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THE UNIVERSITY OF ALBERTA

CONTROL AND ORGANIZATION OF CAT INTERCOSTAL MUSCLES DURING RESPIRATION

BY

JOHN JAMES GREER

A THESIS

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Control and organization of cat intercostal muscles during respiration

submitted by: John James Greer

in partial fulfilment of the requirements for the degree of: Ph.D.

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ABSTRACT

This thesis addresses several questions regarding the motor control of intercostal muscles in anesthetized or decerebrate cats. Specifically, we were interested in how the CNS utilizes the gamma motoneuron system to control the sensitivity of muscle spindles during respiration. This then led to an investigation of the recruitment patterns, length changes and muscle fiber properties of the intercostal muscles. The following experiments were performed: 1) Motor unit recordings with bipolar electrodes. 2) Extracellular recordings of muscle spindle afférents 3) Extracellular recordings of gamma motoneurons 4) Sonomicrometric measurements of muscle length during respiration. 5) Histochemical study of muscle fiber properties.

The results of these studies suggest the intercostal muscles of the cat are compartmentalized into 'respiratory' and 'non-respiratory' areas. The parasternal muscles and external intercostals from the rostroventral quadrant of the ribcage were typically recruited during inspiration. Shortening and lengthening contractions were seen in these muscles, as they worked in concert with the diaphragm to expand intrathoracic volume. There was a relatively high proportion of slowly contracting, fatigue resistant muscle fibers in these areas. The remaining intercostal muscles of the rib cage were usually inactive during respiration. They experienced a complex mixture of passive shortening and

lengthening due to the forces generated by the surrounding muscle and by the elastic recoil of the thoracic cage. The muscle fibers in these areas were largely composed of the faster contracting, lower fatigue resistant types.

The recruitment of the two types of gamma motoneurons, dynamic and static, in the rhythmically active areas of the intercostals was similar to what has been reported for the hindlimb extensors during locomotion. Static gamma motoneurons were active throughout the breathing cycle, while dynamic gamma motoneurons were recruited approximately in phase with alpha motoneurons. Those areas of the rib cage which were not recruited during respiration, received a steady level of input from static gamma motoneurons.

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Many thanks to my supervisor Dick Stein who provided me with an excellent foundation in the neurosciences. Dick has managed to develop a lab of the highest standards while maintaining an atmosphere that is both relaxed and friendly. If only I could have improved his golf game to the degree he did my understanding of physiology.

I am also indebted to Robert Rolf and Kelly James for providing their excellent expertise in the computer, electronic and mechanical engineering skills which were essential for the completion of the work described in this thesis.

The active interest and assistance of Drs. Tessa Gordon and Tom Martin were also greatly appreciated. Ian Gynther was kind enough to offer invaluable suggestions regarding each chapter of the thesis.

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LIST OF ABBREVIATIONS

C	chain fiber
CA	California
CCT	cuneocerebellar tract
cm	centimeter
CNS	central nervous system
CO_2	carbon dioxide
DB_1	dynamic bag fiber
DSCT	dorsal spinocerebellar tract
EMG	electromyogram
FOG	fast twitch, oxidative, glycolytic
FM	frequency modulation
FG	fast twitch, glycolytic
FRC	functional residual capacity
HFO	high frequency oscillation
Hz	hertz
Ia	primary afferent
II	secondary afferent
imp/s	impulses second
kg	kilogram
KHz	kilohertz
L_o	muscle length at which maximum force is produced
L_R	length of muscle at the end of inspiration
Md.	Maryland
mg	milligram
mm	millimeter

mm	micrometer
ms	millisecond
NADH	nicotinamide adenine dinucleotide diaphorase
ns	nanosecond
O_2	oxygen
SB_2	static bag fiber
VSCT	ventral spinocerebellar tract
S.E.	standard error
sec	seconds
SO	slow twitch, oxidative

CHAPTER ONE

INTRODUCTION

The fusiform shaped structures in mammalian muscle, initially thought of as 'muscle buds' or foci of inflammation, were revealed to be sensory organs by the histological studies of Sherrington (1894). Extensive investigations of muscle spindle morphology, starting with the detailed drawings of Ruffini (1898), have demonstrated the intricate structure of this receptor (Barker, 1966; Ovalle & Smith, 1972; Boyd, 1980). Meanwhile, parallel studies of the receptor's physiological properties progressed from the first recordings of muscle spindle afferents by Matthews (1933). Several of these studies in particular, led to an appreciation of the functional complexity of the muscle spindle, including: the establishment of gamma motoneurons as a specific fusimotor system (Leksell, 1945; Hunt & Kuffler, 1951); the functional classification of primary (Ia) and secondary (II) afferents (Cooper, 1961); the subdivision of static and dynamic gamma motoneurons (Matthews, 1962); and the quantitative analysis of the response characteristics of muscle spindle afferents (Matthews & Stein, 1969; Hulliger, Matthews & Noth, 1977). In the last decade, there has been a discernible shift in muscle spindle research towards investigations of the functional role for muscle spindles and gamma motoneurons in

motor control. It was with this emphasis that much of the experimentation described in this text was undertaken.

Specifically, we were interested in studying the role of gamma motoneurons during rhythmically generated movements. Studies of gamma motoneuron and muscle spindle activity in the cat hindlimb muscles during locomotion produced conflicting results from those reported for jaw muscles during chewing (reviewed in Chapter 2). In hopes of shedding some light on this problem, we have recorded from gamma motoneurons and muscle spindles during another rhythmical movement, respiration. Questions regarding the recruitment patterns, length changes and muscle fiber properties of the intercostal muscles were also addressed. Before describing our findings, I will outline the relevant background information regarding muscle spindles, gamma motoneurons and respiratory muscles.

THE MUSCLE SPINDLE AND GAMMA MOTONEURONS

STRUCTURE AND INNERVATION

A simplified illustration of the current view of the mammalian spindle is shown in Fig. 1.1. The spindle consists of an encapsulated group of fine specialized muscle fibers, tapered at each end and expanded at its center in a fluid filled capsule. There are three types of intrafusal fibers, which differ in their morphological, mechanical and electrical properties: bag_1, bag_2 and chain fibers (Barker,

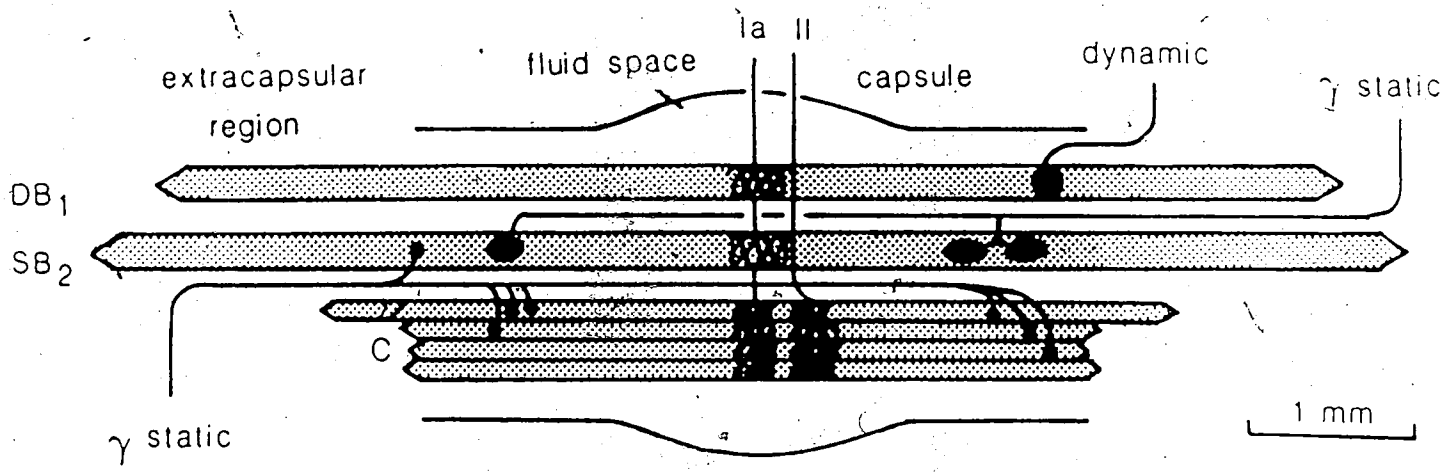

FIG. 1.1. Illustration of the current view of mammalian muscle spindle. There are three types of intrafusal fibers: DB_1, bag1 or dynamic bag fiber; SB_2, bag2 or static bag fiber; C, chain fiber. Each spindle has one Ia afferent and from zero to five II afferents. The motor innervation typically consists of one or two dynamic and three to seven static gamma motoneurons. (Adopted from Boyd, 1985.)

Emonet-Denand, Laporte, Proske & Stacey, 1973; Ovalle & Smith, 1972). The bag_1 intrafusal fibers have a well nucleated central portion while much of the contractile myofibrils are situated at the poles. The chain fibers are distinguished by a single layer of nucleation at the center portion with an almost homogeneous distribution of myofibrils along their length. The bag_1 and chain fibers also differ in their speed of contraction. Bag_1 fibers contract slowly via the spread of local potentials to their polar regions. Chain fibers contract quickly by means of spreading action potentials (Bessou & Pages, 1975). Bag_1 and 2 fibers are alike in that they do not generate action potentials. Otherwise, bag_2 fibers show characteristics intermediate between bag_1 and chain fibers.

The spindle is innervated by two types of sensory neurons: primary (Ia), and secondary (II) afferents. The Ia afferents innervate the central portions of all intrafusal fibers in the spindle, while the II afferents innervate the juxtaequatorial region of chain and 2/3 of all bag_2 fibers (Boyd, Sutherland & Ward, 1985).

The fusimotor, or gamma, nerve fibers can be classified into two types: dynamic and static. Dynamic gamma motoneurons almost exclusively innervate bag_1 fibers. Static gamma motoneurons innervate both bag_2 and chain fibers. There has been suggestion that static gamma motoneurons should be subdivided into those which influence bag_2 and

those which act upon chain fibers (Boyd, 1986).

MUSCLE SPINDLE DISTRIBUTION

The number of muscle spindles within the different skeletal muscles of the cat is quite variable. Those muscles which are involved with the control of precise movements or postural roles typically have the highest density of muscle spindles. At the other extreme are the muscles which are reported to have very few or no muscle spindles, including: parasternal, diaphragm, extraocular muscles, jaw openers, intra-auricular muscles, laryngeal muscles and the striated muscles of the esophagus (Matthews, 1972). Perhaps the reflex activity which is usually produced by muscle spindle activity would be functionally inappropriate for this group of muscles. For instance, reflex activation of phrenic motoneurons by spindle afferents could occur when the diaphragm is passively lengthened either due to trunk rotation or the pressure applied to the muscle by adjacent organs of the abdomen. Likewise, heightened fusimotor activity would also result in increased spindle afferent activity and subsequently to increases in phrenic motoneuron discharge. Clearly, either of these occurences would lead to disturbances in the breathing patterns of the animal.

FUNCTIONAL PROPERTIES

A number of stages are involved between the arrival of the input stimulus, which is the length change of extrafusal

muscle fibers, and the subsequent change in the discharge rate of the Ia and II afferents. Firstly, length changes of extrafusal muscle fibers will, due to the parallel arrangement and mechanical coupling of the spindle and muscle, be transferred to the intrafusal fibers. Subsequently, the localized strain of the intrafusal fibers will result in the generation of a proportional current flux at the Ia and II afferent membranes. How this transition from length to current takes place is unknown, but a possible mechanism could involve a cation-selective channel which is activated by stretch (Guharay & Sachs, 1984). It remains, however, for such a stretch activated channel to be identified in a muscle spindle.

Finally, as a result of the membrane depolarization, the encoder generates a corresponding series of nerve impulses in the afferent terminals. The encoder probably consists of a particular patch of membrane which integrates the generator current reaching it, thereby depolarizing the cell to threshold for impulse production (Poppele & Chen, 1972).

The response of Ia and II afferents to a variety of precisely controlled length changes, over a wide range of amplitudes and frequencies, has been studied over the years (Van Leeuwen, 1949; Harvey & Matthews, 1961; Brown, Engberg & Matthews, 1967; Lennerstrand, 1968; Matthews & Stein, 1969; Kroller, Grusser & Weiss, 1985). The sinusoidal

stretch has been one form of input which has been widely used widely by those studying muscle spindle properties since its initial application in the late 1960's (Matthews, 1981). By applying the sinusoidal stretch over many cycles the response of the afferents to length and velocity components of the length change could be averaged. Three variables can be measured using this technique: mean discharge level, the amplitude of the discharge rate from the mean level, and the phase advance of the afferent response in relation to the length change (Matthews & Stein, 1969). These values and their relation to the afferent discharge rate are illustrated in Fig. 1.2.

It was with the application of various amplitudes of sinusoidal stretch and measuring the average change in modulation of the spindle's response that striking non-linearities of the Ia response became evident. The change in Ia afferent discharge rate for a given length change decreases dramatically when the amplitude of stretch exceeds 0.1% of total muscle length (Matthews & Stein, 1969). Functionally, this non-linearity allows the Ia afferents to be very sensitive to small deviations in muscle length without the signal being saturated during larger length changes. The II afferents respond with a constant, relatively low, sensitivity (modulation amplitude in imp/sec per mm of stretch) to all amplitudes of stretch.

The explanation of these phenomena lies in an

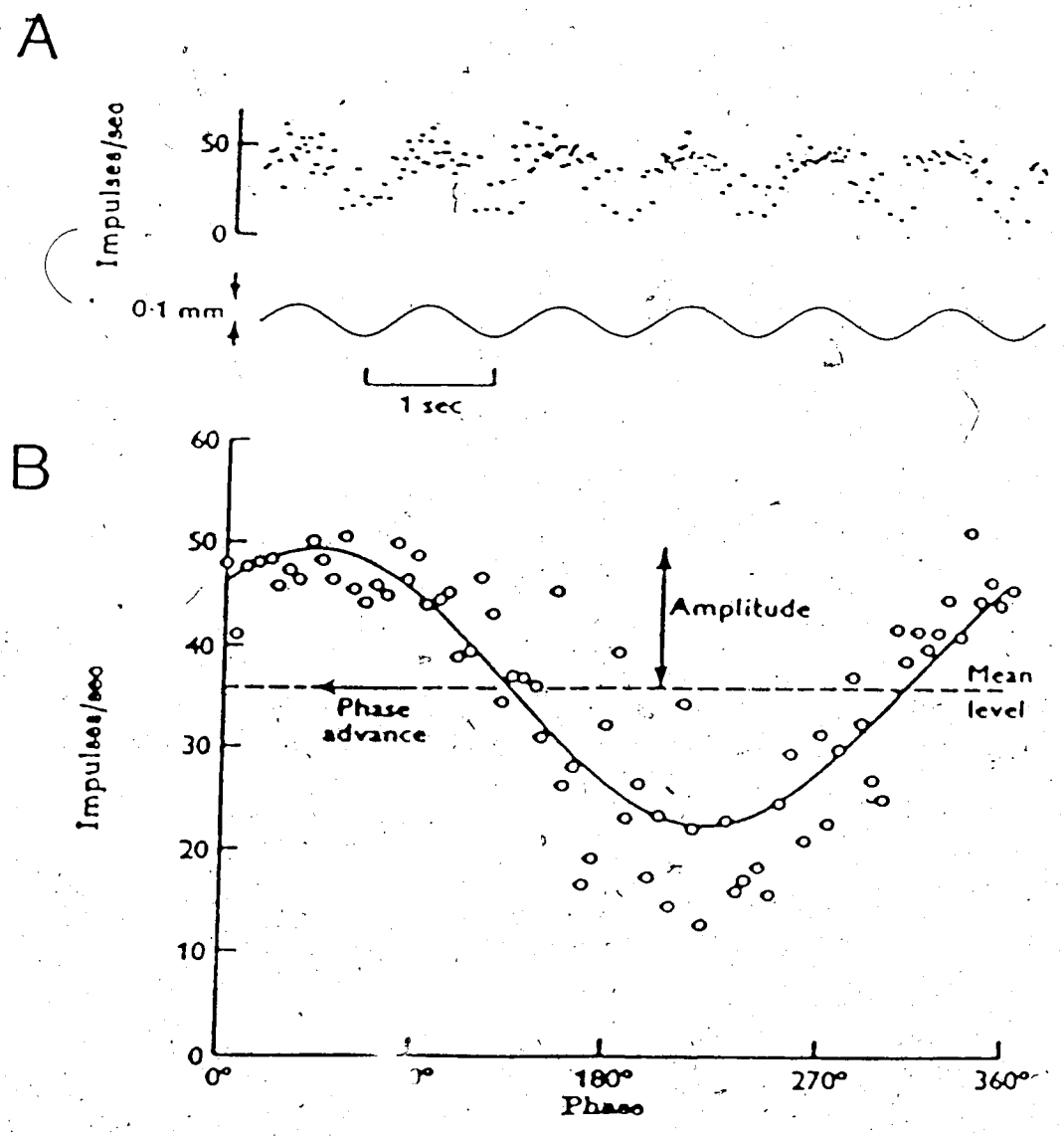

FIG. 1.2. Typical response of Ia afferent to sinusoidal stretch. (A) Display of instantaneous frequency of Ia afferent to a series of sinusoidal length changes. (B) Average response of Ia to the application of 10 stretch cycles, which has been fitted with the best fitting sinusoidal curve. The mean discharge rate (dashed line), the modulation from mean rate (vertical arrow) and the phase advance of the afferent's response with respect to the length change is calculated. Similar analysis of Ia response is used in Chapter 2. (Adapted from Matthews and Stein, 1969).

understanding of the transformation stage of the spindle response. When the intrafusal fibers are at rest there is a fraction of actin-myosin cross bridges that will be attached (Hill, 1968). This will result in a certain degree of stiffness developing in those areas of the intrafusal fibers which are well endowed with myofibrils. The poles of the bag_1 fibers have a high density of myofibrils, while the area under the Ia afferent endings has few (Poppele & Quick, 1985). Therefore, when a small stretch is applied, the area most susceptible to strain is the sensory area of the bag_1 fiber. However, when the size of the stretch reaches a critical limit, the cross bridges rupture, which decreases the stiffness of the poles and the proportion of strain experienced at the Ia ending. The II afferents do not have a similar degree of inhomogeneity in their myofibril distribution and therefore do not exhibit this non-linearity (Matthews & Stein, 1969).

EFFECTS OF GAMMA MOTONEURONS

The activity of muscle spindle afferents is determined by the complex interactions of two inputs: muscle length and gamma motoneuron activity (Hulliger, 1984). A summary of the effects of the two types of gamma motoneurons is illustrated in Fig. 1.3. Static, and to a lesser degree dynamic, gamma motoneurons increase the resting discharge rate of Ia afferents by causing local contractions of the polar regions of intrafusal fibers (Poppele & Quick, 1985). Dynamic gamma

EFFECTS ON MUSCLE SPINDLE AFFERENTS

	Mean Rate	Modulation
Dynamic	+	+
Static	+	−

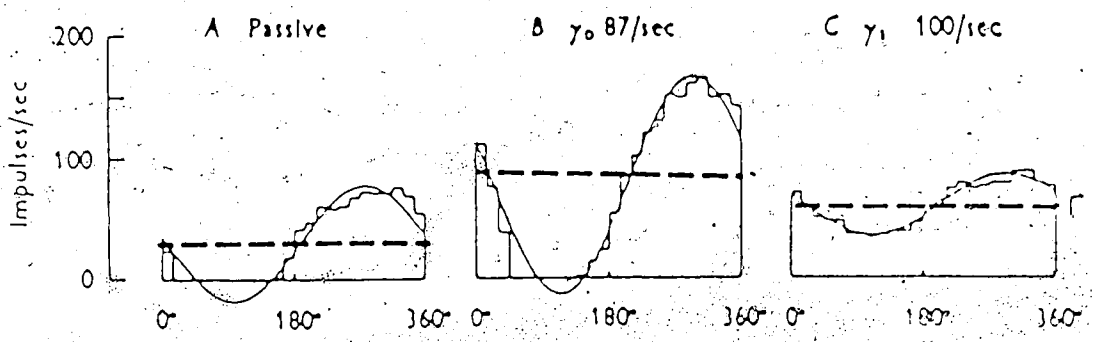

FIG. 1.3 Cycle histograms (explained in Fig. 1.2) illustrating the effects dynamic and static gamma motoneurons have on Ia afferents. Same amplitude of sinusoidal stretch is applied i each case. Both types of gamma motoneuron increase the mean rate (dashed line) of Ia discharge. Dynamic gamma motoneurons increase, while statics, decrease the modulation around the mean rate of Ia afferent. Similar criteria for differentiating between the two types of gamma motoneurons is used in the experiments described in Chapter 2. (Adapted from Hulliger, Matthews and Noth, 1977).

motoneurons, which innervate bag_1 fibers, generally increase the sensitivity of Ia afferents to stretches above 0.1% of muscle length (Hulliger et al., 1977). Presumably, this is due to an enhancement of the stiffness of the polar regions of bag_1 fibers in response to the activity of dynamic gamma motoneurons. Therefore, for a given stretch, there will be an increase in the proportion of local strain at the equatorial region of the bag_1 fiber, where the Ia terminal is located. In contrast, static gamma motoneurons, which innervate bag_2 and chain fibers, generally lower the sensitivity of Ia afferents to stretch by decreasing the stiffness of the polar regions of the intrafusal fibers. The contrasting effects the two types of gamma motoneurons have on the stiffness of the intrafusal fibers is a result of the differing contraction speeds of the myofibrils. The cross-bridges within bag_1 fibers, which turnover slowly, spend the majority of time bound when activated by dynamic gamma motoneurons. Bag_2 and chain fibers have fast contracting myofibrils which, upon activation by static gamma motoneurons, spend a greater proportion of the time with the cross-bridges cycling rather than bound (Mattnews, 1972).

CENTRAL AND PERIPHERAL PROJECTIONS

The projections of spindle afferent information and the source of inputs to gamma motoneurons within the CNS have been well documented (reviewed in Baldissera, Hultborn &

Illert, 1981). 1) Monosynaptic and polysynaptic Ia and II pathways supply excitatory input to alpha motoneurons innervating the homonymous muscle and its functional synergists. 2) In many muscle groups reciprocal Ia inhibition of antagonist motoneurons is transmitted via Ia inhibitory neurons. However, such reciprocal inhibition is not found between adductors and abductors, muscles which open and close the jaw, or inspiratory and expiratory intercostal muscles. 3) Ia spindle afferents also synapse via an inhibitory interneuron with the presynaptic terminals of other Ia afferents, thus lowering the target afferents' excitability. 4) The dorsal spinocerebellar (DSCT) and ventral spinocerebellar (VSCT) tracts in the hindlimb, and the forelimb equivalents, the cuneocerebellar (CCT) and rostral spino-cerebellar (RSCT) tracts, relay information from spindle afferents to the anterior lobe of the cerebellum (Ekerot, Larson, & Oscarsson, 1979). 5) Recordings of evoked potentials and single cells from the cerebral hemispheres have demonstrated the presence of diffuse projections to the somatosensory and motor cortex via brainstem and thalamic nuclei (Oscarsson & Rosen, 1963; Fetz & Cheney 1979; Zarzecki & Asanuma, 1979).

An understanding of the sources of gamma motoneuron recruitment is imperative for the complete elucidation of muscle spindle function. At the peripheral level, gamma motoneurons receive input from skin, joint and muscle

afferents (Murthy, 1978; Murphy, 1981; Ellaway, Murphy and Tripathi, 1982; Johansson & Sojka, 1985; Johansson, Sjolander & Sojka, 1986; Appelberg, Johansson & Sojka, 1986). The effects of these peripheral receptors on gamma motoneuron activity is quite variable and widespread. Furthermore, the strength of these projections seems to be dependent on the experimental preparation and the activity of the animal. For instance, the powerful excitation of gamma motoneurons in response to cutaneous stimulation typically seen in the decerebrate cat appears to be supressed in the intact preparation (Trend, personnel communication). Furthermore, this reflex excitation of gamma motoneurons in the decerebrate animal is totally inhibited when the cat begins to walk (Murphy, Stein & Taylor, 1986). Perhaps the effects of cutaneous and joint receptors on gamma motoneuron activity is surpressed in the freely moving animal. Functionally, this would prevent the gross interference of the centrally programmed control of gamma motoneuron dishcarge by the somewhat unpredictable reflex activation of peripheral receptors.

Supraspinal projections to gamma motoneurons include the reticular formation (Shimazu, Hongo & Kubota, 1962), ventrolateral nucleus of thalamus (Yanagisawa, Narabayashi & Shimazu, 1963), cerebellum (Granit, Holmgren & Merton, 1955; Gilman, 1968), red nucleus (Appelberg, 1981) and the motor cortex (Mortimer & Akert, 1961). There have been suggestions

that a particular type of gamma motoneuron, either dynamic or static, can be selectively activated by the above mentioned supraspinal centers.

FUNCTIONAL ROLE OF MUSCLE SPINDLE

Considering the multitude of efferent and afferent projections involved, it should not be surprising that several roles have been proposed for the muscle spindle and gamma motoneurons. Merton (1953) led the way with his proposition of the follow-up servo hypothesis. The basic premise of this theory states that for all but the fastest movements, muscle activation is driven through a segmental reflex arc. That is to say, the command signal recruits gamma motoneurons, resulting in the excitation of spindle afferents, which in turn excite the alpha motoneurons. However, Vallbo's (1973) recordings from human nerve fibers with fine tungsten electrodes clearly demonstrated that the activation of the muscle preceded spindle afferent discharge. Furthermore, it was apparent that the rhythmical activation of alpha motoneurons during respiration and locomotion was possible in the deafferented preparation (reviewed in Nathan & Sears, 1960).

Nonetheless, Merton's hypothesis generated considerable interest regarding the role of the segmental reflex in motor control. The ensuing investigations eventually led to the vindication of certain aspects of his initial model. For

instance, examination of the motor unit discharge during spontaneous and voluntary movements illustrate that at least part of the drive to alpha motoneurons is dependent on the reflex activity induced by gamma motoneurons (Nathan & Sears, 1960; Hagbarth, Kunesch, Nordin, Schmidt & Wallin, 1986). A related concept known as the 'servo-assistance' theory states that the command signal which is responsible for generating a movement is delivered to gamma and alpha motoneurons. In the presence of unexpected loads or decreased force production by the muscle, the discharge of the gamma motoneurons provides spindle afferents with the appropriate excitation to facilitate the activity of alpha motoneurons (Matthews, 1981). The facilitation by spindle afferents is produced by monosynaptic and polysynaptic segmental pathways, and via what has been termed the 'long-loop' reflex, where the afferent signal may pass through the cortical regions on route to the alpha-motoneurons (Marsden, Rothwell & Day, 1984).

The importance of muscle receptors to motor control in humans is evident from observations of patients with sensory neuropathies. The efferent projections and muscles of these individuals are normal, but due to various disorders, the CNS does not receive proprioceptive information. When tested, these subjects can generate gross movements, but the magnitude and timing of their muscle activation is deficient (Forget & Lamarre, 1987). As well, they have difficulties in

maintaining a steady postural position or performing movements that demand precision (Sanes, Mauritz, Dalakas & Evarts, 1985). All of these deficiencies are accentuated by the application of external loads to the limb being tested.

The reflex effects of Ia and II afferent activity during a movement are not limited to the alpha motoneurons innervating the particular muscle in which the spindle resides. Mendell and Henneman (1971), using the method of spike-triggered averaging, demonstrated monosynaptic projections from spindle afferents to a significant portion (2/3) of agonist motoneurons, while antagonist muscles receive inhibitory projections via spinal interneurons (Eccles, Fatt & Landgren, 1956). Further complexity is introduced by, the still relatively little understood, gating of spinal interneuron and spindle afferent activity. This is produced by cutaneous, joint and other spindle afferents from the periphery, as well as centrally originating projections from the cerebellum, brainstem and cortex (reviewed in Rudomin, 1980). In addition, recent work has shown that perturbations applied during postural states (Nashner, 1982) and speech movements (Abbs & Gracco, 1983) result in compensatory responses which cannot be thought of as simply the resultant action of stretch reflexes. Rather, these studies suggest that proprioceptively mediated responses can occur at those centers within the CNS which program the coordination of movements involving several

muscles and joints. In summary, there is a significant degree of flexibility of reflex responses induced by muscle spindle afferents and they are presumably utilized according to the particular demands imposed on the motor system at any given time (Akazawa, Aldridge, Steeves & Stein, 1982; Capaday & Stein, 1987).

The protracted debate regarding the contribution of muscle spindle afferents to the conscious awareness of limb position appears to be settled. The impetus for the reinvestigation of this problem came from the realization that the signal from joint receptors was not as well coordinated with limb position as had been previously thought (Clark & Burgess, 1975). The initial evidence for involvement of muscle spindle afferents came from the demonstration that vibration of the muscle belly, a known stimulus of Ia afferents, led to the illusion that the joint was moving. This was followed by the report of McCloskey and co-workers, who described the illusion of movement in his big toe when he pulled on the tendon (McCloskey, Cross, Honner & Potter, 1983). Further confirmation has come from the evaluation of position sense during movements while the activity of joint and cutaneous receptors have been eliminated by anesthesia or anoxia (Matthews, 1981). Burgess and his co-workers, have recorded the simultaneous activity of spindle afferents from several muscles surrounding a joint to find that as an ensemble they could provide the CNS

with graded information regarding limb position throughout the physiological range of movements (Simon, Wei, Randic & Burgess, 1984). It has been suggested that the CNS would require information regarding the starting position of muscle length and joint angle to generate the appropriate motor command (Hasan & Stuart, 1988). Furthermore, awareness of the final position attained during a movement and its comparison to the intended position would seem to be necessary during the process of learning motor skills (Brooks, 1986).

An introduction to the specific topic of the role of muscle spindles in respiration is supplied in Chapter 2. The following is a general introduction of the intercostal muscles, which will compliment the information on this topic offered in Chapters 4 and 5.

INTERCOSTAL MUSCLES

At the end of the expiratory phase of the breathing cycle, when the respiratory muscles are inactive, the forces acting on the rib cage are in static equilibrium. At this volume, the elastic recoil of the lungs which tends to collapse the rib cage is balanced by the normal tendency of the chest wall to spring outwards (West, 1974). During inspiration the respiratory muscles function to enhance the forces which expand the rib cage. Normally, the rib cage will return to the equilibrium position during expiration merely by the cessation of activity in inspiratory muscles.

With increased levels of ventilation, however, there is often a recruitment of the expiratory muscles which facilitate the return of the intrathoracic volume to, or occasionally beyond, the equilibrium position (Campbell, Agostoni & Newsom Davis, 1970).

The force produced by the diaphragm is responsible for approximately two-thirds of the increase in intrathoracic volume during inspiration. The synergists of the diaphragm include the parasternal, external intercostal, levator costae and scaleni muscles. On those occasions when there is active expiration, the internal intercostals, triangularis sterni, and abdominal muscles are recruited (Campbell et al, 1970).

There were several reasons for choosing the external intercostal muscles for the study of muscle spindle function during respiration. 1) Only the inspiratory muscles consistently illustrate electromyogram (EMG) activity related to the respiratory cycle in anesthetized or decerebrate cats. 2) The diaphragm is one of the relatively rare mammalian muscles which has very few spindles and does not exhibit an autogenic stretch reflex (Derenne, Macklem & Roussos, 1978). 3) The parasternal muscles also have relatively few spindles and a weak segmental reflex (Duron, 1973). Furthermore, it is technically difficult to isolate its nerve supply for extracellular recordings without inducing a pneumothorax. 4) The external intercostals are

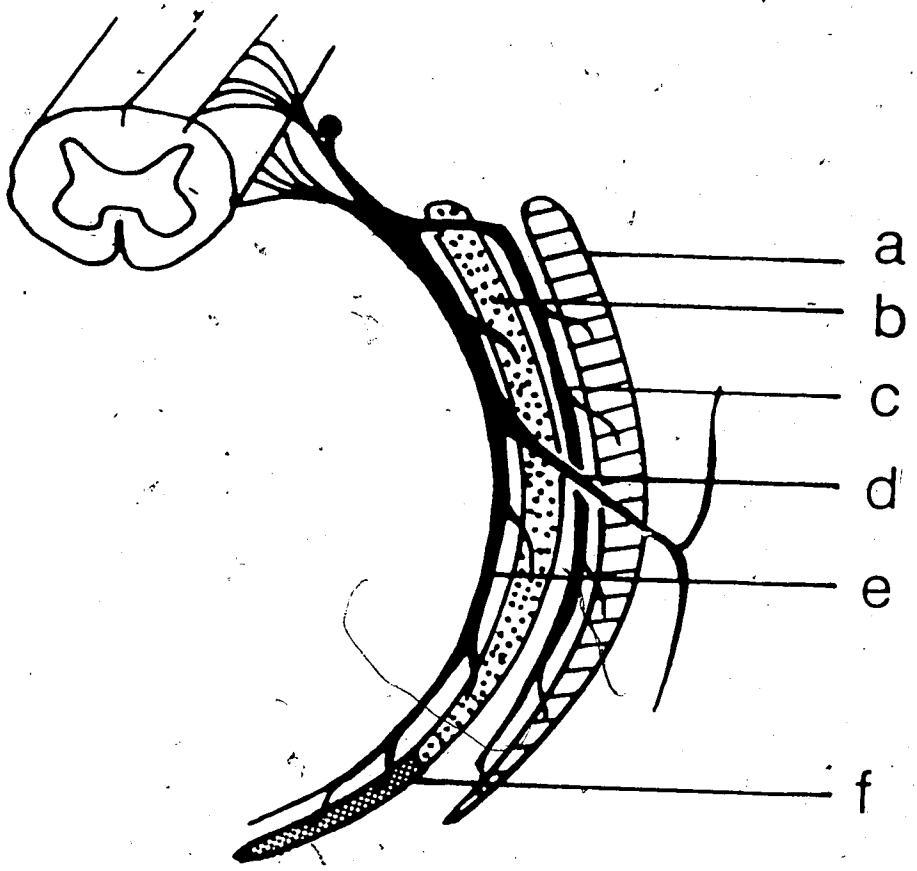

FIG. 1.4. A simplified illustration of the intercostal nerves and muscles. The main intercostal nerve, upon leaving the spinal cord, branches into the internal (e) and external intercostal (c) nerves. The internal intercostal branch innervates the internal intercostal muscle (b), parasternal muscle (f), abdominal muscles and cutaneous sensory receptors (d). The external intercostal nerve runs between the internal and external intercostal muscles (a), innervating the latter. (Adopted from Eccles, Sears and Shealy, 1962).

well supplied with muscle spindles (Duron, 1973). 5) The external nerve is accessible to recording during spontaneous respiration, with the internal intercostal muscle acting as a barrier between the recording site and the thoracic cavity. Moreover, this nerve, unlike the internal intercostal nerve, does not contain cutaneous receptors or efferent fibers to more than one muscle (see Fig. 1.4). 6) It is relatively easy to apply a well controlled stretch to the external intercostal muscle via its insertions into adjacent ribs.

The ribs, when under the influence of forces generated by the action of inspiratory muscles, rotate about their insertion at the vertebral column. Presently, there are two theories regarding the mechanism of action of the intercostal muscles. One is based on the arrangement of the muscle fibers in relation to the ribs (Agostoni, 1964). The fibers of the external intercostal slope obliquely downward and forward from the upper to lower rib. Therefore, the lower insertion is further from the axis of rotation than the upper insertion. Subsequently, when the muscle contracts, more torque is generated on the lower rib, forcing it to move outward and upward. The fibers of internal intercostal muscles slope in the opposite direction and therefore upon contraction they act to move the ribs inward and downward. The parasternals, although aligned in the same direction as the internal intercostals, have the

sternum as their point of reference and therefore they tend to raise the ribs.

DeTroyer and his co-workers have recently suggested a different mechanism for the action of intercostal muscles (DeTroyer, Kelly & Zin, 1983; DeTroyer, Kelly, Macklem & Zin, 1985). They mimicked the recruitment of external intercostals by electrically stimulating the muscles at end tidal volume (i.e at the intrathoracic volume reached at the end of expiration). As predicted by the earlier theory, the cephalad displacement of the lower rib was twice as large as the caudad displacement of the upper rib, and therefore the rib cage expanded. However, when the same muscles were activated at half inspiratory capacity (i.e. volume at which expiration begins), the opposite effect was seen. It appears the movements produced by intercostal muscle activation are dependent on the specific intrathoracic volume present during a given stage of the cycle. Their inspiratory or expiratory function stems from the timing of their recruitment during the respiratory cycle, rather than the mechanical relationship between the muscle and its point of insertion. The proponents of this theory go on to suggest the intercostal muscle action is determined by the relative resistance to movement of the lower and upper rib. At functional residual capacity (FRC), when inspiration starts, there is thought to be passive tension in the neck muscles which act to stabilize the upper rib. In contrast, at high

intrathoracic volumes the abdominal muscles would exert a stabilizing tension on the lower rib. However, the experimental evidence to confirm this model is not yet available.

It became apparent during the course of our investigations that the intercostals were not a homogeneous group of muscles. Firstly, the EMG activity during respiration was limited to well defined areas of the rib cage (Chapter 5). Secondly there were regional differences in the recruitment patterns of gamma motoneurons in different areas of the rib cage (Chapters 2 and 3). Thirdly, length measurements of the muscle fibers in the twelve intercostal spaces revealed the mechanics of the various regions to be different (Chapter 4). These findings led us to investigate whether there may also be regional differences in the muscle fiber properties amongst the intercostals (Chapter 5). As described in the chapters to follow, there seems to be a correlation between the recruitment patterns of alpha and gamma motoneurons, length changes of muscle fibers, and the histochemical profile of the intercostal muscles. An introduction specific to these topics is included in the appropriate chapters.

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CHAPTER TWO

GAMMA MOTONEURON CONTROL OF MUSCLE SPINDLE SENSITIVITY DURING RESPIRATION IN THE CAT

Mammalian muscles contain spindle organs which convey sensory information to the CNS. In turn the CNS can influence the muscle spindle properties via activity in γ-motoneurons. Generally γ-motoneurons are divided into two categories: static and dynamic. The two types of γ-motoneurons have a similar range of discharge frequencies and conduction velocities which makes it impossible to differentiate between them on these bases. They can, however, be differentiated by their effects on muscle spindle afferents (Matthews, 1981). Dynamic γ-motoneurons increase the mean discharge rate and the sensitivity of a primary afferent's response to sinusoidal stretch (above the μm range). Static γ-motoneurons also increase the mean discharge rate but decrease the sensitivity of the spindle afferent's response to sinusoidal stretch (Hulliger, Matthews & Noth, 1977). Therefore, by observing the muscle spindle's response to a length change it is often possible to infer the nature of the γ-motoneuron activity.

Applications of this strategy have been used to

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propose models for how the CNS recruits γ-motoneurons during various rhythmic movements. Recordings of spindle afferents and efferents during rhythmic jaw movements in the cat by Gottlieb and Taylor (1983) suggest that dynamic γ-motoneurons fire tonically throughout chewing movements while static γ-motoneurons are activated phasically with α-motoneurons. Evidence from recordings of muscle spindle afferents in the monkey during isometric biting suggest that dynamic γ-motoneurons are co-activated with α-motoneurons while the static γ-motoneuron activity is minimal (Larson, Smith & Luschei, 1981). Both of these groups rely heavily on their evidence from recordings of what they have classified as secondary endings for their proposals of γ-motoneuron recruitment. However, Larson et al have based their classification on the rather dubious criteria of the afferent's relative sensitivity to stretch, while Taylor's group utilize the more reliable method of observing the afferent's response to suxamethonium. Finally Lund and Olson (1983), after reviewing the evidence, have suggested that the recruitment patterns of dynamic and static γ-motoneurons in jaw muscles varies depending on the particular type of jaw movement.

Evidence from recordings of γ-motoneurons and muscle spindle afferents to leg extensors of the decerebrate cat during locomotion suggest phasic dynamic and tonic static γ-motoneuron activity (Murphy, Stein & Taylor, 1984; Taylor,

Stein & Murphy 1985). Recordings of muscle spindle afferents from the hindlimb muscles of the freely moving cat also suggest that static γ- motoneurons fire tonically during locomotion (Prochazka, Hulliger, Zangger & Appenteng, 1985). These chronic recordings do not show any indication of dynamic γ-motoneuron activity during slow gait, but there is evidence that they are recruited phasically when the speed of gait increases. There have also been suggestions that the levels of dynamic and static γ-motoneuron activity vary amongst the different hindlimb muscles involved in locomotion (Perret & Berthoz, 1973; Cabelguen, 1981; Loeb, 1985).

This chapter describes experiments which were designed to provide information regarding the activity of the two types of γ- motoneurons during another rhythmic activity in the cat, respiration. Muscle spindles have been identified in the intercostal muscles (Huber, 1902; Barker 1962; Duron, 1978) and have been shown to exhibit the same properties as those in the cat hindlimb (Andersson, Lennerstrand & Thoden 1968; Newsom Davis, 1975). Recordings from intercostal nerve filaments have shown that there are both tonically and phasically active γ-motoneurons (Chapter 3; Critchlow & von Euler, 1963; Sears, 1964). Until now these γ-motoneurons have not been satisfactorily identified as being of the dynamic or static type. The functional implications of these findings will be discussed. A brief description of these

results has appeared (Greer & Stein, 1986).

METHODS

Two types of acute experiments were performed on spontaneously breathing adult cats of either sex. The first involved 7 cats which were anesthetized with Halothane (delivered in a mixture of 95% O_2 and 5% CO_2) up until the time they were decerebrated at the intercollicular level. The second group of experiments involved 21 cats which were initially anesthetized with sodium pentobarbital (20mg/kg) and maintained on Halothane anesthesia. While recordings were being made, the level of anesthesia was lowered until the animal illustrated tone in the hindlimbs. The animals were either positioned in the prone position or on their side, depending on which muscles were being studied. The intercostal muscles were exposed and a muscle pool containing warm (37°C) paraffin oil was formed. The muscle pool was formed by suturing the skin, which had been separated from the underlying muscle layers, to a 12 cm diameter metal ring. The temperature of the animal was maintained at 36.0-37.5°C by radiant heat. In all experiments cannulae were inserted in the trachea, carotid artery (for monitoring blood pressure), and in the external jugular vein (for administration of Dextran in the event the animal suffered excessive blood loss during surgery).

Recording Techniques

Peripheral nerves were freed from the surrounding intercostal muscle and extracellular action potentials were recorded triphasically on silver hook electrodes. The tripolar configuration of the recording electrode facilitated the rejection of EMG signals emitted from surrounding muscle. Two sets of electrodes, one proximal and the other distal, were used for each recording. This made it possible to differentiate between efferents and afferents based on direction of propagation of the individual units (Fig. 2.1A). The number of active muscle spindle primary afferents in a given recording was minimized by isolating small areas (0.5-1.5 cm) of muscle which typically contained 1-3 spindles. This was accomplished by denervating all other portions of the muscle distal to the muscle strip of interest.

Primaries and secondaries were differentiated by the amplitude of their spike which, with our recording array, has been demonstrated to be appriximately proportional to the conduction velocity of the afferent squared (Milner, Stein, Gillespie & Hanley, 1981). Therefore differences in axonal size between the two populations of spindle afferents were accentuated. Furthermore, the afferents with the largest spike amplitudes were selected for analysis due to the ease of discriminating these units from amongst the population of action potentials seen in the nerve

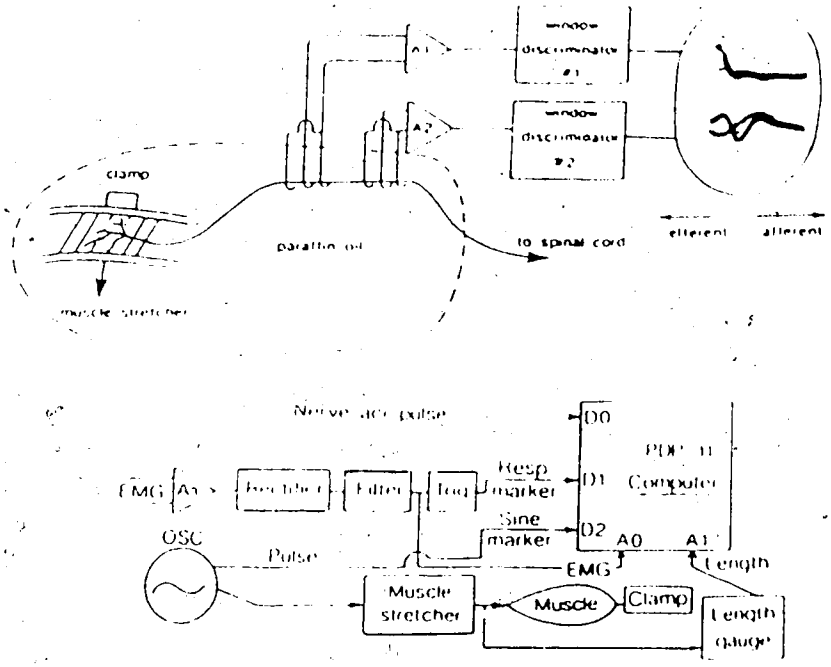

FIG. 2.1 A) Illustration of the technique used to record separately from muscle spindle afferents and efferents. A clamp stabilized the rostral rib while the caudal rib was attached to a torque motor. The intercostal nerve was lifted on hook electrodes into paraffin oil. The recording from the distal electrode was amplified (A1) and led to window discriminator #1 (copy of actual trace from oscilloscope of afferent and efferent units shown within circle). The trigger level and window were set to isolate the large amplitude spikes (dots around spikes are window from discriminator). This selected signal included both muscle spindle primary afferents and alpha motoneurons. Signals from the proximal electrode were amplified (A2) and led to window discriminator #2. To generate an acceptance pulse from window discriminator #2 a signal of the appropriate size and shape had to pass through the window coincidently with the acceptance pulse from window discriminator #1. Since the afferents appeared later and the efferents earlier in time at electrode #2 with respect to the signal at electrode #1, the two components of the mixed signal could be separated (note signal from electrode #2 has been delayed by 0.1 ms in order to facilitate viewing of the signal separation). 1B) The acceptance pulse from window discriminator #2, indicating the occurrence of muscle spindle primary discharge or alpha motoneuron, was led to the computer (D0) along with markers of the respiratory (D1) and stretch (D2) cycles. Analogue signals of the applied length change (A1) and the rectified and integrated diaphragmatic EMG (A0) were also led to the computer. The activity of the spindle afferents and alpha motoneurons with respect to the applied length change and the respiratory cycle were calculated as described in the text.

recordings. Measurements of conduction velocity in preliminary studies demonstrated that the afferents with the largest amplitude spikes were conducting at >80 m/s. The signal-to-noise ratio of our whole nerve recordings was not sufficient to confidently discriminate individual units of small and intermediate size, which presumably included secondary afferents. The units we identified as primary afferents were spontaneously active during the respiratory cycle and acutely sensitive to small length changes applied to the muscle, in contrast to what has been reported for the majority of tendon organs in the intercostal muscles (Bolser, Lindsey & Shannon -87). Our identification of the afferents as deriving from spindle rather than tendon organs was confirmed upon removal of gamma motoneuron activity which resulted in a change in the mean rate and sensitivity of the muscle receptor in response to an applied sinusoidal stretch.

A 4 Hz sinusoidal stretch was applied to the muscle by stabilizing the rostral rib with a clamp and attaching the caudal rib to a servo-controlled torque motor (Fig. 2.2). The size of the stretch was adjusted to be large enough to modulate the primary afferent's discharge, but not so large as to silence the afferent's activity during the release phase of the stretch. It was necessary to apply length changes with amplitudes in the non-linear range (>0.5% of resting length) to differentiate the effects of the two

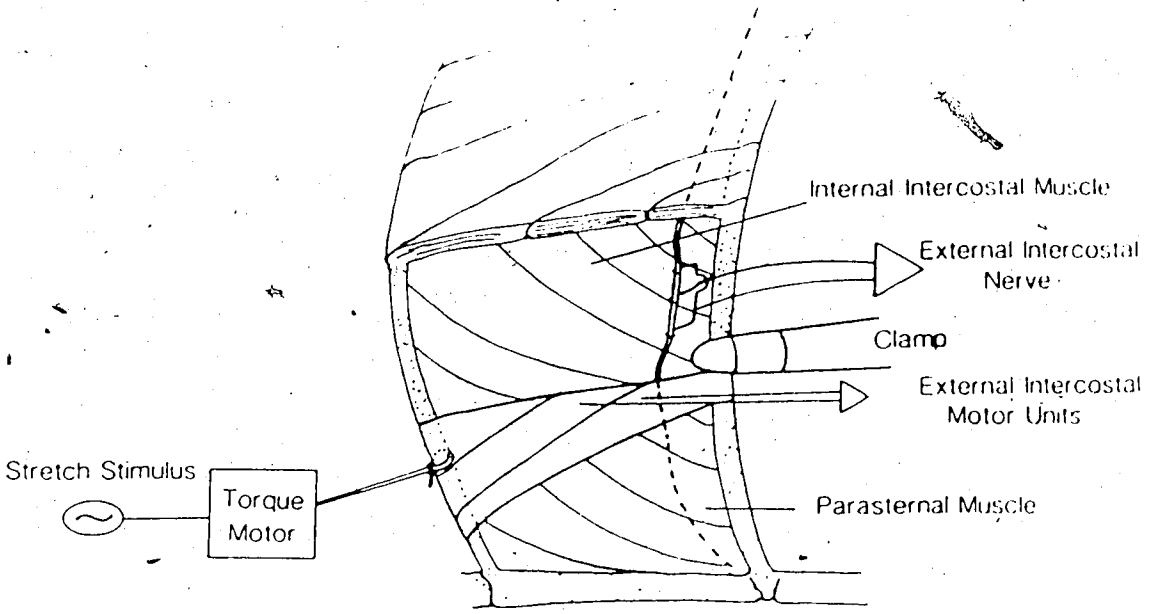

Fig. 2.2 Illustration of the method used to record from muscle spindle afferents while applying a sinusoidal stretch. One rib was stabilized with a clamp (modified hemostat), while the other was attached to a torque motor which imposed the sinusoidal stretch. A section of the external intercostal nerve was exposed for recording by removing the surrounding muscle.

types of γ-motoneuron on the muscle spindle afferents (Taylor et al, 1985). To verify the actual amount of an applied stretch that reached the muscle fibers surrounding the muscle spindle, the technique of sonomicrometry was used (Chapter 4).

In order to monitor the timing and depth of respiration EMG recordings of the diaphragm were made with stainless steel electrodes which were inserted into the caudal surface of the muscle via an incision along the linea alba. The incision was subsequently closed with surgical thread to ensure the integrity of the abdominal wall was maintained. Motor unit recordings of the intercostal muscles were made with a bipolar electrode (a strand of stainless steel wire was inserted into an 18 gauge needle and bent back in the form of a hook which remained in the muscle after the needle was removed).

Diaphragmatic EMG, nerve impulses, length changes and motor unit recordings were amplified and then recorded on an FM tape recorder as well as being monitored on an oscilloscope and chart recorder. The neural signal was filtered (0.2-10 KHz) to improve the signal-to-noise ratio.

Analysis

Response of afferent with respect to sinusoidal stretch. The data was analyzed on a PDP-11 computer. The neural recordings were passed through two window discriminators (Bak Electronics, Md.) cascaded together which allowed for

isolation of afferent and efferent units (Fig. 2.1A). Each selected spike or spikes then triggered a standard pulse which was led to the computer for the generation of cycle histograms. The histogram plotted the discharge rate of the spindle afferent in relation to the sinusoidal length change. Each sweep of the histogram was triggered by a pulse marker which occurred each time the sine wave amplitude reached a designated threshold. A histogram consisted of 256 bins with the width of each bin chosen so that one histogram covered one stretch cycle. The number of spikes in each bin was divided by both the number of cycles and the bin width to convert it to units of impulses per second. The number of stretch cycles for which the afferent's response was averaged was typically 250 (approximately 60 breaths). Each bin of the histogram was averaged with the two neighboring bins on each side (5-point running average) to smooth the histogram. The frequency histogram was then fitted with the best fitting sine wave by the method of least mean squares. From this fitting the afferent's mean rate, modulation around the mean rate and phase advance in relation to the sinusoidal length change could be calculated. The muscle spindles were subsequently deefferented by crushing the nerve proximally and the analysis repeated. In this way the muscle spindle's response to stretch with and without γ-motoneuron activity could be compared.

Response of afferent with respect to respiratory cycle. A more complicated analysis was needed to determine the afferent's discharge rate and modulation with respect to time in the respiratory cycle. Techniques similar to those used by Taylor et al. (1985) for the analysis of spindle afferent response during the step cycle were adopted. The EMG was rectified and low pass filtered with a third-order Paynter filter (30 Hz) and RC filter (50 Hz). This processed EMG was then used to activate a Schmitt trigger and the resultant pulse was sent to the computer, as were the pulses generated by the sine wave and neural signal (Fig. 2.1B). The respiratory cycle was arbitrarily divided into fourteen parts and the afferent's average response to sinusoidal length changes was determined for each part (Fig. 2.3A). The first trace represents the afferent's response to sinusoidal length changes which started 0-214 ms after the marker at the start of the respiratory cycle. The subsequent stretches started after the respiratory marker by increments of 214 ms for each successive histogram. Each trace started at the same phase of the stretch cycle.

To obtain the modulation in afferent rate due solely to the stretch, the average response of the afferent's rate due to respiratory movements (Fig. 2.3B) was subtracted from a sine curve and the modulation in rate determined. The validity of this procedure was tested and confirmed using known inputs to an electronic neural analog (Taylor, 1985).

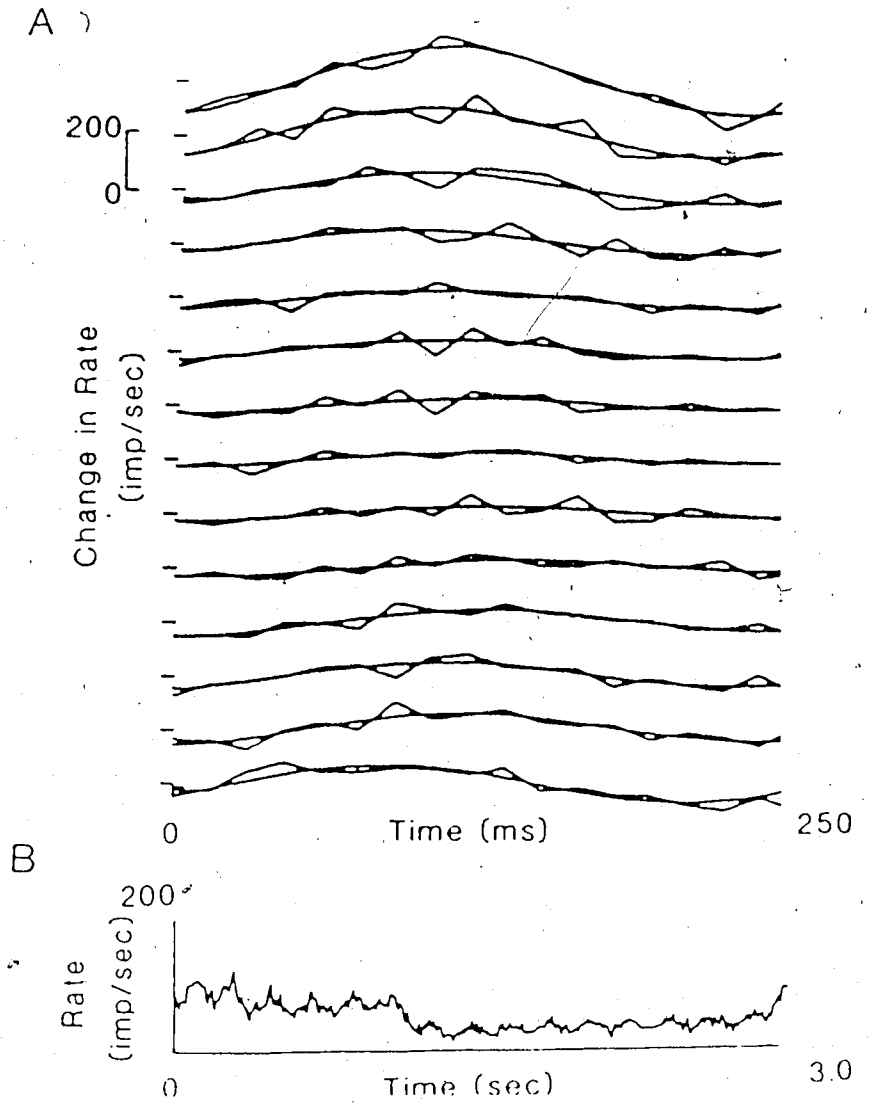

FIG. 2.3 A) Average response of an afferent to sinusoidal stretch during 14 parts of the respiratory cycle. The first histogram represents the afferent's response to a sinusoidal stretch applied during the first 214 ms of the respiratory cycle. The following traces represent the afferent's response during subsequent (214 ms intervals) parts of the respiratory cycle. Each trace starts at the same phase of the stretch cycle. The histograms are then fitted with sine waves from which values of modulation around the mean rate can be calculated. B) The average response of the afferent due to movements of the rib cage associated with respiration. These movements occur when the muscle could not be held isometrically. The modulation of the afferent's response shown in 2B could then be subtracted from the response of the afferents shown in 2A. This procedure allowed the pure response of the afferent to the sinusoidal stretch to be calculated.

The mean rate during each part of the respiratory cycle was calculated from the portion of each histogram corresponding to one stretch cycle before the respiratory movement-induced activity was subtracted away. A plot of mean rate and modulation around mean rate of the muscle spindle afferents versus time in the breathing cycle can then be generated. The points were fitted with a line generated from averaging each point with the neighboring two points (3 - point running average). The activity of the muscle spindle afferents before and after removing the γ-motoneuron activity was compared.

Muscle spindle afferents whose response to the applied sinusoidal stretch did not change after deefferentation were not included in this study. The nerves may have been blocked proximal to the recording electrodes due to damage during dissection. The fact that the majority of these units appeared during the preliminary experiments of this study gives credence to this hypothesis. As well, the level of anesthesia may have been high enough during some recordings to completely inhibit γ-motoneuron activity.

Pattern of α-motoneuron activity. Finally, the intercostal motor unit or α-motoneuron firing rates in relation to the respiratory cycle were determined. Each sweep of the histogram was triggered by the rising phase of the rectified and integrated diaphragmatic EMG. A histogram consisted of 256 bins with the width of each bin chosen so that one

histogram covered one respiratory cycle.

RESULTS

The apparent levels of dynamic and static γ- motoneuron activity varied in muscle spindles from different areas of the intercostal muscles. There was a correlation between the type of γ-motoneuron activity and the presence or absence of respiratory- related EMG activity in the vicinity of the muscle spindle. A full description of the distribution of EMG activity and range of length changes in the intercostal muscles of the cat during respiration will be reported elsewhere (Chapters 4 and 5), but salient points will be included here as necessary.

Areas of external and internal intercostal muscles where α-motoneuron activity is absent.

Fig. 2.4 illustrates the location of the muscle spindles from which the results reported in this section are derived (19 units). EMG activity related to the respiratory cycle is seldom seen in the caudal portions of the rib cage in anesthetized or decerebrate cats (Duron, 1973; Greer, Stein & Martin, 1988). Muscle spindles from both internal (3 units) and external (16 units) intercostal muscles of decerebrate (9 units) and anesthetized cats (10 units) are included in this group.

Fig. 2.5A shows the response of a primary afferent from a muscle spindle located in the external intercostal muscle

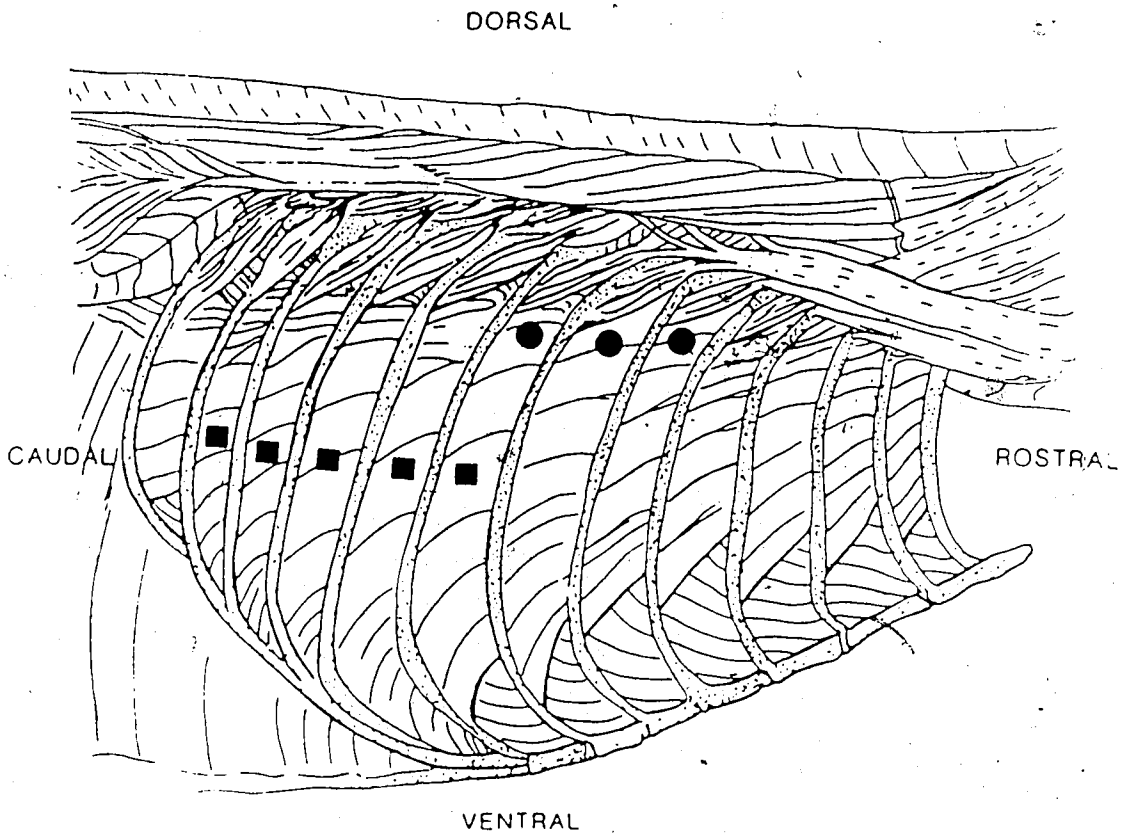

FIG. 2.4 Areas of the intercostal muscles where muscle spindle recordings were made in the absence of respiratory related EMG activity (■) as monitored with bipolar electrodes. Gamma motoneurons innervating these areas appeared to fire tonically and were characterized as being predominantly of the static type. Location of the external intercostal muscles where recordings of muscle spindle afferents were made in association with respiratory related alpha motoneuron activity (●). There is evidence for populations of both tonically and phasically discharging gamma motoneurons in these areas.

to an applied sinusoidal stretch. The histogram represents the average response of the afferent to approximately 250 applications of a 0.1mm, 4 Hz sinusoidal stretch to the area of muscle in which the spindle was located. The peak of the primary afferent's discharge invariably led the length change by approximately 90 degrees indicating that the afferent is mainly responding to velocity (velocity being the first derivative of length).

The response of the same afferent to a stretch of the same size after the muscle spindle has been deefferented can be seen in Fig. 2.5B. The mean rate has decreased and the modulation around mean rate has increased. Therefore, the efferent supply to the muscle spindle which was present in Fig. 2.5A had the effect of increasing the mean rate and decreasing the sensitivity (modulation of mean rate per unit change in length) of the muscle spindle. The changes in mean rate and modulation upon deefferentation of the remaining spindle afferents is shown in Fig. 2.6. All units in this group illustrated characteristics which suggest they were being influenced by the effects of static γ-motoneuron activity.

The above mentioned results describe the average response of the muscle spindle afferents over the whole respiratory cycle. It was also of interest to analyze the responses of the muscle spindle afferents during various parts of the respiratory cycle. Rectified and integrated EMG

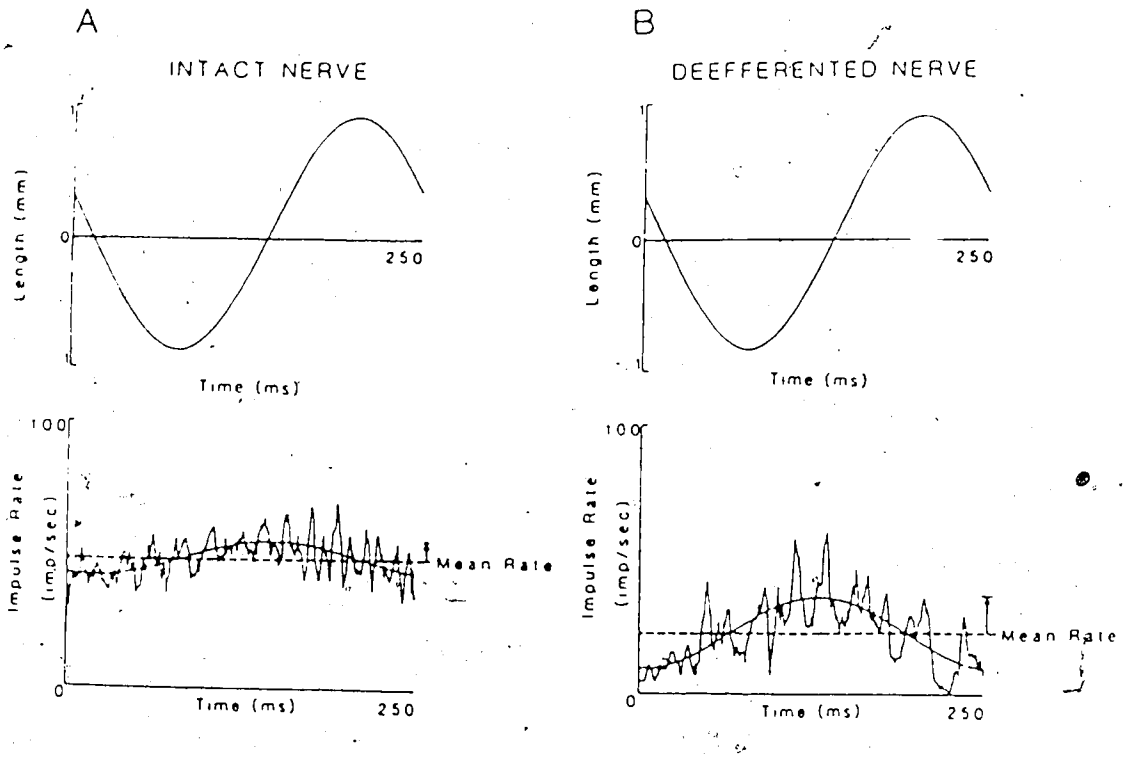

FIG. 2.5 Fitted histogram of primary afferent's average response to a series (typically 250) of 0.1 mm 4 Hz sinusoidal stretches before (A) and after (B) deefferentation. The smoothed histograms are fitted with a sine curve to determine mean rate (as indicated by the dashed horizontal line) and modulation around the mean rate (as indicated by the vertical arrow). The afferent's sensitivity to velocity is evident from the approximately 90 degree phase advance of the afferent's response in relation to the sinusoidal length change. The mean rate decreased and the sensitivity (modulation about the mean rate per mm of applied stretch) increased with removal of gamma motoneuron activity.

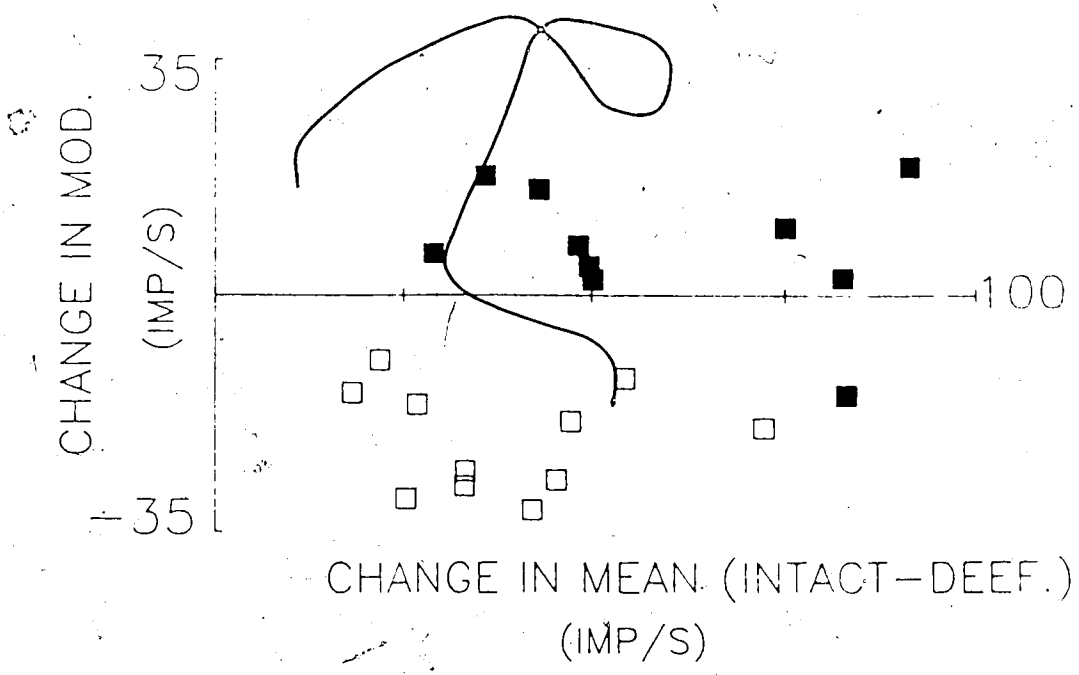

Fig 2.6. Plot of the changes in the mean rate and modulation of the primary afferents due to deefferentation. These values were calculated by subtracting the average mean rate and modulation of the deefferented state from that seen in the intact recordings. The spindle populations were divided according to whether they were located in areas where respiratory related EMG activity was absent (□) or present (■). A change in mean rate was indicative of the presence of gamma motoneuron activity. The change in modulation suggested whether static (decrease modulation) or dynamic (increase modulation) gamma motoneuron effects were dominating the spindle response. In areas where EMG activity was absent there was an increase in mean rate (group mean= 38.1; S.E. 4.8) and a decrease in modulation (group mean=-21.3; S.E. 2.3) when the gamma motoneurons were intact. Areas associated with respiratory EMG activity showed an increase in both mean rate (group mean= 58.8; S.E. 6.9) and modulation (group mean=7.1; S.E. 3.1). The differences between the change in modulation for the two groups is highly significant ($p \leq 0.001$, Student's T-test)

from the diaphragm was used to monitor the timing of the respiratory cycle. Fig. 2.7 illustrates the mean rate and modulation around the mean rate of the primary afferent in response to a sinusoidal stretch during different parts of the respiratory cycle. It is clear that the mean rate decreased and the modulation increased to nearly the same degree throughout the respiratory cycle. The muscle was held approximately isometric so any changes in muscle spindle discharge would be due to the influence of γ-motoneuron activity. The results indicate that the muscle spindle was predominantly receiving a tonic level of static γ-motoneuron input throughout the respiratory cycle. Any phasic component of γ-motoneuron activity would be reflected in differences between the activity of the spindle afferents during inspiration and expiration. Fig 2.8 shows the relative changes between inspiration and expiration of the other spindle afferents in this group. Clearly, they all showed similar responses at both phases of the respiratory cycle, indicating the tonic nature of the static γ-motoneuron activity.

Areas of external intercostal muscle where α-motoneuron activity is present.

The location of the muscle spindles from which the results reported in this section were derived (17 units) is illustrated in Fig. 2.4. Only recordings of muscle spindles from external intercostal muscles are included in this

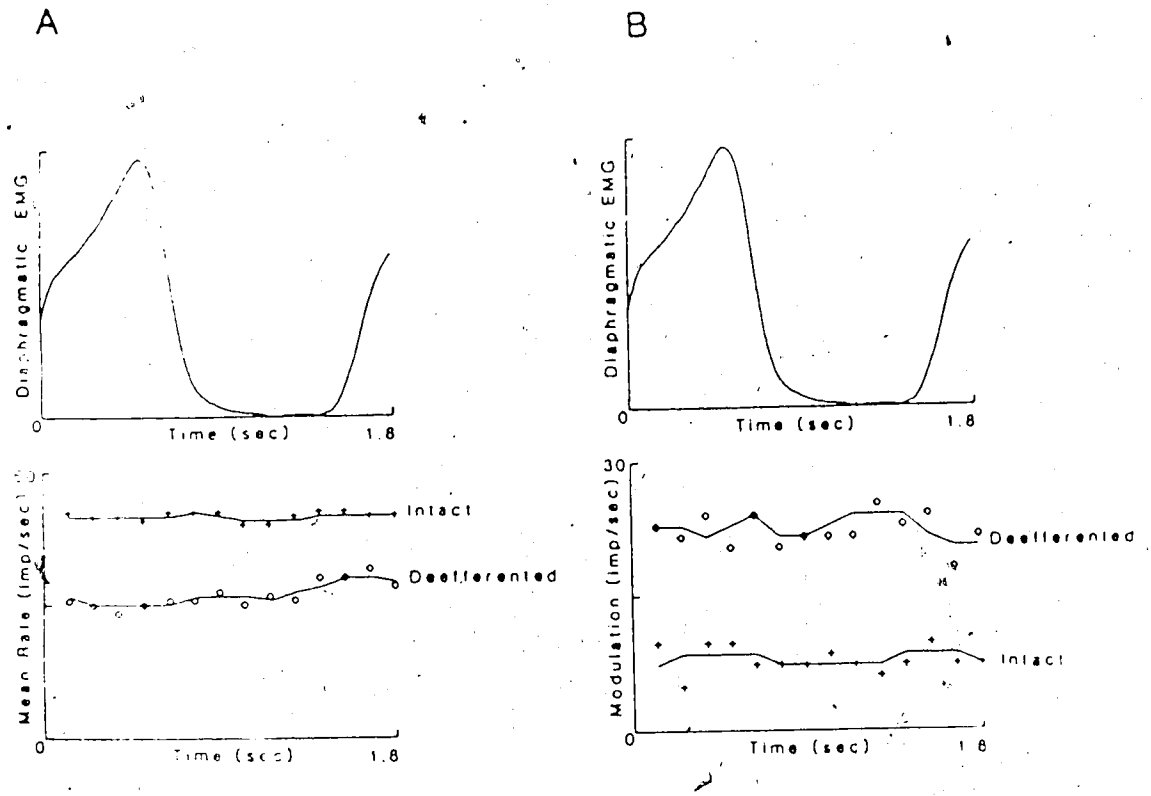

FIG. 2.7 Plots of the afferent's (from Fig. 2.5) mean rate and modulation before and after deefferentation at 14 different parts of the respiratory cycle. These value were derived from histograms of the afferent's response to the applied sinusoidal stretches during successive parts of the breathing cycle as demonstrated in Fig. 2.3. Rectified and filtered diaphragmatic EMG is used to monitor the respiratory cycle (upper panels). The mean rate of the afferent decreases and the modulation increases approximately the same amount throughout the respiratory cycle when the muscle spindle is deefferented.

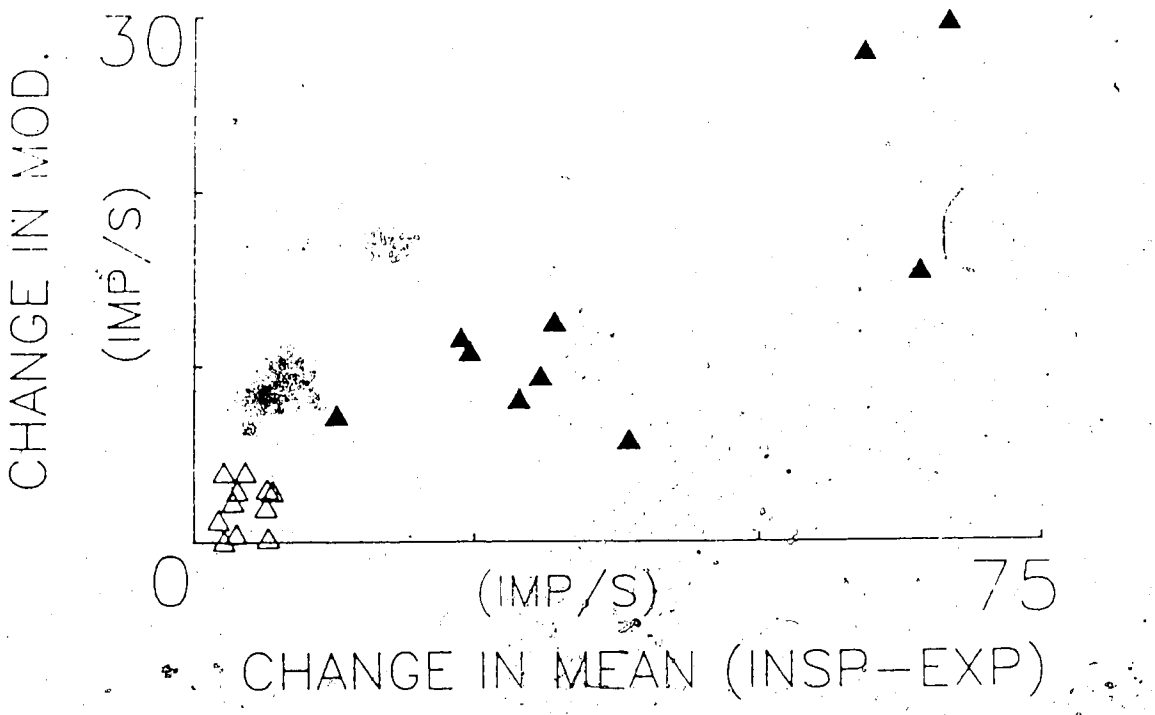

Fig. 2.8. Plot which illustrates the differences between the inspiratory and expiratory values of spindle primaries mean rate and modulation. These values were calculated by subtracting the average mean rate and modulation found during expiration from those values found during inspiration. There was little difference in afferent mean rate (group mean=4.5; S.E. 0.5) and modulation (group mean=2.1; S.E. 0.4) between the two phases of the breathing cycle in areas of the rib cage where respiratory EMG activity is typically absent (△). In contrast, the differences in afferent mean rate (group mean=38.2; S.E.5.9) and modulation (12.3; S.E. 2.0) between inspiration and expiration in the remainder of the rib cage demonstrated the phasic recruitment of dynamic gamma motoneurons approximately in phase with alpha motoneurons(▲).

group. The dorsal areas of the external intercostals in the mid-thoracic spaces are phasically active during respiration (Kirkwood & Sears, 1978; Greer, Stein & Martin, 1988). As shown in Fig. 2.9, the α-motoneuron activity in these areas is recruited in phase with the diaphragmatic EMG.

Fig. 2.10 illustrates the responses of two primary afferents from these areas of the external intercostal muscles to a series of 4Hz sinusoidal stretches before and after deefferentation. In each case the mean rate has dropped and the modulation decreased after the efferent supply has been removed. This is indicative of the effects of dynamic γ-motoneurons. Fig 2.6 shows the changes in mean rate and modulation of all the afferents in this group. Within this group there is evidence for a range of effects from strong static to predominantly dynamic effects. The basis for this variation is evident from studying the response of the afferents during the different parts of the respiratory cycle.

Fig. 2.11A illustrates the mean rate and modulation around the mean rate of the unit from Fig. 2.10A during various parts of the breathing cycle before and after deefferentation. In the intact recording, there is an increase in the afferents mean rate and modulation during inspiration. This result is suggestive of a co-activation of dynamic γ-motoneurons with α-motoneurons.

Fig. 2.11B illustrates the response of the muscle

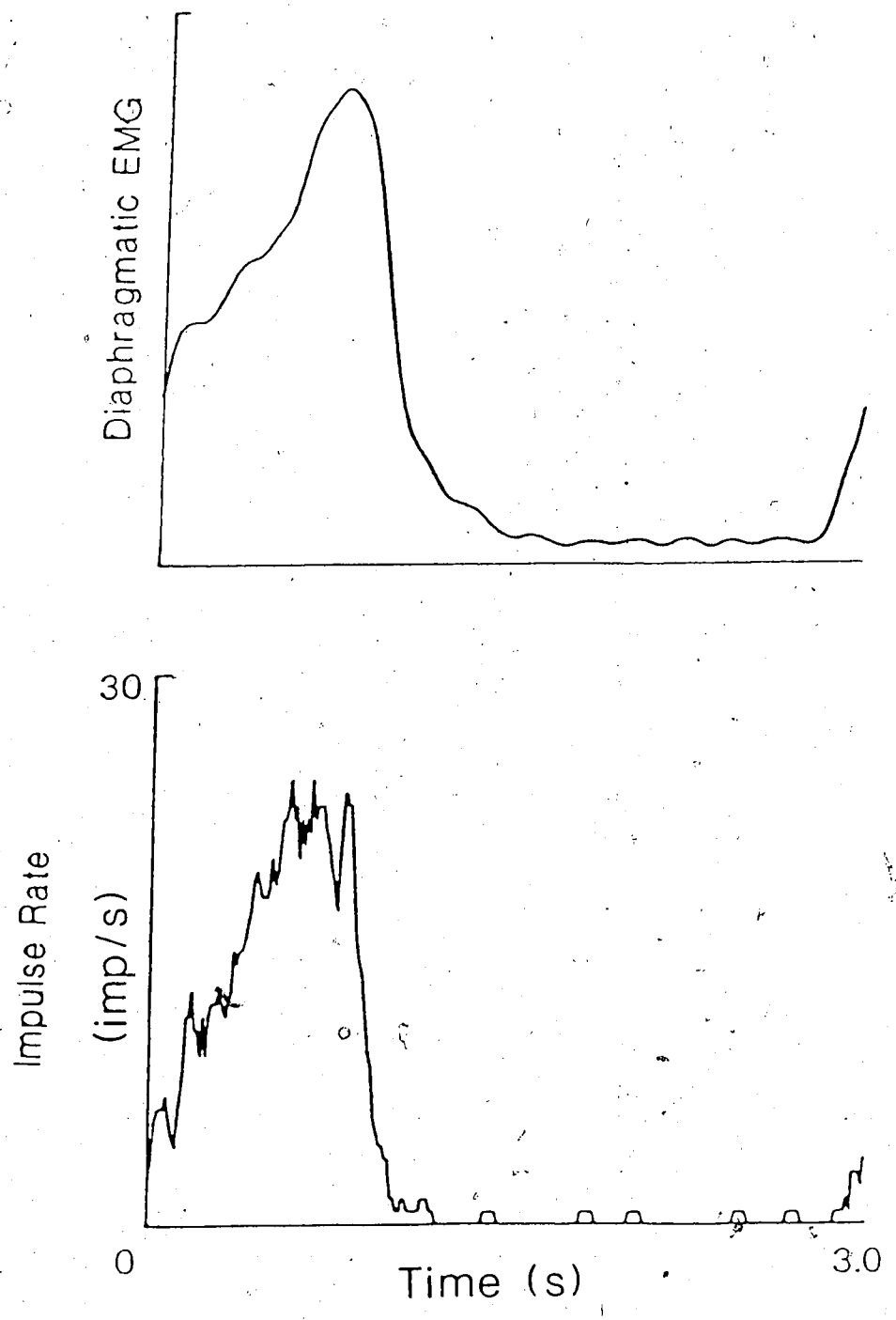

FIG. 2.9 Typical discharge pattern of alpha motoneuron activity recorded from the same external intercostal nerve filaments which contained afferent fibers illustrated in Figs. 2.10 & 2.11. Rectified and integrated diaphragmatic EMG is used to monitor the timing of the respiratory cycle.

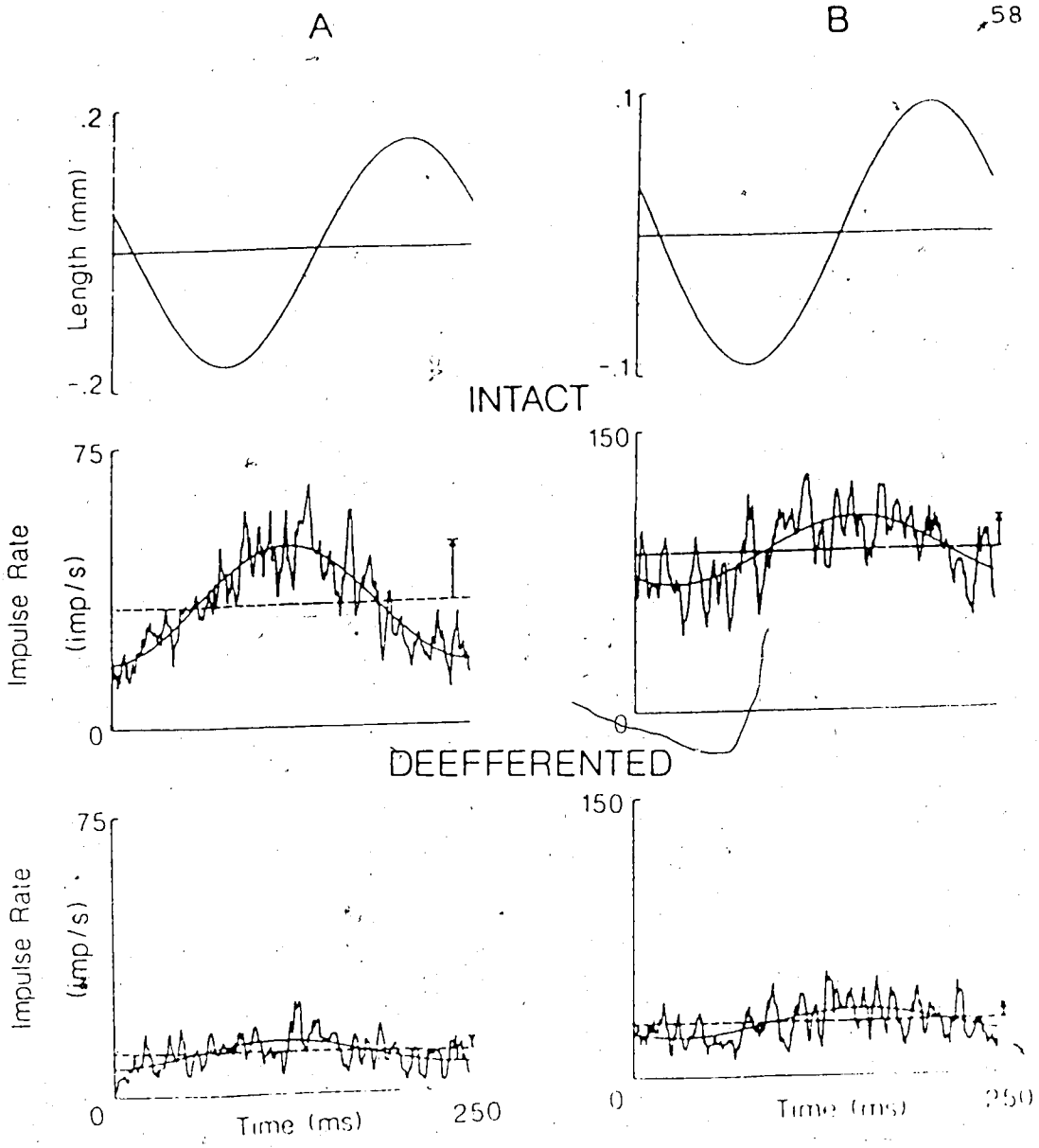

FIG. 2.10. Averages of afferent's responses from recordings of two spindle primaries located within areas specified in Fig. 2.4. Details as in Fig. 2.5. In both examples deefferentation resulted in a decrease in mean rate. The modulation upon removal of gamma motoneuron activity decreased to varying degrees in (A) and (B).

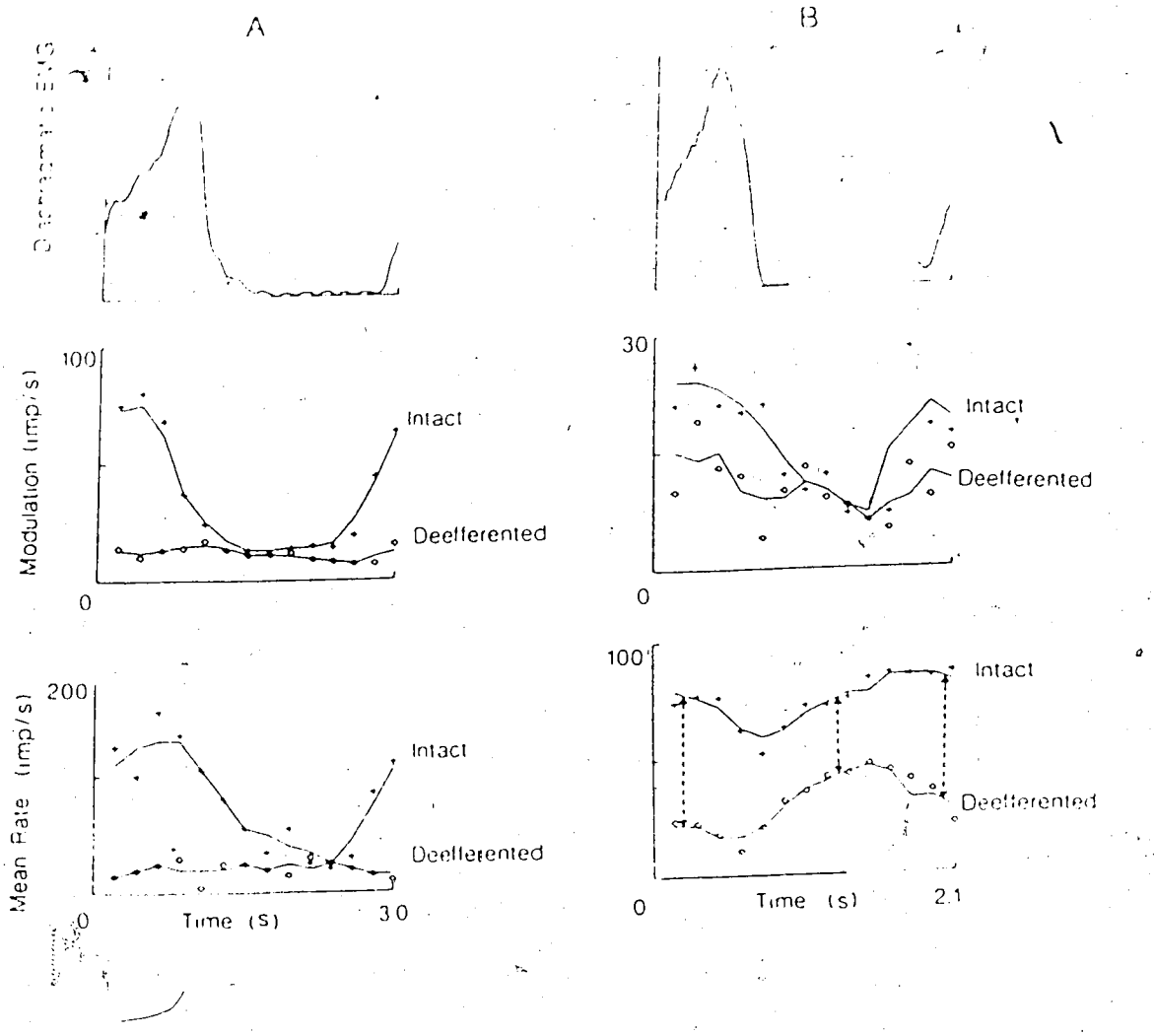

FIG. 2.11. A and B illustrate the changes in mean rate and modulation upon deefferentation during 14 parts of the respiratory cycle of units from Fig. 2.10 A and B respectively. Details as in Fig. 2.7. The afferents in both recordings show evidence of receiving phasically modulated activity from dynamic gamma motoneurons (dashed lines in B illustrate changes in mean rate during inspiration and expiration). The unit shown in B also shows evidence of receiving activity from tonically firing static gamma motoneurons. See text for full description.

spindle afferents from Fig. 2.10B during various parts of the respiratory cycle. The muscle in this example was not held completely isometric, due to the movements imposed on the clamp by the forces generated in the remaining areas of the rib cage. Therefore, the muscle spindle afferents were responding to both the slight length change and, in the case of the intact nerve, the effects of γ-motoneuron activity. Since the forces responsible for the movement of the muscle strip were present in both the recordings of intact and deefferented nerves, any changes in mean rate and modulation between the two recordings were due to the effects of γ-motoneuron activity. Firstly, there is an overall increase in mean rate in the intact recording throughout the respiratory cycle with the largest effect being in phase with inspiration. Secondly, the modulation of the afferent's discharge in response to the sinusoidal stretch is higher during inspiration in the intact as compared with the deefferented nerve. This would suggest the added γ-motoneuron activty seen during inspiration is of the dynamic type. The modulation of the afferent in the intact nerve during expiration is neither increased or decreased significantly from that seen in the deefferented state. This is suggestive of the summation of effects produced by the activity of both static and dynamic γ-motoneurons. Fig. 2.8 shows the differences in the mean rate and modulation between inspiration and expiration for all the afferents in

this group. In every case there was an added increase in mean rate during inspiration accompanied by an increase in the modulation of the afferent discharge. This apparent recruitment of dynamic γ-motoneurons either consisted of purely phasic activity or a waxing and waning of the efferent's discharge. There was also evidence for a steady level of static γ-motoneuron activity throughout the respiratory cycle in 15 of 17 spindles in this group.

Exceptions to the above mentioned pattern of γ-motoneuron activity were seen in two muscle spindles (2 of 17). They were located in the vicinity of α-motoneuron activity, yet showed no signs of receiving any phasic input from γ-motoneurons. Fig. 12 illustrates the response of one such afferent during various parts of the respiratory cycle. The mean rate is greater throughout the cycle while the modulation does not seem to differ much in the intact as compared with the deefferented state. These results could be explained by a mixture of tonic levels of dynamic and static γ-motoneurons influencing the muscle spindle throughout the respiratory cycle, but this would not agree with the majority of our findings. A second possibility is that the muscle spindle is largely under the influence of static γ-motoneuron input which terminates on bag_2 fibers. These static γ-motoneuron terminations are known to increase the discharge rate of spindle primaries without altering the sensitivity (Boyd, 1986). The reason for the dominance of

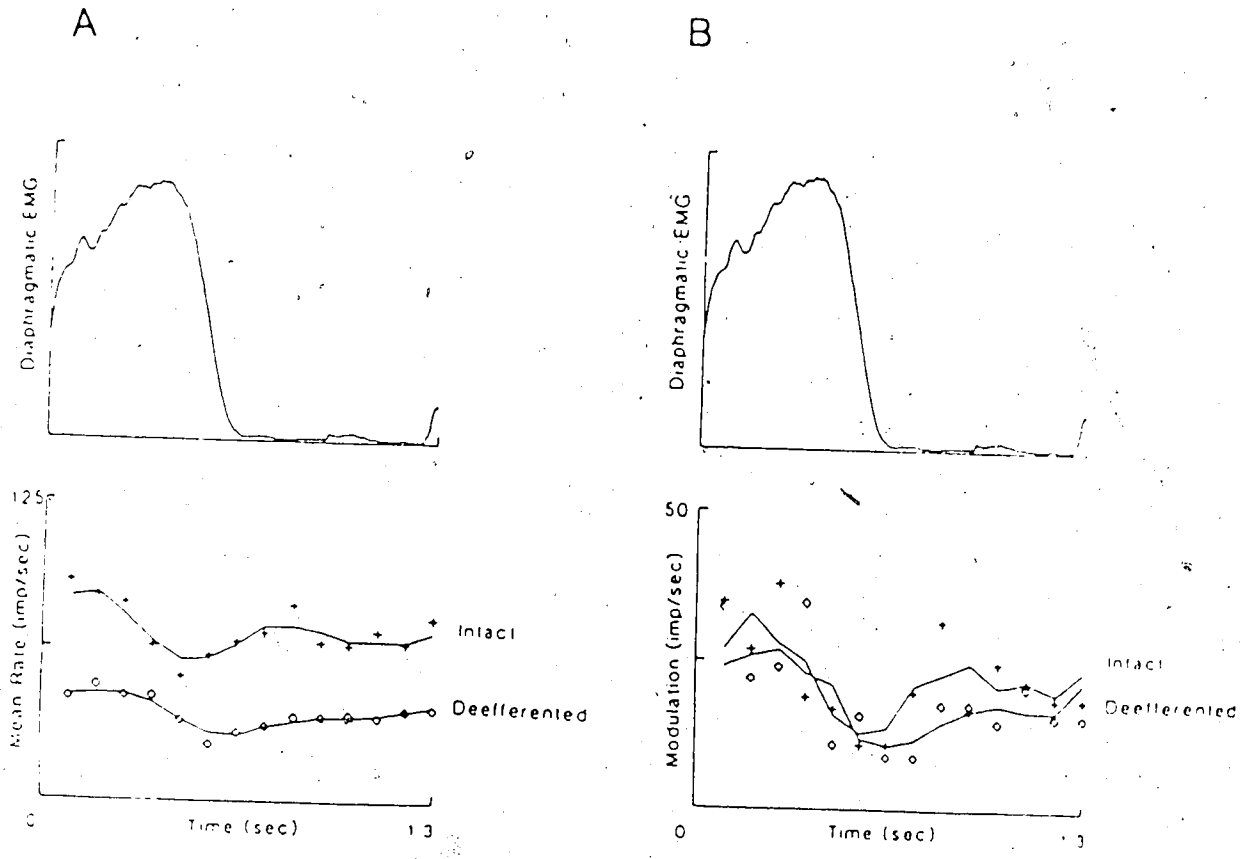

FIG. 2.12 Mean rate and modulation during 14 parts of the respiratory cycle from a recording of an atypical muscle spindle. Details as in Fig. 2.7. Deefferentation led to a decrease in the afferent's mean rate but had little effect on it's modulation (not included in summary Figs. 6 & 8).

effects from the terminals of bag_2 static γ-motoneurons could be a peculiarity of these few spindles or due to selective removal of other γ-motoneur during the isolation of the single muscle spi es as descri in the methods.

DISCU SION

Direct recordings fr γ-mot neurons of interc stal nerves have shown that th e are both ph sica and tonically active units (Crito & von Euler, 196 Sears, 1964; Chapter 3). We have made an at emp a ntifying these γ-motoneurones as being of the dy static types by studying their effects on the response characteristics of muscle spindle primary afferents. These afferents, for the most part, could be divided into two classes according to their response properties. Firstly, there were those afferents which throughout the respiratory cycle displayed the typical characteristics of a muscle spindle under the influence of a constant level of static γ-motoneuron activity. Secondly, there were muscle spindles which behaved as if they were receiving phasically modulated activity from dynamic γ-motoneurons which was usually superimposed on a steady level of static γ-motoneuron activity.

The γ-motoneuron discharge patterns paralleled the differences in α-motoneuron patterns. Areas of intercostal muscle that did not show EMG activity related to respiration appeared to receive only a tonic level of static γ-

motoneuron input. Areas studied that did show EMG activity related to respiration also received phasic activity of dynamic γ-motoneurons. The presence of phasically recruited dynamic γ-motoneuron activity could be seen in these areas at times when the respiratory drive was not sufficient to activate the α-motoneurons. This coincides with the previously reported recruitment threshold of phasically modulated γ-motoneurons in relation to α-motoneurons (Andersen & Sears, 1970). The possibility of β-motoneurons playing a role in altering the spindle response must be considered. Obviously, they were not influencing the spindle activity in those areas where EMG activity was not seen, but recruitment of dynamic and static β-motoneurons could have been recruited where α-motoneuron activity was present.

Both the function of the intercostal muscles and the role of muscle spindles must be considered in explaining the suggested discharge pattern of static and dynamic γ-motoneurons. Certain segments of the external intercostal muscles act as synergists to the diaphragm during inspiration. There is a rostrocaudal and dorsoventral gradient of inspiratory activity in the external intercostal muscles (Kirkwood & Sears, 1978; Greer, Stein & Martin, 1988). While these muscles are active it appears that the γ-motoneuron system is activated in such a way as to increase the mean discharge rate and sensitivity of the muscle spindle afferents. This would be functionally advantageous

considering the proposed importance of spindle afferent input in contributing to α-motoneuron activity during normal and obstructed movements (due to respiratory loading). For instance a deficit in muscle spindle activity in the intercostal muscles can produce lower overall motor unit discharge frequencies (Nathan & Sears, 1960; Sant'Ambrogia & Widdicombe, 1965; Schwieler, 1968), a decrease in reflex compensation in the presence of perturbations (Sears & Newsom Davis, 1970) and, consequently, distortions of the rib cage during inspiration (Chernick, 1981). As well evidence has been put forth to suggest that the CNS sets the muscle spindle sensitivity high during lengthening contractions in order to maximize the reflex contribution to force production (Loeb, 1985, Taylor, Stein & Murphy, 1985). Length measurements of the intercostal muscles have demonstrated the presence of such lengthening contractions in the external intercostals of the the mid-thoracic areas (Greer, Stein & Martin, 1988).

During expiration the inspiratory α-motoneurons are being actively inhibited via a spinal network which receives input from the respiratory center (Aminoff & Sears, 1971). However, passive lengthening of the external intercostal muscle during expiration would result in excitation of the α-motoneurons from spindle afferents. The proposed predominance of static γ-motoneuron activity during expiration would decrease the sensitivity of the muscle

spindle primary afferents and therefore minimize this counter-productive excitation.

The recruitment of the two types of γ-motoneurons which innervate inspiratory intercostal muscles during respiration and extensor muscles during locomotion is similar. Other parallels can also be drawn between the two systems. In both systems there are two well defined periods within the cycles where differential control of the gain of a segmental reflex would seem advantageous. As well both muscle groups experience lengthening contractions and therefore the increased gain of the segmental reflex that results from the phasic activity of dynamic γ-motoneurons would be appropriate.

A role in postural adjustments has also been proposed for the intercostal muscles. Evidence for this proposition is derived from EMG recordings of cat respiratory muscles in which the caudal external intercostals illustrate a tonic level of muscle activity in the awake cat, while the rostral sections show a combination of tonic and phasic activity (Duron, 1973). Studies of the relationship between the intercostal muscles and the cerebellum also support the notion of a postural role. Firstly, stimulation of the anterior lobe of the cerebellum results in selective activation of the tonically firing γ-motoneurons (Corda, von Euler & Lennerstrand, 1966), which we have identified as being of the static type. Secondly, afferents from

intercostal muscles converge with those from limb muscles in the cerebellar cortex and this provides an anatomical basis for the integration of kinesthetic information on which postural adjustments can be made (Coffey, Godwin-Austen, MacGillivray & Sears, 1971). Collectively, these reports suggest that external intercostal muscles and the γ-motoneuron activity in the rostral spaces serve both respiratory and postural functions while those situated caudally are primarily postural.

Our evidence suggests that the tonically active γ-motoneurons associated with control of posture are predominantly of the static type. Static γ-motoneuron activity increases the activity of secondary afferents. Therefore, unless the muscle shortens very rapidly the secondary muscle spindle afferents would provide the CNS with continuous information regarding intercostal muscle length. Obviously, this information would be helpful to the CNS in the control of posture.

Other functions have been proposed for the intercostal muscles including trunk rotation (DeTroyer, Kelly, Macklem & Zen, 1985), shivering (Duron, 1971), purring (Kirkwood, Sears, Stagg & Westgaard, 1987) and vocalization in humans (Draper, Ladefoged & Whitteridge, 1960; Newsom-Davis & Sears, 1973). These functions would involve inputs from a variety of supraspinal centers onto both α and γ-motoneurons. Therefore, during these different tasks the

relative balances between dynamic and static γ-motoneurons could well be altered from those reported in the present study.

In summary, the two types of γ-motoneurons, static and dynamic, can be activated separately. As well, the balance of activity between the two types of γ-motoneurons differs depending on the location of the muscle spindles within the muscle. These regional differences can be explained in terms of the function of the different areas of the intercostal muscles. As well as these spatial differences there are temporal modifications of γ-motoneuron activity during each breath. These differences can be interpreted as an attempt by the CNS to adjust the segmental reflex gain to a level which is appropriate for that particular phase of the respiratory cycle. The recruitment pattern of the two types of γ-motoneurons during respiration is similar to that reported for the hindlimb extensors during locomotion, but disagrees with that reported for jaw movements during chewing. The reasons for the apparent differences in control strategies used by the CNS in different cyclic movements remain to be explained.

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CHAPTER THREE

PATTERNS OF GAMMA MOTONEURON ACTIVITY IN THE EXTERNAL INTERCOSTAL MUSCLES OF THE CAT DURING RESPIRATION

Muscle spindles are unique amongst mammalian proprioceptors in so far as their sensitivity can be directly controlled by the central nervous system. This control is conveyed by the activity of gamma motoneurons which innervate the intrafusal fibers of muscle spindles. The effects the two types of gamma motoneurons, dynamic and static, have on the properties of muscle afferents are well documented (Matthews & Stein, 1969; Hulliger, Matthews & Noth, 1977). However, little is known regarding how the CNS recruits gamma motoneurons to facilitate motor control. Recordings of gamma motoneurons during normal behavior have proved difficult due to the small size of these neurons. Furthermore, dynamic and static gamma motoneurons have similar conduction velocities and ranges of discharge frequencies, making it impossible to differentiate between them based on these criteria (Hulliger, 1984). Therefore, the approach often taken is to record the activity of muscle spindle afferents during a natural movement and then to deduce the type and pattern of gamma motoneuron activity which is influencing the spindle behavior (Larson, Smith &

1. A version of this chapter has been submitted to Brain Research for publication; Greer, J. and Stein R.B.

Luschei, 1981; Gottlieb & Taylor, 1983; Prochazka, Hulliger, Zangger & Appenteng, 1985; Taylor, Stein & Murphy, 1985) Previously, this strategy has been applied to discern the activity of gamma motoneurons during respiration in the cat (Greer & Stein, 1986). We have now extended this investigation by directly recording from gamma motoneurons in the external intercostal muscles of spontaneously breathing cats.

Earlier reports from recordings of gamma motoneurons innervating intercostal muscles have illustrated the presence of tonically and phasically active units (Sears, 1964; Eklund, von Euler & Rutkowski, 1964). The response of muscle spindle afferents suggests tonically firing units are predominantly of the static type, while the majority of dynamic gamma motoneurons discharge with a respiratory rhythm (Greer & Stein, 1986). In contrast to the earlier recordings, however, the study of muscle spindle responses suggests the discharge patterns of gamma motoneurons are different within discrete areas of the intercostal muscles. Direct recordings of gamma motoneurons have now confirmed that these regional specializations are present. Furthermore, there is a correlation between the patterns of gamma motoneuron activity and the previously reported compartmentalization of the EMG activity and motor unit properties found in external intercostal muscles (Greer, Martin & Stein, 1988).

METHODS

Experiments were performed on 5 cats which were initially anesthetized with sodium pentobarbital (20 mg/kg) and maintained on Halothane (delivered in a mixture of 95% O_2 and 5% CO_2). Cannulae were inserted into the trachea, carotid artery (for monitoring blood pressure), and external jugular vein (for administration of Dextran).

With the animals positioned on their side, the external intercostal muscles were exposed, and a muscle pool containing warm (37^{o}C) paraffin oil was formed. The timing and depth of respiration was monitored by recording the diaphragmatic EMG with stainless steel electrodes. Fine branches of the external intercostal nerves were cut distally and lifted onto silver bipolar electrodes. Gamma and alpha motoneurons were differentiated by the amplitude of their spikes and discharge frequencies. The small spikes have been previously classified as gamma motoneurons based on several criteria: conduction velocity, selective blocking with lidocaine, and discharge frequency (Sears, 1964; Eklund et al, 1964). In agreement with these previous findings, the amplitude of the large spikes was 3-4 times greater than that of the smaller units in our recordings. The mean discharge frequencies, averaged over 20-50 breaths, ranged from approximately 15-45 impulses per second for gamma motoneurons and 3-12 impulses per second for alpha

motoneurons.

The neural recordings were passed through a window discriminator (Bak Electronics, Md.) which allowed for the separation of efferent units based on the size and shape of recorded signals. Each selected spike then triggered a standard pulse which was led to a PDP-11 computer along with the rectified and integrated diaphragmatic EMG. Histograms of the efferent's average discharge rate with respect to the timing of the respiratory cycle were generated (averaged over 20-30 breaths).

A spectral analysis of the discharge frequency of the efferent's was also performed. We were interested in knowing if the high frequency oscillation (HFO), of approximately 88 Hz, which has been seen in the activity of other neurons associated with respiration, was evident in our recordings (Feldman, 1986). Its presence in the discharge frequency of a selective population of gamma motoneurons might have been indicative of a discrete input from the brainstem respiratory center. However, the spectral analysis did not reveal any evidence for the presence of HFO in the activity of the alpha or gamma motoneurons studied. The only frequency components seen in the activity of the efferents was related to either the average frequency at which the neurons tended to fire, or the frequency of the respiratory cycle. Perhaps further investigation in different preparations is necessary, as past studies of HFO have shown

that it is rather labile and often depressed by anesthesia (Feldman, 1986).

RESULTS

There were two basic types of gamma motoneuron activity in the external intercostals: tonic and phasic. There was a relationship between the firing patterns of gamma motoneurons and the presence of respiratory EMG activity. The areas of the external intercostals which are recruited during inspiration in the anesthetized cat are illustrated in Fig. 3.1. A similar pattern has been observed in a variety of mammalian species and preparations. In the caudal and ventral areas of the external intercostal muscles, where respiratory EMG activity is seldom seen, only tonically firing gamma motoneurons were found (5 units; Fig. 3.2). In contrast, in the areas of external intercostal muscles which are recruited during inspiration, units with large amplitude spikes (alpha motoneurons) and a population of smaller amplitude spikes (gamma motoneurons) were observed. Selective triggering of the various units illustrates the presence of phasically modulated alpha and gamma motoneurons (8 units) as well as gamma motoneurons which fire tonically (9 units) throughout the respiratory cycle (Fig. 3.3).

DISCUSSION

The earlier studies of gamma motoneuron activity in the intercostal muscles were not designed to investigate

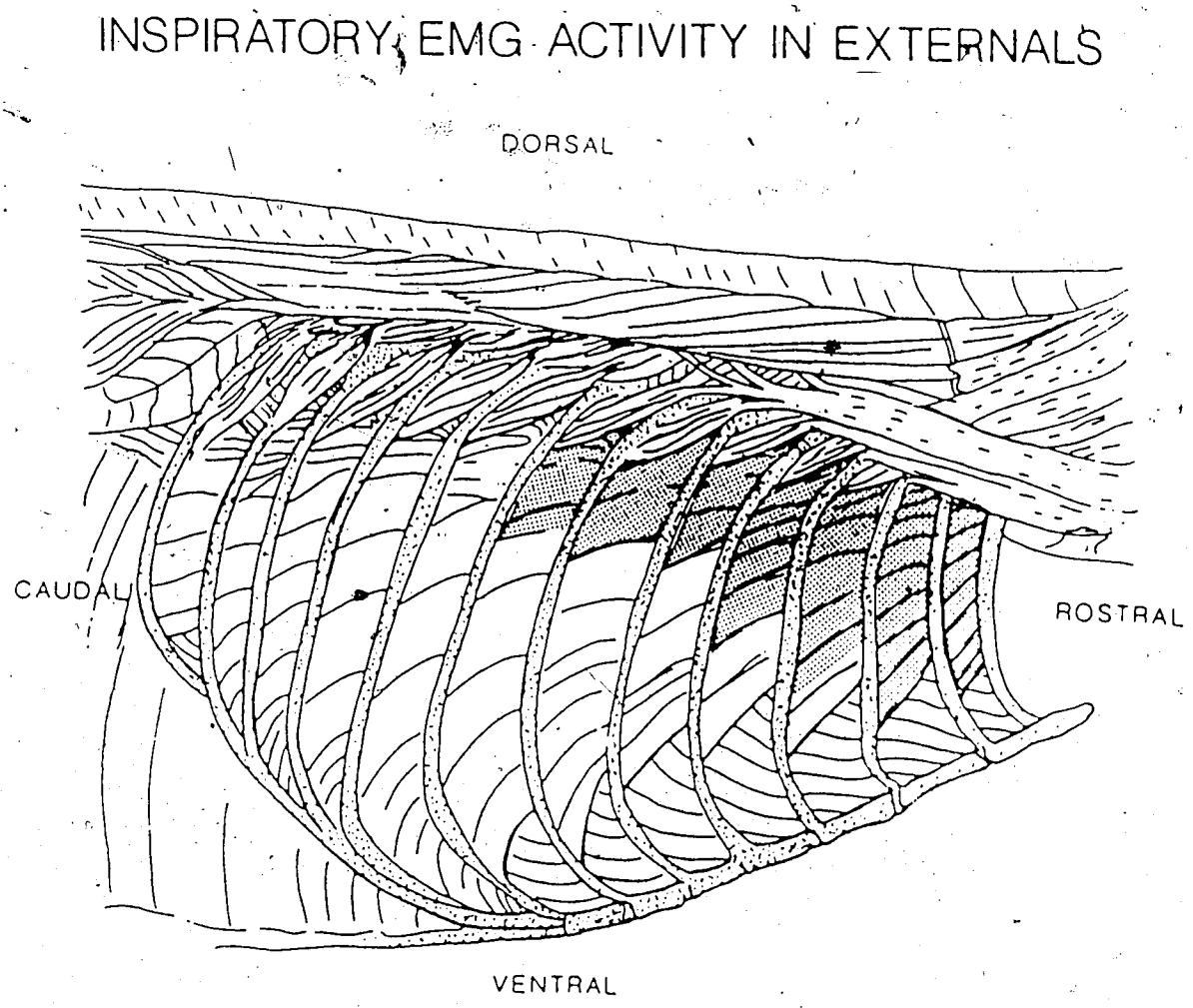

FIG. 3.1 Illustration of the location where EMG activity related to the respiratory cycle is found in the external intercostals of anesthetized cats (shaded areas). These areas received both tonically and phasically active gamma motoneurons. The motor units in the remaining areas were typically silent and were innervated by tonically active gamma motoneurons.

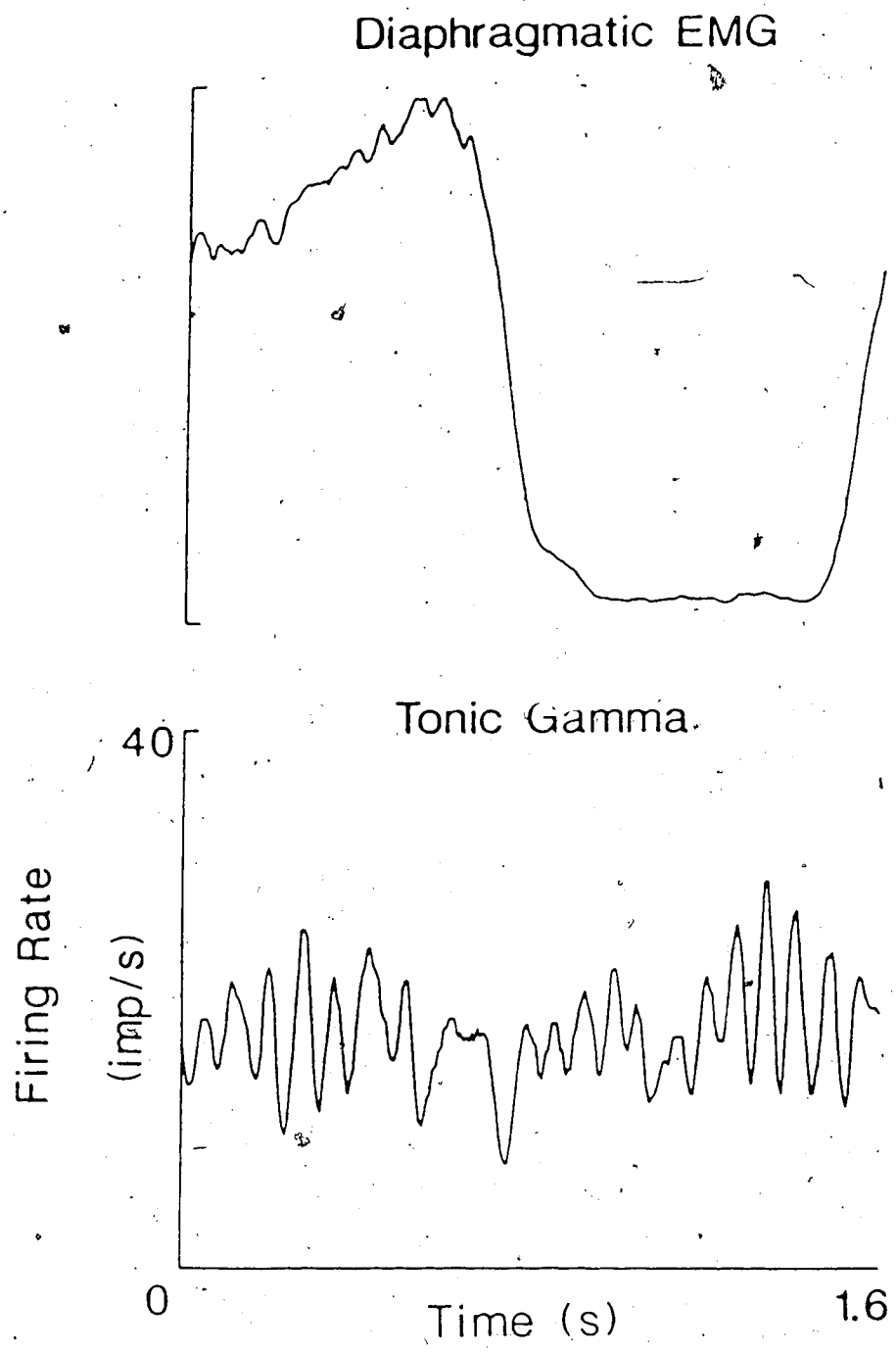

FIG. 3.2 Histogram of the average activity of the gamma motoneurons (lower trace) in the areas of the intercostal muscles which were not recruited during inspiration (averaged over 20-50 breaths) with respect to the diaphragmatic EMG (upper trace).

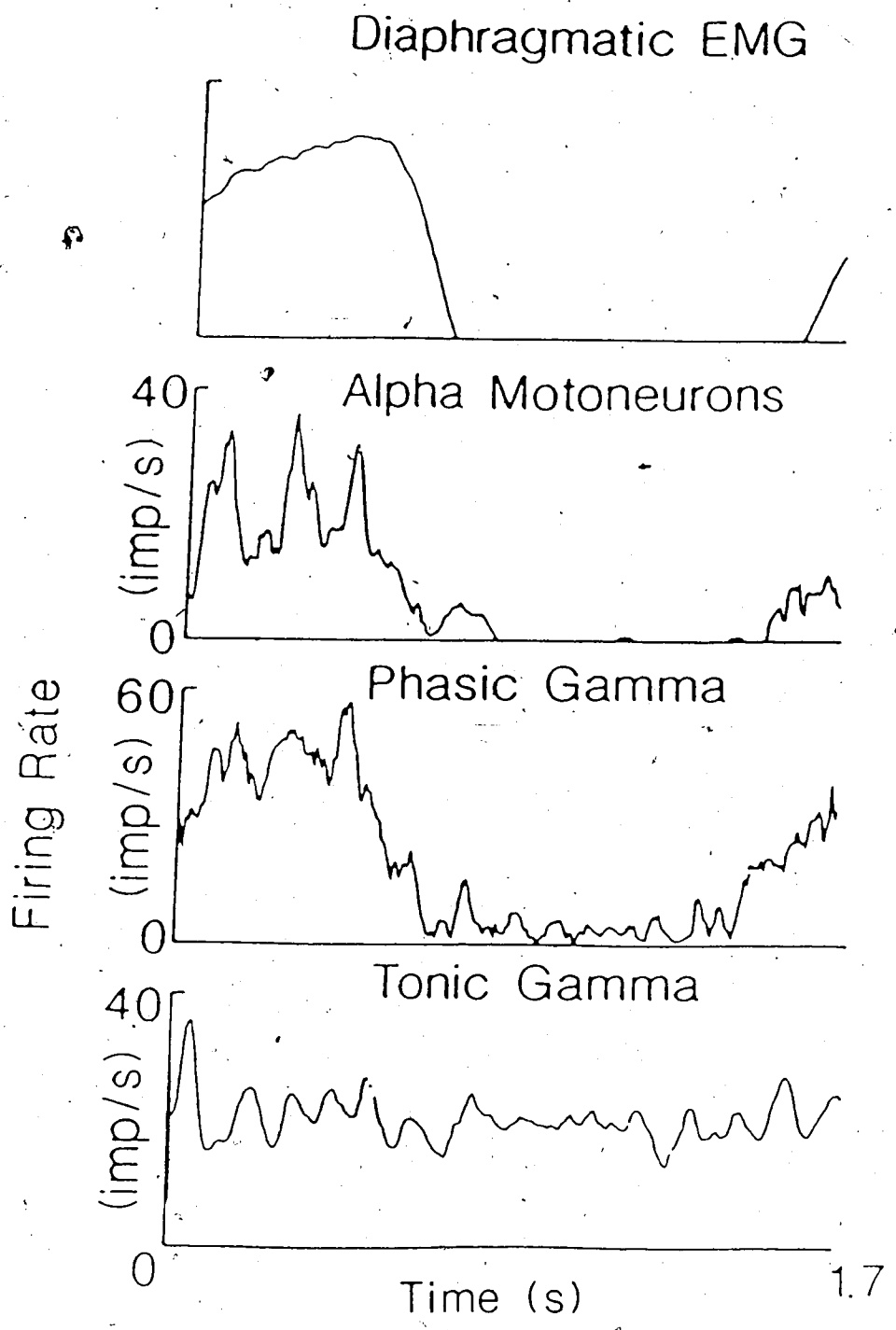

FIG. 3.3 Histograms of the average activity of several alpha motoneurons (second trace), and individual phasically (third trace) and tonically recruited (fourth trace) gamma motoneurons with respect to the diaphragmatic EMG (first trace) in those areas of the intercostal muscles which were active during inspiration.

regional differences in discharge patterns. Recordings were either made from whole dorsal roots or fine filaments in the proximal regions of mid-thoracic spaces (Sears, 1964; Eklund et al, 1964). Both of these populations of nerves would have contained tonically and phasically active units, Only by recording, as in the present study, from selective branches of the external intercostal nerve from areas of the ribcage which are not recruited during respiration do regional differences in gamma motoneuron recruitment became apparent.

In summary, there are two patterns of gamma motoneuron activity in the external intercostal muscles of the cat during respiration. Tonically active units, which we have previously characterized as being predominantly static gamma motoneurons, are found throughout the rib cage. Those areas of the external intercostals which are regularly recruited during inspiration also receive input from phasically active gamma motoneurons, which are thought to be largely composed of the dynamic type.

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CHAPTER FOUR

LENGTH CHANGES OF INTERCOSTAL MUSCLES DURING RESPIRATION IN THE CAT

Traditionally, the parasternal and external intercostal muscles have been thought to actively shorten during inspiration, drawing the lower rib of each space in an outward and cephalad direction. The recruitment of the internal intercostal muscles has been associated with expiration (Campbell, Agostoni & Newsom Davis, 1970). However, there has been considerable disagreement over the years about the function and mechanisms of action of the intercostal muscles (Derenne, Macklem & Roussos 1970; DeTroyer, Kelly, Macklem & Zin, 1985). The matter has been further complicated by the realization that the intercostals are not a homogeneous group of muscles. Several studies of EMG activity in the intercostals, both in man (Taylor, 1960; Newsom Davis & Sears, 1970) and anim ls (Duron, 1973; DeTroyer & Ninane, 1986; Greer, Martin & Stein, 1988), have illustrated that muscle activity related to the respiratory cycle is limited to certain areas of the rib cage. Regional differences in the muscle fiber properties (Greer et al, 1988), distribution of muscle spindles (Duron, 1973), and the recruitment patterns of

1. A version of this chapter has been submitted to Respiration Physiology; Greer, J.J. and Stein, R.B.

gamma motoneurons (Greer & Stein, 1986) have also been reported. We have now measured the regional differences in the length changes of the intercostal muscles. The relevance of these results to the understanding of rib cage mechanics will be discussed. We will also use this information to offer an explanation for the previously suggested role of the muscle spindle system (Greer & Stein, 1986).

Previous measurements of the length changes of external intercostal muscles during respiration have been limited to selected areas of the rib cage. The length excursions of cat intercostal muscles were initially measured using strain-gauge myographs fastened to adjacent ribs (Von Euler & Peretti, 1966; Andersson, Lennerstrand & Thoden, 1968). Both of these groups reported a 10-20% shortening of the mid-thoracic intercostal spaces. Recent reports from sonomicrometric measurements in the dog suggest that muscle fibers in these spaces actually lengthen and any active shortening during inspiration is limited to the rostral areas of the rib cage. (Decramer, Kelly & DeTroyer, 1985). Fitting, Easton & Grassino (1986) could not find a consistent pattern of EMG activity and length changes in the limited number of intercostal spaces they measured in the dog. The fact that parasternal muscles actively shorten has been demonstrated in a number of studies on the dog (Decramer et al, 1985; Van Lunteren & Cherniack, 1986,

Fitting et al, 1986).

In this study sonomicrometry was used to measure the length changes of all the intercostal muscles throughout the rib cage in the spontaneously breathing cat. Sonomicrometry was chosen for two reasons. Firstly, it gives a direct measure of the muscle fiber length, similar to that experienced by the muscle spindles. To simply measure the distance between the two ribs during respiration neglects the independent length perturbations the muscle fibers experience due to the perpendicular forces arising from intrathoracic pressure changes. Secondly, with the use of sonomicrometry there is little or no mechanical impedance of the normal length changes experienced by the muscle during respiration.

METHODS

Experiments were performed on 12 spontaneously breathing adult cats of either sex. The cats were initially anesthetized with sodium pentobarbital (20mg/kg) and maintained on Halothane anesthesia (delivered in a mixture of 95% O_2 and 5% CO_2). Cannulae were inserted into the trachea, carotid artery (for monitoring blood pressure), and external jugular vein (for administration of Dextran). The animals were placed in either a supine position or on their side, depending on which muscles were being studied. The intercostal muscles were exposed and a muscle pool containing warm (37^{o}C) paraffin oil was formed.

EMG Recordings. Diaphragmatic EMG was recorded with stainless steel electrodes which were inserted into the caudal surface of the muscle via an incision along the linea alba. Selective recordings of motor unit potentials were made with bipolar needle electrodes. The electrodes were positioned between the piezoelectric crystals which made it possible to monitor motor unit activity and length changes in the same area of muscle.

Length Measurements. Measurements of the length changes of the intercostal muscles were made with a four channel sonomicrometer (model 120, Triton Technology, San Diego, CA). The sonomicrometer measures the transit time of ultrasound between two transducers (fabricated from plates of ferro-electric ceramic) which are arranged to face each other in alignment with the pennation of the muscle fibers (Fig. 4.1). The transducers were positioned 3-8 mm apart on the surface of the intercostal muscle and the initial distance between them was measured at the end of expiration (defined as L_R). The resolution of the sonomicrometer was approximately +/-0.04 mm.

Analysis. The motor unit activity and length changes of a given area of intercostal muscle were averaged over a number of respiratory cycles (typically 20-50 breaths). The diaphragmatic EMG was rectified and low pass filtered with a

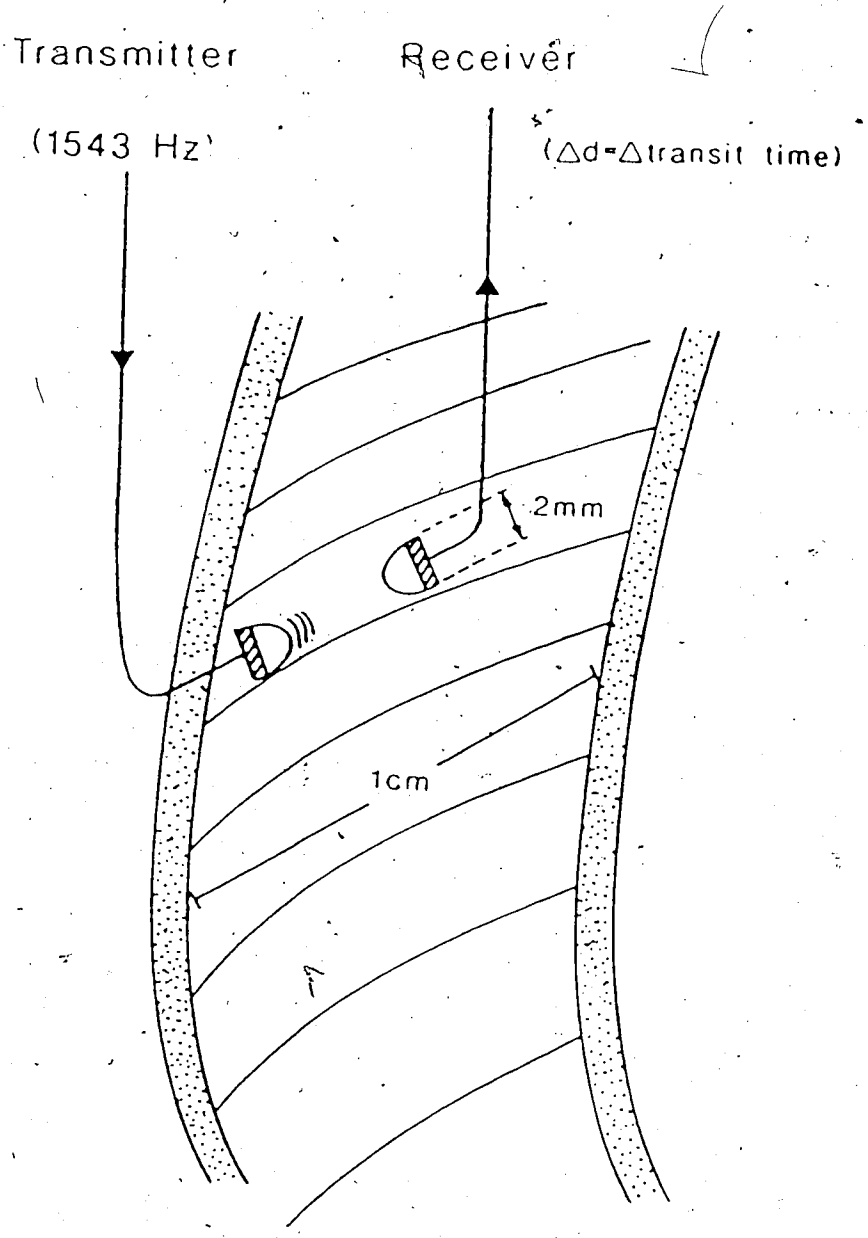

FIG. 4.1. Diagram of preparation for sonomicrometric measuring of length changes of intercostal muscles. Piezoelectric crystals (2 mm diameter) were positioned facing each other in alignment with the pennation of muscle fibers. The crystal which acts as the transmitter resonates at 1543 Hz in response to a 150 volt pulse of 10 nsec duration. The sound waves radiate at a +/- 30 degree angle from the concave surface of the transmitting crystal and are intercepted by the second crystal. The changes in length of intercostal muscle during the respiratory cycle is determined by measuring the transit time between the pair of crystals.

third order Paynter filter (30 Hz) and RC filter (50 Hz). The filtered EMG was used to activate a Schmitt trigger which generated a marker for the respiratory cycle. Single motor unit potentials were isolated from the motor unit recordings with a window discriminator (Bak Electronics, Md.). The standard pulse generated from the window discriminator, the analog signal from the sonomicrometer and the marker for the respiratory cycle were then led to the computer (PDP 11) for averaging.

RESULTS

The length changes of the intercostal muscles occur as a result of forces generated within the active muscle and those imposed from the surrounding structures. From our measurements of muscle length and EMG activity it was possible to infer something about the forces acting on an intercostal space at any given time. The results of these measurements of the internal, parasternal and external intercostals will be discussed in turn.

Internal Intercostals. In our preparation the internal intercostal muscles of the caudal spaces were occasionally active during expiration. An example of the motor unit activity and length changes of one such space is illustrated in Fig. 4.2. The shortening of the caudal spaces which occurs when the diaphragm relaxes is assisted by the force produced upon recruitment of the internal intercostal muscle

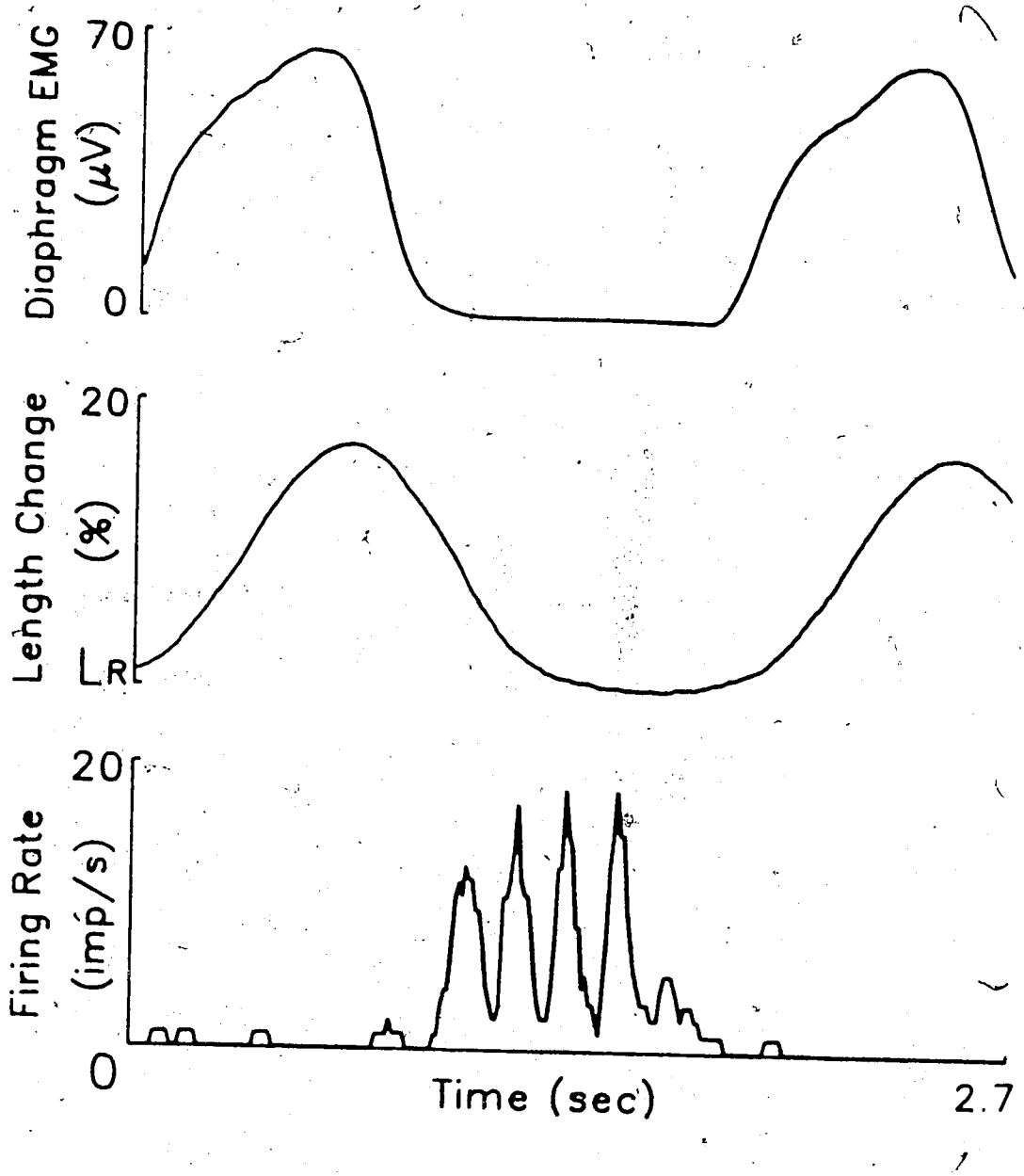

FIG. 4.2. Averages of diaphragmatic EMG (top trace), length change (middle trace) and motor unit discharge frequency (bottom trace) of internal intercostal muscles from a caudally located space (typically averaged over 20-50 breaths). The internal intercostals of the lower rib cage were occasionally recruited during expiration, producing shortening forces within these spaces.

during expiration. However, internal intercostals were generally inactive and their length excursions therefore passively followed those of the external intercostals.

Parasternal muscles. The parasternal muscles actively shortened during inspiration (2.2 - 9.6%; mean 5.5%). A further investigation of these muscles was not undertaken, since as mentioned in the Introduction, the action of the parasternals has been thoroughly studied and described previously.

External Intercostals. The EMG activity and length changes of the external intercostals varied in different areas of the rib cage. There were prominent levels of external intercostal activity in the rostral spaces which resulted in a shortening of the muscles fibers during inspiration (1.6-9.2%). The velocity of shortening in this area ranged from 2.9 to 12.4% L_R/sec with a mean of 6.9% (L_R = length of muscle fiber at the end of expiration). A typical response of an external intercostal from this area of the rib cage is illustrated in Fig. 4.3A. The motor units were recruited in phase with the diaphragmatic activity during inspiration. The forces generated by the activity within these muscles was sufficient to produce shortening of the rostral intercostal spaces.

The external intercostal muscle fibers in the mid-thoracic spaces illustrated a more varied response. In some

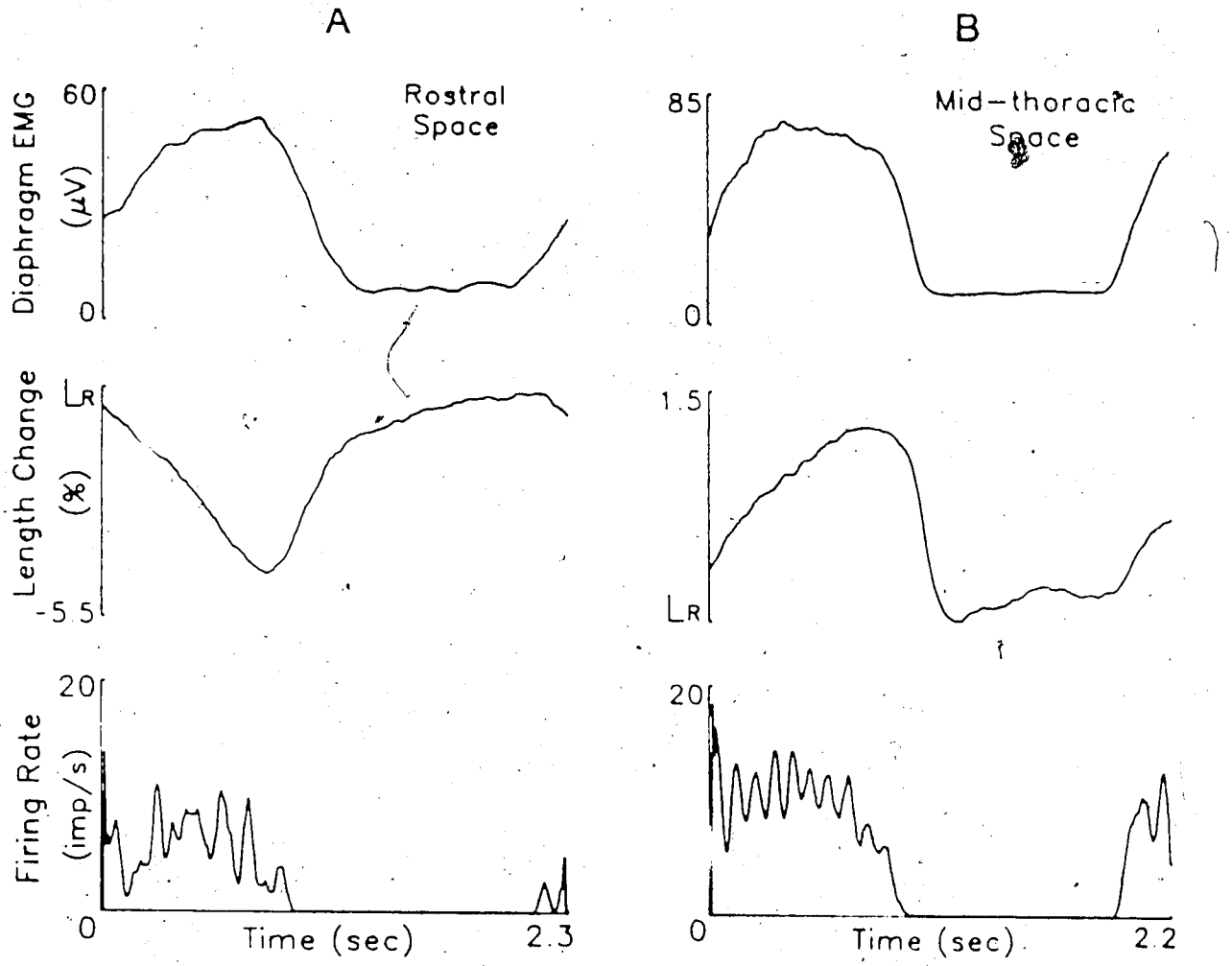

FIG. 4.3. Averages of diaphragmatic EMG, length change and discharge rate of motor units from external intercostal muscle from rostral (space2; A) and mid-thoracic (space 7; B) spaces. Same format as Fig. 4.2. External intercostals in rostral spaces consistently illustrated EMG activity in phase with inspiration, producing sufficient force to shorten the muscle fibers. The external intercostals of the mid-thoracic spaces also actively shortened on occasion, but as illustrated in (B), they often lengthened in spite of the force produced by the motor unit activity.

instances the muscle activity produced sufficient force to shorten the intercostal space (0.7-3%). It was also common, however, to see the muscle fibers of these mid-thoracic spaces lengthen when active (0.4-5.0%). An example of such a recording is illustrated in Fig. 4.3B. Clearly, in this case the forces generated by the surrounding structures were greater than those produced by the intercostal muscle of that space.

The muscle of the external intercostal located in the five most caudal spaces were not active in the preparation. The majority of the muscles in these spaces were passively lengthening (0.5-3.3%). However, all measurements of space nine and one from space ten showed the muscle passively shortening (2.9-6.0%) as these particular intercostal spaces collapsed when the diaphragm, which inserts on the lower ribs, contracted.

A compilation of the data derived from length measurements and EMG recordings of the various external intercostal spaces is illustrated in Fig. 4.4. The EMG activity related to the respiratory cycle was limited to the rostral portion of the external intercostal muscles. There is also a general trend evident in this figure which suggests a lengthening force is acting from the caudal axis of the rib cage, while a shortening force is produced by the contracting muscle. To gain further insight into how these antagonistic forces interact we have measured the length

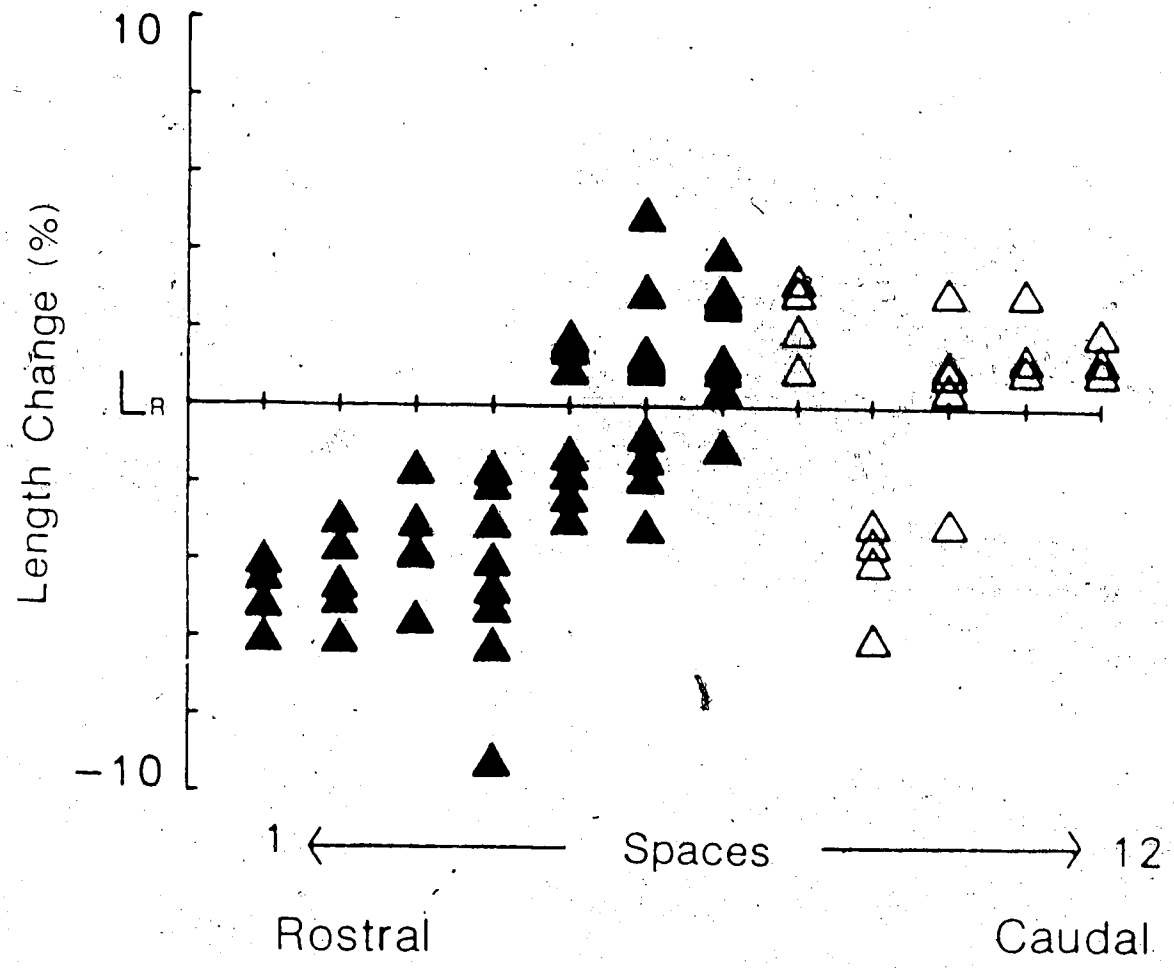

FIG. 4.4. Compilation of the results from length measurements of external intercostal muscles in spaces 1 through 12. Shortening (-ve) and lengthening (+ve) of muscle fibers were calculated as a percentage of the muscle length at the end of expiration (L_R). The presence (▲) or absence (△) of phasic EMG activity during inspiration was also measured. Depending on the location within the rib cage, intercostal muscles showed one of the following: shortening contractions, lengthening contractions, passive shortening or passive lengthening. See text for details.

changes of the intercostal muscles under a variety of conditions.

Increased respiratory drive. The level of respiratory drive was determined by changes in the activity of the diaphragm. As the level of anesthesia was adjusted, the amplitude and periodicity of the diaphragmatic EMG signal changed. Fig. 4.5 illustrates the diaphragmatic EMG and motor unit recordings from the external intercostal muscle during two levels of anesthesia. When the anesthesia was lowered to a level where the cat demonstrated tone in the hindlimbs, the amplitude of activity in the respiratory muscles increased, as did the frequency of breathing. Typically, there was both an increase in the discharge rate of previously active motor units and a recruitment of higher threshold units in the intercostal muscles.

The response of an external intercostal muscle from a rostral space to an increased respiratory drive is illustrated in Fig. 4.5A. The increase in activity of the intercostal muscle was sufficient to produce a greater force on the adjacent ribs resulting in an increased shortening of the intercostal space. Naturally, as the level of respiratory drive increased the activity generated by the diaphragm and surrounding muscles also increased. As illustrated earlier when these forces are greater than those produced by the intercostal muscle lengthening of the space will occur. In the case of augmented respiratory drive the

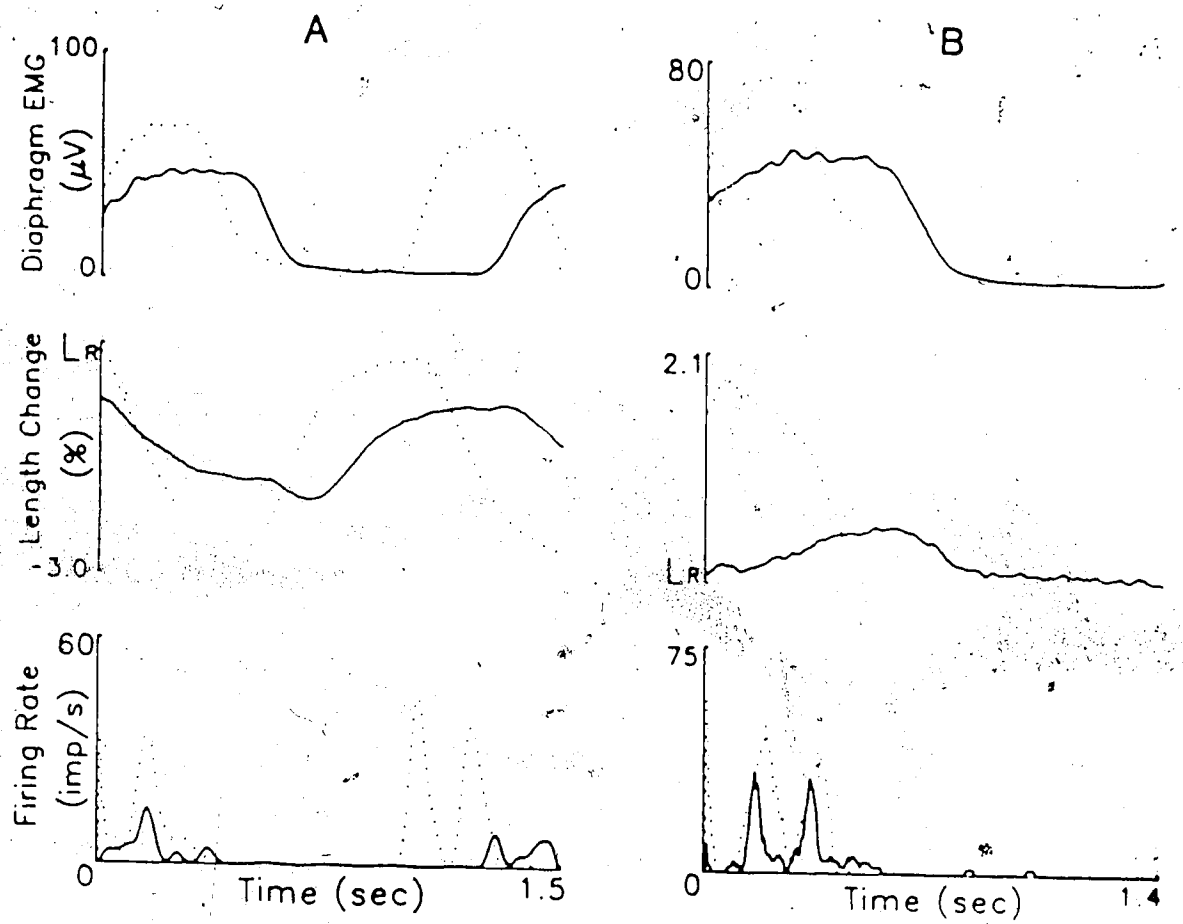

FIG. 4.5. Responses of external intercostal muscles which were actively shortening (A) and lengthening (B) during normal (solid line) and increased respiratory drive (dotted line). Same format as in Fig. 4.2. The increased respiratory drive accentuated the respective shortening and lengthening of the muscle fibers.

discrepancy between the two forces is increased, resulting in further lengthening of the intercostal space during inspiration as shown in Fig. 4.5B.

In summary, increases in respiratory drive result in overall increases in inspiratory muscle activity. The imbalances between the shortening and lengthening forces are subsequently exaggerated.

Effects of Deefferentation. Further evidence for the lengthening forces imposed on the mid-thoracic spaces was obtained by deefferenting the external intercostal muscle (Fig. 4.6). When the muscle is rendered inactive the lengthening of the muscle by the remaining unopposed forces is increased.

Occlusion of trachea. Complete occlusion of the trachea resulted in an increase in the duration of inspiration as illustrated in Fig. 4.7. The activation levels of the motor units in the diaphragm and external intercostal muscles also increased. The intercostal muscles which, prior to occlusion of the trachea, had shortened continue to do so when airflow is prevented, although to a lesser degree (Fig. 4.7A). In contrast, the length excursions of the external intercostal muscles which lengthened during inspiration actually increased when the trachea was occluded (Fig. 4.7B). Presumably, during tracheal occlusion the combination of muscle shortening and lengthening of the various

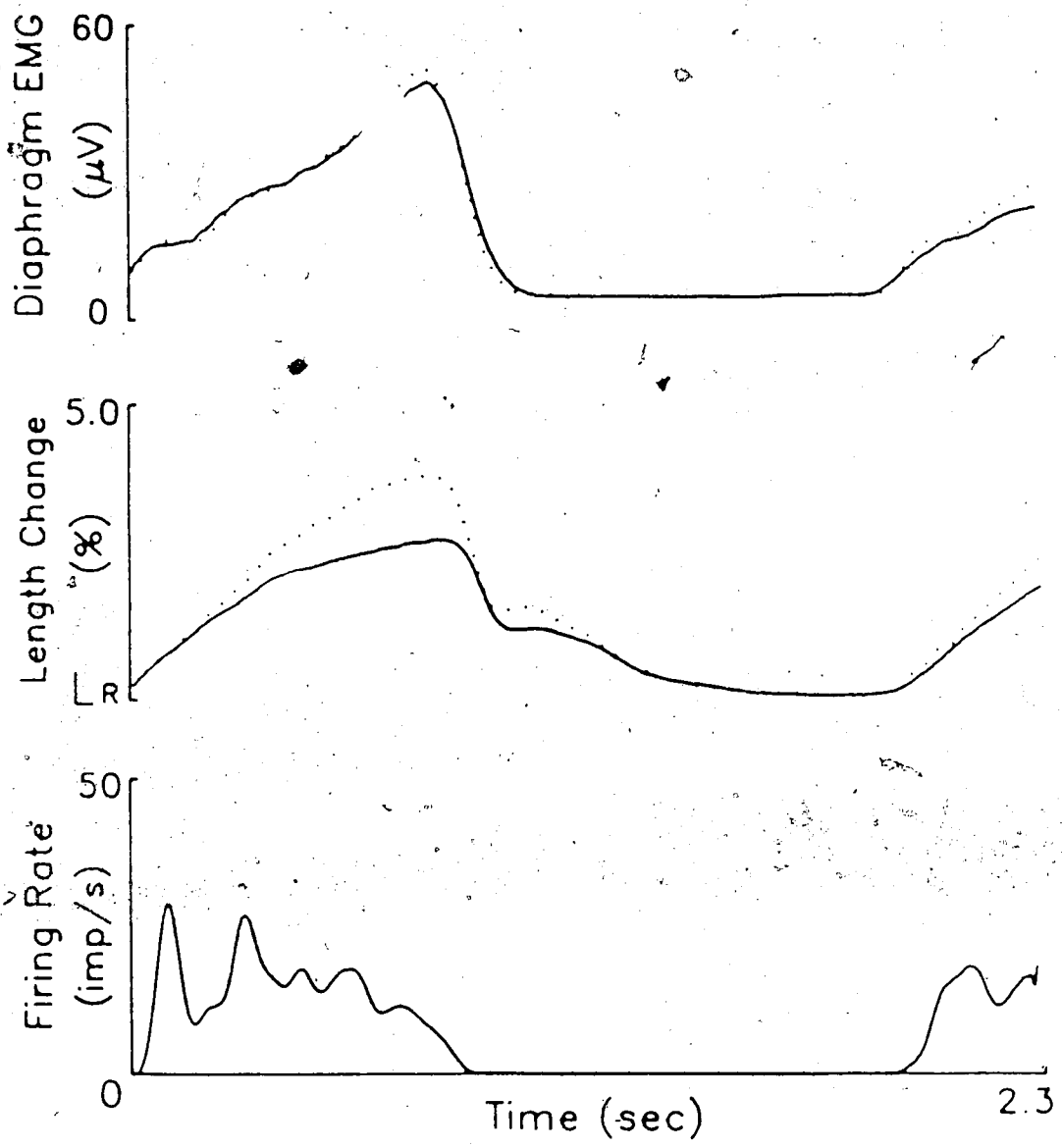

FIG. 4.6. Illustration of the response of deefferenting an external intercostal muscle which was actively lengthening. Same format as Fig. 4.2. Removal of the shortening forces produced by the intercostal muscle activity leads to a further lengthening of the muscle fibers (dotted line).

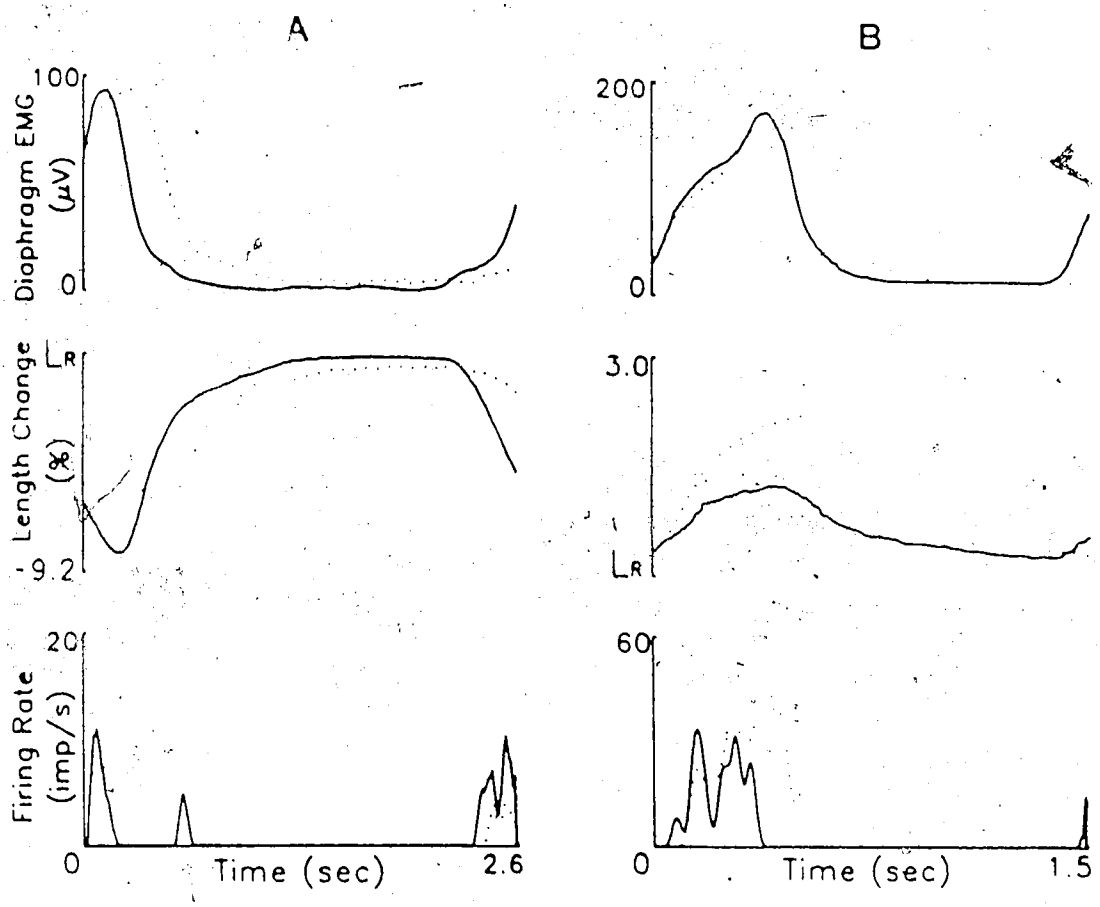

FIG. 4.7. Effects of total occlusion of the trachea (dotted line) on actively shortening (A) and lengthening (B) external intercostals. Same format as Fig. 4.2 (average of 8 breaths). There is a prolongation of the inspiratory period and discharge rate of respiratory muscles. The muscle fibers which were shortening prior to occlusion continued to do so, although to a lesser degree. The length excursions of the mid-thoracic spaces actually increased when the trachea was occluded.

respiratory muscles merely results in a distortion of the rib cage without a significant increase in intrathoracic volume (Van Lunteren & Cherniack, 1986).

DISCUSSION

Distribution of length changes. Clearly, the length changes of the external intercostal muscles vary throughout the rib cage. This variability seems to originate from two sources. Firstly, differing levels of force are being generated by the intercostal muscles in different areas of the rib cage. The force produced by a given intercostal muscle is determined by several factors: degree and distribution of motor unit recruitment, motor unit properties, cross-sectional area of muscle fibers, presence of shortening or lengthening contractions, length of muscle fibers in relation to L_o (length at which optimum force is produced) and the velocity of shortening. These characteristics are not at all consistent throughout the rib cage. Furthermore, the forces produced in adjacent intercostal muscles will interact with those produced within a given space.

Secondly, there are forces generated from outside the intercostal muscle group which affect the muscle's length. The largest peripheral force observed in these experiments was generated at the caudal axis of the rib cage, presumably by the diaphragm. The costal diaphragm has been shown to expand the lower rib cage during inspiration (Eisele, Trenchard, Burki & Guz, 1968). The degree to which this

lengthening force affects the external intercostals is a function of the muscle's proximity to the caudal axis of the rib cage. The intercostal muscles of the rostral spaces produce sufficient muscle force, and are far enough removed from the diaphragm, to continue to shorten during inspiration. The intercostal muscles in the mid-thoracic spaces, which also show inspiratory activity, either shorten or lengthen depending on the particular balance of forces acting on them at a given time. For these spaces to shorten the force produced by the muscle activity will have to be sufficient to overcome the rostrally directed pull from the intercostal muscles above, and the caudally directed pull from the diaphragm below. The external intercostals of the caudal spaces are typically inactive, leaving the forces produced by the diaphragm as the major determinant of their length changes during respiration.

Considering the complexity and variability in the force production of intercostal muscles, the discrepancies apparent in previous reports of the length changes are not surprising. However, when the length changes of all the intercostal spaces are considered together, certain trends are apparent. The external intercostal muscles of the rostral spaces and the parasternals, as the traditional theory implies, do actively shorten during inspiration and function to expand the upper portion of the rib cage. The importance of this activity, and that of the external

intercostals of the mid-thoracic spaces, to respiration becomes apparent when observing the rib cage movements when these muscles are silent. During inspiration the caudal section of the rib cage expands as normal but the upper portion of the rib cage collapses, thereby limiting the increase in intrathoracic volume (Eisele et al, 1968; Knill, Andrews, Bryan & Bryan, 1976). The lack of EMG activity related to the respiratory cycle in the caudal external intercostals has been reported in a variety of preparations (Taylor, 1960; DeTroyer & Ninane, 1986). The costal diaphragm inserts along the underside of these ribs and its activity alone is sufficient to expand the lower portion of the rib cage.

The absolute values of the length excursions reported in this stu likely differ from those which would be found in the intact, unanesthetized animal. However, considering that the recruitment patterns and kinematics of respiratory muscles in anesthetized preparations approximate that of awake animals, the trends are likely to be similar (Duron, 1973; Da Silva, Sayers, Sears & Stagg, 1977). Furthermore, quantitative, but not qualitative differences were observed when respiratory drive was increased.

Length changes during tracheal occlusion. It has been suggested that the length changes of respiratory muscles would be minimal during static contractions (Whitelaw,

Derenne & Milic-Emili, 1975). However, length measurements of the diaphragm and parasternal muscles during closure of the trachea have shown that while these muscle shorten to a lesser degree, they do not contract isometrically (Newman et al 1984; Fitting et al, 1986; Van Lunteren & Cherniack, 1986). We have shown a similar response for the external intercostal muscles of the rostral areas of the rib cage. The shortening of diaphragm, parasternal and external intercostal muscles on their own would act to increase the intrathoracic volume, which clearly does not change significantly in the absence of airflow. A possible explanation for this apparent contradiction is that the rib cage is simply distorting rather than expanding when the trachea is occluded (Van Lunteren & Cherniack, 1986). Our measurements of the external intercostal muscles illustrate that a significant amount of this distortion is taking place in the mid-thoracic spaces which lengthen considerably more than normal when the trachea is occluded.

Relationship between length changes and muscle spindle activity. Given the information regarding the length changes of the intercostal muscles, it is possible to suggest an explanation for the recruitment of gamma motoneurons during respiration. The importance of segmental reflex activation of external intercostal alpha motoneurons from muscle spindle primaries during respiration has been clearly demonstrated. Deprivation of afferent feedback results in a

decrease in the discharge rate of motor unit activity (Sant'Ambrogia & Widdicombe, 1965) and a deficiency in the muscle's ability to compensate for increased loading (Corda, Eklund & Euler, 1965; Sears & Newsom Davis, 1970). Lennerstrand (1968) has shown that muscle spindles, in the absence of gamma motoneuron activity, can be unloaded with contractions of extrafusal fibers at velocities greater than 1% L_R/sec. Considering the range of shortening velocities reported here (2.9-12.4% L_R/sec), muscles spindles deprived of gamma motoneuron input would be unloaded during inspiration and the important reflex activation of the alpha motoneurons would be absent. However, the intercostal muscles receive a tonic input from static gamma motoneurons (Greer & Stein, 1986), which are known to increase the activity of both primary and secondary muscle spindle afferents (Lennerstrand, 1968). In reviewing the discharge properties of muscle spindles from a variety of systems, Prochazka (1979)calculated that the gamma motoneuron system will compensate for unloading effects of extrafusal fibers contracting at rates of less than 20% L_R/sec. Therefore, muscle spindle afferents in the intercostals, when under the influence of gamma motoneuron activity, will continue to relay information to the central nervous system during the respiratory cycle.

In addition to preventing the unloading of muscle spindles, gamma motoneurons can alter the gain of the

segmental reflex. During inspiration the sensitivity of the spindle afferents in the active muscles is heightened by the added recruitment of dynamic gamma motoneurons (Greer & Stein, 1986). Therefore, any perturbations which tend to counteract the shortening of the muscle fibers during inspiration (e.g. increased airway resistance, decrease in chest wall compliance, distorting forces from surrounding structures) will result in a barrage of signals from muscle spindle afferents to the alpha motoneurons of that muscle. The subsequent increase in motor unit activity will serve to counteract the antagonistic forces, thereby ensuring the desired length change is approached.

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CHAPTER FIVE

DISTRIBUTION OF MUSCLE FIBER TYPES AND EMG ACTIVITY IN THE CAT INTERCOSTAL MUSCLES

The intercostal muscles are primarily thought of as respiratory muscles. Several studies of EMG activity have shown the parasternal and external intercostal muscles are generally recruited during inspiration, while the internal intercostals are active during expiration (Newsom Davis & Sears, 1970; Duron, 1973; DeTroyer & Ninane, 1986). However, these recordings also illustrate the variability in the levels and patterns of intercostal muscle activity in different areas of the rib cage. To further characterize these regional differences, we have recorded the activity of motor units from the parasternal, external and internal intercostal muscles of anesthetized cats. In agreement with similar studies on different preparations and species, EMG activity related to the respiratory cycle is restricted to specific areas of the rib cage. Presumably, those muscles which are not recruited during respiration play a role in one of the other functions proposed for the intercostals, which include: postural adjustments (Duron, 1973), trunk rotation (DeTroyer, Kelly, Macklem & Zen, 1985) shivering

1. A version of this chapter has been submitted to Journal of Applied Physiology for publication; Greer, J. and Martin, T.

(Duron and Caillol, 1971), vocalization (Newsom Davis & Sears, 1970) and purring (Kirkwood, Sears, Stagg & Westgaard, 1987).

The regional differences found in the intercostals are not limited to the EMG patterns. Duron (1973) has shown differences in the density of muscle spindles throughout the rib cage, which he interprets as reflecting functional specialization. Furthermore, the response characteristics of muscle spindles in the intercostals are variable due to regional disparities in the recruitment patterns of dynamic and static gamma motoneurons (Greer & Stein, 1986). The spatial organization of the intercostal motoneuron pools are also indicative of regional specializations within the intercostal muscles (Hardman & Brown, 1985).

We have now studied the histochemical profile of the intercostal muscles in different areas of the rib cage to determine if the apparent functional heterogeneity is reflected in the muscle fiber properties. This might be expected, considering the correlation between the motor unit properties and activation patterns of mammalian muscle (Buchthal & Schmalbruch, 1980; Burke, 1981; Gorniak, 1986). Previous studies of the mechanical and histochemical properties of intercostal muscles from both animals (Andersen & Sears, 1964; Maxwell, McCarther, Kuehl & Robotham, 1983; Ogata & Yamasaki, 1985) and man (Sanchez, Derenne, Debesse, Riquet & Monod, 1982) have demonstrated

the presence of the three basic classes of muscle fiber type: slow, oxidative (SO); fast, oxidative, glycolytic (FOG); and fast, glycolytic (FG). In each study, however, only small samples of intercostal muscle from discrete regions of the rib cage were considered. We will show that the distribution of the different muscle fiber types is significantly different throughout the rib cage. In addition, there is a correlation between the recruitment patterns of the intercostal muscles and their histochemical profile.

METHODS

EMG Recordings

The intercostal muscles of 9 adult cats anesthetized with Halothane (delivered in a mixture of 95% O_2 and 5% co_2) were exposed and recordings of their motor unit activity were made with bipolar electrodes. The diaphragmatic EMG was recorded with stainless steel electrodes and the signal then rectified and low pass filtered (as described in Chapter 2). Single motor unit potentials from the bipolar recordings of intercostal muscle were isolated with a window discriminator (Bak Electronics, Md.). A PDP 11 computer was used to average the discharge rate of motor units with respect to the timing of the diaphragmatic EMG (averaged over 20-50 respiratory cycles). The compilation of this data was then used to generate a summary of intercostal muscle recruitment during respiration (Fig. 5.1).

Muscle Fiber Properties

Each of the parasternal, external and internal intercostal muscles were removed in their entirety from the rib cage. The muscle specimens were frozen in isopentane cooled with liquid nitrogen. Cross sections were cut at 10-15 μm in a crysotat at -25°C. Muscle fibers were stained for myofibrillar ATPases under alkaline (pH 10) and acid (pH 4.3) preincubation conditions (Nwoye, Mommaerts, Simpson, Seraydarian & Marusich, 1982), as well as for the determination of nicotinamide adenine dinucleotide(NADH) diaphorase activity (Dubowitz & Brooke, 1973). Muscle fibers were subsequently classified according to the terminology of Peter, Barnard, Edgerton, Gillespie and Sempel (1972) as Types SO, FOG or FG. This classification was based on the relative intensities of staining amongst the muscle fiber types (see Table 5.1). Counts were made of fibers (200-400) from the proximal, middle and distal areas of each muscle.

TABLE 5.1. Criteria for differentiating between fiber types found in intercostal muscles of the cat.

Muscle fiber type	SO	FOG	FF
Histochemical profile			
ATPase (pH 10)	light	dark	dark
ATPase (pH 4.3)	dark	high	medium
NADH	dark	dark	light

STAINING METHODS

1. NADH

10 ml Tris Buffer

10 mg NADH

10 mg NBT

a) add solutions and pH to 7.4-7.6

b) incubate at 38° for 30 minutes

c) rinse slides with distilled water

2. MYOFIBRILLAR ATPase (alkaline pre-incubation)

A. Pre-incubation

4 gms $CaCl_2$

1.9 ml AMP

Bring to 400 ml with distilled water and pH to 10.0

B. Incubation

4.8 ml AMP

500 mg NaN_3

Bring to 500 ml, pH to 9.8

Pre-incubate for 8 min at room temperature

Wash

Incubate at 37° for 20 minutes at room temp.

with 10 ml inc. solution

100 ml $CaCl_2$

12 mg ATP

Wash

Incubate 20 min. with 2% $CoCl_2$ at room temp

Wash

Incubate for 30 sec with 1% $(NH_4)_2S$

Wash

3. Myofibrillar ATPase (Acid pre-incubation)

A. Pre-incubation

1 ml of 1 M KCl

1 ml of 1 M Na-acetate

8 ml distilled water

Add solution for 6 minutes at room temp pH 4.5

B. Incubation

18.1 mg ATP

1 ml .3 M $CaCl_2$

1 ml .55 M NaCl

5 ml .1 M glycine-NaOH

3 ml distilled water

Rinse

Immerse in 1% CaCl

Rinse

Immerse in 1% $CoCl_2$

Rinse

Immerse in 1% $(NH_4)_2S$

Rinse

RESULTS

Parasternal Muscles

The parasternal muscles consistently illustrated EMG activity during the inspiratory phase of the respiratory cycle in the lightly anesthetized cat (Fig. 5.1A). The histochemical profile of the muscle fiber population of the parasternal muscles is shown in Fig. 5.2. Each of the parasternals, regardless of location, had a similar distribution of muscle fiber types: 55% E. 3.7) type SO, 22% (S.E. 4.6) type FOG and 23% (S.E. 3.1) type FG fibers.

External Intercostal Muscles

The areas of the external intercostal muscle typically recruited during inspiration are illustrated in Fig. 5.1B. The presence of phasic EMG activity was limited to the rostral and dorsal areas of the rib cage. During increased respiratory drive (due to increased CO_2 concentration of inspired air or tracheal occlusion) there was an increase in the motor unit discharge of the previously active units and a recruitment of neighboring motor units. However, those regions of the external intercostals which were silent previous to the respiratory challenge, remained so. Tonically firing motor units were occasionally seen in the external intercostal muscles (5 of 84 units).

The muscle fiber properties of the external intercostals varied in different regions of the rib cage. We

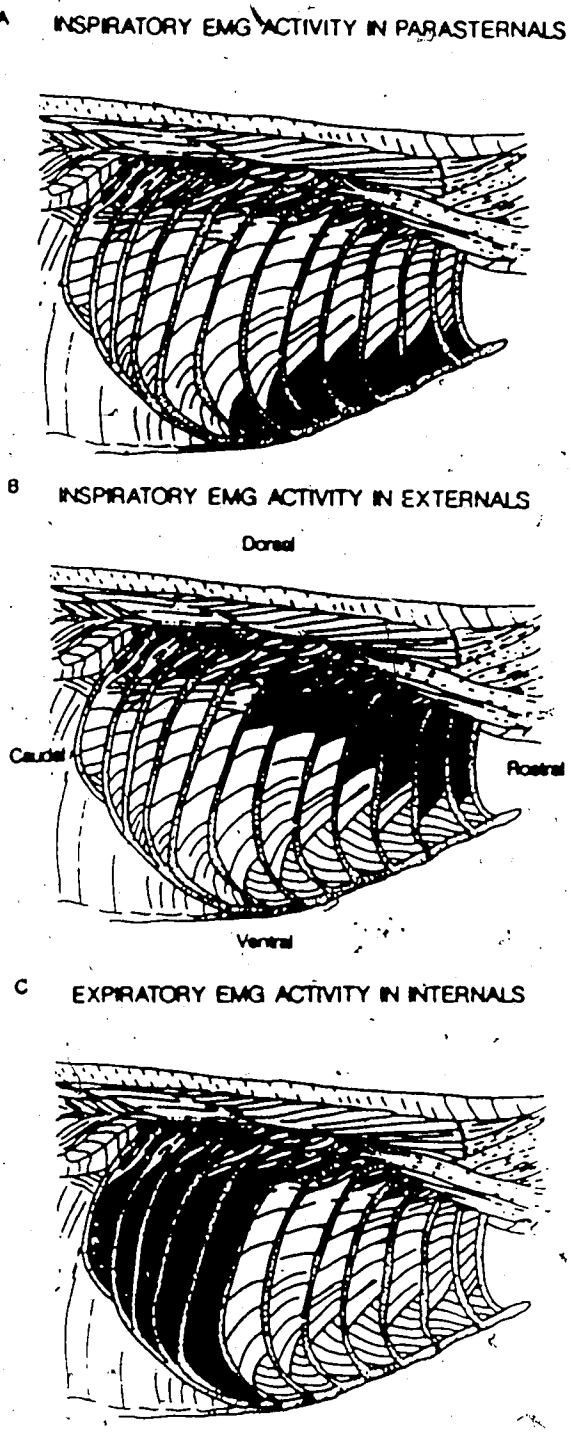

Fig. 5.1 Illustration of the areas of parasternal (A), and external intercostal muscles (B) which were typically recruited during inspiration (shaded areas). Note the decrease in EMG activity in the external intercostals along the rostrocaudal and dorsoventral axis of the rib cage. The internal intercostals were generally inactive in the anesthetized cat, but when there was activity it most often appeared in the shaded areas shown in (C).

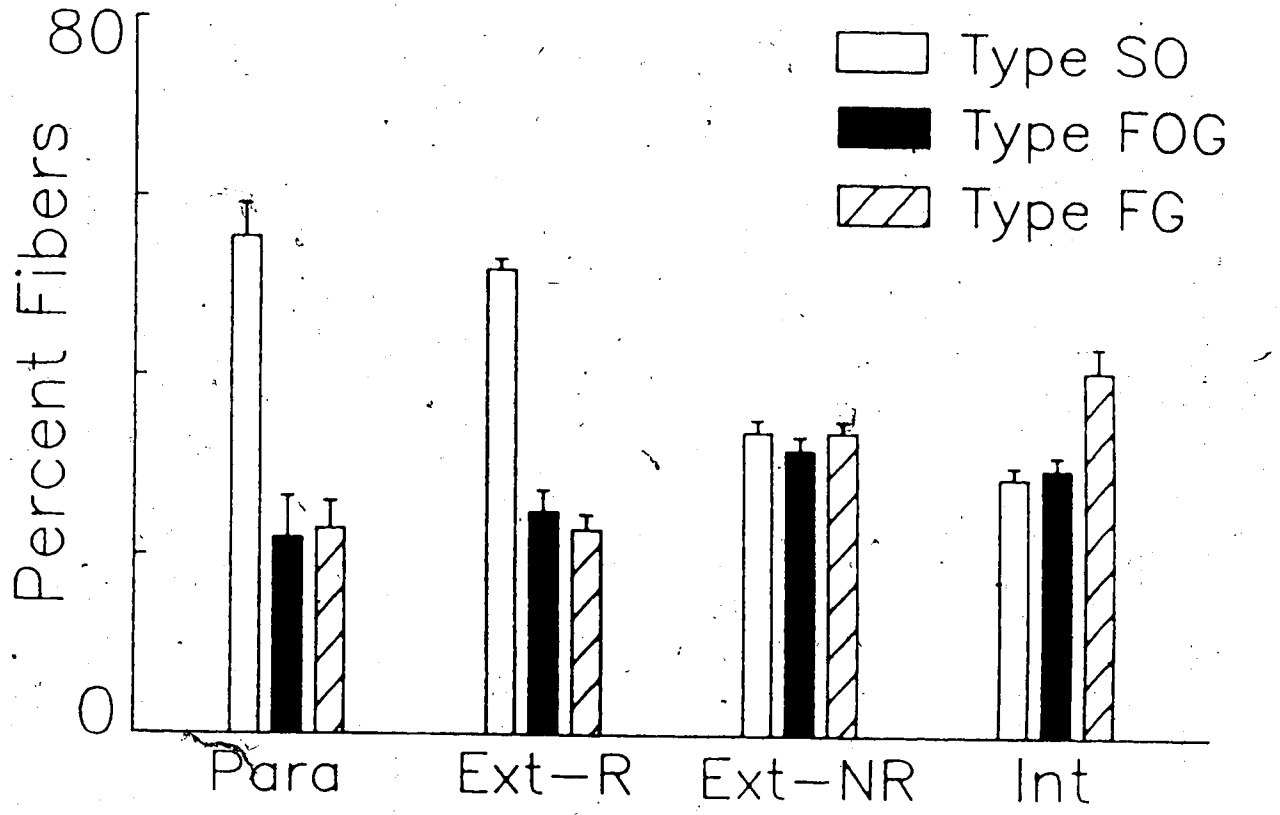

Fig. 5.2 Histograms illustrating the distribution of muscle fiber types in the various intercostal muscles. Parasternal (Para) and those external intercostals which were recruited during inspiration (Ext-R) had a relatively high percentage of Type SO fibers. In contrast, the remaining external (Ext-NR) and internal intercostals (Int) had a lower concentration of Type SO fibers. Error bars indicate standard error of the mean (S.E.).

have arbitrarily divided the external intercostals into two populations: those areas which illustrated EMG activity during inspiration are referred to as 'respiratory' external intercostals (Ext-R), while those which were not recruited for respiration are classed as 'non-respiratory' external intercostals (Ext-NR). As illustrated in Fig. 5.2, areas recruited during inspiration had a considerably higher percentage of Type SO muscle fibers (52∓1.2) as compared with those areas which were silent (34∓1.4). The relationship between the biochemical properties and the recruitment patterns of the external intercostal muscle is further demonstrated in Fig. 5.3. The ratio of Type SO versus type F (Types FOG and FG combined) fibers decreases along the rostrocaudal axis of the rib cage, similar to the trend seen for EMG activity. Furthermore, within a given intercostal space the muscle properties change dramatically along a dorsoventral gradient (Fig 5.4), which again parallels the pattern of EMG activity.

Internal Intercostal Muscles

In our preparation, the internal intercostal muscles of the caudal five spaces were occasionally recruited during expiration (Fig. 5.1C). In contrast to the external intercostals, the internals were recruited with the activity decreasing in a caudal-rostral direction. Tonically firing units were seen in 7 of 59 units recorded.

The histochemical profile of internal intercostals was

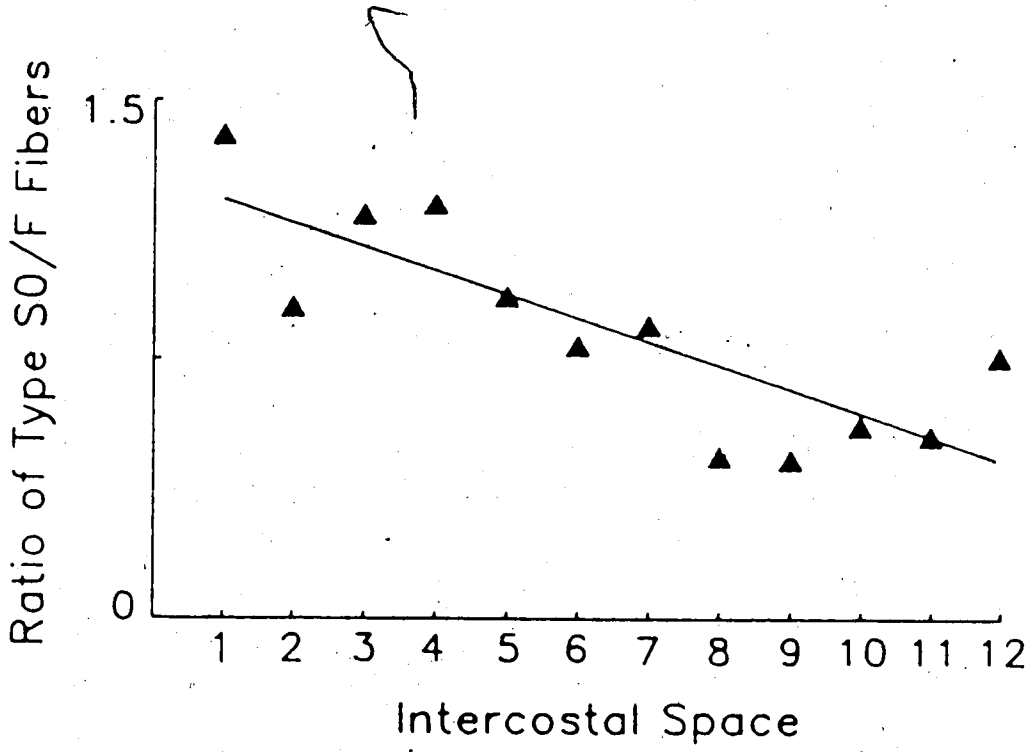

Fig. 5.3 Correlation between the ratio of Type SO/ Type F (FOG + FG) fibers seen in the proximal-middle portions of the external intercostals. The rostrocaudal distribution of muscle fiber type properties parallels the EMG activity patterns in these muscles (see Fig. 5.1B).

Fig. 5.4 Tissue sections of the external intercostal muscle from rostral (top plate), mid-thoracic (middle plate) and caudal spaces (bottom plate) of the rib cage stained for myofibrillar ATPase, preincubation pH 4.3. Darkly stained fibers were classified as Type SO, and the lightly stained fibers as Type F (FOG & FG). Note, the intercostal muscle from the rostrally located space has a higher proportion of Type SO fibers than the muscle from the caudally located space. When the dorsal (top layer) and ventral (bottom layer) areas of the mid-thoracic space are aligned beside each other, as shown here, it is evident that the proportion of Type SO fibers is considerably greater in the dorsal regions.

fairly homogeneous regardless of the location within the rib cage. As illustrated in Fig. 5.2, the predominance of Type F fibers was similar to that seen for 'non-respiratory' external intercostals: Type SO, 29% S.E. 1.3; Type FOG, 30% S.E. 1.5; Type FG, 41% S.E. 2.7.

DISCUSSION

The EMG profile we observed for the intercostal muscles of the anesthetized cat was similar to that reported for a variety of other species and preparations (Koepke, Smith, Murphy & Dickinson, 1958; Newsom Davis & Sears, 1970; Duron, 1973; DeTroyer & Ninane, 1986). The parasternals, and external intercostals of the rostral spaces and ventral portions of the mid-thoracic spaces, appear to be synergists of the diaphragm, acting to increase intrathoracic volume during inspiration. Expiration is either passive or involves the recruitment of the internal intercostals in the caudal spaces of the rib cage. Although these trends have been apparent in the previous studies, there has been some discrepancy regarding exactly which intercostal spaces are typically recruited during respiration. This is likely, in part, due to the differing locations within an intercostal space that the EMG recordings were made. For instance, the inspiratory activity of the external intercostals located in the mid-thoracic spaces would only be noticed if the recording electrode was positioned in the dorsal area of the muscle.

It is interesting to note that there is anatomical, as well as, electrophysiological evidence for the partitioning of intercostal muscles within a given rib cage space. There is a well defined segregation within the thoracic cord between motoneurons innervating the proximal and distal areas of an intercostal muscle (Hardman & Brown, 1985). The intercostal muscles are not unique in this regard, as similar observations regarding the compartmentalization of a muscle into functionally unique regions have been reported for a variety of mammalian muscles (Loeb, 1985; Stuart, Hamm & Vanden Noven, 1988).

The regional differences in the distribution of muscle fiber types in the intercostals may reflect the functional specializations amongst these muscles. Respiratory muscles, which are recruited repetitively for the duration of the animal's life, might be expected to have a high resistance to fatigue. A precedent for this suggestion comes from previous studies of the cat diaphragm, which report approximately 50% of the muscles fibers are Type SO (Riley & Berger, 1979; Sieck, Roy, Powell, Blanco, Edgerton & Harper, 1983). A similar histochemical profile is reported here for the parasternals and those areas of the external intercostals which were recruited during inspiration.

In contrast, the areas of the intercostal muscles which were inactive during respiration demonstrate a lower proportion of fatigue resistant muscle fibers. A possible

exception to this general trend between respiratory and non-respiratory muscles is evident in the internal intercostals. Although the internal intercostals of the caudal spaces were occasionally recruited during expiration, a fairly homogeneous distribution of muscle fiber types existed amongst all the spaces. The differences seen in the EMG activity of these muscles along the caudal-rostral axis of the rib cage were not reflected in the histochemical properties. This could be an indication of the general lack of recruitment of these muscles during respiration. A further possibility is that internal and external intercostals of the rostral spaces are in fact recruited during respiration, but only in the intact, awake cat during periods when the depth and rate of breathing is increased towards the upper limits. It could then be functionally advantageous to recruit muscles with a higher proportion of fast contracting muscle fibers which may produce the necessary increased levels of force.

As discussed previously, there is evidence that the intercostal muscles are recruited for purposes other than respiration. However, further EMG recordings from awake, freely moving animals under a variety of conditions will be necessary before a complete description of the non-respiratory role of the intercostals can be provided. Thus, a precise description of the relationship between the non-respiratory functions of the intercostals and their muscle

fiber properties is difficult at this time. Nevertheless, it does appear that intercostal muscles have a population of muscle fibers which are designed to generate quicker contractions and possibly greater force, without the necessity for the same degree of resistance to fatigue which is seen in those muscle fibers regularly recruited during respiration.

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CHAPTER SIX

GENERAL DISCUSSION

This study was initially designed to investigate the CNS utilization of the gamma motoneuron system during respiration in the cat. The ensuing experiments illustrated that the recruitment patterns of dynamic and static gamma motoneurons in the external intercostals are similar to that reported for the hindlimb extensors of the cat during locomotion (Chapters 2 & 3). Together, these findings might be seen as demonstrating an underlying strategy of gamma motoneuron recruitment common to rhythmical movements in general. However, there is also contrary evidence which suggests this may not be the case. Firstly, Taylor and his colleagues have reported an opposite recruitment pattern of gamma motoneurons in the jaw muscles of the cat during chewing (Taylor & Appenteng, 1981). Secondly, there have been suggestions, based on recordings of muscle spindle afferents in the cat, that the activity of gamma motoneurons varies amongst the different hindlimb muscles involved in locomotion (Perret & Berthoz, 1973; Cabelguen, 1981; Loeb, 1985)

APPROACHES TO THE STUDY OF GAMMA MOTONEURON RECRUITMENT

The methodology used in these studies varies and in some cases the evidence is rather indirect. It may be possible to overcome this problem by applying similar

techniques to the study of a variety of muscles. Methods resembling those used in Stein's laboratory, for instance, could be applied to the investigation of other muscle groups involved in locomotion and perhaps to the jaw muscles during chewing. Taylor's technique is fairly similar, but it does not allow for the direct comparison of muscle spindle response, to a well controlled stretch, before and after the removal of gamma motoneuron input. This is an important factor when one considers that at present the most reliable means for differentiating gamma motor neuron type is the observation of their effects on mean rate and modulation of spindle afferents. Taylor's preparation, nonetheless, does offer the advantage of allowing for recordings from awake, freely moving animals, which removes the potential distortion of gamma motoneuron activity due to the effects of anesthesia. However, comparisons of the results from studies of jaw muscles in the anesthetized and chronic preparations reveal no qualitative differences (Taylor & Appenteng, 1981).

The intricate techniques developed by Hulliger and Prochazka have also proven useful for suggesting the nature of gamma motoneuron activity in the hindlimb extensors of freely moving, unanesthetized cats. Unlike other studies using chronic preparations (Loeb, Hoffer & Pratt, 1985), they do not merely rely on inference in determining which type of gamma motoneuron activity would best explain the

response of spindle afferents during locomotion. Rather, the actual response of spindle afferents is reproduced under similar conditions by introducing a known pattern of gamma motoneuron activity (Prochazka, Hulliger, Zangger & Appenteng, 1985). Unfortunately, this technique depends on precise knowledge of muscle length and spindle location in the chronic preparation, which would be difficult in muscles such as the intercostals (personal communication, Prochazka).

A further technique for determining gamma motoneuron activity was developed several years ago by Gladden and co-workers (Gladden, 1981). Muscle spindles were isolated from the tenuissimus muscle of the cat while leaving the nerve supply and spinal roots intact. Intracellular recordings from intrafusal fibers were performed while specific supraspinal centers were stimulated. Due to the specific innervation of intrafusal fibers by either gamma or dynamic gamma motoneurons, this technique could allow for a precise measure of the activity of these neurons. To my knowledge, this promising strategy has not since been developed or applied to the recording of intrafusal potentials during natural movements. The technical difficulties of stabilizing the preparation sufficiently to allow for intracellular recordings may be one of the problems.

Ideally, a reliable technique for recording directly from gamma motoneurons in the unanesthetized preparation

will be developed. The single largest difficulty is to develop a method of selectively recording the low amplitude signals generated by the small diameter axons of gamma motoneurons (Murphy, Stein & Taylor, 1985). A confounding problem will be to identify the type of gamma motoneuron being recorded. A possible solution to this problem may develop from the identification of discrete supraspinal centers, which when stimulated, selectively affect the activity of either static or dynamic motoneurons (Appelberg, 1981; Gladden, 1981; Wand & Schwarz, 1985).

GENERAL PRINCIPLES OF GAMMA MOTONEURON ACTIVITY

Although a general theory regarding gamma motoneuron activity would be premature at this time, some underlying principles have become apparent. These principles are suggested by the results of this study and the work of others who have investigated the role of gamma motoneurons.

The ability of the CNS to recruit gamma and alpha motoneurons separately. This is a fundamental development in the evolution of the mammalian muscle spindle from the system used in reptiles and amphibians. Muscle spindles, in animals of these classes, are innervated solely by collateral branches of alpha motoneurons, which are referred to as beta motoneurons (Matthews, 1972). Therefore co-activation of intra and extrafusal fibers is inherent in the system, removing the possibility of independently

setting the muscle spindle activity and sensitivity.

Although the human muscle spindle also receives discrete innervation from gamma motoneurons, evidence for separate activation patterns of intrafusal and extrafusal activity is sparse (Hulliger, 1984). A recent report describing direct recordings from gamma motoneurons in the peroneal nerve of humans claims that units were firing tonically, independent of alpha motoneuron activity (Ribot, Roll & Vedel, 1986). However, the number of units were small (six in five years) and the grounds for their identification questionable. Therefore, at this time the nature of gamma motoneuron activity is largely based on microneurographic recordings of muscle spindle afferents from human nerves. The recordings have been made while subjects were performing voluntary tasks which involved either isometric or relatively slow, short range contractions (Vallbo, 1973; Burke, Hagbarth & Lofstedt, 1978) The results suggest that gamma and alpha motoneurons are necessarily recruited together, at least during the limited types of movement so far studied. Intuitively, the general applicability of these findings appears questionable, as it seems unlikely that the separate control of gamma motoneurons which evolved in lower mammals, would have subsequently been lost in humans.

CNS utilization of gamma motoneurons to modulate muscle spindle sensitivity. It appears the mammalian CNS has the ability to utilize the two classes of gamma motoneurons,

dynamic and static, to modulate the sensitivity of muscle spindles. We have suggested the sensitivity is set high while the muscle is recruited, so as to maximize the reflex correction of the muscle activity in the event the intended degree of shortening is not attained (see section on servo assistance in Introduction). Furthermore, the sensitivity of the spindle afferents is kept low during the remaining period of the cycle when any reflex activity would be counter productive. It would appear that there are movements, such as chewing, where it is advantageous to employ the opposite scheme of gamma motoneuron recruitment.

Gamma motoneurons act to prevent muscle spindles afferents from falling silent during muscle shortening. There have been suggestions that static gamma motoneurons are largely responsible for this role during the active shortening of a muscle (Bessou & Pages, 1972; Taylor & Appenteng, 1981). This idea is based on the premise that only static gamma motoneurons can generate intrafusal contractions of sufficient speed to keep up with the shortening of extrafusal fibers. It has therefore been assumed that static gamma motoneurons are typically co-activated with alpha motoneurons. However, there are valid reasons to question this argument. Firstly, Hulliger's reinvestigation of the speed at which the two types of gamma motoneurons can still produce Ia discharge found that the differences are

statistically insignificant (Hulliger, 1979). Secondly, direct recordings of the effects of gamma motoneurons on Ia afferents during muscle shortening have illustrated the ability of both types in preventing the unloading of muscle spindles (Appenteng, Prochazka, Proske & Wand, 1982; Morgan, Prochazka & Proske, 1985). Finally, the argument neglects the fact that the two types of gamma motoneurons are both firing while the muscles are actively shortening during rhythmical movements. The summation of the effects produced by dynamic and static gamma motoneurons will tend to prevent unloading of the spindle afferents, regardless of which type is firing tonically or phasically (Hulliger, Matthews & Noth, 1976).

Muscle spindles, in the absence of gamma motoneuron activity, could also be unloaded by the passive shortening of a muscle. In that instance the CNS would be deprived of information regarding the length of a muscle and body position. It could be argued that the continuous activity of static gamma motoneurons, as reported for intercostals and hindlimb extensors, would be best designed to prevent this occurrence. Static gamma motoneurons, due to their innervation of bag_2 and chain intrafusal fibers, increase the activity of both Ia and II afferents. In contrast, innervation by dynamic gamma motoneurons is limited to bag_1 intrafusal fibers, rendering them unable to prevent the unloading of II afferents.

The level and pattern of gamma motoneuron activity can vary within a given muscle. Our findings in the intercostal muscles are in agreement with this general principle regarding gamma motoneuron recruitment. A similar compartmentalization has been proposed for certain biarticulate muscles of the hindlimb (Loeb, 1985; Stuart, Hamm & Vanden Noven, 1988). It would be interesting to determine if this situation exists in the jaw closing muscles, which have been reported to be compartmentalized based on EMG pattern and motor unit properties (Herring, Grimm & Grimm, 1979; Gorniak, 1986).

ROLE OF MUSCLE SPINDLES

As outlined in the Introduction, information from muscle spindle afferents is transmitted to both the spinal cord and supraspinal nuclei. The following is a brief discussion of how this information from intercostal muscles may used by the these two levels of central nervous system to facilitate motor control.

Spinal connections. The importance of feedback from spindle afferents in the role of servo assisting muscle activation is discussed in Chapters 2 and 4. The source of the reflex activity stems from Ia and II spindle afferents which make monosynaptic and polysynaptic connections with thoracic motoneurons (Kirkwood & Sears, 1974). Previous studies have

shown that muscle spindle afferents synapse with alpha motoneurons innervating the same segment and, to a lesser degree, the adjacent segments (Kirkwood & Sears, 1982). Evidence from human studies have also demonstrated the presence of 'long-loop' reflexes in the thoracic cord (Newsom Davis & Sears, 1970). Whether this reflects polysynaptic routes within the cord or transcortical pathways is unknown. As is the case with the jaw muscles and hip extensors, there are no inhibitory connections between antagonistic muscles of the intercostal muscle group (Aminoff & Sears, 1971).

Modulation of information from muscle spindles at the level of the spinal cord has been demonstrated in the mammalian CNS. This typically takes the form of presynaptic inhibition of the sensory afferents or inhibition of those interneurons which act as relays between the afferent terminal and the target neurons (Brooks, 1986). Presently, this type of modulation of the sensory information from respiratory muscles has yet to be investigated.

Supraspinal connections. The possibility of muscle spindles assisting the cerebellum in postural control is suggested in Chapter 2. Notably, the continuous information regarding muscle length would be available from II afferents when influenced by the tonically firing static gamma motoneurons. This proprioceptive information would also be available to the sensorimotor cortex. This could be important in

providing a reference regarding the position of the thorax which then could be used in generating appropriate motor commands.

Activity of muscle spindle afferents can also modify the output of centers within the CNS which coordinate motor programs (Nashner, 1982; Abbs & Gracco, 1983). It is unclear whether spindle afferents from intercostal muscles play such a role by affecting the activity of the respiratory pattern generator. Shannon (1986) and his colleagues have addressed this problem and suggest they do not. However, while the medullary cells they studied were respiratory neurons, it is not certain whether those cells were part of the pattern generator, or merely premotor cells which act as relays to the spinal cord (Feldman, 1986).

An argument, on theoretical grounds, can be put forth for questioning the importance of muscle spindle input to the respiratory center. Information regarding the degree of ribcage expansion is already provided by stretch receptors located within the bronchi and lung parenchyma (West, 1974). These receptors, which signal lung expansion, have a direct impact on the timing of the transition from inspiration to expiration. Unlike the muscle spindle in the intercostal muscles, however, they are not as likely to be affected by non-respiratory movements of the thorax which could reflexly influence the respiratory center in an inappropriate way.

INTERCOSTAL MUSCLES

Questions arose from this initial study of muscle spindles during respiration regarding the architecture of the intercostal muscles. Electrophysiological, histochemical and kinematic evidence was presented to illustrate the functional compartmentalization of this muscle group. As a possible extension of this study, one could investigate whether a similar correlation between the level of respiratory related activity and muscle fiber properties exists in the levator costae muscles. These small muscles, which insert between each rib and the vertebrae, show a clear decrease in inspiratory EMG activity in a caudal-rostral direction (Hilaire, Nicholls & Sears, 1983).

To conclude, the intercostals are designed with very clear regional differences which correlate well with the functional demands imposed on the muscles. The regional specializations are also reflected in the recruitment patterns of gamma motoneurons. In turn, this results in the temporal and spatial optimization of muscle spindle properties to facilitate the motor control of the intercostal muscles.

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