Regulation of Muscle Stiffness During Periodic Length Changes in the Isolated Abdomen of the Hermit Crab

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Chapple, William. Regulation of muscle stiffness during periodic length changes in the isolated abdomen of the hermit crab. J. Neurophysiol. 78: 1491–1503, 1997. Reflex activation of the ventral superficial muscles (VSM) in the abdomen of the hermit crab, Pagurus pollicarus, was studied using sinusoidal and stochastic longitudinal vibration of the muscle while recording the length and force of the muscle and the spike times of three exciter motoneurons. In the absence of vibration, the interspike interval histograms of the two larger motoneurons were bimodal; cutting sensory nerves containing most of the mechanoreceptor input removed the short interval peak in the histogram, indicating that the receptors are important in maintaining tonic firing. Vibration of the muscle evoked a reflex increase in motoneuron frequency that habituated after an initial peak but remained above control levels for the duration of stimulation. Motoneuron frequency increased with root mean square (rms) stimulus amplitude. Average stiffness during stimulation was about two times the stiffness of passive muscle. The reflex did not alter muscle dynamics. Estimated transfer functions were calculated from the fast Fourier transform of length and force signals. Coherence was >0.9 for the frequency range of 3–35 Hz. Stiffness magnitude gradually increased over this range in both reflex activated and passive muscle; phase was between 10 and 20°. Reflex stiffness decreased with increasing stimulus amplitudes, but at larger amplitudes, this decrease was much less pronounced; in this range stiffness was regulated by the reflex. The sinusoidal frequency at which reflex bursts were elicited was ~6 Hz, consistent with previous measurements using ramp stretch. During reflex excitation, there was an increase in amplitude of the short interval peak in the interspike interval histogram; this was reduced when the majority of afferent pathways was removed. A phase histogram of motoneuron firing during sinusoidal vibration had a peak at ~110 ms, also suggesting that an important component of the reflex is via direct projections from the mechanoreceptors. These results are consistent with the hypothesis that a robust feedforward regulation of abdominal stiffness during continuous disturbances is achieved by mechanoreceptors signaling the absolute value of changing forces; habituation of the reflex, its high threshold for low frequency disturbances and the activation kinetics of the muscle further modify reflex dynamics.

INTRODUCTION

Sensory control of posture and movement has been a major area of interest in motor control for many years. Although the maintenance of a posture can be achieved by passive mechanical mechanisms, the requirement that an animal be flexible enough to move requires the active control of posture by the activity of the CNS. This control requires an interaction between signals originating within the CNS and those derived from various sensory systems. If appropriate corrective action is to be taken, information is required about the direction and magnitude of external forces. Among the sensory modalities that provide this information, local mechanoreceptors are known to be particularly important. Because posture is by definition a static process, the time constraints that might operate in the control of rapid movement do not necessarily apply. Negative feedback is one strategy that is believed to regulate posture; that is, to maintain a variable such as joint angle, muscle length or stiffness within narrow limits (Houk and Rymer 1981). Alternatively, open loop or feedforward regulation might be used to increase the stiffness of agonist and antagonist muscles (Hogan 1984; Polit and Bizzi 1979).

Postural control has been studied most intensively in animals with exoskeletons or endoskeletons. Much less is known about postural control in animals with hydrostatic skeletal systems. In these animals, the predominant mechanoreceptors associated with muscle are phasic receptors that signal local stress and that often are activated by increases and decreases in local stress. This rectification of muscle stress presents considerable problems for any control scheme. Because hydrostatic skeletal systems are common in both vertebrates and invertebrates, it is important to understand how their posture is controlled.

The abdomen of the hermit crab, Pagurus pollicarus, grasps and supports the hermit crab’s shell during movement. Hermit crabs live in shells of different weights and dimensions, and the shell itself is an eccentric load that can topple the animal unless its position remains stable (Chapple 1973b). Unlike other decapod crustaceans, the abdomen of the hermit crab is a hydrostat: longitudinal, circular, and helical muscles surround an incompressible volume of tissue, and their cocontraction stiffens the abdomen so that it can better grasp and support the shell (Chapple 1973a, 1995). The hermit crab abdomen executes this control with 13 motoneurons on each side of each segment, and this small number of motoneurons provides an opportunity for analyzing its postural control in detail.

Previous experiments have demonstrated that longitudinal stretch or release of the ventral superficial muscles (VSM), which lift the columnella of the shell, activates mechanoreceptors that phasically excite all of the exciter motoneurons of the postural muscles of the posterior abdomen (Chapple 1993). Coactivation of antagonist sets of muscles long has been known to be important in movements (Feldman 1966; Polit and Bizzi 1979) and has been studied as a mechanism for controlling the mechanical impedance around a joint (Hogan 1984). Under static conditions, muscles have spring-like properties; by increasing their activation, muscle stiffness is increased. In the hermit crab, this increase in stiffness reduces the deflection of the abdomen in response to external forces.
forces. The long latency of the VSM reflex and its activation only during rapid stretch and release of the muscle indicate that the reflex cannot operate as a negative feedback controller to regulate stiffness. An alternative is that mechanoreceptor firing due to continuous external disturbances is averaged by the CNS to activate the muscles in a feedback manner, increasing muscle stiffness and reducing the effects of the disturbances on the animal’s stability. The present experiments were designed to explore this hypothesis by examining the correlated changes in stiffness and motoneuron frequency during vibration of the longitudinal muscle fibers of the VSM. Because the circular and helical muscle fibers of the VSM lie between the longitudinal fibers and the cuticle, they are less accessible and were not studied.

METHODS

The preparation has been described previously (Chapple 1993). Briefly, P. pollicatus was collected throughout the year from sublittoral protected sandy areas in Fishers Island Sound off the coast of Connecticut. The right fourth segment of the VSM was exposed by opening the abdomen through a longitudinal incision along the medial dorsal surface, and the abdominal nervous system was exposed by removing the large abdominal flexor muscles of the third, fourth, and fifth segments from around it. The first and third ganglionic roots were left intact, but the second ganglionic roots of the third and fourth segments, innervating the dorsal surface of the abdomen, were cut to reduce the complexity of the dissection. Control experiments did not show any difference in the response of the preparation under these conditions. Two acrylic plastic (Plexiglas) plates with stainless steel wire rings inserted in them were glued to the external ventral surface of the third and fifth abdominal segments with a cyanoacrylate adhesive. The muscle, together with the abdominal nervous system, was suspended between a force transducer and an electromagnetic stretcher, controlled by a computer, and submerged in a saline bath maintained at 12.5°C. The inner ventral surface of the abdomen was mounted uppermost so the VSM, homologous to macruran slow flexors, was accessible to microelectrodes.

The VSM is composed of several layers (Chapple 1969), a medial layer of longitudinal muscle fibers innervated by three exciters and an inhibitor motoneuron on each side of each segment; more superficial layers of circular and helical muscle fibers are innervated by two exciters and the inhibitor. The inhibitor is normally silent in the isolated abdomen, although it fires in the intact animal at the end of the activation of the abdominal fast flexor muscles by the medial giant interneurons. A nomenclature was adopted for identifying the abdominal ganglionic roots and their motoneurons. First, the abdominal segment of the ganglionic root (from 2 to 6) was specified, then its side (left or right) and, finally, the number of the ganglionic root (from 1 to 3). For a motoneuron, an identifying letter (medial, central, or lateral) or sequence of letters (circular) was appended that identifies the region of muscle innervated. Thus: 4r1 is the right first root of the fourth segmental ganglion; 4r3m is the third ganglionic root medial motoneuron of the right fourth segment. The motoneurons of the longitudinal layer innervate discrete regions, the largest motoneuron, 4r3l, a lateral strip of muscle fibers; a small motoneuron, 4r3c, a central region; and the second largest motoneuron, 4r3m, a medial strip of muscle fibers.

The response of each VSM motoneuron was studied using intracellular microelectrodes to record the excitatory junction potentials (EJPs) generated by a single motoneuron in the muscle fibers. A “floating electrode” technique reduced movement artifacts. The tip of a microelectrode, filled with 3 M KCl or 2 M K acetate, was broken off and mounted on a 0.005-in.-diam silver wire; the junction was sealed with a dab of Dow Corning High Vacuum Grease to prevent the electrolyte from evaporating. The “floating” microelectrode then was lowered onto the desired region of the muscle to confirm a muscle fiber. Under normal conditions, stable recordings could be maintained for hours. More importantly, the electrodes remained in the muscle fibers during mechanical stretch of the muscle and vibration amplitudes ±0.5 mm (±4% of optimum muscle length). EJPs in a muscle fiber were associated with a particular motoneuron by correlating the EJP with the extracellular potentials in the ganglionic roots, recorded with conventional suction electrodes. With experience, the motoneuron generating an EJP could be identified by its tonic frequency, amplitude, and the location of the electrode in the muscle.

The EJPs were amplified with conventional intracellular DC amplifiers. Spike times were obtained by differentiating the EJPs before sending them to a comparator circuit that produced a uniform 10 μs pulse for each EJP. These pulses were logically ORed and latched; the output of the OR gate generated an interrupt in a real-time clock board (CIO-CTR05, Computer Boards), which recorded the time of each spike with a 10-kHz clock. The output of the digital latch was read by the CIO-CTR05 board’s digital input, identifying which motoneuron had triggered the interrupt. During each experiment, the threshold for the logic pulses was periodically compared with the EJPs to ensure that the trigger level remained stable.

The general form of these experiments was to record intracellularly from two muscle fibers, each innervated by a single motoneuron, and to perturb the VSM with a test waveform while recording the muscle force, length, and spike times of the individual motoneurons. Each test waveform was repeated 10 times; force and length information as well as spike times were stored on disk for each trial. Force, length, and spike times were recorded for 10 s before the start of the test waveform and for 10 s after its end, to provide an estimate of the tonic frequency of the motoneuron before and after stimulation. Force and length were sampled at 10-ms intervals using a Data Translation 3831 I/O board, mounted in a Gateway 4DX2-66V computer; input and output channels were prefiltered with 8-pole low-pass Bessel filters at 20 Hz to avoid aliasing artifacts. Three test waveforms were used: sinewaves of different frequencies and amplitudes, low-pass filtered (20 Hz) Gaussian white noise, and a varying frequency sinewave (“sweep”) stimulus that started with a 10-s interval at 1.0 Hz, then increased linearly during 10 s to 10 Hz, and remained at 10 Hz for an additional 10 s. In all three stimulus waveforms, the amplitude of the stimulus waveform was linearly increased from zero to a constant value during 4 s; during the last 4 s of stimulation the amplitude was linearly decreased to zero. This procedure was used to minimize start-up transients.

Spike times were used to construct peristimulus time histograms (PSTH), probability spike density functions (PSDF) (Richmond et al. 1987), interspike interval histograms, autocorrelograms, and cross-correlograms (Perkel et al. 1967a,b). The PSDF was calculated by convolving the time of each spike with a Gaussian distribution kernel with a fixed standard deviation of 0.005 s and a sampling frequency of 0.001 s. The PSDF then was normalized by dividing by the integral of the kernel (so that the kernel’s cumulative distribution was equal to 1.0). A mean PSDF with its standard deviations was calculated for each series of ten trials and then multiplied by 1,000 to give the PSDF in units of frequency (impulses per second—ips). The PSDF provides a continuous rather than discrete estimate of the probability of firing (Silverman 1986). Force and length signals during noise vibration were analyzed by calculating spectral density functions, as well as their cross-spectral densities and coherence, using routines in the Matlab (The Mathworks) Signal Processing Toolbox. The cumulative sum test (Schwartz et al. 1988) was used to determine the time at which
the reflex firing of a motoneuron becomes significantly greater than the control period. The PSTH was calculated for 10 trials, and the mean for the control period then was calculated and subtracted from the PSTH. The cumulative sum of this normalized PSTH is calculated and compared with the 95% confidence limits derived from the standard deviation of the control period (Armitage 1975), the assumption being that random fluctuations would vary around a mean value and would not show a progressive increase or decrease exceeding the confidence limits.

RESULTS

**Tonic firing of the motoneurons**

Because muscle activation in hermit crab VSM is produced by a combination of the tonic firing of the motoneurons as well as reflex activation by mechanoreceptors, the tonic frequencies of the three motoneurons innervating the longitudinal muscle fibers of the VSM of the fourth right segment were examined first in the absence of vibration. Spike times of pairs of VSM motoneurons were recorded simultaneously under isometric conditions. From these spike times interspike interval histograms, autocorrelation histograms, and cross-correlation histograms then were calculated.

Each motoneuron of the VSM has its characteristic firing pattern. Figure 1A shows these spike time statistics in one preparation for 4r3m, which innervates the ventromedial region of the VSM, and 4r3l, which innervates the region of muscle at the lateral margin of the VSM. The mean interval, sampled over fifty seconds, for 4r3m was $0.178 \pm 0.157$ s (mean $\pm$SD, $n = 282$, median $= 0.165$ s) and for 4r3l during the same period was $0.263 \pm 0.237$ s ($n = 191$, median $= 0.253$ s). In the left column are the interspike interval histograms, which are bimodal for both units, with one interval peak at intervals $< 20$ ms and a second broader peak at $\sim 200$ ms for 4r3m and at $\sim 400$ ms for 4r3l. The short interval peaks arise from bursts of two or three spikes that are superimposed on a much lower tonic frequency, corresponding to the long interval region in the interval histograms. Figure 1B shows interspike interval histograms for 4r3m and for 4r3c, which innervates a region of muscle between those of the other two motoneurons. The mean interval $> 50$ s for 4r3m was $0.123 \pm 0.117$ s ($n = 408$, median $= 0.088$ s) and for 4r3c was $0.093 \pm 0.05$ s ($n = 538$, median $= 0.084$ s). In contrast to 4r3m, which has a distribution qualitatively similar to that in Fig. 1A, 4r3c has a sharply skewed distribution that is not obviously bimodal.

In contrast to the autocorrelograms of pacemaker neurons that exhibit prominent periodic peaks and valleys (Perkel et al. 1967a), the autocorrelograms for the VSM motoneurons do not exhibit marked periodicities and reach constant values after $\sim 0.4$ s. Cross-correlation histograms between pairs of
motoneurons exhibit peaks at delays of 5–10 ms, suggesting that the motoneurons are activated simultaneously by a common process and that the time delay may be due to differences in the time of arrival of motoneuron action potentials at the different recording sites in the muscle.

Although there was considerable variability in the frequencies of the motoneurons in different preparations, their frequencies in a single preparation were correlated. Figure 2A shows a plot of the mean interspike intervals of 4r3l (n = 20) and 4r3c (n = 8) as functions of the simultaneously recorded mean interspike interval of 4r3m. Both relationships are approximately linear: for the interspike interval of 4r3l (I_{4r3l}) = ±0.01 + 3.48 · I_{4r3m} [r = 0.6952, standard error of estimate (s.e.e.) = 0.33] and for I_{4r3c} = 0.08 + 0.24 · I_{4r3m} (r = 0.8545, s.e.e. = 0.05). Three preparations in which the frequency of 4r3l was <0.5 Hz were excluded from the graph and the calculations; in these preparations, the tonic firing was measured at the end of an experiment and 4r3l was firing at a very low rate (mean intervals of 3–5 s). The graphed experiments appear to be subdivided into a group in which 4r3l is firing at ~1 Hz and another in which it is firing at ~3 Hz for the same average frequency of 4r3m. However, a larger sample would be needed to establish this. For the majority of preparations, the motoneurons fired in a ratio of 1.45:5.0:8.38 Hz (4r3l:4r3m:4r3c). This relationship was not invariant; in a number of preparations, 4r3m and 4r3l would fire in a relatively constant ratio for several hours, but then 4r3l suddenly shifted to a much lower frequency while 4r3m continued to fire at the same frequency.

**Origin of the bimodal interspike interval distribution**

The bimodal interspike interval distributions of 4r3m and 4r3l were composed of two distributions: a short interval peak that resembled an exponential distribution and a broad longer interval peak that was approximately Gaussian. A histogram (Fig. 2B, top left) of the interspike intervals of 4r3m sampled during a period of 100 s was similar to the interspike interval histograms in Fig. 1. When the interspike interval histogram between 0 and 0.1 s was calculated, using a shorter binwidth of 0.001 s (Fig. 2B, bottom left), the short interval peak was seen to be composed of a cluster of intervals between ~0.005 and 0.02 s, comprising 21 ± 12% (n = 10) of the total intervals during the sampling period.

Such bimodal interval distributions might arise because the motoneuron is changing its firing rate during the sampling period, or from the simultaneous action of different presynaptic sources, or as a result of the intrinsic properties of the motoneurons themselves, or as some combination of these factors. The first alternative, nonstationarity during the recording period, was examined by analyzing sequences of spike times of 4r3m from four preparations using the run and the reverse arrangement test (Bendat and Piersol 1986). During short time periods (20 s) motoneuron frequencies were stationary, but as the sampling period increased, motoneuron frequencies frequently became nonstationary (P < 0.05). During stationary periods, the interspike interval histogram had the same bimodal shape as that calculated from 100-s samples. Moreover, in several long sequences, interspike interval histograms were calculated for short segments of the sequence; although there were changes in the position of the longer interval peak, there was no change in the shape of the distribution. Thus the bimodal distribution of spike times is not due to nonstationary alterations in motoneuron frequency.

The second alternative, that the short interval peak in the interspike interval histogram may arise from a different presynaptic source than the source for tonic excitation, seemed plausible because earlier experiments showed a peak in the cross-correlogram between the sensory afferents that excite the motoneurons and 4r3m (Chapple 1993). Figure 2B, right, shows the results of an experiment in which most of the ganglionic roots, containing the majority of sensory fibers, were cut, leaving intact only the third right ganglionic...
root of the fourth segment, which carries the motoneuron axons. The interspike interval histogram on the top left, the control, has both the short interval and the broad longer interval peak. After removing most of the afferent input (the 3rd ganglionic root contains a small population of afferents), the interspike interval histogram on the top right shows a loss of the short interval peak and the longer interval peak becomes narrower and shifts toward shorter intervals; this change was significant at the 1% confidence level ($\chi^2$). The bottom histograms show the change in the short interval peak over the narrow interval from 0 to 0.1 s. The loss of the short interval peak was observed in two other preparations; in one there was no shift of the long interval peak toward shorter intervals. Removing most of the sensory afferents generally silences 4r3l. However, the peak in the cross-correlogram between 4r3m and 4r3l (Fig. 1) suggests that both motoneurons receive correlated synaptic input from the afferents and that the interval histogram of 4r3l probably also arises from the sum of a tonic input and random activation of sensory afferents.

**Reflex activation of the motoneurons by vibration**

Previous experiments (Chapple 1993) employed a transient ramp-hold–release length change to activate the reflex. To determine whether long-lasting disturbances also produce increases in motoneuron frequency and muscle stiffness and to see whether these increases last throughout the period of stimulation, several different types of mechanical vibration were used. Only two (4r3m and 4r3l) of the three motoneurons of the VSM were studied in detail using vibration because it was difficult to record the EJPs of 4r3c without dislodging the microelectrode from the muscle fiber.

Vibration of the VSM, using either sinusoidal or band-pass limited Gaussian ("noise") waveforms, results in an increase in frequency of the motoneurons usually lasting for the duration of the stimulus. Figure 3A shows a photograph of an oscilloscope trace from an experiment on an isolated abdomen preparation in which a 5-Hz waveform was used to evoke the reflex. At ~3 s after the beginning of stimulation, muscle force (top of record) shows a transient upward shift of the baseline and an increase in the amplitude of the force sinusoid, both correlated with an increase in frequency in 4r3m EJPs (bottom) and 4r1 mechanoreceptors (second from bottom). The amplitude of the force waveform at this point is ~15% greater than later in the record, and the transient increase in the basal force level is 0.03 N. Figure 3B shows force and length records from a comparable experiment on an intact animal whose abdomen was vibrated with a 10-Hz sinusoid. As in the isolated preparation, after the transient increase in stiffness and force there is a subsequent decline in motoneuron frequency and stiffness to a "steady state" that lasts for the duration of stimulation. Figure 3C shows a record in which low-pass filtered Gaussian noise was used to stimulate the fourth right VSM in the isolated preparation; Fig. 3D shows a similar experiment in the intact animal. Noise activation of the reflex is more effective than sinusoidal vibration, due presumably to its additional frequency components, but the qualitative features of reflex activation are similar to sinusoidal stimulation. Figure 3E shows a threshold coactivation of the VSM and the dorsal superficial muscles in response to noise vibration. Thus the response to sustained vibration is qualitatively the same in both the isolated abdominal preparation and the intact animal.

The changes in force and stiffness produced by mechanical vibration of the VSM are mirrored in the frequencies of the motoneurons that are elevated for the duration of stimulation, followed by a rapid return to tonic firing levels at its end. In seven animals, the 40-s period of a trial was separated into four 10-s intervals (the prestimulus control, the first 10 s of stimulation, the last 10 s of stimulation, and the poststimulus control period) and the number of EJPs was counted for each interval. Significant ($P < 0.05$; two-sided analysis of variance) elevation of 4r3m frequency during noise stimulation was observed in 23 blocks of 10 trials.
Motoneuron frequency increases with stimulus amplitude

Figure 4 shows PSDF of the average motoneuron frequency for 10 trials at each of four noise amplitudes. The stimulus noise amplitude is below threshold for the plot on the upper left (Fig. 4A); as the stimulus amplitude increases (Fig. 4B), there is an increase in the initial frequency of 4r3m, followed by an irregular decline. 4r3l does not show a significant increase in frequency until the noise has an amplitude of 0.064 mm (Fig. 4C). In Fig. 4D, both motoneurons fire throughout the duration of stimulation.

The relationship between the amplitude of muscle force and peak frequency was examined quantitatively in four preparations. The regression between force and frequency accounted for more of the variation than did a regression between peak frequency and length changes. This is consistent with earlier observations (Chapple 1993) that the mechanoreceptors that evoke the reflex are responding to local stress of the VSM. At different initial muscle lengths, peak frequency varied with force amplitude, not with muscle length, which is also consistent with the interpretation of this reflex as being activated by changes in muscle force. However, the slopes of the relationship between force and peak frequency of 4r3m and 4r3l varied between preparations (Table 1) and the ratio of the slopes of this relationship for the two motoneurons, recorded simultaneously, varied between preparations. For example, in one preparation, 4r3l was three times as sensitive to mean force as 4r3m, in another it was one-third as sensitive.

Latency of the reflex decreases with stimulus amplitude

To measure latency, the cumulative sum of the intervals (Schwartz et al. 1988) was used to determine the time at which motoneuron frequency exceeded the 95% confidence level of the PSDF.

TABLE 1. Relationship between force and peak motoneuron frequency

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Regression coefficients for the relationship between force (N) and peak motoneuron frequency (pulses/sec). Each series number represents a different preparation.
which the stiffness and phase angle of the muscle as a function of frequency may be calculated) from the discrete fast Fourier transforms (FFTs) of length and force (Bendat and Piersol 1986). Figure 6A shows a plot of the magnitude of stiffness versus frequency for the reflex preparation and for the isolated muscle. Both show a minimum at the lowest frequencies, an increase in stiffness that flattens at ~20 Hz and then a more gradual increase to ~35 Hz. From these magnitude estimates in four preparations, the reflex preparation was calculated to be 2.06 ± 0.24 times as stiff as the isolated muscle.

The slope of the low frequency increase in stiffness as a function of frequency in the magnitude plot, estimated by calculating the ratio of stiffnesses at 5 and 20 Hz, did not vary significantly with stimulus amplitude within the reflex or passive muscle groups. However, in three out of the four animals, this ratio was significantly greater in the reflex condition than in isolated muscle, suggesting that the reflex muscle was slightly stiffer (18%) than passive muscle at low frequencies. This difference was significantly greater than the variation in the individual estimates of stiffness, which averaged ~6% for reflex and isolated muscle conditions. Figure 6B shows a plot of the phase angle versus frequency derived from the transfer function estimate. Over

Reflex stiffness consists of two components

The transient increase in stiffness illustrated in Fig. 3A is superimposed on a tonic stiffness produced by the tonic firing of the motoneurons. In a similar experiment, using a 20-Hz sinusoid (to increase the reflex activation of the VSM), the transient increase in stiffness was 18.2 ± 5.3% greater (0.001 < P, t-test) than stiffness at the end of stimulation. The nervous system then was removed from the VSM; the difference in stiffness between the beginning of stimulation and the end (not significant at the 0.05 level, t-test) was 3.2% and average stiffness dropped to 60% of that in the intact preparation. In the intact animal, shown in Fig. 3B, the transient stiffness was 15.3 ± 8.7% greater (0.01 < P, t-test) that at the end of stimulation.

The average total stiffness during reflex activation was calculated in several different ways. An initial estimate was obtained by calculating the ratio of the standard deviation of force and length changes during stimulation by noise of different average amplitudes. In four preparations, using 33 blocks of 10 trials, the relationship between length and force was highly significant; the reflex muscle was 2.0 ± 0.52 times as stiff as isolated muscle. A second estimate was obtained by calculating the estimated transfer function (from

FIG. 5. Cumsum determination of reflex threshold. --- cumulative sum of the interspike intervals, ⋯⋯ 95% confidence limits. 4r3m and 4r3l are recorded simultaneously.

limits. Figure 5 shows cumulative sum plots for 4r3m and 4r3l for a noise amplitude of 0.087 mm. In four preparations, there was a significant inverse relationship between force amplitude and reflex latency for 4r3m; 4r3l had a significant relationship in three preparations.

greater than ~0.075 mm, it asymptotically approaches a line horizontal to the abscissa. One possible explanation for this is that the high-frequency components of the noise might be disrupting the crossbridges; at more physiological vibration frequencies, stiffness might not decrease. However, a similar decrease in stiffness was observed using a 5-Hz sinusoidal vibration. Plotting force as a function of length during sinusoidal vibration shows that at small amplitudes (Fig. 8A) the stiffness during stretch is higher than at greater amplitudes (Fig. 8B), but there is no indication of a prominent short range yield. As in other muscles that have been examined by this method, the work performed on the muscle increases with amplitude (Josephson 1985; Rack and Westbury 1974). During bursts (Fig. 8B), the stiffness during the later portions of stretch increases with activation more than in the early portions of stretch.

**Reflex activation of the motoneurons is frequency dependent**

To study this, a “sweep” frequency stimulus was used to evoke the reflex. After an initial 10-s period of 1.0 Hz sinusoidal stimulation, the stimulus frequency was increased during a period of 10 s to 10 Hz and maintained for an additional 10 s. Figure 9A (isolated abdomen) and B (intact animal), shows the results of this experiment. At the onset of stimulation, there is little change in the tonic frequency of the motoneurons, but as the sinusoidal frequency increases to ~5–7 Hz, there is often an abrupt increase in the spike frequency of 4r3m, followed by a decrease over the remaining period of stimulation. The onset of this increase is variable (Fig. 9C), depending on the excitability of the preparation and the tension of the VSM. When the motor...
bursts and often there were two or three bursts, lasting from 1 to 2 s, separated by 2- to 3-s intervals. The histogram of burst times had a broad peak. In three experiments, the relationship between tonic frequency, in the 1-s interval before stretch, and burst frequency, in response to a constant ramp stretch, was investigated to determine if higher levels of tonic firing resulted in a larger number of spikes in the motoneuron reflex burst. However, there was no significant (0.05 < P) relationship between them.

**Reflex activation increases short interspike intervals**

The increase in peak frequency as a function of stimulus amplitude could arise either from an increase in tonic frequency or an increase in the number of short interspike intervals. To estimate this, the number of short (between 0.006 and 0.015 s) and long (>0.1 s) intervals was calculated (Fig. 10) for the 10-s prestimulus period and the first 10 s of stimulation. In each of four preparations, there was a significant increase in the number of short intervals during stimulation. In two preparations, there was a shift in the long interval peak toward shorter intervals as well, whereas in the other two preparations the position of the long interval peak was unchanged.

One explanation for the relative increase in the number of short interspike intervals during stimulation is that they are generated by mechanoreceptors firing in phase with the frequency. To examine this, histograms of the average spike times within the sinusoid cycle were calculated and compared with the null hypothesis that spike times were distributed uniformly throughout the cycle. Figure 11A shows an example of such a 5-Hz sine wave cyclogram; there is a significant peak ($\chi^2; P < 0.05$) at ~110 ms. Figure 11B shows that this peak is reduced significantly when sensory afferents are reduced by cutting the left and right first roots of the fourth ganglion. Three other experiments showed similar results; in two additional experiments significant peaks were observed only in the last 10 s of stimulation.

**Fig. 9.** Frequency threshold of reflex burst during increasing sinusoidal frequency. **A:** an oscilloscope photograph of beginning of the increase in frequency. Vertical calibration: force, 0.25 N; length, 0.25 mm; EJPs of 4r3m, 20 mV; EJPs of 4r3l, 10 mV. **B:** a digitized record of a sweep frequency experiment in an intact animal. **C:** a histogram of burst latency from the beginning of the increase in frequency from 1 to 10 Hz; 121 bursts in 6 preparations.

**Fig. 10.** Increase in short interval bursts of 4r3m during vibration. **Top:** interspike interval histograms with binwidths of 0.01 s. **Bottom:** interspike interval histograms with binwidths of 0.001 s of intervals <0.1 s from the same data as displayed in the top panels. I. region of the top histograms from which the bottom histograms are taken.
activated by stretch or release. During continuous sinusoidal or A curious feature of the VSM reflex is that it is most the circular and longitudinal layers of the VSM as well as ation may be a mechanism for regulating the gain of the motoneurons, the system cannot be acting as a conventional mechanoreceptors and muscles that generate an initial eleva-

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by increasing the coactivation of all the muscles of the posterior abdomen. Muscle stiffness thus is regulated by a combination of nonlinear muscle properties, reflex activation of the muscle, and habituation of mechanoreceptor activation of the motoneurons.

The evidence that the VSM reflex operates in this fashion derives from experiments in this paper as well as previous work (Chapple 1985, 1993). Ramp longitudinal stretch and release of the VSM produces short bursts in the motoneurons that are proportional to changes in force rather than its static level. Because length changes in each direction activate the motoneurons, the system cannot be acting as a conventional negative feedback controller. Motoneurons innervating both the circular and longitudinal layers of the VSM as well as those innervating the dorsal superficial muscles are coacti-

vated by stretch or release. During continuous sinusoidal or stochastic length changes, the longitudinal VSM motoneu-

rons increase their average frequency as the amplitude of the disturbance increases and their frequency decreases rap-

didly to control levels at the end of stimulation.

The mechanical effects of the combination of reflex and tonic activation of the muscle are an average stiffness during vibration between 2 and 2.5 times that of passive muscle. Both passive and reflexive muscle are more compliant at frequencies <10 Hz and have slight phase leads of between 10 and 20°. Although there are changes in the relative gains and phases at different frequencies as a function of muscle activation, these changes are relatively modest. The dynamics of the reflex preparation are also consistent with the dynamics of isolated muscle activated by electrical stimulation of the motor nerve (Chapple 1989). Thus there is no evidence that the reflex modifies muscle dynamics significantly, in contrast to cat and human muscle (Jacks et al. 1988; Poppele and Terzuolo 1968). It is interesting that, despite the absence of any effect of the reflex on overall dynamics, the length-force transfer function measured for the VSM is similar to that obtained for cat gastrocnemius with intact reflex pathways (Berthoz et al. 1971; Kirsch et al. 1994) and for postural reflexes in standing humans (Fitzpatrick et al. 1992, 1996). Over the physiological range, all these systems approximate a linear elastic substance.

A second feature of the mechanical response of the system to continuous stimulation is that VSM stiffness decreases with average stimulus amplitude. This decrease in muscle stiffness in response to stochastic perturbations of different amplitudes is also observed in cat (Kirsch et al. 1994) and human (Kearney and Hunter 1982). As in cat and human muscle, hermit crab VSM is linear and elastic for a particular stimulus amplitude, but as the average amplitude increases, stiffness decreases. The major portion of the amplitude-dependent decrease in stiffness occurs at small stimulus amplitudes; at greater amplitudes changes in stiffness are relatively modest. For rms amplitudes >0.075 mm, rela-

tively modest increases in motoneuron activation can in-

crease total stiffness enough to counteract the length-depend-

dent decreases. Thus the sharp decreases in stiffness at small vibration amplitudes may be physiologically irrelevant; for larger disturbances of more concern to the animal, muscle stiffness fluctuates around an average value.

Motoneuron activation is not constant throughout the pe-

riod of stimulation. At the onset of stimulation, there is a transient peak in motoneuron frequency and, correlated with this, a stiffness 20–30% greater than that at the end of stimulation. Because intact animals, as well as the isolated abdomen preparation, show similar decreases in motoneuron frequency and stiffness during stimulation, this decrease cannot be the result of the isolation of the abdominal motor centers from control by the higher CNS. One possibility is that this habituation of the response is purely central, possibly in the synapses between mechanoreceptors and motoneu-

rons. Alternatively, the initial peak in motoneuron frequency and muscle stiffness could be due to the coupling between mechanoreceptors and muscles that generate an initial elevation of motoneuron firing as a result of feedback. This habituation may be a mechanism for regulating the gain of the reflex, similar to many other systems (Laughlin 1989).

A curious feature of the VSM reflex is that it is most sensitive to mechanical frequencies >5 Hz; the present ex-

periments provide additional evidence for observations first made using ramp stretches of different velocities. Because many of the environmental stimuli that might be of potential importance in eliciting a load compensation reflex have sig-

nificant components only below this frequency [e.g., ocean waves (Denny 1988)], it may be that the reflex is activated by a more restricted stimulus such as turbulence within the

**FIG. 11.** Decrease in phase locked motoneuron firing during vibration with a 5-Hz sinusoid. Histograms are the mean events/bin (binwidth 18°) for 10 trials. Error bars are ±1 standard error of mean. A: 4rl1 and 5rl1 sensory roots intact. B: 4rl1 and 5rl1 sensory roots cut.

**DISCUSSION**

The results of the present experiments are consistent with the hypothesis that prolonged random disturbances activate mechanoreceptors signalling the absolute value of changing force to produce a time varying representation of disturbance intensity that activates motoneurons in a feedforward fash-

ion. This activation of the motoneurons increases abdominal stiffness by increasing the coactivation of all the muscles of the posterior abdomen. Muscle stiffness thus is regulated by a combination of nonlinear muscle properties, reflex activation of the muscle, and habituation of mechanoreceptor acti-

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dent decreases. Thus the sharp decreases in stiffness at small vibration amplitudes may be physiologically irrelevant; for larger disturbances of more concern to the animal, muscle stiffness fluctuates around an average value.

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nificant components only below this frequency [e.g., ocean waves (Denny 1988)], it may be that the reflex is activated by a more restricted stimulus such as turbulence within the
shell. Another possibility is that the higher threshold for low-frequency forces is a mechanism for reducing the positive feedback between mechanoreceptors and muscle activation, reminiscent of the presynaptic inhibition of mechanoreceptors by the lateral giant fiber in the crayfish during the escape response (Kirk and Govind 1990). Motoneuron activation of the muscles produces force changes with time constants on the order of 1–2 Hz (Chapple 1989). What is more, EMG recordings during movement in intact animals show few bursts in the motoneurons (Chapple 1973a). Thus a reflex that is activated by vibration with frequencies >5 Hz would limit the positive feedback from centrally generated activation of the muscles to the mechanoreceptors and back to the motoneurons. This explanation is also consistent with the apparent lack of correlation between tonic firing and burst frequency in response to stretch. A further implication of this argument is that these mechanoreceptors are not specialized for proprioception, despite the coupling between muscle activation and their frequencies. Although they are located beneath the integument, they respond to external forces as well as muscle activation; indeed, in a hydrostatic skeletal system such as the hermit crab abdomen, it is difficult to distinguish between the two.

Such an explanation is also consistent with the observations on the interspike interval histograms of tonic firing of 4r3m and 4r3l. It is likely from the experiments in which the majority of afferent pathways were removed that the short interval peaks are due to direct activation of the motoneurons by the mechanoreceptors. Cross-correlograms between sensory afferents and 4r3m have a peak at a latency of ~30 ms (Chapple 1993), similar to that observed in crayfish cheliped motoneurons by Wiens and Gerstein (1976). During rest and walking in the intact animal (Chapple 1973a), these short interval peaks were absent in 4r3m interspike interval histograms; in contrast light touch to the abdomen produced high-frequency bursts in the EMG.

A combination of feedback and feedforward enhancement of active muscle stiffness is found in both postural and locomotor systems in mammals. Although force feedback from Golgi tendon organs is inhibitory in nonlocomoting cats, during locomotion this force feedback changes to excitation (Conway et al. 1987; McCrea et al. 1995). Force feedback compensates for fatigue-induced changes in muscle stiffness during perturbations of elbow angle in humans (Kirsch and Rymer 1992). During human standing, Fitzpatrick et al. (1996) concluded that negative feedback alone is not adequate to maintain stability and must be supplemented by feedforward control. Thus postural regulation in hermit crab abdomen shares some features with mammalian motor systems.

A difficulty in determining the relative contributions of feedforward activation of the motoneurons and their feedback activation by the mechanoreceptors responding to muscle activation is that there are afferents in the third ganglionic motor root that reflexly excite the VSM motoneurons, in contrast to the homologous purely motor roots in crayfish and lobster. Thus it is not possible to open the feedback loop to study the feedforward gain of the reflex. This gain is important, not only because it might provide insight into the utility of the reflex for load compensation, but also because it may indicate other roles of the reflex in posture. Recent work in mammals (Horak and MacPherson 1996) suggests that there is a complex interaction between information from different modalities and regions of the body combining to form an internal model that then is used to control posture dynamically. It is well established that reflex sign and gain vary with behavioral state in a variety of animals (Prochazka 1989), including crustaceans (DiCaprio and Clarac 1983; Skorupski 1996). Moreover, signals from statocysts and visual centers are combined with mechanoreceptor signals to control postural orientation in crustaceans. If local mechanoreceptor signals are indeed combined with more global estimates of orientation, a reflex with a high gain might overwhelm signals from other sources.

The evidence, therefore, is that the reflex gain due to feedforward and feedback pathways is quite low, only twice the stiffness of passive muscle. However, longitudinal length changes of the abdomen are likely to underestimate total abdominal stiffness, due to the complexity of hydrostatic skeletons; longitudinal stretch of the VSM is opposed by forces from other muscle layers as well as the longitudinal muscle fibers. There are circular and helical muscle layers that are located between the cuticle and the longitudinal muscle layer. Each is composed of a single layer of small muscle fibers. In the isolated abdomen preparation, longitudinal length changes would, in theory, not affect the circular muscle fibers (that are orthogonal to the direction of stretch). However, the helical muscle fibers, which make an angle of 60–70° to the longitudinal axis, would contribute to longitudinal stiffness. These two muscle layers have not been studied in these experiments because they are difficult to record from with floating electrodes. The circular layer muscle fibers are coactivated during transient longitudinal stretch (Chapple 1993), so that it is likely that all three muscle layers cocontract during reflex activation. In the intact abdomen, this cocontraction of the three muscle layers would increase the stiffness of the abdomen by a greater amount than would be predicted on the basis of a longitudinal stretch of the isolated abdomen because of the mechanical coupling between muscle layers produced by the hydrostatic pressure.

The fixed ratio of the tonic frequencies of the three motoneurons suggests that there is some common source of presynaptic excitation. Under some conditions, these ratios are not maintained, however, suggesting that this source of excitation is labile. The relative increases in motoneuron frequency with stimulus amplitude also differ between preparations, suggesting a diffuse and labile source of presynaptic excitation. Moreover, the autocorrelograms do not have prominent periodicities but flattens out at longer delays, a feature of motoneuron firing in crayfish cheliped motoneurons well (Lindsey and Gerstein 1979). This implies that a number of presynaptic elements may contribute to motoneuron excitability. This is consistent with the investigations of premotor interneurons by Larimer and his colleagues in crayfish (Larimer 1988; Murphy et al. 1989) and Page and his colleagues in lobster (Kotak et al. 1988; Sukhdeo and Page 1992). In crayfish, a number of premotor interneurons with different properties activate the superficial flexors (the homologues in macrurans of the VSM) and superficial extensor motoneurons. Such a population of randomly firing interneurons converging on the motoneurons would be expected to produce random firing in the motoneu-
rons themselves. Changes in the levels of activation of different members of the premotor interneuronal population might alter the frequency ratio of the individual motoneurons, a possible explanation for the different ratios of motoneuron firing calculated in Table 1.

The VSM reflex works in concert with the mechanical properties of the abdominal muscles to increase substantially muscle force to resist external disturbances more rapidly than can be achieved by negative feedback. Because the mechanical dynamics of the muscle do not change with activation level, an increase in average motoneuron frequency increases stiffness without affecting the stability of the control. Although this exploitation of muscle elastic properties is a common feature of postural systems (Horak and MacPherson 1996; Rack 1981), it is particularly advantageous in a system in which the speed of contraction of the muscles is quite low. Thus these results are consistent with the hypothesis that a robust feedforward regulation of abdominal stiffness during continuous disturbances is achieved by mechanoreceptors signalling the absolute value of changing forces; habituation of the reflex, its high-threshold for low frequency disturbances, and the activation kinetics of the muscle further shape reflex dynamics.  

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