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FUSIMOTOR CONTROL OF MUSCLE SPINDLE SENSITIVITY DURING RESPIRATION IN THE CAT

BY JOHN J. GREER* AND RICHARD B. STEIN

From the Department of Physiology, University of Alberta, Edmonton, Alberta, Canada T6G 2H7

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SUMMARY

1. The two types of fusimotor neurones, dynamic and static, can be differentiated by their effects on muscle spindle afferents. We have recorded the activity of muscle spindle primary afferents from the intercostal nerves of anaesthetized or decerebrate cats. A 4 Hz sinusoidal stretch was applied to the muscle containing the spindles of interest before and after crushing the nerve proximal to the recording site to eliminate fusimotor effects. The relative activity of the dynamic and static fusimotor neurones was inferred from the change in the spindle afferents' response.

2. Some areas of intercostal muscle normally showed phasic activity linked to respiration, where as other areas of intercostal muscle showed no EMG activity under our experimental conditions. In areas of intercostal muscle lacking EMG activity, the afferents' mean rate was higher and the modulation around the mean was lower at all phases of the breathing cycle when the efferent supply was intact. This result suggests the muscle spindles were receiving a steady level of static fusimotor activity.

3. Spindle primary afferents from regions of intercostal muscle that were typically recruited during respiration had an additional increase in mean rate and modulation around the mean rate in phase with the EMG activity. This is suggestive of phasic activation of dynamic fusimotor neurones in addition to static fusimotor discharge.

4. Thus, the two types of fusimotor neurones can be activated separately by the CNS to control the sensitivity of muscle spindles. The regional differences in the recruitment patterns of fusimotor neurones parallels the functional specializations of different areas of the intercostal muscles. The temporal modifications of fusimotor activity during each respiratory cycle means that the segmental reflex gain will vary in those intercostal muscles that are active during respiration.

5. These findings regarding the CNS recruitment of the two types of fusimotor neurones during respiration are similar to those reported for the hindlimb extensors during locomotion, but differ from those reported for jaw muscles during chewing. This may reflect differing control strategies being used by the CNS to meet the unique demands of the various rhythmical movements.

* Present address: Systems Neurobiology Laboratory, Department of Kinesiology, UCLA, 405 Hilgard Avenue, Los Angeles, 90024 CA, USA.

MS 6989

INTRODUCTION

Mammalian muscles contain spindle organs which convey sensory information to the central nervous system (CNS). In turn the CNS can influence the muscle spindle properties via fusimotor activity. Fusimotor neurones are divided into two categories: static and dynamic. The two types of fusimotor neurones 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 fusimotor activity increases the mean discharge rate and the sensitivity of a primary afferent's response to sinusoidal stretch (above a certain amplitude). Static fusimotor activity also increases the mean discharge rate but decreases the sensitivity of the spindle afferent's response to sinusoidal stretch (Hulliger, Matthews & Noth, 1977*a*). Various combinations of static and dynamic activity have also been studied and give intermediate effects (Hulliger, Matthews & Noth, 1977*b*; Appelberg, Hulliger, Johannson & Sojko, 1982). Therefore, by observing the muscle spindle's response to a length change it is often possible to infer the nature of the fusimotor activity.

Applications of this strategy have been used to propose models for how the CNS recruits fusimotor neurones during various rhythmic movements. Recordings during rhythmic jaw movements in the cat by Gottlieb & Taylor (1983) suggest that dynamic fusimotor neurones fire tonically throughout chewing movements while static fusimotor neurones are activated phasically with α-motoneurones. However, recordings from muscle spindle afferents in the monkey during isometric biting suggest that dynamic fusimotor neurones are co-activated with α-motoneurones while the static fusimotor activity is minimal (Larson, Smith & Luschei, 1981). Finally, Lund & Olson (1983) have suggested that the recruitment patterns of dynamic and static fusimotor neurones in jaw muscles varies depending on the particular type of jaw movement.

Evidence from recordings of fusimotor neurones and muscle spindle afferents to leg extensors of the decerebrate cat during locomotion suggest phasic dynamic and tonic static fusimotor 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 fusimotor neurones fire during locomotion (Prochazka, Hulliger, Zangger & Appenteng, 1985). These chronic recordings do not show any indication of dynamic fusimotor 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 fusimotor activity vary amongst the different hindlimb muscles involved in locomotion (Perret & Berthoz, 1973; Cabelguen, 1981; Loeb, 1985).

This paper describes experiments which were designed to provide information regarding the activity of the two types of fusimotor neurones during another rhythmic activity in the cat, respiration. Muscle spindles have been identified in the intercostal muscles (Huber, 1902; Barker, 1962; Duron, Jung-Caillol & Marlot, 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 fusimotor neurones (Critchlow & von Euler, 1963; Sears, 1964; Greer & Stein, 1989). Until now these fusimotor neurones 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 seven cats which were anaesthetized 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, after which they breathed room air. The second group of experiments involved twenty-one cats which were initially anaesthetized with sodium pentobarbitone (20 mg/kg) and maintained on halothane anaesthesia with 95% O_2 and 5% CO_2. While recordings were being made, the level of anaesthesia 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 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 external jugular vein (for administration of Dextran as a blood volume expander in animals where there was considerable blood loss, for example, during decerebration).

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 generated by surrounding muscle (Stein, Nichols, Jhamandas, Davis & Charles, 1977). 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. 1*A*). The number of active muscle spindle primary afferents in a given recording was minimized by isolating small strips of muscle (0·5–1·5 cm wide) which typically contained one to three spindles. This was accomplished by denervating all other portions of the muscle distal to the muscle strip of interest. When the signals from each of these units could be isolated they were analysed separately; otherwise the responses of two or occasionally three spindle primary afferents from adjacent spindles were averaged together.

Primaries and secondaries were differentiated by the amplitude of their spikes which, with our recording method, has been demonstrated to be approximately proportional to the conduction velocity of the afferent squared (Stein *et al.* 1977; 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 amplitude were selected for analysis due to the ease of discriminating these units from amongst the population of action potentials seen in the nerve recordings. Measurements of conduction velocity in preliminary studies demonstrated that the afferents with the largest amplitude spikes conducted 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, 1987). Our identification of the afferents as deriving from spindle rather than tendon organs was confirmed upon removal of fusimotor activity which resulted in a change in the mean rate and sensitivity of the afferents response to be 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. The size of the stretch was adjusted to be large enough to modulate the primary afferents' discharge, but not so large as to silence the afferents' 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

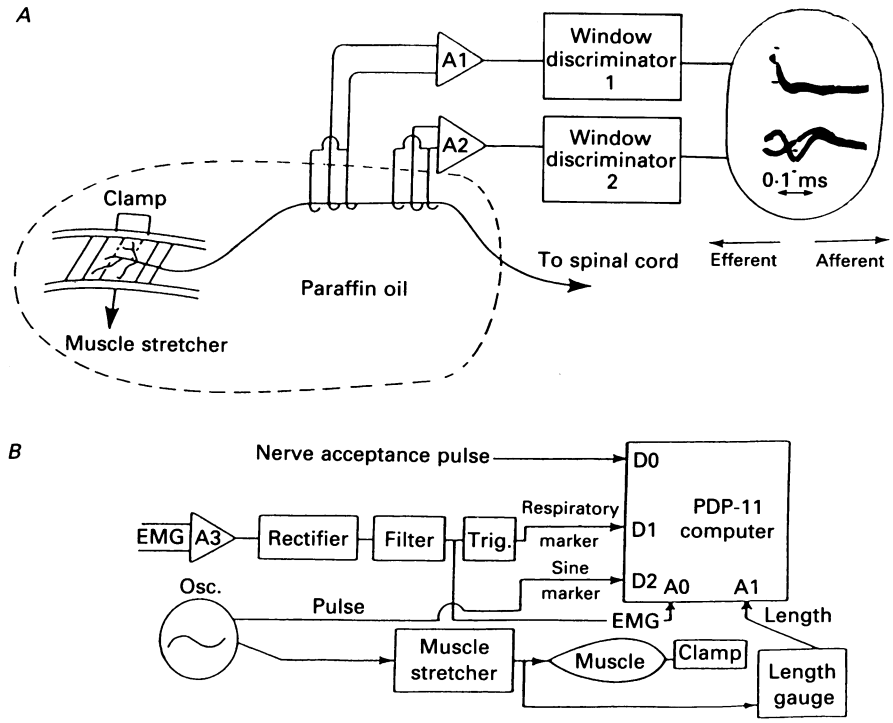

Fig. 1. *A*, illustration of the technique used to record separately from muscle afferents and efferents. A clamp stabilized the rostral rib while the caudal rib was attached to a torque motor. The intercostal nerve was lifted on hook electrodes into paraffin oil. The recording from the distal electrode was amplified (A1) and led to window discriminator 1 (copy of actual trace of afferent and efferent units from oscilloscope 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 α-motoneurones. 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 coincidentally 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 to facilitate viewing of the signal separation and the window adjusted to discriminate afferent spikes). *B*, the acceptance pulse from the window discriminator 2, indicating the occurrence of muscle spindle primary discharge, 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 afferent with respect to the applied length change and the respiratory cycle was calculated as described in the text. (Osc., oscilloscope; Trig., trigger).

the effects of the two types of fusimotor neurone on the muscle spindle afferents (Taylor *et al.* 1985). The applied length change was continuously monitored by the length change of the servo-torque motor. The accuracy and reliability of the torque motor was verified by sonomicrometric measurements (Greer, Stein & Martin, 1988). The sonomicrometer (model 120, Triton Technology, San Diego, CA, USA) measures the transit time of ultrasound between two transducers which are

arranged to face each other in alignment with the pennation of the muscle fibres. The change in transit time was proportional to changes in length of the muscle fibres.

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 that the integrity of the abdominal wall was maintained. Motor unit recordings of the intercostal muscles were made with a bipolar electrode.

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 (200 Hz–10 kHz) to improve the signal-to-noise ratio.

Analysis

Response of afferent with respect to sinusoidal stretch. The data were analysed on a Digital Equipment Co. PDP-11 computer (Marlboro, MA, USA). The neural recordings were passed through two window discriminators (Bak Electronics, Rockville Pike, MD, USA) cascaded together which allowed for isolation of afferent and efferent units (Fig. 1*A*). 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 afferents in relation to the sinusoidal length change. Each sweep of the histogram was triggered by a pulse marker which occurred whenever 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 afferents' response was averaged was typically 250 (approximately thirty breaths). Each bin of the histogram was averaged with the two neighbouring bins on each side (five-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 afferents' 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 de-efferented by crushing the appropriate branch of the intercostal nerve (i.e. external or internal, depending on which muscle the spindle resided) proximal to the recording electrode and the analysis repeated. In this way the muscle spindles' response to stretch with and without fusimotor activity could be compared.

Response of afferent with respect to respiratory cycle. A more complicated analysis was needed to determine the afferents' 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 diaphragm 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. 1*B*). The respiratory cycle was arbitrarily divided into fourteen parts and the efferent's average response to sinusoidal length changes was determined for each part (Fig. 2*A*). 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 following traces represent stretches starting with successive subsequent periods of 214 ms in the respiratory cycle. Each trace started at the same phase of the stretch cycle, and was plotted at the middle of the cycle in graphs such as Fig. 6. For example the first trace would be plotted at a time of $(214+250)/2 = 232$ ms.

To obtain the modulation in afferent rate due solely to the stretch, the average response of the afferent's rate over the breathing cycle (Fig. 2*B*) was substracted from each histogram. Each of the histograms was then fitted with 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 analogue (Taylor, 1985). The mean rate during each part of the respiratory cycle was calculated from each histogram before the activity locked to respiration was subtracted away. A plot of mean rate and modulation around mean rate of the muscle spindle afferents *versus* time in the breathing cycle could then be generated. The points were fitted with a line generated from averaging each point with the neighbouring two points (three-point running average). The activity of the muscle spindle afferents before and after removing fusimotor activity was compared.

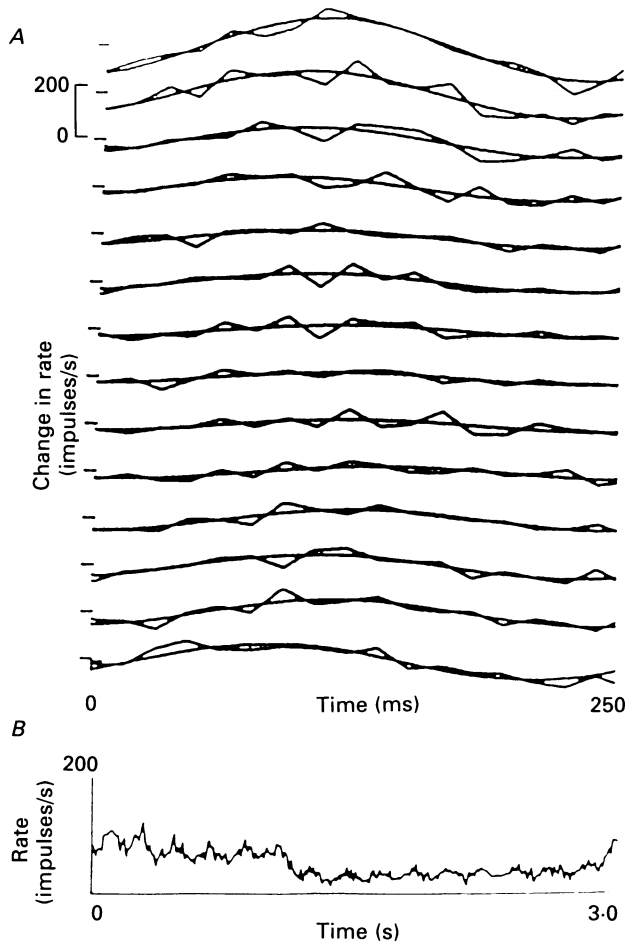

Fig. 2. *A*, average response of an afferent to sinusoidal stretch during fourteen parts of the respiratory cycle. The first histogram represents the afferent's response to a sinusoidal stretch applied during the first 214 ms of the respiratory cycle. The following traces represent the afferent's response 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 associated with respiration. The modulation of the afferent's response shown in *B* could then be subtracted from the response of the afferents shown in *A*. This procedure allowed the pure response of the afferent to the sinusoidal stretch to be calculated. The horizontal marks in *A* represent the zero value for each trace after the subtraction. All data in *A* are plotted on the same vertical scale as in *B*.

Muscle spindle efferents whose response to the applied sinusoidal stretch did not change after de-efferentation 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 anaesthesia may have been high enough during some recordings to completely inhibit fusimotor activity.

Pattern of α-motoneurone activity. Finally, the intercostal motor unit or α-motoneurone 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 fusimotor activity varied in muscle spindles from different areas of the intercostal muscles. There was a correlation

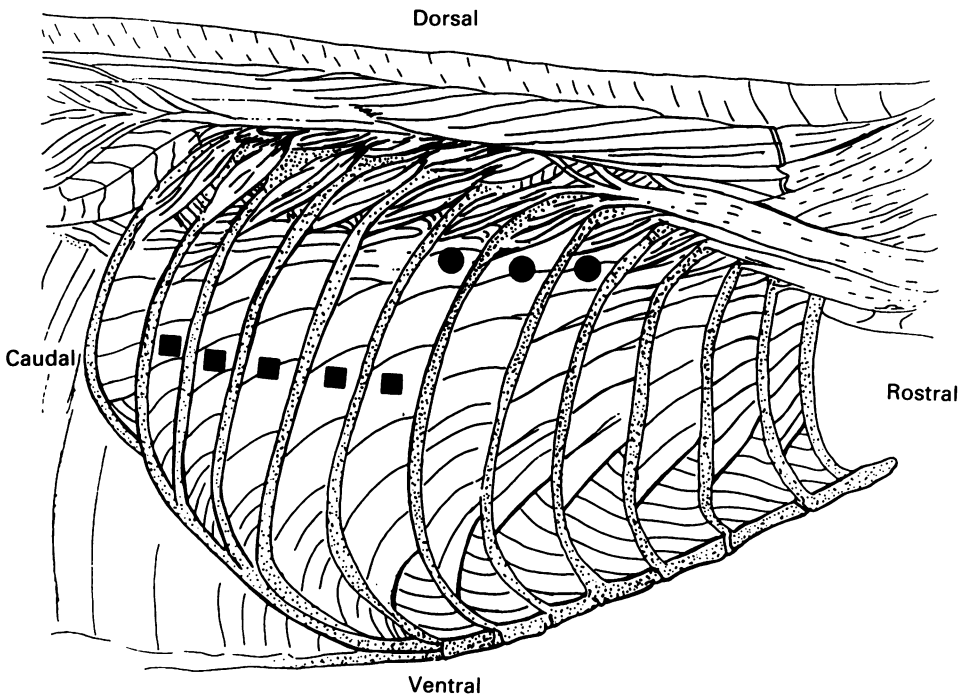

Fig. 3. Recordings of muscle spindle afferents were made from areas of intercostal muscle where EMG activity related to the respiratory cycle was normally absent (■) or present (●).

between the type of fusimotor activity a muscle spindle received and the typical recruitment pattern of the surrounding muscle. A full description of the distribution of EMG activity and range of length changes in the intercostal muscles of the cat during respiration are reported elsewhere (Greer, 1988), but salient points will be included here as necessary.

Recordings of spindle primaries in areas of external and internal intercostal muscles where α-motoneurone activity is normally absent

Figure 3 illustrates the location of the muscle spindles from which the results reported in this section are derived (nineteen units). EMG activity related to the respiratory cycle is seldom seen in the caudal portions of the rib-cage in anaesthetized or decerebrate cats (Duron & Marlot, 1987; Greer *et al.* 1988). Muscle spindles from

both internal (three units) and external (sixteen units) intercostal muscles of decerebrate (nine units) and anaesthetized cats (ten units) are included in this group.

Figure 4*A* shows the response of a primary afferent from a muscle spindle located in the external intercostal muscle to an applied sinusoidal stretch. The histogram

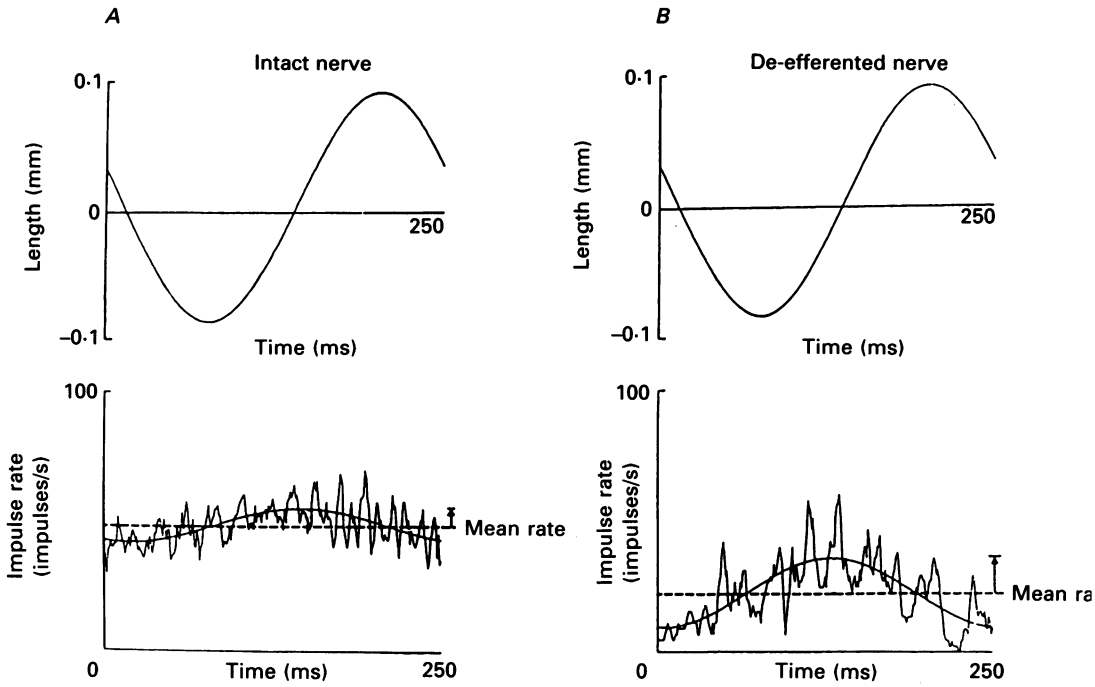

Fig. 4. Fitted histogram of a primary afferent's average response to a series (typically 250) of 0·1 mm 4 Hz sinusoidal stretches before (*A*) and after (*B*) de-efferentation. The smoothed histograms are fitted with a sine curve to determine mean rate (as indicated by the dashed horizontal line) and modulation around the mean rate (as indicated by the vertical arrow). The afferent's sensitivity to velocity is evident from the approximately 90 deg 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 millimetre of applied stretch) increased with removal of fusimotor activity.

represents the average response of the afferent to approximately 250 applications of a 0·1 mm, 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 deg indicating that the afferent is mainly responding to velocity (velocity being the first derivative of length). In eleven intact nerves (containing nineteen primary muscle spindle afferents) the mean rate was 95·7 impulses/s (S.E.M. = 11·5). The sinusoidal length change was set to give approximately 20–25% modulation (22·6 impulses/s; S.E.M. = 4·0).

The response of the afferent of Fig. 4*A* to a stretch of the same size after the muscle spindle has been de-efferented can be seen in Fig. 4*B*. 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. 4*A* had the effect of increasing the mean rate and decreasing the sensitivity (modulation from mean rate per unit change

in length) of the muscle spindle. The changes in mean rate and modulation upon de-efferentation of the remaining spindle afferents is shown in Fig. 5. In areas where EMG activity was absent there was an increase in mean rate (38·1 impulses/s; S.E.M. = 4·8) and a decrease in modulation (21·3 impulses/s; S.E.M. = 2·3) when the

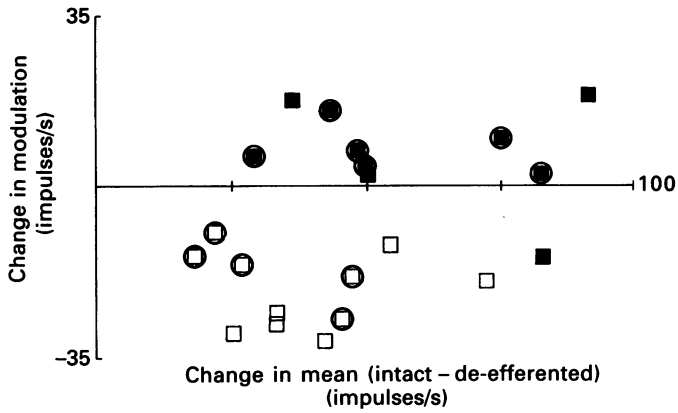

Fig. 5. Plot of the changes in the mean rate and modulation of the primary afferents due to de-efferentation. These values were calculated by subtracting the average mean rate and modulation of the de-efferented state from that seen in the intact recordings. The spindle populations were divided according 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 fusimotor activity. The change in modulation suggested whether static (decreased modulation) or dynamic (increased modulation) fusimotor neurones were dominating the spindle's response. Points circled are from recordings of single muscle spindle primary afferents.

fusimotor neurones were intact. Thus, all units in this group illustrated characteristics which suggest they were being influenced by the effects of static fusimotor activity.

The results mentioned above describe the average response of the muscle spindle afferents over the whole respiratory cycle. It was also of interest to analyse the responses of the muscle spindle afferents during various parts of the respiratory cycle. Rectified and integrated EMG from the diaphragm was used to monitor the timing of the respiratory cycle. Figure 6 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, except for the applied sinusoids, so any changes in muscle spindle discharge would be due to the influence of fusimotor activity. The results indicate that the muscle spindle was predominantly receiving a tonic level of static fusimotor activity throughout the respiratory cycle. Phasic components of fusimotor activity are most likely to be reflected in differences between the activity of the spindle afferents during inspiration and expiration. Figure 7 shows the relative changes between inspiration and expiration of all spindle afferents in this group. There was little difference in mean rate (4·5 impulses/s; S.E.M. = 4·5) and modulation (2·1; S.E.M. = 0·4) between the two phases of the breathing

cycle in these areas of the rib-cage. This clearly indicates the tonic nature of the static fusimotor activity.

Recordings of spindle primaries from areas of external intercostal muscle where α-monotoneurone activity is normally present

The location of the muscle spindles from which the results reported in this section were derived (seventeen units) is illustrated in Fig. 3. Only recordings of muscle

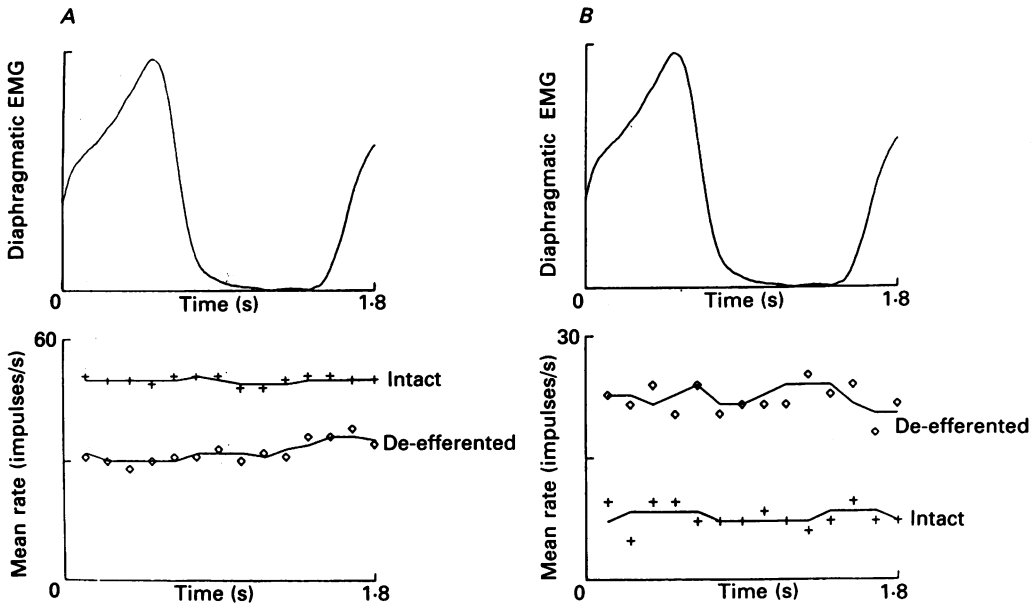

Fig. 6. Plots of the mean rate and modulation before and after de-efferentation of the afferent from Fig. 4 at fourteen different parts of the respiratory cycle. These values were derived from histograms of the afferent's response to the applied sinusoidal stretches during successive parts of the breathing cycle as demonstrated in Fig. 2. 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 de-efferented.

spindles from external intercostal muscles are included in this group. Typically, the dorsal areas of the external intercostals in the mid-thoracic spaces are phasically active during respiration (Kirkwood & Sears, 1978; Greer *et al.* 1988). As shown in Fig. 8, the α-motoneurone activity in these areas is recruited in phase with the diaphragmatic EMG. The mean rate for ten nerves (containing fifteen muscle spindle primary afferents) was 105·0 impulses/s (S.E.M. = 12·4) and the length change produced a modulation of 22·5 impulses/s (S.E.M. = 2·6).

Figure 9 illustrates the responses of two primary afferents from these areas of the external intercostal muscles to a series of 4 Hz sinusoidal stretches before and after de-efferentation. 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 fusimotor activity. Figure 5 shows the changes in mean rate and modulation of all the afferents in this group. Areas associated with respiratory EMG

activity showed an increase in both mean rate (58·8 impulses/s; S.E.M. = 6·9) and modulation (7·1 impulses/s; S.E.M. = 3·1) when the fusimotor neurones were intact. The differences between the change in modulation for the respiratory and non-respiratory areas is highly significant ($P < 0{\cdot}001$, Student's t test). Nevertheless, within this group there is evidence for a range of effects from strong dynamic to predominantly static effects. The basis for this variation is evident from studying the response of the afferents during the different parts of the respiratory cycle.

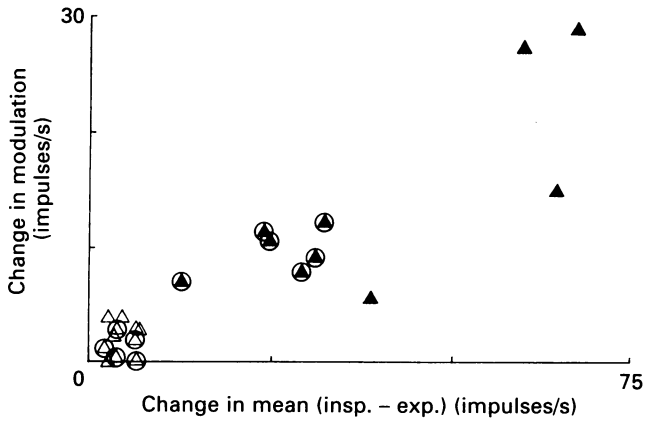

Fig. 7. Plot which illustrates the differences between the inspiratory and expiratory values of the mean rate and modulation of spindle primaries in respiratory (▲) and non-respiratory (△) areas of intercostal muscle. These values were calculated by subtracting the average mean rate and modulation found during expiration from those values found during inspiration. Data points circled are from recordings of single muscle spindle afferents. Inspiration and expiration were defined in relation to the presence or absence of significant levels of diaphragm EMG activity. Although the timing of this activity may differ somewhat from the periods of inspiration and expiration defined mechanically and from the periods of phasic fusimotor activity, the data clearly fall into two distinct clusters with this definition.

Figure 10*A* illustrates the mean rate and modulation around the mean rate of the unit from Fig. 9*A* during various parts of the breathing cycle before and after de-efferentation. The change in the afferent's response is largely during the inspiratory phase, thus offering an opportunity to study the phasic component of the γ-motoneurone activity without any interfering tonic activity. In the intact recording, there is an increase in the afferent's mean rate and modulation during inspiration. This result is suggestive of a co-activation of dynamic fusimotor neurones with α-motoneurones.

There is some evidence that activation of a single static fusimotor neurone at low stimulation rates with short muscle lengths can enhance, rather that reduce, primary afferent sensitivity to sinusoidal stretch (Hulliger, Bauman & Emonet-Denand, 1983). Since the spindles are probably innervated by several fusimotor neurones (Matthews, 1981) which have been reported to discharge at peak frequenceis of 20–100 impulses/s (Sears, 1964; Greer & Stein, 1989) and since we maintained the muscle taut to insure continuous firing of the afferent, such paradoxical effects are unlikely in our preparation.

Figure 10*B* illustrates the response of the muscle spindle afferents from Fig. 9*B*

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

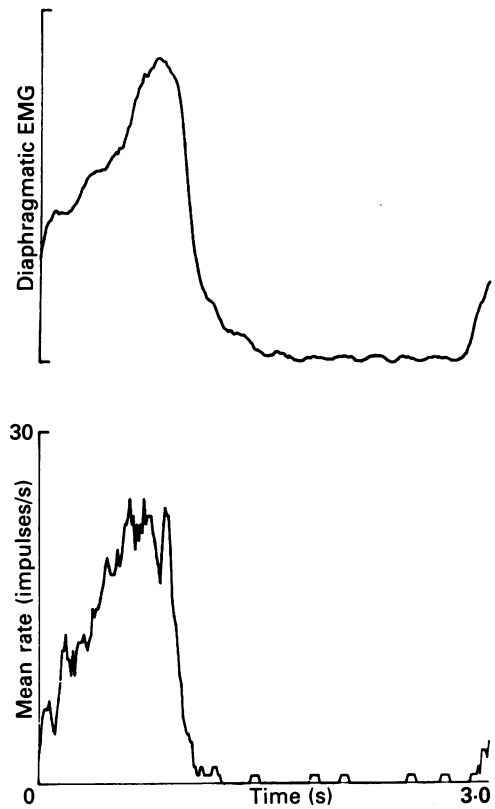

Fig. 8. Typical discharge pattern of activity from α-motoneurones recorded from the same external intercostal nerve which contained afferent fibres illustrated in Figs 9 and 10. Rectified and integrated diaphragmatic EMG is used to monitor the timing of the respiratory cycle.

intact nerve, the effects of fusimotor activity. Since the forces responsible for the movement of the muscle strip were present in both the recordings of intact and de-efferented nerves, any changes in mean rate and modulation between the two recordings were due to the effects of fusimotor activity. Firstly, there is an overall increase in mean rates 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 de-efferented nerve. This would suggest the added fusimotor activity 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 de-efferented state. This is suggestive of the summation of effects produced by the activity of both static and dynamic fusimotor neurones. A similar response was seen during the expiratory phase in nine of the

spindles studied, while modulation of the remaining eight was lower in the intact *versus* de-efferented state (presumably when the effect of the static fusimotor activity was not significantly occluded by dynamic fusimotor activity).

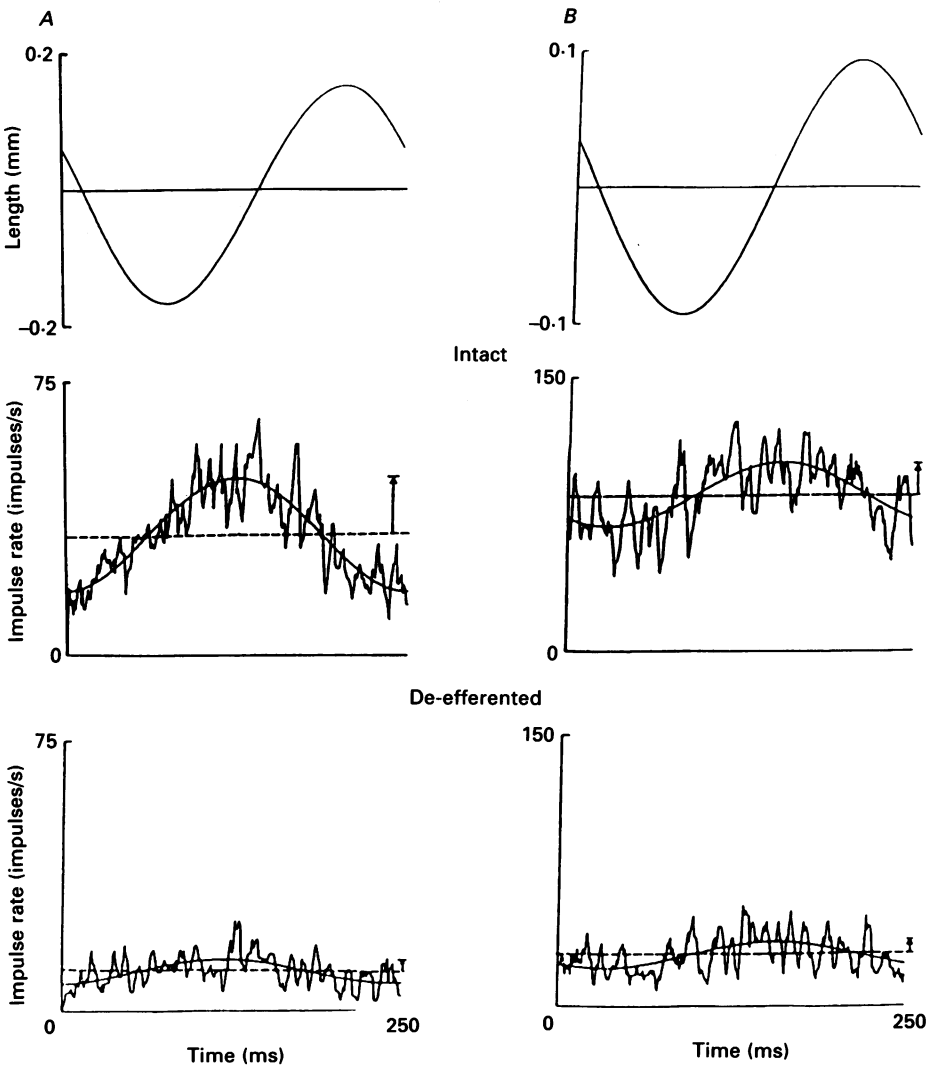

Fig. 9. Average response of afferents from two different recordings within areas specified in Fig. 3. Details as in Fig. 4. In both examples de-efferentation resulted in a decrease in mean rate. The modulation upon removal of fusimotor activity increased in *A* and *B*.

Figure 7 shows the differences in the mean rate and modulation between inspiration and expiration for all the afferents in this group. Where EMG activity is typically present differences in afferent mean rate (38·2 impulses/s; S.E.M. = 5·9) and modulation (12·3 impulses/s; S.E.M. = 2·0) between inspiration and expiration in the

active portions of the rib-cage demonstrated the phasic recruitment of dynamic fusimotor neurones approximately in phase with α-motoneurones. The differences between the changes in mean rate and modulation in respiratory and non-respiratory

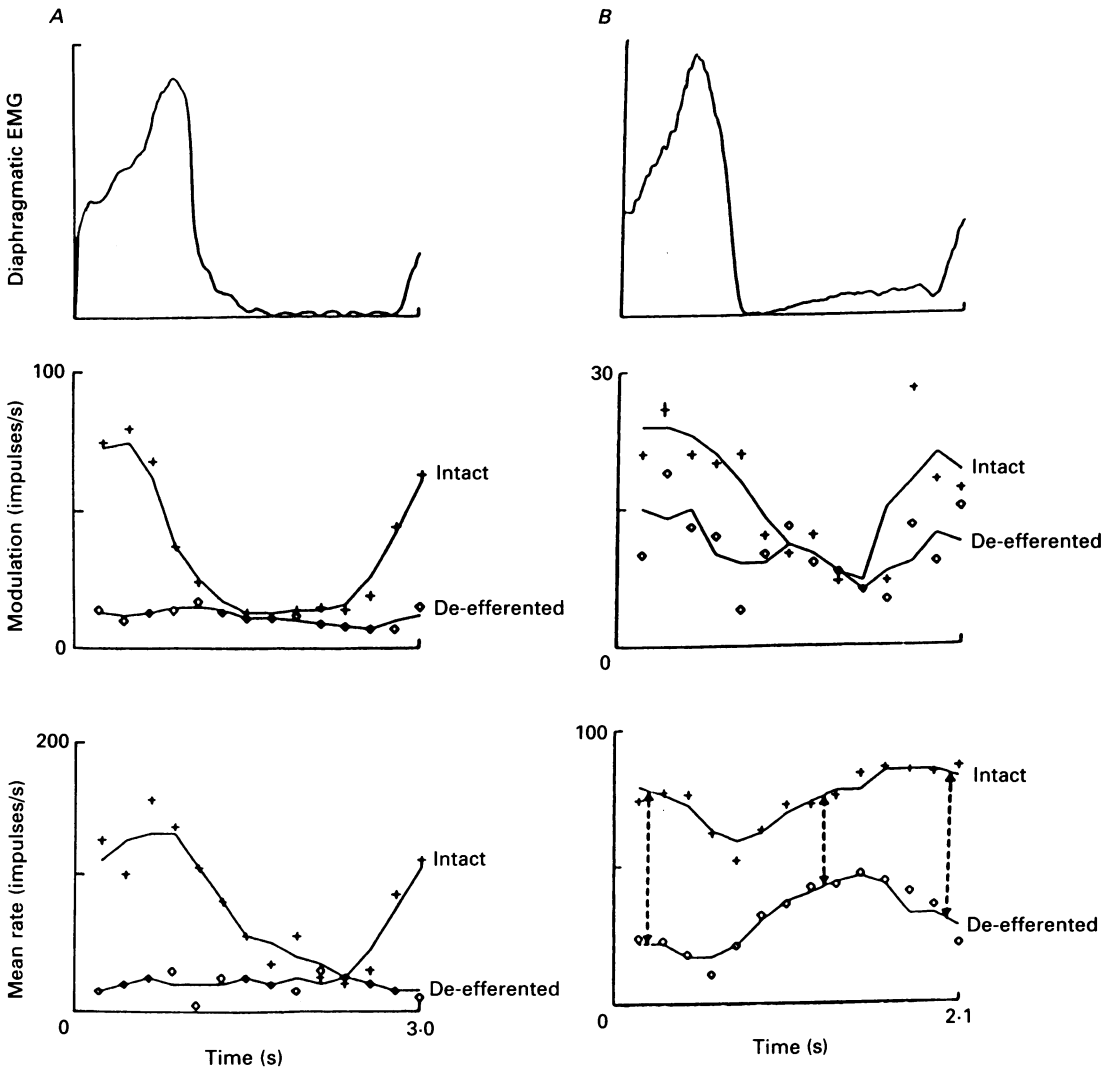

Fig. 10. *A* and *B* illustrate the changes in mean rate and modulation upon de-efferentation during fourteen parts of the respiratory cycle of units from panels *A* and *B* respectively of Fig. 9. Details as in Fig. 6. The afferents in both recordings show evidence of receiving phasically modulated activity of dynamic fusimotor neurones. Afferents in *B* also seem to be receiving a steady level of static γ-motoneurone activity. See text for full description.

areas are both highly significant ($P < 0{\cdot}001$, Student's t test). This apparent recruitment of dynamic fusimotor neurones either consisted of purely phasic activity or a waxing and waning of the efferent's discharge. Results from the analysis of single or multi-unit (two to three spindles) afferents from adjacent spindles combined were

qualitatively similar in all areas of the rib-cage (Figs 5 and 7). This should not be surprising, considering the afferents averaged together came from the same small area of muscle and received the identical stretch.

Exceptions to the above mentioned pattern of fusimotor activity were seen in two

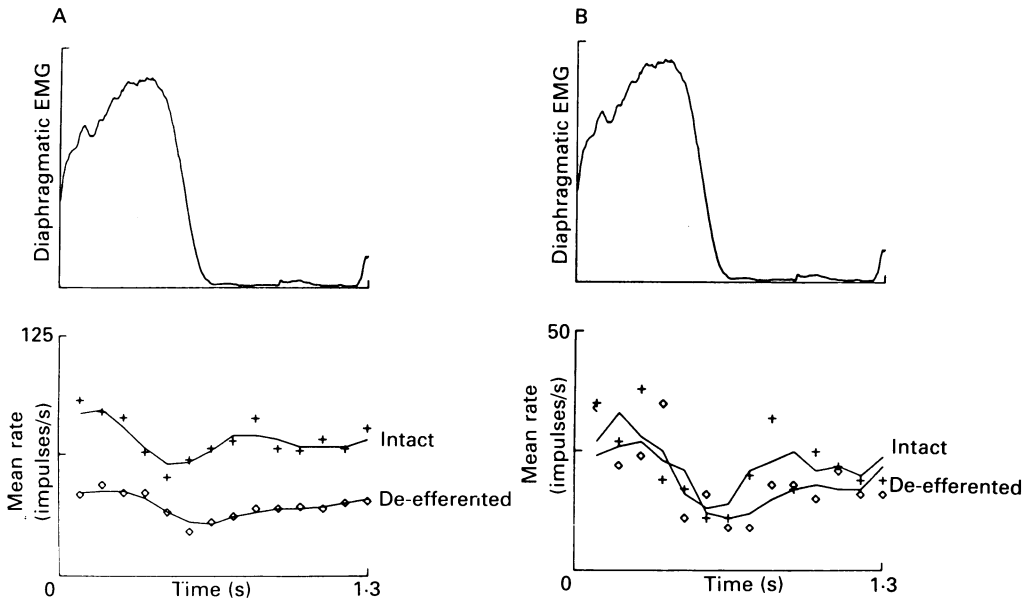

Fig. 11. Mean rate and modulation during fourteen parts of the respiratory cycle from a recording of an atypical muscle spindle. Details as in Fig. 6. De-efferentation led to a decrease in the afferent's mean rate but had little effect on its modulation.

muscle spindles (2/17). They were located in the vicinity of α-motoneurone activity, yet showed no signs of receiving significant phasic input from fusimotor neurones. Figure 11 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 de-efferented state.

These results could be explained by a mixture of tonic levels of dynamic and static fusimotor neurone 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 fusimotor activity which terminates on bag2 fibres. These static fusimotor neurone terminations are known to increase the discharge rate of spindle primaries without altering the sensitivity (Boyd, 1986). The reason for the dominance of effects from the terminals of bag2 static fusimotor neurones could be a peculiarity of these few spindles or due to selective removal of other fusimotor activity during the isolation of the single muscle spindles as described in the Methods.

DISCUSSION

Direct recordings from fusimotor neurones of intercostal nerves have shown that there are both phasically and tonically active units (Critchlow & von Euler, 1963; Sears, 1964; Greer & Stein, 1989). We have made an attempt at identifying these

fusimotor neurones as being of the dynamic or 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 fusimotor activity. Secondly, there were muscle spindles which behaved as if they were receiving phasically modulated activity from dynamic fusimotor neurones in addition to static fusimotor activity. Whether the static fusimotor neurones are phasic or tonic in the second group of neurones cannot be accurately determined from these recordings of muscle spindle afferents. However, we recorded fusimotor neurones under the same conditions in the same area of the rib-cage (Greer & Stein, 1989) and found some which fired tonically while others fired phasically. Since the dynamic fusimotor neurones were phasically active, the simplest suggestion form these data is that many of the static fusimotor neurones were tonically active, even in the respiratory areas.

The fusimotor discharge patterns paralleled the difference in α-motoneurone patterns. Areas of intercostal muscle that did not show EMG activity related to respiration appeared to receive a tonic level of predominantly static fusimotor activity. Areas studied that did show EMG activity related to respiration also received phasic activity of dynamic fusimotor neurones. The presence of phasically recruited dynamic fusimotor activity could be seen at times when the respiratory drive was not sufficient to activate α-motoneurones. Thus, the recruitment threshold of phasically modulated fusimotor neurones is lower than that of α-motoneurones (Andersen & Sears, 1970). The possibility of β-motoneurones 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-motoneurones could have been recruited where α-motoneurone 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 fusimotor neurones. 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 *et al.* 1988). While these muscles are active the fusimotor system increases 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-motoneurone activity during normal and obstructed movements. For instance a deficit in muscle spindle activity in the intercostal muscles can produce lower overall motor unit discharge frequencies (Nathan & Sears, 1960; Sant'Ambrogio & Widdicombe, 1965; Schwieler, 1968), a decrease in reflex compensation in the presence of perturbations (Newsom Davis & Sears, 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 the hindlimb (Loeb, 1985; Taylor *et al.* 1985). Length measurements of the intercostal muscles

have illustrated that sections of the external intercostals are also lengthening while active during respiration which offers a further explanation for the pattern of fusimotor activity found (Greer *et al.* 1988).

During expiration the inspiratory α-motoneurones are being actively inhibited via a spinal network which receives input from the respiratory centre (Aminoff & Sears, 1971). However, passive lengthening of the external intercostal muscle during expiration would result in excitation of the α-motoneurones from spindle afferents. The proposed predominance of static fusimotor activity during expiration would decrease the sensitivity of the muscle spindle primary efferents and therefore minimize this counter-productive excitation.

The recruitment of the two types of fusimotor neurones 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 fusimotor neurones 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 fusimotor neurones (Corda, von Euler & Lennerstrand, 1966), which we have identified as being predominantly of the static type. Secondly, afferents from intercostal muscles project to the same areas of the cerebellar cortex as those from limb muscles and thus provide an anatomical basis for the integration of kinaesthetic information on which postural adjustments can be made (Coffey, Godwin-Austen, MacGillivray & Sears, 1971). Collectively, these reports suggest that external intercostal muscles and the fusimotor 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 fusimotor neurones associated with control of posture are predominantly of the static type. Static fusimotor 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 muscles including trunk rotation (DeTroyer, Kelly, Macklem & Zen, 1985), shivering (Duron & Caillol, 1971), purring (Kirkwood, Sears, Stagg & Westgaard, 1987) and vocalization in humans (Draper, Ladefoged & Whitteridge, 1960; Newsom-Davis & Sears, 1970). These functions would involve inputs from a variety of supraspinal centres onto both

α- and fusimotor neurones. Therefore, during these different tasks the relative balance between dynamic and static fusimotor neurones could well be altered from those reported in the present study.

In summary, the two types of fusimotor neurones, static and dynamic, can be activated separately. As well, the balance of activity between the two types of fusimotor neurones 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 fusimotor 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 fusimotor neurones 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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