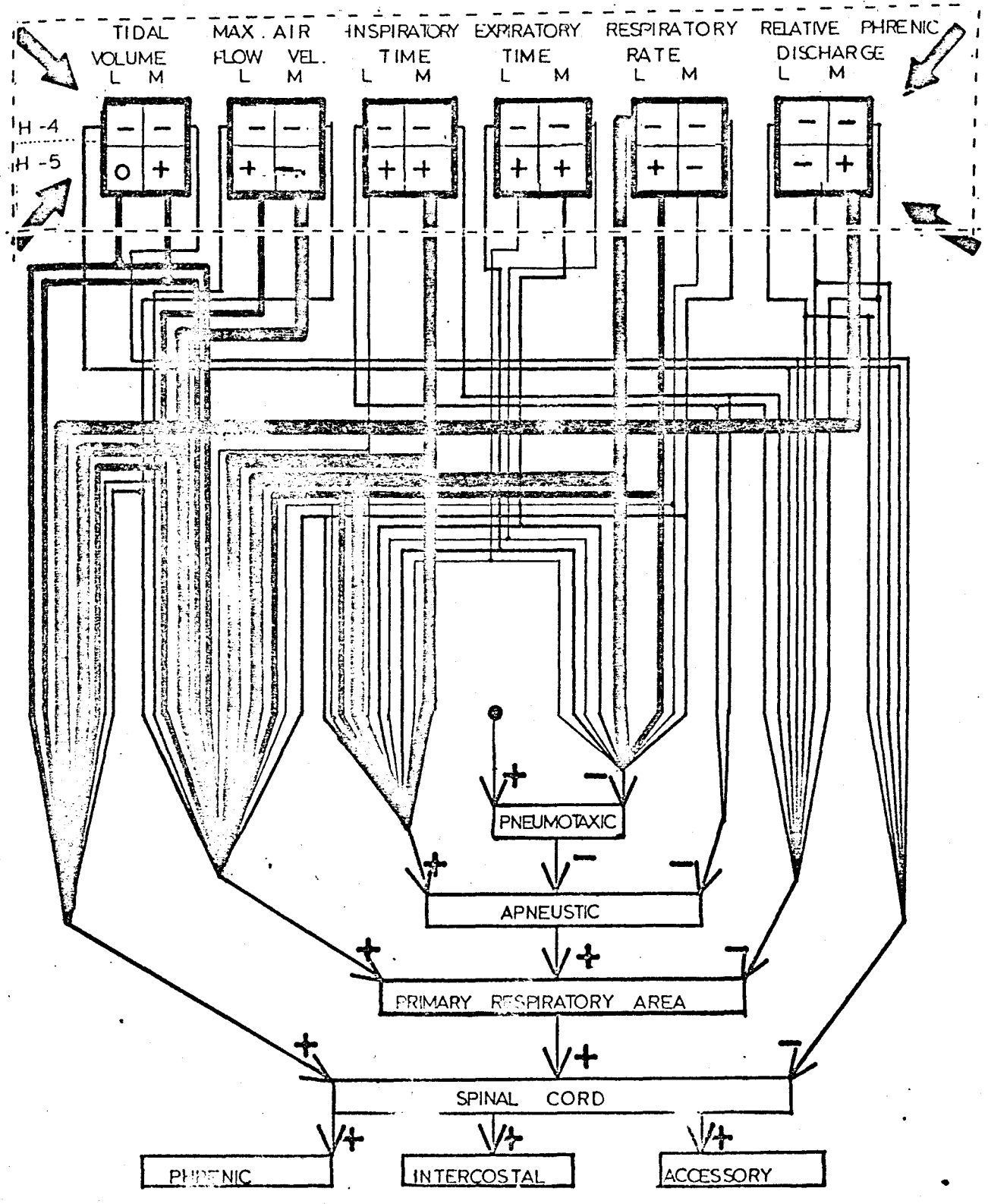


Figure 54.
A proposed model of the hypothalamic-respiratory apparatus interactions. See following page for details.

Figure 54: Model of Respiratory Control

A proposed model of the interactions between the hypothalamus and the respiratory apparatus. Blocks enclosed by the broken lines indicate specific functions within the hypothalamus. The upper left square in each dark box represents site L4, the upper right box M4. Site L5 is indicated by the lower left box, and M5 by the lower right. Large arrows denote that the system under consideration is tonically modulated by both higher and lower brain influences. The plus and minus signs indicate whether the action of the vagus inhibits (-) or facilitates (+) the indicated response. Lines connect the hypothalamic areas for each parameter to brain stem and spinal cord sites where possible interaction may occur. Light lines indicate relatively small contribution of that site for a given parameter, while heavy lines signify profound changes in the measured parameter during stimulation. To reduce the complexity, facilitatory interactions are diagrammed on the left, inhibitory on the right. No inference is made as to the relative position of the proposed descending pathways. Note especially the proposed direct connections to spinal cord. In some cases these outflows may actually inhibit the phrenic discharge, yet increase the tidal volume, MAF, and rate, which would be indicative of a final common pathway involving accessory muscles of respiration and intercostals.

Because of the complexity of interactions involved, the classical method of designating inputs to the system has been abandoned. Vagal interactions are shown directly in the control blocks in terms of whether or not the vagus inhibits (-) or facilitates (+) the response of each system. Because in some cases the polarity of vagal interaction is ambiguous, certain assumptions are necessary. If the tidal volume is depressed below control more after vagotomy than before, the vagus must be considered facilitatory with respect to tidal volume. Maximal air flow (MAF) was always increased above control. A greater increase before vagotomy than after would indicate a facilitatory vagal effect. With respect to inspiratory and expiratory times, the vagus is assumed to have a positive effect if the time of each was lengthened after vagotomy. Thus, even though the length of time required for each segment of the respiratory cycle may be shortened below control, the vagus can be considered facilitatory if it were reduced less after vagotomy. This presents some problems relative to rate calculations. If the effect of the vagus were to facilitate the shortening of both the inspiratory and expiratory phases of respiration, rate would increase and the vagus could be considered facilitatory on rate as well. Since the relation between rate and segment time is not linear, the magnitude of the rate response depends on the relative change in cycle components as related to control. Thus, the effect of the

vagus has been considered facilitatory if, following its removal, the respiratory rate did not increase as much above control level. Finally, the relative phrenic discharge as measured by the "integrated" method (Figure 53) is considered facilitated if the relative discharge is increased less above control, or decreased less below control prior to vagotomy.

Let us consider, a parameter at a time, the interactions between the hypothalamus and the balance of the respiratory system. Data are average values taken from Tables I and II.

Tidal volume was reduced below control in both H-4 sites and elevated above control by both M5 sites. Vagotomy reduced the depression in the H-4 sites and produced little change in M5 response. Site L5, however, was strongly inhibited after vagotomy, even though this same site was strongly facilitated before vagotomy was performed. Increases in tidal volume (L5 and M5) may be explained by a tonic facilitatory influence on either the primary respiratory area or on spinal cord integrative areas. Of itself, the latter choice may not be obvious, yet the changes noted concurrently with phrenic discharge are difficult to explain if such a mechanism is not possible. In view of the fact that vagotomy does not significantly alter the response to M5 stimulation, it also seems likely that some degree of input attributable to the vagus exists

at the hypothalamic level, at least for the other four sites studied. Regarding the inhibitory influence of the H-4 sites, these also may be explained primarily by interconnections at two levels. Interaction with the primary respiratory area as well as a direct inhibition at the spinal cord level are possible.

It was observed that the average MAF was increased in all cases. The magnitude of the increase at H-4 sites was considerably less than that of the L5 site. The greatest increase in MAF was demonstrated by stimulation of site M5. The vagus was inhibitory at all but site L5. In the latter case, removal of the vagus resulted in a slight decrease in MAF, while at all other sites vagotomy resulted in a further enhancement of the increased MAF. Mediation of these responses might be considered as facilitation of both the primary respiratory area and the spinal cord directly. Direct inhibition of the pneumotaxic or apneustic centers will not result in the measured response for either tidal volume or MAF. It is of course also likely that the systems represented within the hypothalamus interact with themselves. For simplicity these have been omitted from the model in Figure 54. However, tidal volume is influenced by changes in rate (Figure 8), by expiratory time (Figure 9), and by inspiratory time (Figure 10). Maximal air flow velocity is also interrelated with rate (Figure 13), inspiratory time (Figure 14), and is a function

of the ratio of expiratory to inspiratory time ratio (Figure 15). Presumably, there is also a relationship between the amount of input to these areas and the respiratory response. Numerous examples of the effect of inspiratory and expiratory phase stimuli on both these parameters have been presented.

Measurement of the changes in inspiratory and expiratory time relationships is relatively easy. Speculation on the interactions involved is not. H-4 site stimulation shortened inspiratory time on the average below control. Inhibition of either the apneustic or primary respiratory areas would be most consistent here, although it is possible that the effect may also be mediated by the spinal cord directly via inhibition of motor discharge early in inspiration, thus preventing full development of the inspiratory response. Since phrenic discharge was also inhibited, this does not seem an unlikely prospect. Lengthening of the inspiratory phase was noted for the H-5 sites, with the strongest interaction at M5. Lengthened inspiratory time can best be explained by facilitation of the apneustic and primary respiratory centers. After vagotomy, all inspiratory times were less than control, indicating the inhibitory drive of the H-4 sites was itself depressed by the vagus. The facilitatory effect of the H-5 sites was reversed following vagotomy. The vagus therefore facilitated the increased inspiratory time, and the intrinsic

effect of the H-5 sites must therefore have been inhibition of inspiratory time.

Expiratory time was shortened from control values. This could result either from early inhibition of the pneumotaxic center or from direct facilitation of the apneustic center. This would override the normal inhibitory influence of the pneumotaxic, and initiate a new inspiration. After vagotomy, M5 site produced little change in the control expiratory time, indicating that the primary cause of shortening was of vagal origin. Site L5 actually produced a slight lengthening of expiratory time. Both H-4 sites produced further inhibition of expiratory time following vagotomy, which indicates that the vagus was exerting a tonic restraining influence on these sites.

Rate responses were complicated by the inverse relationship between cycle time and rate. It is probable that no rate determining sites exist, and that the ultimate rate is determined by a complex interaction of inspiratory and expiratory components. Yet it is helpful to view the system as if a rate determining system were present. Since rates were invariably increased by the stimulations, one must presuppose either inhibition of the pneumotaxic center, or facilitation of apneustic and/or primary respiratory centers. With the exception of L5, the responses were greater after vagotomy, indicating that the vagus exerted a restraining influence on

the rate determining system. The possibility also exists that the rate response did originate within the hypothalamus, was mediated via a direct spinal route, and possessed little interaction with lower brainstem respiratory centers.

The relative phrenic discharge data is a mixed problem. The prominent effect of inhibited total discharge for all except the M5 site indicates either an inhibitory site in the primary respiratory areas or a direct cord interaction. M5 probably possesses a direct pathway because of the strong interactions it shows. Vagotomy reduces this facilitation to nearly control values, signifying a vagal facilitatory effect at this site, and an inhibitory role for the vagus at all other sites.

Various combinations of interaction can thus be examined. However, it has been pointed out above that the response of the hypothalamic system exhibits differing gain depending upon the nature as well as the site of stimulation. Also, the relative amount of stimulation delivered in each phase of the respiratory cycle determines to some extent the tidal volume (Figure 11), and the ratio of inspiratory to expiratory time (Figure 7). The exact physiological meaning of this observation is difficult to assess. It is tempting to speculate that such a system could enable the processing of information utilizing the same neuronal networks, and possibly the same transducers, through a multiplexing arrangement. The nature

of these signals is of course pure speculation. Integration of temperature regulation and cardiovascular function is known to exist in the same area of the hypothalamus. Large volumes of sensory information are also processed and/or passed through this region. The bulk of this input is relatively static in that it does not possess information dealing with the change in status of a given system as a result of ongoing activity in the respiratory system. Yet, some degree of "seeking" apparently exists to explain the response of the cardiovascular system throughout a respiratory cycle. Such a hypothalamic site would be a logical choice for integration and regulation of a variety of systems with some degree of regulation as the cumulative effects of regulatory changes are assessed. The fine, cycle to cycle, control would still reside in the shorter loop brainstem centers, leaving longer term adjustments to the hypothalamic sites. Failure to provide the short term adjustment due to pathology involving the "primary centers" would then leave the hypothalamus as a backup system, which could provide an almost normal function. This function would involve relatively poor reflex control because of the slow response time of the system, but would retain the rapid response characteristic of conscious or volitional control. In the absence of any input or output by the brainstem areas, any hypothalamic pathways bypassing lower brainstem integrative sites would be unaffected.

Absence of tonic inputs might render them ineffective except by direct conscious control, or by experimental activation (Figure 48).

Several questions remain. Vagotomy experiments provide only a rudimentary look at potential interactions with hypothalamic areas. In the normal animal, is inhibition of classic lower brainstem areas necessary to achieve activation of the more rostral hypothalamic areas? What, if any, are the pathways involved in reciprocal control between the brainstem and the hypothalamus? A more precise definition of the descending pathways seems essential, and should be possible using the techniques presented here together with extensive degeneration studies. It would be instructive to examine the respiratory response obtained from hypothalamic stimulation compared before and after bilateral phrenic section. The chordotomy data of Belmusto (10) provides a good starting point for an additional study utilizing cord lesions to define the spinal pathways involved in this response. Microelectrode recording from the hypothalamus in these highly reactive areas has great potential for revealing interactions between respiratory and cardiovascular systems. This study is currently being completed.

This study, then, has given some small insight into the overall system. Yet, it has provided a quantification of some portions of the interaction, and demonstrated a

useful technique for studying the system as a whole. Further questions, some of which might yield useful information, have been suggested as a result of this study.

It appears that the possibility exists for direct rate and depth control from the hypothalamus. Based on the model presented, in some cases it is possible to tentatively narrow down the possible sites of interaction. Yet, if the system described involves significant bypass pathways, more experiments are needed to establish the existence and functional role of these interconnections. It is entirely possible that hypothalamic intermodulation is mediated at the spinal cord level, with little or no integration in the lower brainstem.

Chapter VII

CONCLUSION

1) On the basis of stimulation experiments, the hypothalamus possesses the ability to modify maximum air flow velocity, tidal volume, inspiratory and expiratory times.

2) Respiratory rate can be directly controlled by selective stimulation of specific hypothalamic sites.

3) Changes in phrenic discharge do not necessarily correlate with changes in tidal volume or maximum air flow velocity. These observations are suggestive of an accessory respiratory pathway not primarily involving the phrenic motor nerve.

4) Maximum air flow velocity is increased as a result of anterior hypothalamic stimulation. The minimum increase seen was an average of 130%, while the average maximum increase seen was 600% for site M5.

5) Tidal volume is increased by stimulation in the H-5 plane, and decreased by stimulation in the H-4 plane.

6) Vagotomy produces a complex series of changes in the response to stimulation in the hypothalamus, with most

responses inhibited by the vagus.

7) Each component of the stimulus train used to evoke respiratory responses from the hypothalamus is important in determining the final response. Train repetition rate determines primarily the respiratory rate, much as if the animal was paced. The frequency slope of the crescendo modifies the MAF. The number of stimuli delivered in each phase of respiration governs the time of the phases.

8) The stimulation effects are difficult to disrupt by lesions which collectively occupy a large portion of the medullary tissue, indicating a diffuse descending pathway.

9) Although found in only one animal, a site was found in the hypothalamus of the cat from which respiratory activity sufficient to maintain life may be evoked in an animal which promptly expired due to the complete absence of spontaneous respiration following termination of stimulation.

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APPENDIX I

SUMMARY

The following four Figures are provided to assist the reader in interpretation of the data handling procedures used and the computer program which follows immediately after Figure A4.

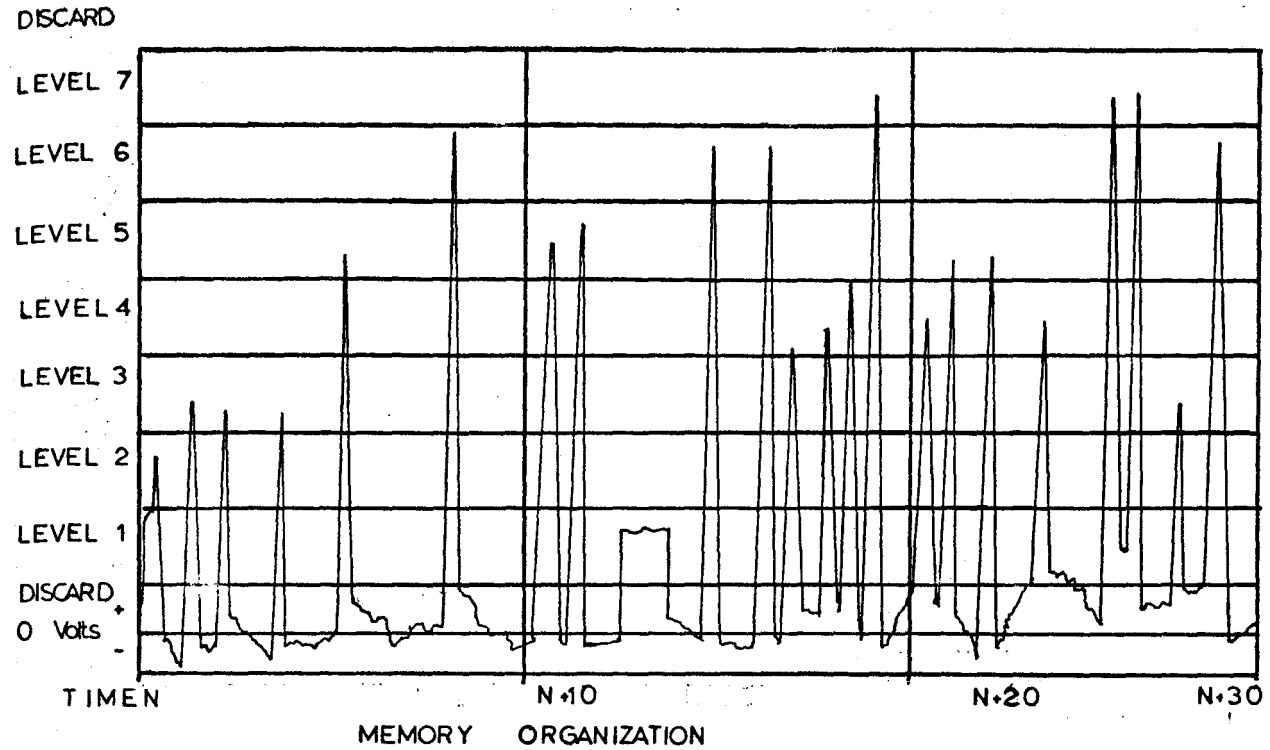
Figure A1 describes the equipment used to generate the stimulus trains used in this study. The basic rate at which trains were applied was established by the Grass S-4 stimulator in the upper left. Its output, represented by A, was a square wave whose frequency and duration could be controlled from the S-4. A small bypass capacitor was provided across the output to assist in closing relay K1. When not activated, the 7 volt battery was applied across the 2000 Mfd timing capacitor. When activated, the relay applied the stored charge of the capacitor across the voltage controlled generator portion of the Model 134 Wavetek. A trigger was also provided from the S-4 output. Output point B describes the voltage curve of the capacitor. Its discharge curve may be adjusted with the 1000 ohm variable resistor provided. Larger voltages applied to the Wavetek provide lower frequency outputs. Thus, as the voltage across

the capacitor decreases, the output frequency of the Wavetek increases exponentially. Point C represents this output. Point D represents the signal after differentiation across .1 mfd. The isolated grass S5D stimulator triggers only on the positive going pulses, and gives output E. The duration and amplitude of each pulse are determined independently by the settings of S5D. These pulses are then fed to the bipolar hypothalamic electrodes through an appropriate switchbox. Signal D is also directly recorded on magnetic tape as a gate signal for analysis of phrenic firing, and as a stimulus mark.

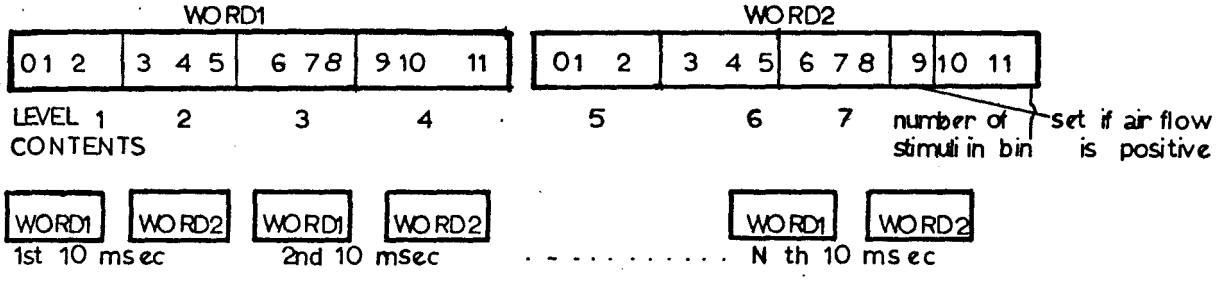
In Figure A2, a graphical presentation of the computer data handling method employed is presented. Only points marked by an asterick are counted as peaks. Two successive 10 msec time blocks are portrayed, and the equivalent stored data for the example pictured are indicated on the right. At the bottom, a memory allocation table is presented. The first group indicates organization within each word of computer memory, and the lower portion shows how two memory words are used to store 9 pieces of information for each 10 msec bin.

Figure A3 is a flow diagram for data sampling. References to retrieval program refer to Figure A4, which details the options available to the operator following data acquisition and which is controlled by teletype input.

DATA REDUCTION EXAMPLE



MEMORY ORGANIZATION



IN EXAMPLE ABOVE:

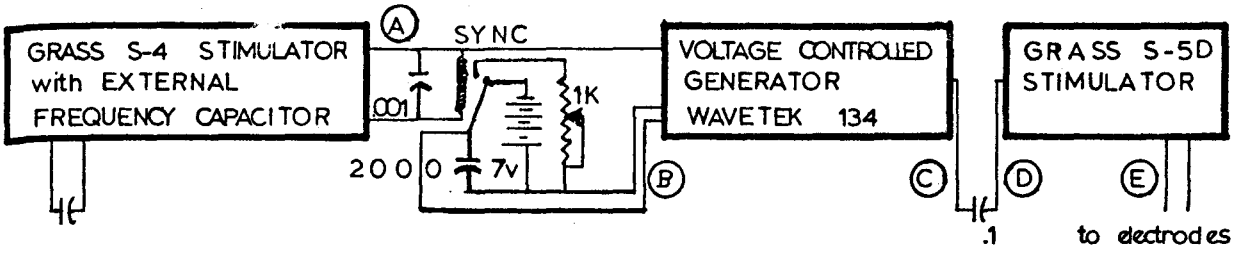
WORD1	000 001 011 000 (0130)	000 000 000 100 (0004)	000 000 001 010 (0012)
WORD2	001 000 001 --- (101_)	001 011 001 --- (131_)	010 001 010 --- (212 -)
TIME	N+10	N+20	N+30

OCTAL EQUIVALENTS ( )

Figure A1.

Summary of computer conventions used to express data obtained from hypothetical sample.

EQUIPMENT AND TIMING



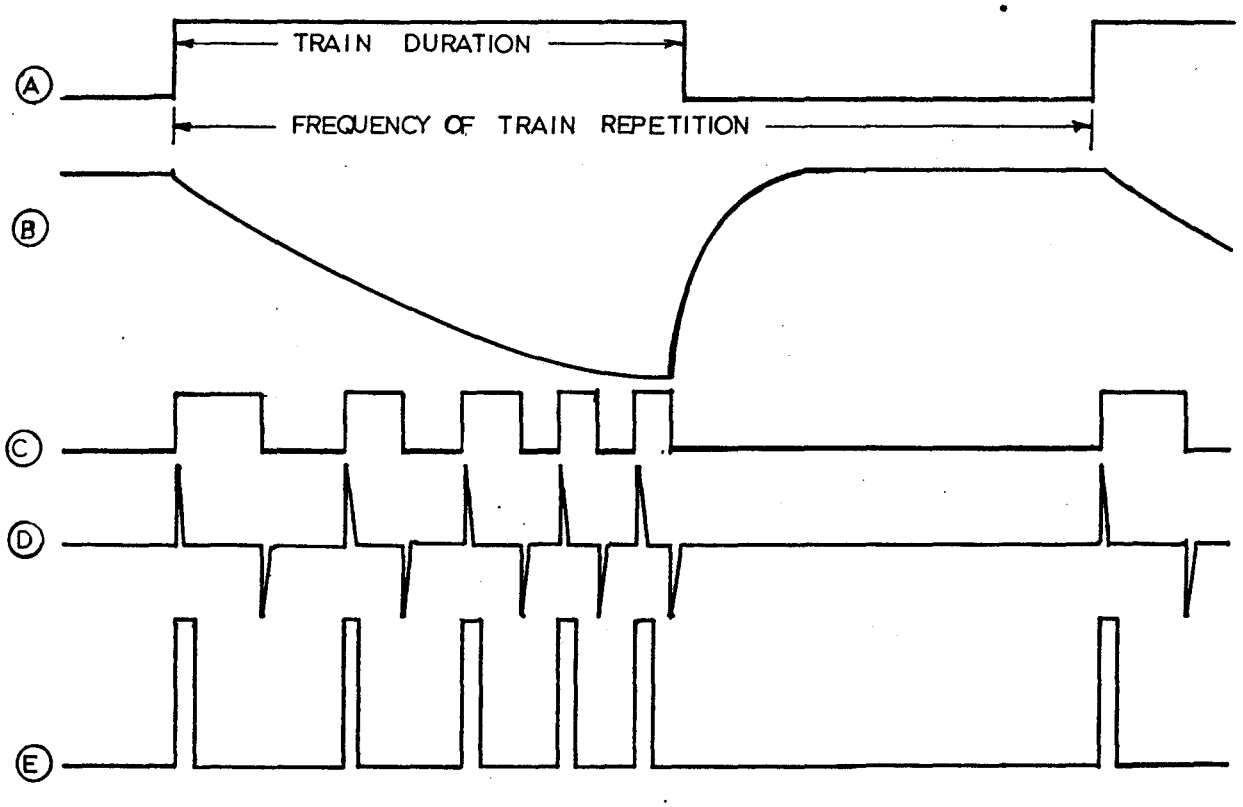


Figure A 2.

Equipment and timing diagram for stimulator system used.

SAMPLING FLOW DIAGRAM

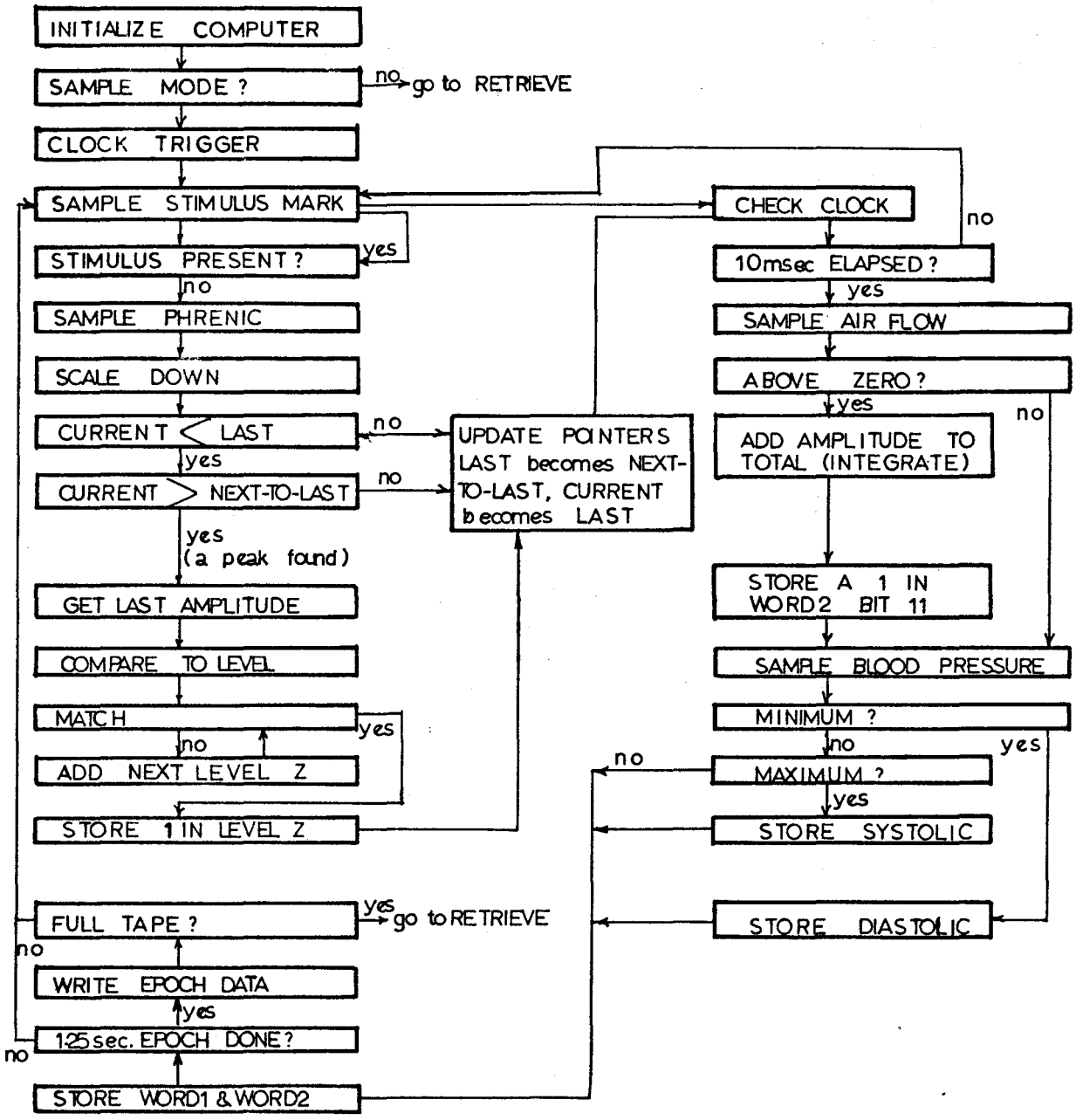


Figure A 3 .

Flow diagram for computer data sampling. Exits to RETRIEVE refer to Figure following.

DATA RETRIEVAL OPTIONS
operator controlled

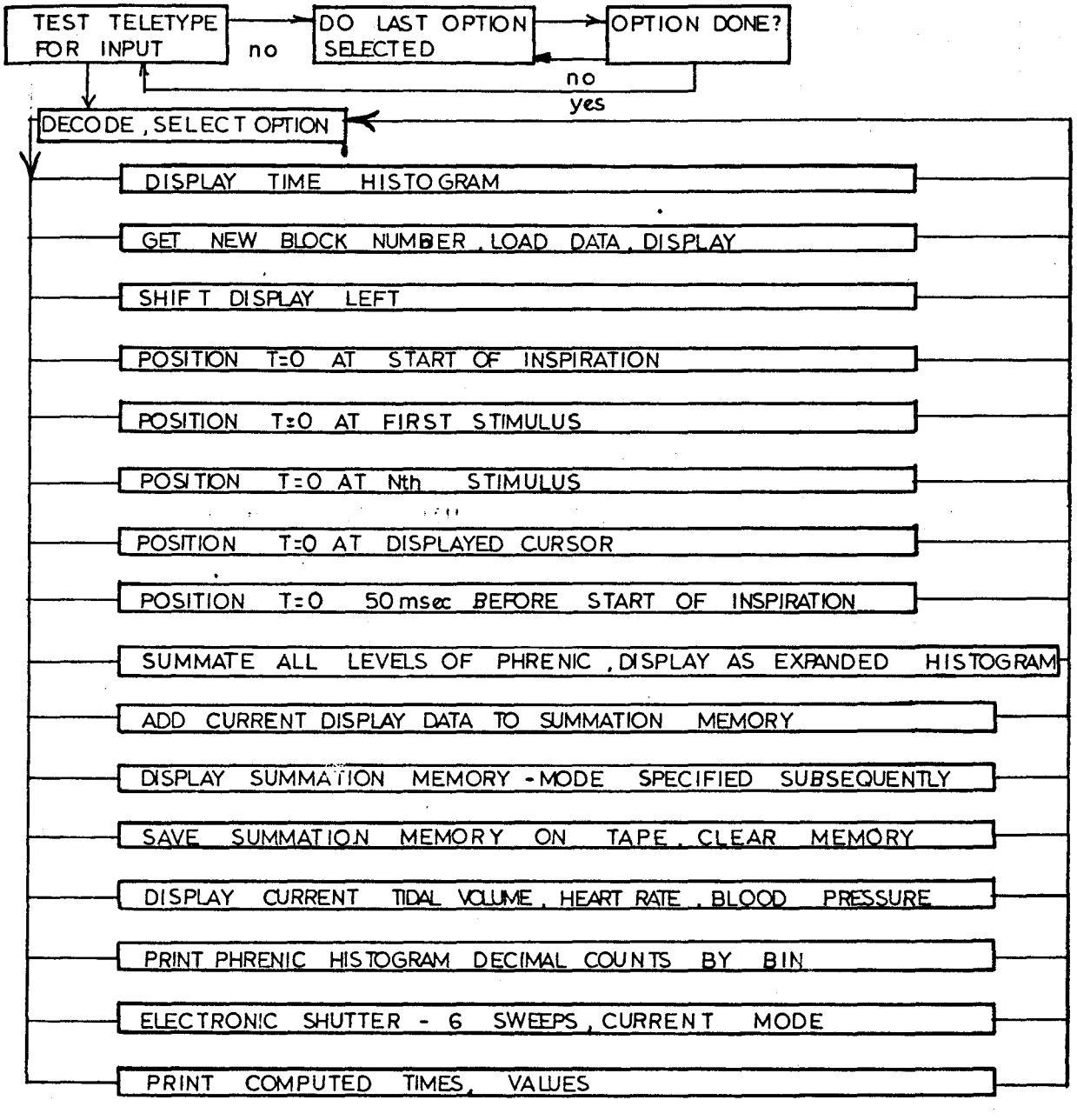


Figure A4.

Operator controlled options available. Each option selected is recursive until a new option is chosen from the keyboard.

```
0000                    *20
0001                        *2
0002    0002    0000    TIME,   0               /TIME TALLEY
0003                        *20
0004    0020    0500            IOB
0005    0021    6002            6002            /IOF,DISABLE INT
0006    0022    1020            LDA I
0007    0023    0120            120             /I-O PRESET
0010    0024    0004            ESF             /SPEC. FUNCT
0011    0025    0710            RDC 10
0012    0026    4000            4000            /INITIALIZE TAPE
0013    0027    1020            LDA I
0014    0030    0010            10
0015    0031    0001            AXO             /TAPE NO PAUSE
0016    0032    0067            SET I 7
0017    0033    7761            -16             /TYPE LINE COUNT
0020    0034    0062            SET I 2
0021    0035    0000            0               /TIME TALLEY
0022    0036    0063            SET I 3
0023    0037    2000            2000            /INITIAL ADDRESS
0024    0040    0011            CLR
0025    0041    4762            STC BLOCK
0026    0042    6046            JMP .+4
0027    0043    0300    ENABLE, 300             /CLOCK SETUP
0030    0044    4100    CONTROL,4100            /1KC,FREE,PRESET
0031    0045    7766    PRESET, -11             /PRESET COUNTS
0032    0046    0002    CLOCK,  PDP             /CLOCK INITIAL
0033                            PMODE           /ROUTINES
0034    4047    6135            CLSA
0035    4050    1245            TAD PRESET
0036    4051    6133            CLAB
0037    4052    7200            CLA
0040    4053    1244            TAD CONTROL
0041    4054    6132            CLLR
0042    4055    7200            CLA
0043    4056    1243            TAD ENABLE
0044    4057    6134            CLEN
0045    4060    6131            CLSK
0046    4061    5260            JMP .-1
0047    4062    6135            CLSA
0050    4063    7200            CLA
0051    4064    6141            LINC
0052                            LMODE
0053    0065    0440            SNS 0           /1=READ,0=INPUT
0054    0066    6073            JMP .+2
0055    0067    7314            JMP READ
0056    0070    0441            SNS 1           /INPUT/CAL
0057    0071    6073            JMP .+2
0060    0072    6670            JMP CAL
0061    0073    0100            SAM 0           /VAR RESP BASELN
0062    0074    2754            ADD BASE4       /ZERO INPUT
0063    0075    4244            STC RESP+7      /CORRECTION VALS
0064    0076    1020            LDA I
0065    0077    0100            100
0066    0100    0004            ESF             /FAST SAMPLE
0067    0101    0500    SAMP,   IOB
0070    0102    6131            6131            /CLSK(SKIP INTER
0071    0103    6105            JMP INDIR       /INDIRECT NERVE
0072    0104    6107            JMP SAMPLE      /FLAG SET
0073    0105    6332    INDIR,  JMP NSAMP       /SAMP NERVE
0074    0106    6101            JMP SAMP        /CHECK CLOCK
0075    0107    0500    SAMPLE, IOB
```

```
0076    0110  6135          6135            /DROP FLAG
0077    0111  6332          JMP NSAMP
0100    0112  1000          LDA             /STORE PREVIOUS
0101    0113  0766          WORD1           /INTERVAL NERVE
0102    0114  1063          STA I 3         /SAMPLES
0103    0115  1000          LDA
0104    0116  0767          WORD2
0105    0117  1063          STA I 3
0106    0120  0011          CLR             /INITIALIZE INT
0107    0121  4766          STC WORD1       /ERVAL VALS TO 0
0110    0122  4767          STC WORD2
0111    0123  6332          JMP NSAMP       /SAMPLE NERVE
0112    0124  1022          LDA I 2         /BUMP TIME TALLY
0113    0125  1020          LDA I
0114    0126  0112          112             /SAM 12 CODE
0115    0127  4363          STC NERN-2
0116    0130  6332          JMP NSAMP
0117    0131  0112          SAM 12          /BP SAMPLE
0120    0132  0340          SCR 0           /TEMPORARY SCALE
0121    0133  2753          ADD BASE3       /CAL BASELINE
0122    0134  1040          STA
0123    0135  0771          TBP             /TEMP BP STORE
0124    0136  0017          COM             /INVERT TO -BP
0125    0137  1100          ADA
0126    0140  0773          LOBP            /PREV. LOW BP
0127    0141  0451          APO             /IF +,NEW LOW
0130    0142  6150          JMP .+6         /NOT+,NO NEW LOW
0131    0143  6332          JMP NSAMP       /NEW LOW BP
0132    0144  1000          LDA
0133    0145  0771          TBP
0134    0146  4773          STC LOBP        /UPDATE LO BP
0135    0147  6165          JMP HR          /MINIMA,GET RATE
0136    0150  6332          JMP NSAMP
0137    0151  1000          LDA             /NEW MAXIMA BP
0140    0152  0771          TBP             /CURRENT SAMP
0141    0153  0017          COM             /INVERT TO -BP
0142    0154  1100          ADA             /ADD PREVIOUS
0143    0155  0776          HIBP            /HIGH BP
0144    0156  0451          APO             /-,NEW HI
0145    0157  6161          JMP .+2         /YES ,NEW HI
0146    0160  6165          JMP HR          /NOT HI,TEST HR
0147    0161  6332          JMP NSAMP
0150    0162  1000          LDA             /GET CURRENT BP
0151    0163  0771          TBP             /VALUE
0152    0164  4776          STC HIBP        /UPDATE NEW HI
0153    0165  6332  HR,     JMP NSAMP
0154    0166  1020          LDA I
0155    0167  0000  LIMIT,  0
0156    0170  1120          ADA I
0157    0171  0040          40
0160    0172  0017          COM
0161    0173  2771          ADD TBP
0162    0174  0471          APO I
0163    0175  6226          JMP GATECLR
0164    0176  6332          JMP NSAMP
0165    0177  1000          LDA
0166    0200  0771          TBP
0167    0201  2777          ADD HLAST
0170    0202  0451          APO
0171    0203  6230          JMP HRESET
0172    0204  6332          JMP NSAMP
0173    0205  1020          LDA I
0174    0206  0000  HGATE,  0
```

```
0175    0207  1460          SAE I
0176    0210  0000          0   :
0177    0211  6230          JMP HRESET
0200    0212  6332          JMP NSAMP
0201    0213  1000          LDA
0202    0214  0002          TIME
0203    0215  4775          STC HRATE
0204    0216  6332          JMP NSAMP
0205    0217  0062          SET I 2
0206    0220  0000          0
0207    0221  6332          JMP NSAMP
0210    0222  1020          LDA I
0211    0223  0002          2
0212    0224  4206          STC HGATE
0213    0225  6230          JMP HRESET
0214    0226  0011  GATECLR,       CLR
0215    0227  4206          STC HGATE
0216    0230  6332  HRESET, JMP NSAMP
0217    0231  1000          LDA
0220    0232  0771          TBP             /CURRENT BP
0221    0233  0017          COM             /INVERT
0222    0234  4777          STC HLAST       /UPDATE LAST
0223    0235  6332  RESP,   JMP NSAMP
0224    0236  1020          LDA I
0225    0237  0113          113             /SAM 13 CODE
0226    0240  4363          STC NERN-2
0227    0241  6332          JMP NSAMP
0230    0242  0113          SAM 13          /RESP INPUT
0231    0243  1120          ADA I           /GET BASELINE
0232    0244  0000          0               /CORRECTION
0233    0245  1040          STA             /TEMPORARY STORE
0234    0246  1002          TRESP
0235    0247  0451          APO             /IF +,SAVE SKIP
0236    0250  6252          JMP .+2
0237    0251  6255          JMP RPOLAR      /INCOMING -
0240    0252  4757          STC RLAST       /UPDATE
0241    0253  6332          JMP NSAMP
0242    0254  6535          JMP RTEST       /IS FIELD FULL?
0243    0255  6332  RPOLAR,JMP NSAMP
0244    0256  1000          LDA             /KNOWN+
0245    0257  0757          RLAST           /WAS LAST+-
0246    0260  0451          APO             /LAST +,NO 0CROS
0247    0261  6263          JMP RTIME       /ZERO CROSS,TIME
0250    0262  6272          JMP RINTEG      /VOLUME PROGRAM
0251    0263  6332  RTIME,  JMP NSAMP
0252    0264  1000          LDA
0253    0265  0767          WORD2           /NTALLY STORE
0254    0266  1620          BSE I           /FOR INSPIRATION
0255    0267  0004          0004            /STORE A 4
0256    0270  4767          STC WORD2
0257    0271  6332          JMP NSAMP
0260    0272  6332  RINTEG, JMP NSAMP
0261    0273  1000          LDA             /SAMP OF AIR
0262    0274  1002          TRESP           /FLOW TO RINTEG
0263    0275  0344          SCR 4           /SCALE 32:1
0264    0276  1140          ADM
0265    0277  0760          TRVOL           /TEMP RESP VOL
0266    0300  6332          JMP NSAMP
0267    0301  6332  SLOPE,  JMP NSAMP       /DV/DT BY MAX CH
0270    0302  1000          LDA             /ANGE PER SAMPLE
0271    0303  0757          RLAST           /INTERVAL
0272    0304  0017          COM             /LAST SAM,-
0273    0305  1100          ADA             /CURRENT AF
```

```
0274    0306  1002          TRESP
0275    0307  0451          APO  .          /+,RISING
0276    0310  6535          JMP RTEST       /FALLS,CK FIELD
0277    0311  1040          STA             /RISING,GET DIFF
0300    0312  0761          TDIF            /TEMP STORECOM
0301    0313  6332          JMP NSAMP
0302    0314  1000          LDA             /POINT DIFF LAST
0303    0315  0761          TDIF            /TWO SAMPLES
0304    0316  0017          COM             /INVERT
0305    0317  1100          ADA             /PREVIOUS MAX
0306    0320  0765          LDIF            /DV/DT
0307    0321  0451          APO             /+,LDF WAS BIGGR
0310    0322  6324          JMP .+2         /CURRENT BIGGER
0311    0323  6535          JMP RTEST       /PREVIOUS BIGGER
0312    0324  6332          JMP NSAMP
0313    0325  1000          LDA             /PRESENT BIGGER,
0314    0326  0761          TDIF            /UPDATE DV/DT
0315    0327  4765          STC LDIF
0316    0330  6332          JMP NSAMP
0317    0331  6535          JMP RTEST
0320    0332  0111  NSAMP,  SAM 11          /INSURES FAST
0321    0333  1000          LDA             /SAMPLE SYNC.
0322    0334  0743          HOLD            /PREV NSAMPLE
0323    0335  0017          COM             /INVERT
0324    0336  4416          STC LAST        /UPDATE LAST
0325    0337  1000          LDA             /GET RETURN
0326    0340  0000          0               /ADDRESS
0327    0341  4742          STC RETURN
0330    0342  0110          SAM 10          /HAS 11 DATA
0331    0343  0341          SCR 1
0332    0344  0451          APO
0333    0345  6351          JMP .+4
0334    0346  1120          ADA I           /GATING LEVEL
0335    0347  7727          -50             /FOR STIM ARTIFT
0336    0350  0451          APO
0337    0351  6365          JMP NERN        /NO STIM,GET N
0340    0352  1020          LDA I           /CHECK STIM GATE
0341    0353  7776  SGATE,  -1              /-1=CLEAR
0342    0354  0471          APO I           /ANY +,SET
0343    0355  6742          JMP RETURN      /+,RETURN
0344    0356  1020          LDA I           /ADD A 1 TO STIM
0345    0357  0001          1               /STORE SITE THIS
0346    0360  1140          ADM             /BIN
0347    0361  0767          WORD2
0350    0362  4353          STC SGATE       /SET +,CLOSED S
0351    0363  0110          SAM 10          /SYNC ONLY,MAY
0352    0364  6742          JMP RETURN      /BE UPDATED
0353    0365  1000  NERN,   LDA             /GET CHAN TO SAM
0354    0366  0363          NERN-2          /FROM STORAGE
0355    0367  4373          STC .+4         /SET IT UP
0356    0370  1020          LDA I
0357    0371  7776          -1              /IF ENTERED,NO
0360    0372  4353          STC SGATE       /STIM,CLEAR GATE
0361    0373  0111          SAM 11          /CONTAINS 10 SAM
0362    0374  0342          SCR 2           /SCALE 1:4
0363    0375  0451          APO
0364    0376  6742          JMP RETURN      /-,DISCARD
0365    0377  1040          STA             /+,STORE A LEVEL
0366    0400  0743          HOLD
0367    0401  2416          ADD LAST        /PREV AMPLITUDE
0370    0402  0471          APO I           /SKIP IF -
0371    0403  6411          JMP RISE        /SAMPLE RISES
0372    0404  1020          LDA I
```

```
0373    0405  7776  NGATE,  -1              /-1 CLEAR,+ SET
0374    0406  0451          APO
0375    0407  6415          JMP LEVEL       /CLOSED GATE
0376    0410  6742          JMP RETURN
0377    0411  1020  RISE,    LDA I           /IF RISING,CLEAR
0400    0412  7776          -1              /THE GATE
0401    0413  4405          STC NGATE
0402    0414  6742          JMP RETURN
0403    0415  1020  LEVEL,   LDA I
0404    0416  0000  LAST,    0               /PREV SAMPLE
0405    0417  0017          COM             /REINVERT
0406    0420  1040          STA             /SET GATE +
0407    0421  0405          NGATE
0410    0422  1120          ADA I
0411    0423  7724          -53             /GROSS LEVEL
0412    0424  0471          APO I           /ASSIGNMENTS
0413    0425  6451          JMP STORE
0414    0426  1000          LDA
0415    0427  0416          LAST            /GET PEAK VALUE
0416    0430  0017          COM
0417    0431  1120          ADA I
0420    0432  7770          -7
0421    0433  0451          APO             /TOO SMALL
0422    0434  6742          JMP  RETURN     /LESS THAN 7
0423    0435  1120          ADA I
0424    0436  7763          -14
0425    0437  0451          APO             /SMALLEST?
0426    0440  6472          JMP TEM0        /LEVEL 7
0427    0441  1120          ADA I
0430    0442  7763          -14
0431    0443  0451          APO
0432    0444  6477          JMP TEM1        /LEVEL 6
0433    0445  1120          ADA I
0434    0446  7763          -14
0435    0447  0451          APO
0436    0450  6504          JMP TEM2        /LEVEL 5
0437    0451  1120  STORE,   ADA I
0440    0452  7763          -14
0441    0453  0451          APO
0442    0454  6511          JMP TEM3        /LEVEL 4
0443    0455  1120          ADA I
0444    0456  7763          -14
0445    0457  0451          APO
0446    0460  6516          JMP TEM4        /LEVEL 3
0447    0461  1120          ADA I
0450    0462  7763          -14
0451    0463  0451          APO
0452    0464  6523          JMP TEM5        /LEVEL 2
0453    0465  1120          ADA I
0454    0466  7707          -70
0455    0467  0451          APO
0456    0470  6530  JMP TEM6          /LEVEL 1,       HIGHEST
0457    0471  6742          JMP  RETURN     /OVERFLOW ERROR
0460    0472  1020  TEM0,    LDA I
0461    0473  1000          1000            /XXX000000000
0462    0474  1140          ADM             /SMALLEST LEVEL
0463    0475  0766          WORD1
0464    0476  6742          JMP RETURN
0465    0477  1020  TEM1,    LDA I           /000XXX000000
0466    0500  0100          100
0467    0501  1140          ADM
0470    0502  0766          WORD1
0471    0503  6742          JMP RETURN
```

```
0472    0504  1020  TEM2,   LDA I          /000000XXX000
0473    0505  0010          10
0474    0506  1140          ADM
0475    0507  0766          WORD1
0476    0510  6742          JMP RETURN
0477    0511  1020  TEM3,   LDA I          /000000000XXX
0500    0512  0001          1               /FULL WORD1
0501    0513  1140          ADM
0502    0514  0766          WORD1
0503    0515  6742          JMP RETURN
0504    0516  1020  TEM4,   LDA I          /XXX000000000
0505    0517  1000          1000
0506    0520  1140          ADM
0507    0521  0767          WORD2
0510    0522  6742          JMP RETURN
0511    0523  1020  TEM5,   LDA I          /000XXX000000
0512    0524  0100          100
0513    0525  1140          ADM
0514    0526  0767          WORD2
0515    0527  6742          JMP RETURN
0516    0530  1020  TEM6,   LDA I          /000000XXX000
0517    0531  0010          10              /LARGEST LEVEL
0520    0532  1140          ADM
0521    0533  0767          WORD2
0522    0534  6742          JMP RETURN
0523    0535  6332  RTEST,  JMP NSAMP
0524    0536  1000          LDA             /FULL MEMORY?
0525    0537  0003          3
0526    0540  1560          BCL I
0527    0541  7000          7000            /STRIP TO QUARTR
0530    0542  1120          ADA I
0531    0543  7410          -367            /FILLED NUMBER
0532    0544  0451          APO             /FILLED?
0533    0545  6101          JMP SAMP        /NO,RETURN
0534    0546  6332          JMP NSAMP
0535    0547  1000          LDA
0536    0550  0773          LOBP            /LOW BP IN EPOCH
0537    0551  1063          STA I 3         /AT 2371
0540    0552  4167          STC LIMIT       /HR SLOPE WINDOW
0541    0553  6332          JMP NSAMP
0542    0554  1000          LDA
0543    0555  0776          HIBP            /HI BP IN EPOCH
0544    0556  1063          STA I 3         /AT 2372
0545    0557  6332          JMP NSAMP
0546    0560  1000          LDA             /EPOCH HEART RAT
0547    0561  0775          HRATE           /E AS INTERVAL
0550    0562  1063          STA I 3         /AT 2373
0551    0563  6332          JMP NSAMP
0552    0564  1000          LDA
0553    0565  0765          LDIF            /MAX DV/DT
0554    0566  1063          STA I 3
0555    0567  6332          JMP NSAMP
0556    0570  1000          LDA             /EPOCH TOTAL
0557    0571  0760          TRVOL           /RESP VOL
0560    0572  1063          STA I 3         /AT 2375
0561    0573  6332          JMP NSAMP
0562    0574  1000  LOAD,   LDA             /BN COUNTER
0563    0575  0762          BLOCK
0564    0576  0014          ATR             /RELAY LOAD
0565    0577  1460          SAE I
0566    0600  0777  AEND,   777             /LAST BN TO LOAD
0567    0601  6603          JMP .+2         /NO,CHANGE ON
0570    0602  7314          JMP READ        /YES,READ
```

```
0571    0603  6332          JMP NSAMP
0572    0604  1000          LDA             /LAST ADDRESS
0573    0605  0003          3               /IT FILLED
0574    0606  1560          BCL I           /TO DET MEM QTR
0575    0607  0777          0777            /LEAVE 2 OR 3
0576    0610  1460          SAE I
0577    0611  2000          2000            /Q4 AT 2377
0600    0612  6647          JMP L3000       /Q6 AT 3377
0601    0613  6332          JMP NSAMP
0602    0614  0063          SET I 3
0603    0615  3000          3000            /Q6 INITIAL
0604    0616  6332          JMP NSAMP
0605    0617  1000          LDA
0606    0620  0762          BLOCK           /TAPE BN
0607    0621  1120          ADA I           /ADVANCE BY 1
0610    0622  0001          1
0611    0623  1040          STA
0612    0624  0762          BLOCK           /BN READY
0613    0625  1120          ADA I
0614    0626  4000          4000            /WRITE 04,2000+
0615    0627  4632          STC .+3
0616    0630  6332          JMP NSAMP
0617    0631  0716          WRI 10          /WRITE TAPE 1
0620    0632  0000          0               /BN/QARTER
0621    0633  6332  CLEAR,  JMP NSAMP
0622    0634  0011          CLR             /INITIALIZES ALL
0623    0635  4776          STC HIBP        /TEMP STORES
0624    0636  4775          STC HRATE
0625    0637  4765          STC LDIF
0626    0640  4760          STC TRVOL
0627    0641  6332          JMP NSAMP
0630    0642  1020          LDA I
0631    0643  0777          777
0632    0644  4773          STC LOBP
0633    0645  6332          JMP NSAMP
0634    0646  6101          JMP SAMP
0635    0647  6332  L3000,  JMP NSAMP       /LOAD DATA FROM
0636    0650  0063          SET I 3         /QARTER 6,3000+
0637    0651  2000          2000            /START Q 4 POINT
0640    0652  6332          JMP NSAMP
0641    0653  1000          LDA
0642    0654  0762          BLOCK
0643    0655  1120          ADA I
0644    0656  0001          1               /ADVANCE TAPE BN
0645    0657  1040          STA
0646    0660  0762          BLOCK           /READY BN
0647    0661  1120          ADA I
0650    0662  6000          6000            /WRITE Q6,3000+
0651    0663  4666          STC .+3
0652    0664  6332          JMP NSAMP
0653    0665  0716          WRI 10          /WRITE TAPE 1
0654    0666  0000          0               /BN/ON
0655    0667  6633          JMP CLEAR       /RESET STORES
0656    0670  1020  CAL,    LDA I
0657    0671  4000          -3777           /VERY SLOW CLOCK
0660    0672  4045          STC PRESET      /SLOWDOWN
0661    0673  0004          ESF             /CLEAR FAST SAM
0662    0674  0500          IOB
0663    0675  6133          6133            /CLAB
0664    0676  0500          IOB
0665    0677  6131          6131            /CLSK
0666    0700  6704          JMP .+4
0667    0701  1023          LDA I 3         /FLAG.INCREMENT
```

```
0670    0702  0500          IOB
0671    0703  6135          6135            /CLEAR FLAG
0672    0704  0011          CLR             /0 BASELINE
0673    0705  0143          DIS 3           /REFERENCE
0674    0706  0110          SAM 10          /NERVE CHANNEL
0675    0707  0143          DIS 3
0676    0710  0342          SCR 2           /SCALE 4:1
0677    0711  0017          COM             /INVERT
0700    0712  4751          STC BASE1       /NERVE BASE
0701    0713  0111          SAM 11          /STIM MARK CH.
0702    0714  0143          DIS 3
0703    0715  0343          SCR 3           /SCALE 1:16
0704    0716  0017          COM             /INVERT
0705    0717  4752          STC BASE2       /STIM BASELINE
0706    0720  0112          SAM 12          /BP CHANNEL
0707    0721  0143          DIS 3
0710    0722  0017          COM             /INVERT
0711    0723  4753          STC BASE3       /BP 0 LINE
0712    0724  0113          SAM 13          /RESP
0713    0725  0143          DIS 3
0714    0726  0343          SCR 3           /SCALE 1:16
0715    0727  0017          COM             /INVERT
0716    0730  4754          STC BASE4       /RESP BASELINE
0717    0731  0100          SAM 0           /AF BASELINE
0720    0732  0143          DIS 3           /VARIABLE
0721    0733  0442          SNS 2           /CAL EXIT
0722    0734  6670          JMP CAL         /NO EXIT
0723    0735  1020          LDA I
0724    0736  7766          -11             /INPUT PRESET
0725    0737  4045          STC PRESET      /REPLACE VALUE
0726    0740  6020          JMP 20          /START
0727    0741  0016          NOP
0730    0742  0000  RETURN,  0
0731    0743  0000  HOLD,    0
0732    0744  0000  CURSER,  0
0733    0745  0000  CURSPT,  0
0734    0746  0000  SECOND,  0
0735    0747  0000  STEM,    0
0736    0750  5007  DIFF,    -2770
0737    0751  0000  BASE1,   0
0740    0752  0000  BASE2,   0
0741    0753  0000  BASE3,   0
0742    0754  0000  BASE4,   0
0743    0755  6000  DELAY,   -1777
0744    0756  0133  TMTB,    133
0745    0757  0000  RLAST,   0
0746    0760  0000  TRVOL,   0
0747    0761  0000  TDIF,    0
0750    0762  0000  BLOCK,   0
0751    0763  0000  IHRA,    0
0752    0764  0000  DIF,     0
0753    0765  0000  LDIF,    0
0754    0766  0000  WORD1,   0
0755    0767  0000  WORD2,   0
0756    0770  0000  GARBAGE, 0
0757    0771  0000  TBP,     0
0760    0772  0000  BPL,     0
0761    0773  0777  LOBP,    777
0762    0774  0000  YAXIS,   0
0763    0775  0000  HRATE,   0
0764    0776  0001  HIBP,    1
0765    0777  0000  HLAST,   0
0766    1000  0000  HSECOND,        0
-
```

```
0767    1001  0000  GATE,   0
0770    1002  0000  TRESP,  0
0771    1003  0000  TIME1,  0
0772    1004  0000  TIME2,  0
0773    1005  0000  LBLOCK, 0
0774    1006  0000  INRESP, 0
0775    1007  0323  DIREKT, 323            /S LOADED
0776    1010  0000  SENSE,  0
0777    1011  0000  WPRINT, 0
1000    1012  0000  WDISP,  0
1001    1013  0000  WINDOW, 0
1002    1014  1120  PRINT,  ADA I          /PRINTOUT OCTAL
1003    1015  0260        260             /NERVE BY LEVELS
1004    1016  0006        DJR             /2 WORDS/INTERVL
1005    1017  0500        IOB
1006    1020  6041        6041            /TSF
1007    1021  7016        JMP .-3
1010    1022  0500        IOB
1011    1023  6046        6046            /TLS PRINT
1012    1024  6000        JMP 0
1013    1025  0500  NTYPE, IOB
1014    1026  6032        6032            /CLEAR AC & FLAG
1015    1027  0500        IOB
1016    1030  6042        6042            /CLEAR PRINT FLG
1017    1031  1000        LDA
1020    1032  0003        3
1021    1033  1460        SAE I
1022    1034  2371        2371
1023    1035  7045        JMP .+10
1024    1036  1020        LDA I
1025    1037  7734        -43
1026    1040  7014        JMP PRINT
1027    1041  1020        LDA I
1030    1042  7731        -46
1031    1043  7014        JMP PRINT
1032    1044  7314        JMP READ
1033    1045  1023        LDA I 3
1034    1046  1040        STA
1035    1047  1011        WPRINT          /TEMP STORE
1036    1050  1560        BCL I           /STRIP 1ST CHAR
1037    1051  0777        777             /ACTER
1040    1052  0311        ROR 9           /NUMBER 0-7
1041    1053  7014        JMP PRINT       /PRINT IT
1042    1054  1000        LDA
1043    1055  1011        WPRINT          /RETREIVE WORD
1044    1056  1560        BCL I           /STRIP 2ND CHAR
1045    1057  7077        7077            /ACTER
1046    1060  0306        ROR 6           /NUMBER 0-7
1047    1061  7014        JMP PRINT       /PRINT IT
1050    1062  1000        LDA
1051    1063  1011        WPRINT
1052    1064  1560        BCL I           /STRIP 3ED CHAR
1053    1065  7707        7707            /ACTER
1054    1066  0303        ROR 3
1055    1067  7014        JMP PRINT
1056    1070  1000        LDA
1057    1071  1011        WPRINT
1060    1072  1560        BCL I
1061    1073  7770        7770            /STRIP 4TH CHAR
1062    1074  7014        JMP PRINT       /ACTER
1063    1075  1020        LDA I
1064    1076  7757        -20             /SPACE
1065    1077  7014        JMP PRINT
```

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1066    1100  0500          IOB
1067    1101  6031          6031            /KSF FOR BREAK-
1070    1102  7104          JMP .+2         /OUT,QUIT PRINT
1071    1103  7314          JMP READ
1072    1104  0227          XSK I 7         /WORDS PER LINE
1073    1105  7025          JMP NTYPE
1074    1106  1020          LDA I
1075    1107  7734          -43             /CR
1076    1110  7014          JMP PRINT
1077    1111  1020          LDA I
1100    1112  7731          -46             /LF
1101    1113  7014          JMP PRINT
1102    1114  0067          SET I 7
1103    1115  7761          -16             /16 WORDS/LINE
1104    1116  7025          JMP NTYPE       /GET NEXT CHARTR
1105    1117  1000  DISP,    LDA             /8 LEVEL DISPLAY
1106    1120  0000          0               /RETURN ADDR
1107    1121  4742          STC RETURN
1110    1122  1000          LDA             /WANT A CURSOR?
1111    1123  0744          CURSER
1112    1124  1460          SAE I
1113    1125  0007          7               /CURSOR IF 7
1114    1126  7147          JMP CDISP       /OMIT CURSOR
1115    1127  0104          SAM 4           /HORIZ CURSOR
1116    1130  1120          ADA I
1117    1131  0400          400             /MAKE POSITIVE
1120    1132  0341          SCR 1           /SCALE 2:1
1121    1133  1040          STA
1122    1134  0745          CURSPT          /CURSOR ADDRESS
1123    1135  4001          STC 1           /LOCATION TO
1124    1136  1020          LDA I           /DISPLAY CURSOR
1125    1137  0274          274             /V,TOP TRACE
1126    1140  0141          DIS 1           /DISPLAY IT
1127    1141  1020          LDA I           /SERIES FORMS A
1130    1142  0275          275             /CURSOR LINE
1131    1143  0141          DIS 1
1132    1144  1020          LDA I
1133    1145  0276          276
1134    1146  0141          DIS 1
1135    1147  0011  CDISP,   CLR             /NO CURSOR
1136    1150  1040  DISPY,   STA             /Y CALIB. SCALE
1137    1151  0774          YAXIS
1140    1152  7154          JMP .+2         /SKIP IF AXIS
1141    1153  1023  DISPI,   LDA I 3
1142    1154  5012          STC WDISP       /ROUTINE FOR
1143    1155  1000          LDA             /DISPLAY OF 8
1144    1156  1012          WDISP           /LINES UNPACKED
1145    1157  1560          BCL I           /NERVE LEVELS
1146    1160  0777          0777            /STRIP 1ST
1147    1161  0307          ROR 7
1150    1162  1120          ADA I
1151    1163  7400          -377            /V COORD 1ST LVL
1152    1164  0143          DIS 3
1153    1165  1000          LDA
1154    1166  1012          WDISP
1155    1167  1560          BCL I
1156    1170  7077          7077            /STRIP 2ND
1157    1171  0304          ROR 4
1160    1172  1120          ADA I
1161    1173  7500          -277
1162    1174  0143          DIS 3
1163    1175  1000          LDA
1164    1176  1012          WDISP
```

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1165    1177  1560    BCL I
1166    1200  7707    7707
1167    1201  0301    ROR .1.
1170    1202  1120    ADA I
1171    1203  7600    -177
1172    1204  0143    DIS 3
1173    1205  1000    LDA
1174    1206  1012    WDISP
1175    1207  1560    BCL I
1176    1210  7770    7770
1177    1211  0242    ROL 2
1200    1212  1120    ADA I
1201    1213  7700    -77
1202    1214  0143    DIS 3
1203    1215  1000    LDA             /IS THIS AXIS
1204    1216  0774    YAXIS           /DISPLAY?
1205    1217  1460    SAE I
1206    1220  7777    7777
1207    1221  7225    JMP .+4         /Y AXIS POINTS
1210    1222  7223    JMP .+1         /DATA POINTS
1211    1223  1023    LDA I 3         /GET SECOND WORD
1212    1224  5012    STC WDISP
1213    1225  1000    LDA
1214    1226  1012    WDISP
1215    1227  1560    BCL I
1216    1230  0777    0777
1217    1231  0307    ROR 7
1220    1232  0143    DIS 3
1221    1233  1000    LDA
1222    1234  1012    WDISP
1223    1235  1560    BCL I
1224    1236  7077    7077
1225    1237  0304    ROR 4
1226    1240  1120    ADA I
1227    1241  0077    77
1230    1242  0143    DIS 3
1231    1243  1000    LDA
1232    1244  1012    WDISP
1233    1245  1560    BCL I
1234    1246  7707    7707
1235    1247  0301    ROR 1
1236    1250  1120    ADA I
1237    1251  0177    177
1240    1252  0143    DIS 3
1241    1253  1000    LDA
1242    1254  1012    WDISP
1243    1255  1560    BCL I
1244    1256  7770    7770
1245    1257  0242    ROL 2
1246    1260  1120    ADA I
1247    1261  0277    277
1250    1262  0143    DIS 3
1251    1263  1000    LDA             /Y AXIS CHECK
1252    1264  0774    YAXIS
1253    1265  1460    SAE I
1254    1266  7777    7777            /7777=AXIS DONE
1255    1267  7271    JMP .+2         /AXIS LOOP
1256    1270  7274    JMP .+4         /DATA
1257    1271  1120    ADA I           /INCREMENT Y AXS
1260    1272  1111    1111
1261    1273  7150    JMP DISPY
1262    1274  1020    LDA I
1263    1275  7777    7777            /SKIP Y AXIS COD
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1264    1276  4774          STC YAXIS
1265    1277  0500          IOB
1266    1300  6031          6031            /KSF
1267    1301  7303          JMP .+2
1270    1302  7314          JMP READ        /KSF BREAKOUT
1271    1303  1000          LDA
1272    1304  0003          3               /CURRENT ADDRESS
1273    1305  1560          BCL I
1274    1306  7000          7000
1275    1307  1120          ADA I           /CHECK FOR END
1276    1310  7407          -370            /OF DISP LINE
1277    1311  0451          APO             /DONE YET
1300    1312  7153          JMP DISPI       /NO
1301    1313  6742          JMP RETURN      /YES
1302    1314  0442  READ,   SNS 2           /KEYBOARD CONTRL
1303    1315  7333          JMP .+16
1304    1316  0237          XSK I 17        /PHOTO TIMING
1305    1317  7333          JMP .+14                /LOOP
1306    1320  0077          SET I 17
1307    1321  7771  APHOT,  -6              /NUMBER OF SCANS
1310    1322  0061          SET I 1
1311    1323  0000          0
1312    1324  0076          SET I 16
1313    1325  0001          1
1314    1326  0011          CLR
1315    1327  1756          DSC 16
1316    1330  0500          IOB             /RESET COUNTER
1317    1331  6031          6031            /KSF
1320    1332  7322          JMP .-10
1321    1333  0500          IOB
1322    1334  6031          6031            /KSF
1323    1335  7344          JMP .+7
1324    1336  0500          IOB
1325    1337  6036          6036            /KRB
1326    1340  1040          STA
1327    1341  1007          DIREKT          /EXECUTIVE
1330    1342  0601          LIF 1           /CHANGE TO IF 1
1331    1343  7403          JMP GET         /MODE TO DISP
1332    1344  0500          IOB
1333    1345  6032          6032            /CL AC&FLAG
1334    1346  0500          IOB
1335    1347  6042          6042            /CLEAR PRNT FLAG
1336    1350  1000          LDA             /BLOCK NO.TO
1337    1351  1005          LBLOCK          /DISPLAY FIELD
1340    1352  0601          LIF 1           /IN IF 1
1341    1353  7406          JMP VALUES      /DISP DIGITAL
1342    1354  1000  VREAD,  LDA
1343    1355  1007          DIREKT          /WHICH MODE?
1344    1356  1460          SAE I
1345    1357  0302          302             /B ENTRY, BN
1346    1360  7362          JMP .+2
1347    1361  7522          JMP BEN         /SET LBLOCK
1350    1362  1460          SAE I
1351    1363  0306          306             /F,FOREWARD
1352    1364  7366          JMP .+2
1353    1365  7574          JMP FORE
1354    1366  1460          SAE I
1355    1367  0322          322             /R, REVERSE
1356    1370  7372          JMP .+2
1357    1371  7564          JMP BACK
1360    1372  1460          SAE I
1361    1373  0310          310             /H,STIMULUS HIST
1362    1374  7376          JMP .+2
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```
1363    1375  7650      JMP STIM1
1364    1376  1460      SAE I
1365    1377  0304      304           /D,DELAY ZCROSS
1366    1400  7405      JMP .+5
1367    1401  1020      LDA I
1370    1402  5767      -2010         /50 MSEC DELAY
1371    1403  4755      STC DELAY
1372    1404  7622      JMP ZCROSS
1373    1405  1460      SAE I
1374    1406  0332      332           /Z,0CROSS
1375    1407  7414      JMP .+5
1376    1410  1020      LDA I
1377    1411  6000      -1777         /LOOK AT 1ST ADR
1400    1412  4755      STC DELAY
1401    1413  7622      JMP ZCROSS
1402    1414  1460      SAE I
1403    1415  0323      323           /S,SOURCE MODE
1404    1416  7420      JMP .+2
1405    1417  7616      JMP INBLOCK   /DISP 8 LEVELS
1406    1420  1460      SAE I
1407    1421  0303      303           /C,CLEAR MEM
1410    1422  7426      JMP .+4
1411    1423  7746      JMP SLOAD     /SET S MODE
1412    1424  0601      LIF 1         / TO IF 1
1413    1425  6250      JMP ERASE     /ERASE MEMORY
1414    1426  1460      SAE I
1415    1427  0301      301           /ADD HISTOS,A
1416    1430  7434      JMP .+4
1417    1431  7746      JMP SLOAD     /SET S MODE
1420    1432  0601      LIF 1         /SUMMATE CURRENT
1421    1433  6123      JMP ADDRUN    /TO MEMORY CONTS
1422    1434  1460      SAE I
1423    1435  0324      324           /TTYPE BP ETC.
1424    1436  7445      JMP .+7
1425    1437  0416      STD           /SKIP TAPE DONE
1426    1440  7437      JMP .-1       /WAITE FOR TAPE
1427    1441  0011      CLR
1430    1442  0500      IOB           /CHUG TTY
1431    1443  6046      6046          /TLS
1432    1444  7752      JMP TEMP      /TYPE NERVE VALS
1433    1445  1460      SAE I
1434    1446  0316      316           /N,DO NTYPE
1435    1447  7466      JMP .+17
1436    1450  1020      LDA I
1437    1451  0000      0             /CLEAR DIREKT
1440    1452  5007      STC DIREKT    /AND TYPE N
1441    1453  0063      SET I 3       /VALUES START AT
1442    1454  2000      2000
1443    1455  0067      SET I 7
1444    1456  7761      -16           /LINE LENGTH
1445    1457  0500      IOB
1446    1460  6042      6042          /CLEAR FLAG
1447    1461  0416      STD           /SKIP TAPE DONE
1450    1462  7461      JMP .-1       /WAIT FOR TAPE
1451    1463  0500      IOB
1452    1464  6046      6046          /TLS....WHY?
1453    1465  7025      JMP NTYPE     /TYPE N VALUES
1454    1466  1460      SAE I
1455    1467  0315      315           /M,RETREIVE MEMR
1456    1470  7474      JMP .+4
1457    1471  7746      JMP SLOAD     /SET S MODE
1460    1472  0601      LIF 1
1461    1473  6323      JMP RET       /BEGIN RETREIVAL
```

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1462    1474  1460          SAE J.
1463    1475  0314          314             /L,LETTERS
1464    1476  7504          JMP .+6
1465    1477  1020          LDA I           /ROUTINE FOR A
1466    1500  0000          0               /CAPTION ENTRY
1467    1501  5007          STC DIREKT      /ALSO ENTER VIA
1470    1502  0601          LIF 1           /SNS 1=0
1471    1503  6020          JMP 20
1472    1504  1460          SAE I
1473    1505  0326          326             /V,VARIABLE
1474    1506  7510          JMP .+2
1475    1507  7677          JMP CURSOR      /DISPLAY CURSOR
1476    1510  1460          SAE I
1477    1511  0305          305             /E,EXECUTE STRIP
1500    1512  7514          JMP .+2
1501    1513  7704          JMP EXECUTE     /UPDATE TO CURSR
1502    1514  1460          SAE I
1503    1515  0313          313             /K,KOMPOSITE
1504    1516  7521          JMP .+3
1505    1517  0601          LIF 1           /ADD ALL SAMS EA
1506    1520  6420          JMP KOMPOS      /BIN AS COMPOSIT
1507    1521  7314          JMP READ        /NO ENTRY
1510    1522  1020  BEN,     LDA I
1511    1523  0306          306             /FOREWARD
1512    1524  5007          STC DIREKT
1513    1525  0500          IOB
1514    1526  6031          6031            /KSF
1515    1527  7525          JMP .-2
1516    1530  0500          IOB
1517    1531  6036          6036            /KRB
1520    1532  1560          BCL I
1521    1533  7770          7770            /1ST BLOCK NO.
1522    1534  0246          ROL 6
1523    1535  5005          STC LBLOCK
1524    1536  0500          IOB
1525    1537  6031          6031            /KSF
1526    1540  7536          JMP .-2
1527    1541  0500          IOB
1530    1542  6036          6036            /KRB
1531    1543  1560          BCL I           /GET 2ND BN
1532    1544  7770          7770
1533    1545  0243          ROL 3
1534    1546  1140          ADM
1535    1547  1005          LBLOCK
1536    1550  0500          IOB
1537    1551  6031          6031            /KSF
1540    1552  7550          JMP .-2
1541    1553  0500          IOB
1542    1554  6036          6036            /KRB
1543    1555  1560          BCL I           /GET 3ED BN
1544    1556  7770          7770
1545    1557  1120          ADA I
1546    1560  7776          -1              /SUBTRACT 1
1547    1561  1140          ADM
1550    1562  1005          LBLOCK
1551    1563  7314          JMP READ
1552    1564  1020  BACK,    LDA I
1553    1565  0000          0               /RETURN ADDRESS
1554    1566  5007          STC DIREKT
1555    1567  1000          LDA
1556    1570  1005          LBLOCK
1557    1571  1120          ADA I
1560    1572  7776          -1              /MOVE TAPE BACK
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1561    1573  7603          JMP .+10          /ONE BLOCK
1562    1574  1023  FORE,    LDA I
1563    1575  0000          0                 /RETURN ADDRESS
1564    1576  5007          STC DIREKT
1565    1577  1000          LDA
1566    1600  1005          LBLOCK
1567    1601  1120          ADA I             /MOVE TAPE FORE-
1570    1602  0001          1                 /WARD 1 BLOCK
1571    1603  1040          STA
1572    1604  1005          LBLOCK
1573    1605  1120          ADA I
1574    1606  4000          4000              /ON
1575    1607  5611          STC .+2
1576    1610  0712          RDE 10            /READ TAPE
1577    1611  0000          0                 /ON/BN
1600    1612  0011          CLR
1601    1613  4744          STC CURSER        /CLEAR CURSOR
1602    1614  7746          JMP SLOAD         /???????
1603    1615  7314          JMP READ
1604    1616  0063  INBLOCK, SET I 3          /DISPLAY AS SAM-
1605    1617  2001          2001              /PLED,8 LEVELS
1606    1620  7117          JMP DISP
1607    1621  7314          JMP READ
1610    1622  0063  ZCROSS,  SET I 3          /DISPLAY START-
1611    1623  2000          2000              /ING W 0 AF CROS
1612    1624  1000          LDA
1613    1625  0003          3
1614    1626  1460          SAE I
1615    1627  2371          2371              /NO CROSS IN BN
1616    1630  7633          JMP .+3
1617    1631  7746          JMP SLOAD
1620    1632  7314          JMP READ
1621    1633  1023          LDA I 3           /DISCARD
1622    1634  1023          LDA I 3           /WORD2
1623    1635  1560          BCL I             /STRIP TO AIR
1624    1636  7770          7770
1625    1637  1120          ADA I
1626    1640  7774          -3                /REMOVE STIM
1627    1641  0451          APO               /AIR 0 CROSS?
1630    1642  7624          JMP .-16          /NO
1631    1643  1000          LDA               /YES
1632    1644  0003          3
1633    1645  2755          ADD DELAY         /DELAY,IF ANY
1634    1646  4745          STC CURSPT        /UPDATE TO ADDRS
1635    1647  7704          JMP EXECUTE       /PERFORM UPDATE
1636    1650  0063  STIM1,   SET I 3          /DISP STARTING
1637    1651  2000          2000              /AT 1ST STIM
1640    1652  1000          LDA
1641    1653  0003          3
1642    1654  1460          SAE I             /LAST ADDRESS TO
1643    1655  2371          2371              /CHECK
1644    1656  7661          JMP .+3
1645    1657  7746          JMP SLOAD         /SET S MODE
1646    1660  7314          JMP READ
1647    1661  1023          LDA I 3           /DISCARD
1650    1662  1023          LDA I 3           /WORD2
1651    1663  1560          BCL I             /REMOVE AIR MARK
1652    1664  7774          7774
1653    1665  1120          ADA I
1654    1666  7777          7777              /-0
1655    1667  0451          APO               /+ IF A STIM
1656    1670  7652          JMP .-16          /NO STIM,RE-TRY
1657    1671  1000          LDA               /ARRD OF STIM
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1660    1672  0003          3
1661    1673  1120          ADA I
1662    1674  6000          -1777          /CURSOR IS 0-777
1663    1675  4745          STC CURSPT     /STORE CURSOR
1664    1676  7704          JMP EXECUTE    /UPDATE DISPLAY
1665    1677  1020  CURSOR, LDA I          /GATE FOR A
1666    1700  0007          7              /CURSOR DISPLAY
1667    1701  4744          STC CURSER     /YES IF 7
1670    1702  7746          JMP SLOAD      /SET S MODE
1671    1703  7314          JMP READ
1672    1704  7746  EXECUTE,JMP SLOAD      /SET S MODE
1673    1705  1000          LDA            /REMOVE LEFT OF
1674    1706  1005          LBLOCK         /CURSOR,MOVE TO
1675    1707  1120          ADA I          /LEFT,FILL IN
1676    1710  5001          5001           /FROM NEXT BN
1677    1711  5713          STC .+2        /05,BN +1
1700    1712  0712          RDE 10         /READ NEXT BN
1701    1713  0000          0              /INTO2400+ BUFFR
1702    1714  0065          SET I 5        /RESTORAGE
1703    1715  2001          2001           /ADDRESS
1704    1716  1000          LDA            /UPDATE BEGIN AT
1705    1717  0745          CURSPT         /ADDRESS
1706    1720  1560          BCL I
1707    1721  0001          1              /START EVEN WORD
1710    1722  1120          ADA I
1711    1723  2001          2001           /WORD1
1712    1724  4004          STC 4          /GET FROM ADDRSS
1713    1725  0416          STD            /TAPE DONE?
1714    1726  7725          JMP .-1        /WAIT FOR TAPE
1715    1727  1000  CUTE,   LDA            /TEST FOR LAST
1716    1730  0004          4              /ADDRESS 04
1717    1731  1460          SAE I
1720    1732  2371          2371
1721    1733  7736          JMP .+3        /04 NOT DONE
1722    1734  0064          SET I 4        /04 DONE
1723    1735  2401          2401           /FILL IN FROM
1724    1736  1024          LDA I 4        /05,NEXT BLOCK
1725    1737  1065          STA I 5        /MOVE 4 TO 5
1726    1740  1000          LDA            /CURRENT LOAD
1727    1741  0005          5              /ADDRESS
1730    1742  1460          SAE I          /BALANCE FILLED
1731    1743  2371          2371
1732    1744  7727          JMP CUTE       /NOT DONE
1733    1745  7314          JMP READ       /UPDATE DONE
1734    1746  1020  SLOAD,  LDA I          /SET S DISPLAY
1735    1747  0323          323
1736    1750  5007          STC DIREKT
1737    1751  6000          JMP 0
1740    1752  0601  TEMP,   LIF 1
1741    1753  7264          JMP TEMPNT
1742    1754  0002          PDP
1743                        PMODE
1744                        *1000
1745    1000  0000          0
1746    1001  7200          CLA
1747    1002  6046          TLS
1750    1003  1034          TAD K302       /B
1751    1004  4020          JMS TYPO
1752    1005  1044          TAD K320       /P
1753    1006  4020          JMS TYPO
1754    1007  1041          TAD K315       /M
1755    1010  4020          JMS TYPO
1756    1011  1037          TAD K311       /I
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1757    1012  4020    JMS TYPO
1760    1013  1042    TAD K316        /N
1761    1014  4020    JMS TYPO
1762    1015  1031    TAD K240        /SPACE
1763    1016  4020    JMS TYPO
1764    1017  1460    TAD I 60        /2371 BP LOAD
1765    1020  4065    JMS BINDEC      /DECIMAL CONVERT
1766    1021  1031    TAD K240        /SP
1767    1022  4020    JMS TYPO
1770    1023  1031    TAD K240        /SP
1771    1024  4020    JMS TYPO
1772    1025  1034    TAD K302        /B
1773    1026  4020    JMS TYPO
1774    1027  1044    TAD K320        /P
1775    1030  4020    JMS TYPO
1776    1031  1041    TAD K315        /M
1777    1032  4020    JMS TYPO
2000    1033  1033    TAD K301        /A
2001    1034  4020    JMS TYPO
2002    1035  1051    TAD K330        /X
2003    1036  4020    JMS TYPO
2004    1037  1031    TAD K240        /SPACE
2005    1040  4020    JMS TYPO
2006    1041  1461    TAD I 61        /2372,BPMAX
2007    1042  4065    JMS BINDEC      /DECIMAL CONVERT
2010    1043  1031    TAD K240        /SP
2011    1044  4020    JMS TYPO
2012    1045  1031    TAD K240        /SP
2013    1046  4020    JMS TYPO
2014    1047  1036    TAD K310        /H
2015    1050  4020    JMS TYPO
2016    1051  1045    TAD K322        /R
2017    1052  4020    JMS TYPO
2020    1053  1031    TAD K240        /SP
2021    1054  4020    JMS TYPO
2022    1055  1131    TAD RAT         /PRESET CODE
2023    1056  7040    CMA             /COM AC(PRESET)
2024    1057  7421    MQL             /LOAD MQ
2025    1060  1462    TAD I 62        /2373,HR
2026    1061  3263    DCA .+2
2027    1062  7405    MUY             /MULTIPLY
2030    1063  0000    0               /PRESET X HR INT
2031    1064  7501    MQA             /ERVAL
2032    1065  7000    NOP
2033    1066  7000    NOP             /ROD FOR PAGING
2034    1067  7000    NOP
2035    1070  3275    DCA .+5
2036    1071  1127    TAD LOW         /CONVERSION FOR
2037    1072  7421    MQL             /MSEC TO MINUTE
2040    1073  1130    TAD HI
2041    1074  7407    DVI
2042    1075  0000    0               /OCTAL RATE
2043    1076  7200    CLA
2044    1077  7501    MQA
2045    1100  4065    JMS BINDEC      /DECIMAL CONVERT
2046    1101  1031    TAD K240        /SPACE
2047    1102  4020    JMS TYPO
2050    1103  1031    TAD K240        /SPACE
2051    1104  4020    JMS TYPO
2052    1105  1041    TAD K315        /M
2053    1106  4020    JMS TYPO
2054    1107  1033    TAD K301        /A
2055    1110  4020    JMS TYPO
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2056    1111  1037          TAD K311        /I
2057    1112  4020          JMS TYPO
2060    1113  1045          TAD K322        /R
2061    1114  4020          JMS TYPO
2062    1115  1031          TAD K240        /SPACE
2063    1116  4020          JMS TYPO
2064    1117  1046          TAD K323        /S
2065    1120  4020          JMS TYPO
2066    1121  1040          TAD K314        /L
2067    1122  4020          JMS TYPO
2070    1123  1043          TAD K317        /O
2071    1124  4020          JMS TYPO
2072    1125  1044          TAD K320        /P
2073    1126  4020          JMS TYPO
2074    1127  1035          TAD K305        /E
2075    1130  4020          JMS TYPO
2076    1131  1031          TAD K240        /SPACE
2077    1132  4020          JMS TYPO
2100    1133  1463          TAD I 63        /2374,LDIF
2101    1134  4065          JMS BINDEC      /DECIMAL CONVERT
2102    1135  1031          TAD K240        /SPACE
2103    1136  4020          JMS TYPO
2104    1137  1031          TAD K240        /SPACE
2105    1140  4020          JMS TYPO
2106    1141  1047          TAD K324        /T
2107    1142  4020          JMS TYPO
2110    1143  1050          TAD K326        /V
2111    1144  4020          JMS TYPO
2112    1145  1031          TAD K240        /SPACE
2113    1146  4020          JMS TYPO
2114    1147  1464          TAD I 64        /2375,TV
2115    1150  4065          JMS BINDEC      /DECIMAL CONVERT
2116    1151  1030          TAD K215        /CR
2117    1152  4020          JMS TYPO
2120    1153  1027          TAD K212        /LF
2121    1154  4020          JMS TYPO
2122    1155  6141          LINC
2123                        LMODE
2124    1156  0643          LDF 3
2125    1157  0602          LIF 2
2126    1160  7746          JMP SLOAD       /SET S MODE
2127    1161  7314          JMP READ        /GET COMMAND
2130    1162  0002          PDP
2131                        PMODE
2132                        *20
2133    0020  0000  TYPO,     0
2134    0021  6041          TSF             /CK PRINT FLAG
2135    0022  5021          JMP .-1         /NO FLAG
2136    0023  6046          TLS             /PRINT
2137    0024  7200          CLA
2140    0025  5420          JMP I TYPO
2141    0026  0000  REMAIN,   0
2142    0027  0212  K212,     212           /LF
2143    0030  0215  K215,     215           /CR
2144    0031  0240  K240,     240           /SPACE
2145    0032  0260  K260,     260           /ASCII CODE
2146    0033  0301  K301,     301           /A
2147    0034  0302  K302,     302           /B
2150    0035  0305  K305,     305           /E
2151    0036  0310  K310,     310           /H
2152    0037  0311  K311,     311           /I
2153    0040  0314  K314,     314           /L
2154    0041  0315  K315,     315           /M
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2155    0042  0316  K316,   316          /N
2156    0043  0317  K317,   317          /O
2157    0044  0320  K320,   320          /P
2160    0045  0322  K322,   322          /R
2161    0046  0323  K323,   323          /S
2162    0047  0324  K324,   324          /T
2163    0050  0326  K326,   326          /V
2164    0051  0330  K330,   330          /X
2165    0052  0740  K740,   740
2166    0053  0000  DEVIS,  0
2167    0054  1000  TEMB,   1000
2170                        *60
2171    0060  6371          6371         /2371,BPMIN
2172    0061  6372          6372         /2372,BPMAX
2173    0062  6373          6373         /2373,HR
2174    0063  6374          6374         /2374,MAX AF
2175    0064  6375          6375         /2375,TV
2176    0065  0000  BINDEC,  0            /OCTAL TO DECI-
2177    0066  7100          CLL          /MAL CONVERSION
2200    0067  7421          MQL
2201    0070  7407          DVI
2202    0071  1750          1750         /DIV BY 1750
2203    0072  3026          DCA REMAIN   /STORE REMAINDER
2204    0073  7200          CLA
2205    0074  7501          MQA          /1ST DIGIT
2206    0075  1032          TAD K260     /ADD ASCII CODE
2207    0076  4020          JMS TYPO     /PRINT IT
2210    0077  1026          TAD REMAIN   /GET REMAINDER
2211    0100  7100          CLL
2212    0101  7421          MQL
2213    0102  7407          DVI
2214    0103  0144          144          /DIVIDE BY 144
2215    0104  3026          DCA REMAIN
2216    0105  7200          CLA
2217    0106  7501          MQA          /2ND DIGIT
2220    0107  1032          TAD K260
2221    0110  4020          JMS TYPO
2222    0111  1026          TAD REMAIN
2223    0112  7100          CLL
2224    0113  7421          MQL
2225    0114  7407          DVI
2226    0115  0012          12           /DIVIDE BY 12
2227    0116  3026          DCA REMAIN
2230    0117  7200          CLA
2231    0120  7501          MQA          /3ED DIGIT
2232    0121  1032          TAD K260
2233    0122  4020          JMS TYPO
2234    0123  1026          TAD REMAIN   /4TH DIGIT
2235    0124  1032          TAD K260
2236    0125  4020          JMS TYPO
2237    0126  5465          JMP I BINDEC
2240                        MQA=7501
2241                        DVI=7407
2242                        MQL=7421
2243                        MUY=7405
2244    0127  1060  LOW,    1060         /171060 OCTAL
2245    0130  0015  HI,     15           /INTERVAL-RATE
2246    0131  7766  RAT,    -12
2247                        PMODE
2250                        *2020        /IF 1
2251                        LMODE        /TITLE ROUTINE
2252    0020  0075          SET I 15     /INCOMING LETTER
2253    0021  1000          1000         /STORAGE ADDRESS
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2254    0022  0074          SET I 14        /DISPLAY STARTS
2255    0023  1000          1000;           /AT
2256    0024  0500          IOB
2257    0025  6046          6046            /ECHO,CLEAR
2260    0026  0445  LETTERS, SNS 5           /CHARACTER SIZE
2261    0027  6033          JMP .+4
2262    0030  1020          LDA I
2263    0031  0000          0               /FULL SIZE
2264    0032  6035          JMP .+3
2265    0033  1020          LDA I
2266    0034  0200          200             /HALF SIZE
2267    0035  0004          ESF
2270    0036  6037          JMP LISN
2271    0037  0500  LISN,    IOB
2272    0040  6031          6031            /KSF
2273    0041  6060          JMP TITLE       /NO INPUT,DISPLA
2274    0042  0500          IOB
2275    0043  6036          6036            /KRB,READIN
2276    0044  0500          IOB
2277    0045  6046          6046            /ECHO
2300    0046  1560          BCL I           /STRIP
2301    0047  7700          7700
2302    0050  0241          ROL 1           /DOUBLE
2303    0051  1120          ADA I           /PATTERN WORD
2304    0052  0600          600             /STARTING ADDRSS
2305    0053  1075          STA I 15        /STORE AT
2306    0054  1460          SAE I           /NUMBER SIGN
2307    0055  0706          706             /ERASE CODE
2310    0056  6037          JMP LISN        /GET NEXT LETTER
2311    0057  6106          JMP EXIT        /ERASE
2312    0060  0074  TITLE,   SET I 14        /READ FROM
2313    0061  1000          1000
2314    0062  0061          SET I 1         /DISP LEFT SIDE
2315    0063  4000          4000            /CH 2 DISPLAY
2316    0064  1034  INTITL,  LDA I 14        /BUMP
2317    0065  1000          LDA             /LAST ADDRESS
2320    0066  0015          15              /FILLED W INPUT
2321    0067  0017          COM             /INVERT
2322    0070  1100          ADA             /LAST ADDRESS
2323    0071  0014          14              /DISPLAYED
2324    0072  0451          APO             /DONE DISPLAY?
2325    0073  6075          JMP .+2         /NO
2326    0074  6116          JMP END         /YES
2327    0075  1014          LDA 14          /HORIZ DISP PT
2330    0076  4100          STC .+2
2331    0077  0076          SET I 16        /HORIZ FOR DSC
2332    0100  0000          0
2333    0101  1020          LDA I
2334    0102  0370          370             /V FOR DSC
2335    0103  1756          DSC 16
2336    0104  1776          DSC I 16
2337    0105  6064          JMP INTITL      /NEXT CHARACTER
2340    0106  0075  EXIT,    SET I 15        /ERASE TABLE
2341    0107  1000          1000            /OF CHARACTERS
2342    0110  0500          IOB
2343    0111  6042          6042            /TCF,CLEAR FLAG
2344    0112  0500          IOB
2345    0113  6244          6244            /RMF
2346    0114  7354          JMP VREAD       /CONTINUE EXECUT
2347    0115  6037          JMP LISN
2350    0116  0441  END,     SNS 1           /ENTER CHARACTR?
2351    0117  6122          JMP .+3         /YES
2352    0120  0602          LIF 2           /NO
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2353    0121  7354          JMP VREAD              
2354    0122  6037          JMP LISN       /GO GET CHARACTR
2355    0123  1026  ADDRUN, LDA I 6       /N COUNTER
2356    0124  6126          JMP IADDR      /FOR SUMMATION
2357    0125  6144          JMP ICLEAR     /IN COMPOSITE
2360    0126  1000  IADDR,  LDA            /RETURN ADDRESS
2361    0127  0000          0
2362    0130  4246          STC IRETRN
2363    0131  0067          SET I 7
2364    0132  2000          2000           /GET FROM ADR
2365    0133  0062          SET I 2
2366    0134  2000          2000           /BIT 1
2367    0135  0063          SET I 3
2370    0136  2400          2400           /BIT 2
2371    0137  0064          SET I 4
2372    0140  3000          3000           /BIT 3
2373    0141  0065          SET I 5
2374    0142  3400          3400           /BIT 4
2375    0143  6246          JMP IRETRN
2376    0144  1027  ICLEAR, LDA I 7        /GET PACKED WORD
2377    0145  1040          STA
2400    0146  0247          IWORD          /FIRST WORD
2401    0147  0644          LDF 4
2402    0150  1000          LDA
2403    0151  0247          IWORD          /GET WORD1
2404    0152  1560          BCL I          /STRIP TO THE
2405    0153  0777          0777           /FIRST CHARACTER
2406    0154  0311          ROR 11         /NUMBER 0-7
2407    0155  1162          ADM I 2        /BIT 1,WORD1
2410    0156  1000          LDA
2411    0157  0247          IWORD
2412    0160  1560          BCL I          /STRIP TO 2ND
2413    0161  7077          7077           /CHARACTER
2414    0162  0306          ROR 6          /NORMALIZE
2415    0163  1163          ADM I 3        /BIT 2,WORD1
2416    0164  1000          LDA
2417    0165  0247          IWORD
2420    0166  1560          BCL I          /3ED CHARACTER
2421    0167  7707          7707
2422    0170  0303          ROR 3
2423    0171  1164          ADM I 4        /BIT 3,WORD1
2424    0172  1000          LDA
2425    0173  0247          IWORD
2426    0174  1560          BCL I
2427    0175  7770          7770
2430    0176  1165          ADM I 5        /BIT 4,WORD1
2431    0177  0643          LDF 3
2432    0200  1027          LDA I 7        /GET PACKED WRD2
2433    0201  1040          STA
2434    0202  0247          IWORD
2435    0203  0645          LDF 5
2436    0204  1000          LDA
2437    0205  0247          IWORD
2440    0206  1560          BCL I          /STRIP TO 1ST
2441    0207  0777          0777           /CHARACTER
2442    0210  0311          ROR 11         /NORMALIZE
2443    0211  1142          ADM 2          /BIT 5,WORD2
2444    0212  1000          LDA
2445    0213  0247          IWORD
2446    0214  1560          BCL I
2447    0215  7077          7077           /STRIP 2ND CHAR.
2450    0216  0306          ROR 6
2451    0217  1143          ADM 3          /BIT 6,WORD2
```

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2452    0220  1000          LDA
2453    0221  0247          IWORD
2454    0222  1560          BCL I.          /STRIP 3ED CHAR.
2455    0223  7707          7707
2456    0224  0303          ROR 3
2457    0225  1144          ADM 4           /BIT 7,WORD2
2460    0226  1000          LDA
2461    0227  0247          IWORD
2462    0230  1560          BCL I           /BIT 8,WORD2
2463    0231  7770          7770            /STIM MK,A.F.
2464    0232  1145          ADM 5
2465    0233  0643          LDF 3
2466    0234  1000          LDA             /LAST RETREIVAL
2467    0235  0007          7               /ADDRESS
2470    0236  1460          SAE I
2471    0237  2370          2370            /DONE YET?
2472    0240  6242          JMP .+2         /NO
2473    0241  6244          JMP .+3         /YES
2474    0242  6144          JMP ICLEAR      /NOT DONE,NXT WD
2475    0243  0643          LDF 3           /RESTORE MEMORY
2476    0244  0602          LIF 2           /FIELDS &RETURN
2477    0245  7314          JMP READ        /TO CONTROL
2500    0246  0000  IRETRN, 0               /INTERNAL RETURN
2501    0247  0000  IWORD,  0               /WORD TO STRIP
2502    0250  0644  ERASE,  LDF 4           /CLEARS MEMORY
2503    0251  0067          SET I 7         /SET DF 4&5=0
2504    0252  2000          2000
2505    0253  0011          CLR             /FOR DF 4
2506    0254  1067          STA I 7         /STORE ZEROS
2507    0255  0207          XSK 7           /SKIP ON 3777
2510    0256  6253          JMP .-3         /DF 4 NOT DONE
2511    0257  0645          LDF 5           /NOW CLEAR DF 5
2512    0260  0067          SET I 7
2513    0261  2000          2000
2514    0262  0011          CLR
2515    0263  1067          STA I 7         /FILL WITH 0
2516    0264  0207          XSK 7           /SKIP OUT 3777
2517    0265  6262          JMP .-3         /FIELD NOT DONE
2520    0266  0066          SET I 6
2521    0267  0000          0               /RESET N COUNTER
2522    0270  0643          LDF 3           /RESTORE MEMORY
2523    0271  0602          LIF 2           /FIELDS & RETURN
2524    0272  7314          JMP READ        /TO CONTROL
2525    0273  0000  DEDEND,  0               /DIVIDEND
2526    0274  0000  DEVID,   0               /DIVISOR
2527    0275  4273  DEVY,    STC DEDEND
2530    0276  1000          LDA
2531    0277  0000          0               /RETURN ADDRESS
2532    0300  4246          STC IRETRN      /INTERNAL
2533    0301  0002          PDP             /ROUTINE DIVIDES
2534                        PMODE           /SUM OF N SAMPLS
2535    2302  7200          CLA             /BY N(AVERAGES)
2536    2303  1274          TAD DEVID       /DIVISOR,N
2537    2304  3310          DCA .+4
2540    2305  1273          TAD DEDEND      /DIVIDE STORAGE
2541    2306  7421          MQL             /CONTENTS BY N
2542    2307  7407          DVI
2543    2310  0000          0
2544    2311  7430          SZL             /SKIP IF 0
2545    2312  5316          JMP .+4
2546    2313  7200          CLA
2547    2314  7501          MQA
2550    2315  5317          JMP .+2
```

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2551    2316  7200          CLA             /INSURANCE!
2552    2317  6141          LINC
2553                        LMODE
2554    0320  1560          BCL I           /INSURES 1 DIGIT
2555    0321  7770          7770            /ONLY
2556    0322  6246          JMP IRETRN      /NEXT?
2557    0323  1000  RET,    LDA
2560    0324  0006          6
2561    0325  4274          STC DEVID
2562    0326  6126          JMP IADDR
2563    0327  0644          LDF 4
2564    0330  6331          JMP OCLEAR
2565    0331  1022  OCLEAR, LDA I 2
2566    0332  6275          JMP DEVY
2567    0333  0251          ROL 11
2570    0334  4247          STC IWORD
2571    0335  1023          LDA I 3
2572    0336  6275          JMP DEVY
2573    0337  0246          ROL 6
2574    0340  1140          ADM
2575    0341  0247          IWORD
2576    0342  1024          LDA I 4
2577    0343  6275          JMP DEVY
2600    0344  0243          ROL 3
2601    0345  1140          ADM
2602    0346  0247          IWORD
2603    0347  1025          LDA I 5
2604    0350  6275          JMP DEVY
2605    0351  1140          ADM
2606    0352  0247          IWORD
2607    0353  1000          LDA
2610    0354  0247          IWORD
2611    0355  0643          LDF 3
2612    0356  1067          STA I 7
2613    0357  0645          LDF 5
2614    0360  1002          LDA 2
2615    0361  6275          JMP DEVY
2616    0362  0251          ROL 11
2617    0363  4247          STC IWORD
2620    0364  1003          LDA 3
2621    0365  6275          JMP DEVY
2622    0366  0246          ROL 6
2623    0367  1140          ADM
2624    0370  0247          IWORD
2625    0371  1004          LDA 4
2626    0372  6275          JMP DEVY
2627    0373  0243          ROL 3
2630    0374  1140          ADM
2631    0375  0247          IWORD
2632    0376  1005          LDA 5
2633    0377  6275          JMP DEVY
2634    0400  1140          ADM
2635    0401  0247          IWORD
2636    0402  1000          LDA
2637    0403  0247          IWORD
2640    0404  0643          LDF 3
2641    0405  1067          STA I 7
2642    0406  1000          LDA
2643    0407  0007          7
2644    0410  1460          SAE I
2645    0411  2370          2370
2646    0412  6416          JMP .+4
2647    0413  7230          JMP SAVIT
-
```

```
2650    0414  0602          LIF 2
2651    0415  7314          JMP READ
2652    0416  0644          LDF 4
2653    0417  6331          JMP OCLEAR
2654    0420  0646  KOMPOS,  LDF 6
2655    0421  0062          SET I 2
2656    0422  2000          2000              /SOURCE
2657    0423  0063          SET I 3
2660    0424  3000          3000              /STORE
2661    0425  0064          SET I 4
2662    0426  3001          3001              /DISPLAY
2663    0427  0065          SET I 5
2664    0430  3175          3175              /S&AF MARKS
2665    0431  0011          CLR
2666    0432  1063          STA I 3
2667    0433  0203          XSK 3
2670    0434  6432          JMP .-2
2671    0435  0063          SET I 3
2672    0436  3000          3000
2673    0437  0643  IKOMPS,  LDF 3
2674    0440  1022          LDA I 2
2675    0441  0646          LDF 6
2676    0442  1040          STA
2677    0443  0247          IWORD
2700    0444  1560          BCL I
2701    0445  0777          777
2702    0446  0311          ROR 11
2703    0447  1163          ADM I 3
2704    0450  1000          LDA
2705    0451  0247          IWORD
2706    0452  1560          BCL I
2707    0453  7077          7077
2710    0454  0306          ROR 6
2711    0455  1143          ADM 3
2712    0456  1000          LDA
2713    0457  0247          IWORD
2714    0460  1560          BCL I
2715    0461  7707          7707
2716    0462  0303          ROR 3
2717    0463  1143          ADM 3
2720    0464  1000          LDA
2721    0465  0247          IWORD
2722    0466  1560          BCL I
2723    0467  7770          7770
2724    0470  1143          ADM 3
2725    0471  0643          LDF 3
2726    0472  1022          LDA I 2
2727    0473  0646          LDF 6
2730    0474  1040          STA
2731    0475  0247          IWORD
2732    0476  1560          BCL I
2733    0477  0777          777
2734    0500  0311          ROR 11
2735    0501  1143          ADM 3
2736    0502  1000          LDA
2737    0503  0247          IWORD
2740    0504  1560          BCL I
2741    0505  7077          7077
2742    0506  0306          ROR 6
2743    0507  1143          ADM 3
2744    0510  1000          LDA
2745    0511  0247          IWORD
2746    0512  1560          BCL I
```

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2747    0513  7707          7707
2750    0514  0303          ROR 3
2751    0515  1143          ADM 3
2752    0516  1000          LDA
2753    0517  0247          IWORD
2754    0520  1560          BCL I
2755    0521  7770          7770
2756    0522  0242  A,       ROL 2
2757    0523  1120          ADA I
2760    0524  7400          -377
2761    0525  1065          STA I 5
2762    0526  1000          LDA
2763    0527  0002          2
2764    0530  1460          SAE I
2765    0531  2370          2370
2766    0532  6437          JMP IKOMPS
2767    0533  0063          SET I 3
2770    0534  3000          3000
2771    0535  0062          SET I 2
2772    0536  7400          -377
2773    0537  0065          SET I 5
2774    0540  3175          3175
2775    0541  1000          LDA
2776    0542  0002          2
2777    0543  0143          DIS 3
3000    0544  1120          ADA I
3001    0545  0036          36              /V AXIS SPACING6
3002    0546  1040          STA
3003    0547  0002          2
3004    0550  1460          SAE I
3005    0551  0377          377
3006    0552  6541          JMP .-11
3007    0553  1023  DKOMPS,  LDA I 3
3010    0554  0245          ROL 5           /V MULT FACTOR
3011    0555  1120          ADA I
3012    0556  7433          -344
3013    0557  0144          DIS 4
3014    0560  1020          LDA I
3015    0561  7433          -344
3016    0562  0144          DIS 4           /ZERO DISPLAY
3017    0563  1025          LDA I 5
3020    0564  0144          DIS 4
3021    0565  1000          LDA
3022    0566  0003          3
3023    0567  1460          SAE I
3024    0570  3175          3175
3025    0571  6575          JMP .+4
3026    0572  0643          LDF 3
3027    0573  0602          LIF 2
3030    0574  7314          JMP READ
3031    0575  1560          BCL I
3032    0576  7000          7000            /DIS CH 0
3033    0577  0242          ROL 2
3034    0600  4004          STC 4
3035    0601  6553          JMP DKOMPS
3036                        PMODE
3037                        *3400           /1400 IF 1
3040                        LMODE
3041    1400  0320  VERT,    320
3042    1401  0000  BN,      0
3043    1402  0323  MODE,    323
3044    1403  5402  GET,     STC MODE
3045    1404  0602          LIF 2
```

```
3046        1405  6000            JMP 0
3047        1406  5401  VALUES,   STC BN
3050        1407  1000            LDA .
3051        1410  2371            2371
3052        1411  7462            JMP DECODE
3053        1412  7535            JMP BYPASS
3054        1413  1020  SETUP,    LDA I
3055        1414  0320            320
3056        1415  5400            STC VERT
3057        1416  0061            SET I 1
3060        1417  4450            4450              /H COORD
3061        1420  0071            SET I 11          /BETA PATTERN
3062        1421  0000            0
3063        1422  0072            SET I 12
3064        1423  1117            1117              /TABLE AT 1120
3065        1424  1032  IVALUE,   LDA I 12
3066        1425  1460            SAE I
3067        1426  1000            1000
3070        1427  7431            JMP .+2
3071        1430  7443            JMP VADVAN
3072        1431  1460            SAE I
3073        1432  1001            1001
3074        1433  7435            JMP .+2
3075        1434  7453            JMP VALEXIT
3076        1435  4011            STC 11            /PATTERN WORD
3077        1436  1000            LDA
3100        1437  1400            VERT
3101        1440  1751            DSC 11
3102        1441  1771            DSC I 11
3103        1442  7424            JMP .-16
3104        1443  1000  VADVAN,   LDA
3105        1444  1400            VERT
3106        1445  1120            ADA I
3107        1446  7737            -40
3110        1447  5400            STC VERT
3111        1450  0061            SET I 1
3112        1451  4450            4450
3113        1452  7424            JMP IVALUE
3114        1453  6060  VALEXIT,JMP TITLE
3115        1454  0600  K600,600
3116        1455  0000  P1,       0
3117        1456  0000  P2,       0
3120        1457  0000  P3,       0
3121        1460  0000  P4,       0
3122        1461  0000  LEFT,     0
3123        1462  0002  DECODE,   PDP
3124                              PMODE
3125        3463  7100            CLL
3126        3464  7421            MQL
3127        3465  7407            DVI
3130        3466  1750            1750
3131        3467  3261            DCA LEFT
3132        3470  7200            CLA
3133        3471  7501            MQA
3134        3472  7100            CLL
3135        3473  7004            RAL
3136        3474  1052            TAD K740
3137        3475  3255            DCA P1
3140        3476  1261            TAD LEFT
3141        3477  7100            CLL
3142        3500  7421            MQL
3143        3501  7407            DVI
3144        3502  0144            144
-
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```
3145    3503  3261          DCA LEFT
3146    3504  7200          CLA
3147    3505  7501          MQA
3150    3506  7100          CLL
3151    3507  7004          RAL
3152    3510  1052          TAD K740
3153    3511  3256          DCA P2
3154    3512  1261          TAD LEFT
3155    3513  7100          CLL
3156    3514  7421          MQL
3157    3515  7407          DVI
3160    3516  0012          12
3161    3517  3261          DCA LEFT
3162    3520  7200          CLA
3163    3521  7501          MQA
3164    3522  7100          CLL
3165    3523  7004          RAL
3166    3524  1052          TAD K740
3167    3525  3257          DCA P3
3170    3526  1261          TAD LEFT
3171    3527  7100          CLL
3172    3530  7004          RAL
3173    3531  1052          TAD K740
3174    3532  3260          DCA P4
3175    3533  6141          LINC
3176                        LMODE
3177    1534  6000          JMP 0
3200    1535  1000  BYPASS, LDA
3201    1536  1455          P1
3202    1537  5126          STC B1
3203    1540  1000          LDA
3204    1541  1456          P2
3205    1542  5127          STC B2
3206    1543  1000          LDA
3207    1544  1457          P3
3210    1545  5130          STC B3
3211    1546  1000          LDA
3212    1547  1460          P4
3213    1550  5131          STC B4
3214    1551  1000          LDA
3215    1552  2372          2372
3216    1553  7462          JMP DECODE
3217    1554  1000          LDA
3220    1555  1455          P1
3221    1556  5141          STC B5
3222    1557  1000          LDA
3223    1560  1456          P2
3224    1561  5142          STC B6
3225    1562  1000          LDA
3226    1563  1457          P3
3227    1564  5143          STC B7
3230    1565  1000          LDA
3231    1566  1460          P4
3232    1567  5144          STC B8
3233    1570  0002          PDP
3234                        PMODE
3235    3571  1131          TAD RAT
3236    3572  7040          CMA
3237    3573  7421          MQL
3240    3574  1462          TAD I 62
3241    3575  7000          NOP
3242    3576  7000          NOP
3243    3577  7000          NOP
```

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3244    3600  3202        DCA .+2
3245    3601  7405        MUY
3246    3602  0000        0
3247    3603  7501        MQA
3250    3604  3211        DCA .+5
3251    3605  1127        TAD LOW
3252    3606  7421        MQL
3253    3607  1130        TAD HI
3254    3610  7407        DVI
3255    3611  0000        0
3256    3612  7200        CLA
3257    3613  7501        MQA
3260    3614  6141        LINC
3261                      LMODE
3262    1615  7462        JMP DECODE
3263    1616  1000        LDA
3264    1617  1455        P1
3265    1620  5151        STC H1
3266    1621  1000        LDA
3267    1622  1456        P2
3270    1623  5152        STC H2
3271    1624  1000        LDA
3272    1625  1457        P3
3273    1626  5153        STC H3
3274    1627  1000        LDA
3275    1630  1460        P4
3276    1631  5154        STC H4
3277    1632  1000        LDA
3300    1633  2374        2374
3301    1634  7462        JMP DECODE
3302    1635  1000        LDA
3303    1636  1455        P1
3304    1637  5167        STC A1
3305    1640  1000        LDA
3306    1641  1456        P2
3307    1642  5170        STC A2
3310    1643  1000        LDA
3311    1644  1457        P3
3312    1645  5171        STC A3
3313    1646  1000        LDA
3314    1647  1460        P4
3315    1650  5172        STC A4
3316    1651  1000        LDA
3317    1652  2375        2375
3320    1653  7462        JMP DECODE
3321    1654  1000        LDA
3322    1655  1455        P1
3323    1656  5177        STC T1
3324    1657  1000        LDA
3325    1660  1456        P2
3326    1661  5200        STC T2
3327    1662  1000        LDA
3330    1663  1457        P3
3331    1664  5201        STC T3
3332    1665  1000        LDA
3333    1666  1460        P4
3334    1667  5202        STC T4
3335    1670  1000        LDA
3336    1671  1402        MODE
3337    1672  1560        BCL I
3340    1673  7700        7700
3341    1674  0241        ROL 1
3342    1675  1120        ADA I
-
```

```
3343    1676  0600          600
3344    1677  5220          STC X
3345    1700  1000          LDA
3346    1701  0006          6
3347    1702  7462          JMP DECODE
3350    1703  1000          LDA
3351    1704  1456          P2
3352    1705  5224          STC N1
3353    1706  1000          LDA
3354    1707  1457          P3
3355    1710  5225          STC N2
3356    1711  1000          LDA
3357    1712  1460          P4
3360    1713  5226          STC N3
3361    1714  1000          LDA
3362    1715  1401          BN
3363    1716  1560          BCL I
3364    1717  7077          7077
3365    1720  0305          ROR 5
3366    1721  1120          ADA I
3367    1722  0740          740
3370    1723  5207          STC BK1
3371    1724  1000          LDA
3372    1725  1401          BN
3373    1726  1560          BCL I
3374    1727  7707          7707
3375    1730  0302          ROR 2
3376    1731  1120          ADA I
3377    1732  0740          740
3400    1733  5210          STC BK2
3401    1734  1000          LDA
3402    1735  1401          BN
3403    1736  1560          BCL I
3404    1737  7770          7770
3405    1740  0241          ROL 1
3406    1741  1120          ADA I
3407    1742  0740          740
3410    1743  5211          STC BK3
3411    1744  7413          JMP SETUP
3412                        PMODE
3413                        *3120
3414                        LMODE
3415    1120  0604          604             /B
3416    1121  0640          640             /P
3417    1122  0632          632             /M
3420    1123  0622          622             /I
3421    1124  0634          634             /N
3422    1125  0700          700             /SPACE
3423    1126  0740    B1,   740
3424    1127  0740    B2,   740
3425    1130  0740    B3,   740
3426    1131  0740    B4,   740
3427    1132  1000          1000            /LINE COMMAND
3430    1133  0604          604             /B
3431    1134  0640          640             /P
3432    1135  0632          632             /M
3433    1136  0602          602             /A
3434    1137  0660          660             /X
3435    1140  0700          700             /SPACE
3436    1141  0740    B5,   740
3437    1142  0740    B6,   740
3440    1143  0740    B7,   740
3441    1144  0740    B8,   740
```

```
3442    1145  1000          1000      /LINE COMMAND
3443    1146  0620          620       /H
3444    1147  0644          644       /R
3445    1150  0700          700       /SPACE
3446    1151  0740  H1,     740
3447    1152  0740  H2,     740
3450    1153  0740  H3,     740
3451    1154  0740  H4,     740
3452    1155  1000          1000      /LINE COMMAND
3453    1156  0632          632       /M
3454    1157  0700          700       /SPACE
3455    1160  0602          602       /A
3456    1161  0622          622       /I
3457    1162  0644          644       /R
3460    1163  0700          700       /SPACE
3461    1164  0646          646       /S
3462    1165  0630          630       /L
3463    1166  0700          700       /SPACE
3464    1167  0740  A1,     740
3465    1170  0740  A2,     740
3466    1171  0740  A3,     740
3467    1172  0740  A4,     740
3470    1173  1000          1000      /LINE COMMAND
3471    1174  0650          650       /T
3472    1175  0654          654       /V
3473    1176  0700          700       /SPACE
3474    1177  0740  T1,     740
3475    1200  0740  T2,     740
3476    1201  0740  T3,     740
3477    1202  0740  T4,     740
3500    1203  1000          1000      /LINE COMMAND
3501    1204  0604          604       /B
3502    1205  0634          634       /N
3503    1206  0700          700       /SPACE
3504    1207  0740  BK1,    740
3505    1210  0740  BK2,    740
3506    1211  0740  BK3,    740
3507    1212  1000          1000      /LINE COMMAND
3510    1213  0632          632       /M
3511    1214  0636          636       /O
3512    1215  0610          610       /D
3513    1216  0612          612       /E
3514    1217  0700          700       /SPACE
3515    1220  0740  X,      740
3516    1221  1000          1000      /LINE COMMAND
3517    1222  0634          634       /N
3520    1223  0772          772       /=
3521    1224  0740  N1,     740
3522    1225  0740  N2,     740
3523    1226  0740  N3,     740
3524    1227  1001          1001      /TERMINATOR
3525    1230  0443  SAVIT,  SNS 3
3526    1231  6000          JMP 0
3527    1232  1000          LDA
3530    1233  1252          AKBLOK
3531    1234  0014          ATR
3532    1235  0000          HLT
3533    1236  1120          ADA I
3534    1237  0001          1
3535    1240  1040          STA
3536    1241  1252          AKBLOK
3537    1242  0014          ATR
3540    1243  0000          HLT
```

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3541    1244  1120          ADA I
3542    1245  4000          4000
3543    1246  5250          STC .+2
3544    1247  0706          WRI 0
3545    1250  0000          0
3546    1251  6000          JMP 0
3547    1252  0000  AKBLOK, 0
3550    1253  1120  PRINTIT,ADA I
3551    1254  0260          260
3552    1255  0006          DJR
3553    1256  0500          IOB
3554    1257  6041          6041            /TSF
3555    1260  7255          JMP .-3
3556    1261  0500          IOB
3557    1262  6046          6046            /TLS PRINT
3560    1263  6000          JMP 0
3561    1264  1020  TEMPNT, LDA I
3562    1265  7734          -43
3563    1266  7253          JMP PRINTIT
3564    1267  1020          LDA I
3565    1270  7731          -46
3566    1271  7253          JMP PRINTIT
3567    1272  1000          LDA
3570    1273  1401          BN
3571    1274  1560          BCL I
3572    1275  7077          7077
3573    1276  0306          ROR 6
3574    1277  7253          JMP PRINTIT
3575    1300  1000          LDA
3576    1301  1401          BN
3577    1302  1560          BCL I
3600    1303  7707          7707
3601    1304  0303          ROR 3
3602    1305  7253          JMP PRINTIT
3603    1306  1000          LDA
3604    1307  1401          BN
3605    1310  1560          BCL I
3606    1311  7770          7770
3607    1312  7253          JMP PRINTIT
3610    1313  1020          LDA I
3611    1314  7757          -20
3612    1315  7253          JMP PRINTIT.
3613    1316  0002          PDP
3614                        PMODE
3615    3317  4454          JMS I TEM8
3616                        PMODE
3617                        *2602
3620    2602  4477          4477
3621    2603  7744          7744            /A
3622    2604  5177          5177
3623    2605  2651          2651            /B
3624    2606  4136          4136
3625    2607  2241          2241            /C
3626    2610  4177          4177
3627    2611  3641          3641            /D
3630    2612  4577          4577
3631    2613  4145          4145            /E
3632    2614  4477          4477
3633    2615  4044          4044            /F
3634    2616  4136          4136
3635    2617  2645          2645            /G
3636    2620  1077          1077
3637    2621  7710          7710            /H
```

```
3640    2622  7741    7741
3641    2623  0041    0041        /I
3642    2624  4142    4142
3643    2625  4076    4076        /J
3644    2626  1077    1077
3645    2627  4324    4324        /K
3646    2630  0177    0177
3647    2631  0301    0301        /L
3650    2632  3077    3077
3651    2633  7730    7730        /M
3652    2634  3077    3077
3653    2635  7706    7706        /N
3654    2636  4177    4177
3655    2637  7741    7741        /O
3656    2640  4477    4477
3657    2641  3044    3044        /P
3660    2642  4276    4276
3661    2643  0376    0376        /Q
3662    2644  4477    4477
3663    2645  3146    3146        /R
3664    2646  5121    5121
3665    2647  4651    4651        /S
3666    2650  4040    4040
3667    2651  4077    4077        /T
3670    2652  0177    0177
3671    2653  7701    7701        /U
3672    2654  0176    0176
3673    2655  7402    7402        /V
3674    2656  0677    0677
3675    2657  7701    7701        /W
3676    2660  1463    1463
3677    2661  6314    6314        /X
3700    2662  0770    0770
3701    2663  7007    7007        /Y
3702    2664  4543    4543
3703    2665  6151    6151        /Z
3704    2666  4177    4177
3705    2667  0000    0000        /(
3706    2670  2040    2040
3707    2671  0410    0410        /REVER SL
3710    2672  0000    0
3711    2673  7741    7741        /)
3712    2674  2000    2000
3713    2675  2077    2077        /*
3714    2676  3410    3410
3715    2677  1010    1010        /BACK ARROW
3716    2700  0000    0
3717    2701  0000    0           /SPACE
3720    2702  7500    7500
3721    2703  0000    0           /!
3722    2704  6006    6006
3723    2705  0060    60          /"
3724    2706  3614    3614
3725    2707  1436    1436        /NUMBER SIGN
3726    2710  7721    7721
3727    2711  4677    4677        /DOLLAR SIGN
3730    2712  1446    1446
3731    2713  6130    6130        /%
3732    2714  5166    5166
3733    2715  0523    0523        /&
3734    2716  0500    500
3735    2717  0006    6           /APOSTROPHY
3736    2720  4163    4163
```

```
3737        2721  0000          0                 /(
3740        2722  0000          0
3741        2723  6341          6341              /)
3742        2724  2050          2050
3743        2725  0050          50                /*
3744        2726  0404          404
3745        2727  0437          437               /+
3746        2730  0605          605
3747        2731  0000          0                 /,
3750        2732  0404          404
3751        2733  0404          404               /-
3752        2734  0001          1
3753        2735  0000          0                 /.
3754        2736  0601          601
3755        2737  4030          4030              //
3756        2740  4136          4136
3757        2741  3641          3641              /0
3760        2742  2101          2101
3761        2743  0177          177               /1
3762        2744  4523          4523
3763        2745  2151          2151              /2
3764        2746  4122          4122
3765        2747  2651          2651              /3
3766        2750  2414          2414
3767        2751  0477          477               /4
3770        2752  5172          5172
3771        2753  0651          651               /5
3772        2754  1506          1506
3773        2755  4225          4225              /7
3774        2756  4443          4443
3775        2757  6050          6050              /7
3776        2760  5126          5126
3777        2761  2651          2651              /8
4000        2762  5120          5120
4001        2763  3651          3651              /9
4002        2764  4200          4200
4003        2765  0000          0                 /:
4004        2766  2601          2601
4005        2767  0000          0                 /;
4006        2770  2410          2410
4007        2771  0042          0042              /<
4010        2772  1212          1212
4011        2773  1212          1212              /=
4012        2774  4200          4200
4013        2775  1024          1024              /?
4014        2776  2055          2055
4015        2777  4020          4020              /?
4016        3000  0000          0000
4017        3001  0000          0000              /IGNORED
4020                            *2015
4021        2015  1000          1000
4022                    /GATED PEAKREADING VERSION WITH SCREEN
4023                    /ON SYSTEM TAPE AS PEAK S&B
4024                    /CHECKED VERSION
4025                    /TIMINGS CHECKED.2/14/71

NO ERRORS

A       2522
ADDRUN  2123
AEND    4600
AKBLOK  3252
APHOT   5321
-
```

```
A1      3167
A2      3170
A3      3171
A4      3172
BACK    5564
BASE1   4751
BASE2   4752
BASE3   4753
BASE4   4754
BEN     5522
BINDEC  0065
BK1     3207
BK2     3210
BK3     3211
BLOCK   4762
BN      3401
BPL     4772
BYPASS  3535
B1      3126
B2      3127
B3      3130
B4      3131
B5      3141
B6      3142
B7      3143
B8      3144
CAL     4670
CDISP   5147
CLEAR   4633
CLOCK   4046
CONTRO  4044
CURSER  4744
CURSOR  5677
CURSPT  4745
CUTE    5727
DECODE  3462
DEDEND  2273
DELAY   4755
DEVID   2274
DEVIS   0053
DEVY    2275
DIF     4764
DIFF    4750
DIREKT  5007
DISP    5117
DISPI   5153
DISPY   5150
DKOMPS  2553
DVI     7407
ENABLE  4043
END     2116
ERASE   2250
EXECUT  5704
EXIT    2106
FORE    5574
GARBAG  4770
GATE    5001
GATECL  4226
GET     3403
HGATE   4206
HI      0130
HIBP    4776
HLAST   4777
```

```
HOLD    4743
HR      4165
HRATE   4775
HRESET  4230
HSECON  5000
H1      3151
H2      3152
H3      3153
H4      3154
IADDR   2125
ICLEAR  2144
IHRA    4763
IKOMPS  2437
INBLOC  5616
INDIR   4105
INRESP  5006
INTIIL  2064
IRETRN  2245
IVALUE  3424
IWORD   2247
KOMPOS  2420
K212    0027
K215    0030
K240    0031
K260    0032
K301    0033
K302    0034
K305    0035
K310    0036
K311    0037
K314    0040
K315    0041
K316    0042
K317    0043
K320    0044
K322    0045
K323    0046
K324    0047
K326    0050
K330    0051
K600    3454
K740    0052
LAST    4416
LBLOCK  5005
LDIF    4765
LEFT    3461
LETTER  2026
LEVEL   4415
LIMIT   4167
LISN    2037
LOAD    4574
LOBP    4773
LOW     0127
L3000   4647
MODE    3402
MQA     7501
MQL     7421
MUY     7405
NERN    4365
NGATE   4405
NSAMP   4332
NTYPE   5025
N1      3224
-
```

N2	3225
N3	3226
OCLEAR	2331
PRESET	4045
PRINT	5014
PRINTI	3253
P1	3455
P2	3456
P3	3457
P4	3460
RAT	0131
READ	5314
REMAIN	0026
RESP	4235
RET	2323
RETURN	4742
RINTEG	4272
RISE	4411
RLAST	4757
RPULAR	4255
RTEST	4535
RTIME	4263
SAMP	4101
SAMPLE	4107
SAVIT	3230
SECOND	4746
SENSE	5010
SETUP	3413
SGATE	4353
SLOAD	5746
SLOPE	4301
STEM	4747
STIM1	5650
STORE	4451
TBP	4771
TDIF	4761
TEMP	5752
TEMPNT	3264
TEM0	4472
TEM1	4477
TEM2	4504
TEM3	4511
TEM4	4516
TEM5	4523
TEM6	4539
TEM8	0054
TIME	4002
TIME1	5003
TIME2	5004
TITLE	2060
TMTB	4756
TRESP	5002
TRVOL	4760
TYPO	0020
T1	3177
T2	3200
T3	3201
T4	3202
VADVAN	3443
VALEXI	3453
VALUES	3406
VERT	3400
VREAD	5354

WDISP	5012
WINDOW	5013
WORD1	4766
WORD2	4767
WPRINT	5011
X	3220
YAXIS	4774
ZCROSS	5622

APPROVAL SHEET

This dissertation submitted by Harold A. Spurgeon has been read and approved by the following five members of the Dissertation Committee:

Clarence N. Peiss, Ph.D. (Chairman of the Committee)

Ruy Lourenco, M.D.

G. Keith Matheson, Ph.D.

Walter C. Randall, Ph.D.

Robert D. Wurster, Ph.D.

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

This dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

May 18, 1973
Date

Clarence N. Peiss
Signature of Advisor