

Neuromuscular Strategies Underlying Different Behavioral Acts in a Multifunctional Crustacean Leg Joint

Joseph Ayers* and François Clarac**

CNRS, INP-10, 31, chemin Joseph Aiguier, F-13274 Marseille Cedex 2, France

Accepted July 5, 1978

Summary. 1. The movements of the merocarpopodite (M-C) joint of the rock lobster were recorded simultaneously with electromyograms from the extensor, flexor and accessory flexor muscles during unrestrained walking in all directions. In optimal recordings it was possible to distinguish the activity of several identifiable motor neurons.

2. During lateral walking, the M-C joint makes vigorous movements (Table 1), due in large part to strong alternating discharge in the antagonistic muscles (Fig. 2, Table 2). During forward and backward walking, the movements of the M-C joint occur over a more restricted angular range (Table 1). Here the antagonistic muscles are co-activated (Fig. 2, Table 2), so that the joint functions more as a rigid strut.

3. Extreme variability was observed in the timing of the discharge of several identified motor neurons between steps during walking in a given direction (Table 3). Implications with regard to the nature of the pattern generator are discussed.

4. Major differences were observed in the discharge frequency of these identified motor neurons between walking modes when the corresponding muscles served a power stroke, return stroke or postural function (Fig. 8, Table 4). Variability occurred in the discharge of some units between steps in some walking directions, but was not directly related to step period (Figs. 9, 10; Table 4).

Introduction

Animals commonly employ the same locomotory appendages to produce many fundamentally different

* Supported by US-France Exchange of Scientists Fellowship FR-016

Current address: Department of Biology and Marine Science Institute, Northeastern University, Boston, Massachusetts 02115, USA

** Supported by ATP INSERM No. 29.76.61

Current address: Laboratoire de Neurobiologie Comparée, Institut de Biologie Marine, 2, rue du Professeur Jolyet, F-33120 Arcachon, France

behavioral acts. For example, most vertebrates can use their hindlimbs to stand, walk, run, swim, jump, copulate and scratch. Furthermore, many animals can locomote with equal facility in all directions. A simple invertebrate model for this latter phenomenon is provided by walking behavior in decapod crustacea. Decapods, such as lobsters and crayfish, apparently utilize the same neuronal oscillator system to produce cyclic walking movements in all directions (Ayers and Davis, 1977a; Clarac and Ayers, 1977). In this system, some joint movements which are synergistic for walking in one direction become antagonistic for walking in the opposite direction. It has been concluded that in the overall organization of walking behavior in Crustacea, the difference in the nature of the behavior results from a shift in the coordination of some bifunctional basalar joint motor units relative to a common return stroke pacemaker (Ayers and Davis, 1977a).

A similar organization is observed in the control of the more peripheral merocarpopodite (M-C) joint of the crustacean walking leg (Clarac, 1977; Clarac and Ayers, 1977). During lateral walking on the leading side, propulsive forces are provided by a vigorous flexion of the M-C joint, whereas, an extension movement of this same joint underlies the power stroke on the trailing side (Clarac and Coulmance, 1971). In contrast, during forward and backward walking, propulsive forces are provided by movements of the more basalar thoraco-coxal (THC) joint. Here, the M-C joint apparently functions only as a strut with little vigorous movement (Ayers and Davis, 1977a; Clarac and Ayers, 1977). The M-C joint motor neurons are therefore presumably controlled in several distinct coordination modes, each of which is specific for a different locomotory act.

It is of considerable interest to determine the neuromuscular mechanisms which the central nervous system uses to elicit these different coordination modes, and the M-C joint system is quite amenable to such analysis (Clarac and Ayers, 1977a). In con-

trast to vertebrate motor systems, decapod Crustacea offer considerable simplicity in their functional organization. Extension movements of the M-C joint are achieved by contractions of the merocarpopodite extensor muscle which is controlled by only two exciters and a peripheral inhibitor motor neuron. Flexion movements of this joint are produced by two muscles which operate in parallel: the flexor (F) muscle and the accessory flexor (AF) muscle. The flexor muscle is controlled by a phasic flexor motor neuron, three tonic flexor motor neurons and a peripheral flexor inhibitor motor neuron (Wiersma and Ripley, 1952). The accessory flexor muscle is controlled by the accessory flexor motor neuron and the peripheral inhibitor which it shares with the extensor muscle (Angaut-Petit et al., 1974). Due to this relative paucity of motor innervation it is possible to distinguish the activity of several identified excitatory motor neurons in favorable extracellular myograms in freely behaving animals.

The present study utilizes techniques which enable us to chronically record the activity of four identified motor neurons: the tonic extensor (t-E), the phasic extensor (p-E), the phasic flexor (p-F), the accessory flexor (AF) and the tonic flexor pool simultaneously with the angle of the M-C joint in freely behaving lobsters during walking in all directions. Through quantitative analysis we address the following questions: (1) What overall patterns of synergistic and antagonistic discharge characterize the motor output in the four walking modes? (2) What are the sources of variability within a walking mode in the relative timing of the discharge of identified motor neurons? and (3) How does the organism grade motor output in intensity in different identified motor neurons when it walks at varying stepping frequencies in the four directions? These experiments are aimed at characterizing normal motor output in unrestrained animals. In a subsequent report (Clarac and Ayers, 1978), we examine the effects of proprioceptive perturbations on the motor output of these motor neurons to ascertain, in part, the role of proprioceptive feedback in the production of the final motor score.

Materials and Methods

Rock lobsters, *Palinurus vulgaris*, were used in all experiments. Animals were obtained from a local distributor (Sommepron, S.A., Marseille), maintained in a closed filtered sea water system and fed live mussels on a sporadic schedule. All experiments were performed on the fourth right walking leg as the normal axis of movement of this leg is nearly perpendicular to the longitudinal body axis and its movements consist of a simple rowing action during forward and backward walking. The basalar axis of the more anterior walking legs of this species are oriented more ros-

trally such that they tend to push or pull during walking in these directions.

Recording Techniques

Movements. The angle of the M-C joint was monitored with a capacitive movement transducer (Sandeman, 1968) which was arranged to measure the movements of a signal wand attached to the carpopodite between two antennae which were attached to a wire frame mounted on the distal end of the meropodite (Fig. 1b). As this type of transducer is somewhat non-linear under the conditions of the present experiments, the apparatus was calibrated frequently between walking bouts with regard to the DC voltage produced over the full range of joint angles.

Electrophysiology. Techniques for electromyographic recording from lobster walking leg muscles can be found in Ayers and Davis (1977a). In general, several implantations were made into each muscle and the best pair of electrodes was selected during the course of the experiment. Poor recordings could often be greatly improved by passage of DC current from a battery through the recording electrodes. In the present study, recordings were made from freely behaving animals in an aquarium. To insure complete mobility, the recording leads were attached to a mini-connector which was mounted on a small float (Fig. 1). The lobster and attached wiring harness could then be removed from the recording apparatus for implantation or storage between recording sessions. The float was apparently frictionless on the surface and provided no obvious restraint on the animals movements.

Interpretation of Records. Electrodes of the type used in the present experiments record field potentials generated by muscle EJP's. In favorable recordings, the potentials generated by different identifiable motor units of the same muscle are distinguishable on the basis of the number of inflections and relative amplitude of positive and negative components. In addition, some potentials exhibit cross talk on the electrodes which record from different muscles. Thus, if the muscle activity is recorded at high enough speeds (ca. 5–10 cm/s), it is typically possible to distinguish the activity of several identifiable motor neurons. For example, the extensor muscle is innervated by only two excitatory elements, the tonic and phasic extensors, which typically can be differentiated on the basis of impulse amplitude. Similarly, the accessory flexor muscle receives only one excitatory axon. Recordings from the flexor muscle often presented ambiguity. The flexor receives three tonic excitatory axons which can only be differentiated in the most fortuitous recordings. Our description in this case will treat these units as a pool. The activity of the phasic flexor was typically unambiguous for its activity was commonly observed as cross talk on the accessory flexor recording whereas the activity of tonic units was not. No attempt was made to interpret the activity of the peripheral inhibitors.

Experimental Strategy

In the present study, an attempt was made to characterize the normal locomotor output underlying walking in the four directions. In contrast to the treadmill situation (Ayers and Davis, 1977a), where both the speed and direction of locomotion are under direct experimental control, considerable difficulty was experienced in eliciting long sequences of walking in a given direction. Animals could often be induced to walk by a generalized mechanical stimulation or by application of weak electrical fields on the side of the body opposite to the intended direction of locomotion. Such stimulation was produced by bipolar silver wires which were attached to a light wand and connected to a small battery.

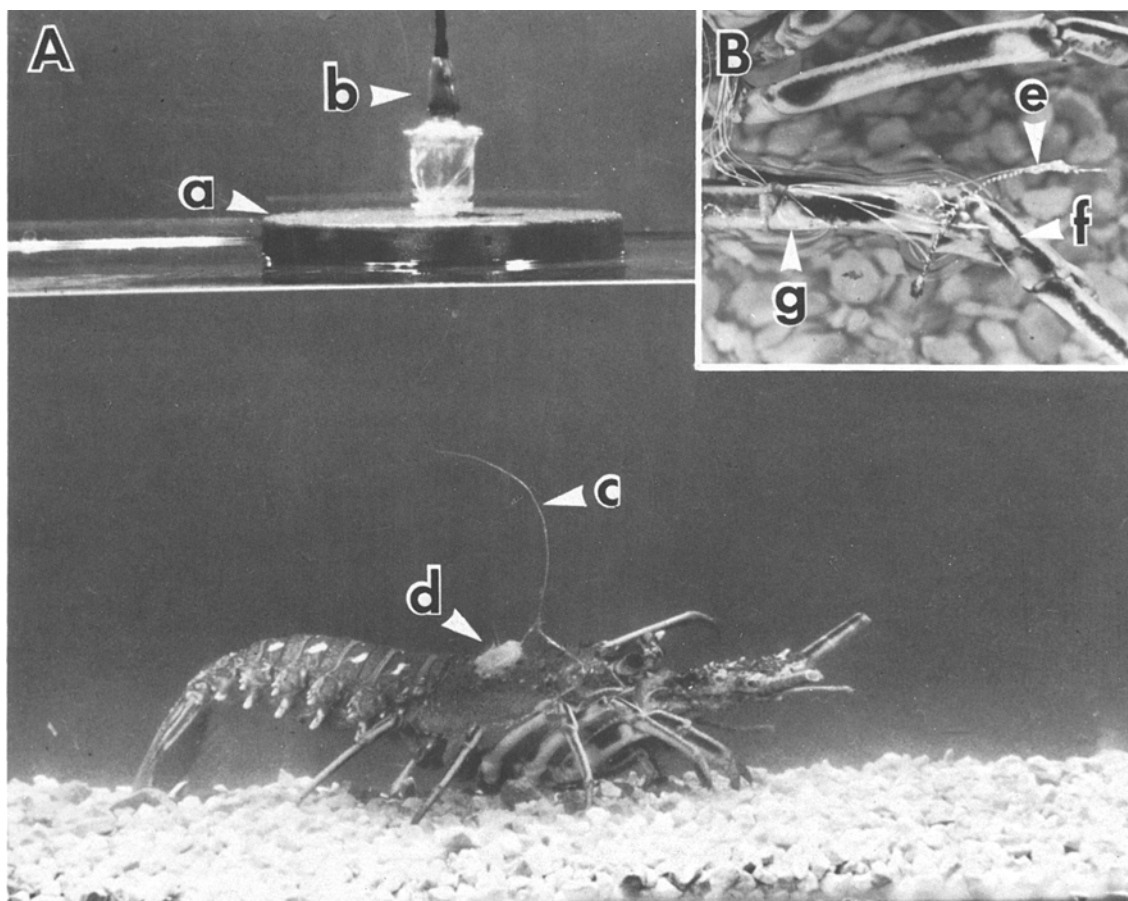


Fig. 1 A and B. Experimental system utilized for recording in vivo. **A** Overall view of the recording system. **B** Detail showing movement transducer system and a typical electromyographic implantation. Recording leads (*c*) were mechanically attached to the carapace with dental cement (*d*) and lead off to a mini-connector (*b*) which was supported by a small float (*a*). Electromyographic recording leads were sealed to the overlying cuticle with wax (*g*). Inputs to the movement transducer consisted of a signal wand attached to the carpopodite (*f*) which moved between two antennae mounted on a wire frame (*e*) affixed to the meropodite

The electromyographic and positional activity was recorded on FM tape simultaneously with a vocal log. The general experimental strategy was for one investigator to initiate locomotion while recording a step by step vocal log of the direction of evoked locomotion. The vocal log was then utilized to define sequences of walking in a given direction during subsequent filming.

Quantitative Analysis

Quantitative analysis of the activity of identified units was performed on steps selected from the middle of longer sequences of walking in a given direction. A step is defined as the time interval from the mid-point of a burst in the anterior basipodite elevator (see Ayers and Davis, 1977a), to the mid-point of the subsequent elevator burst. The data were digitized directly from film records using a magnetic tablet which punched the x-y coordinates of each spike on paper tape. The raw data were then converted from magnetic tablet coordinates to time for subsequent analysis on a PDP 11-45 digital computer.

Three types of graphical displays are used in our analysis:

Phase Histograms. In phase histograms, phase is defined as the time interval from the origin of the step to the time of occurrence of a muscle spike divided by the period of the step cycle. The

histogram thus expresses where muscle impulses of a unit occur in the step cycle, independent of the cycle period, and allows the comparison of the relative timing of events in steps of different periods.

Since the bursts of muscle activity which occur during walking are periodic it is necessary to utilize circular statistics to describe the distributions (Batschelet, 1965). Two statistics were computed in the present experiments. Mean angle of a circular distribution is the average phase position of motor neuron spikes and is analogous to the arithmetic mean of a continuous distribution (Zar, 1974). Angular deviation of a circular distribution is analogous to the standard deviation of a continuous distribution, and thus expresses the amount of variation in the circular sample.

Interval Histograms. In interval histograms, the abscissa is the interval between successive impulses in the step cycle. This quantity is thus the inverse of the instantaneous discharge frequency within the step.

Frequency-Burst Period Plots. In frequency-burst period plots, the average instantaneous discharge frequency is calculated for each step and plotted versus the burst period (referenced to elevator burst midpoints) of that step. These diagrams thus indicate whether the intensity of discharge is a function of step frequency.

A Question of Semantics. Many crustacean motor neurons have been classified as either tonic or phasic based upon their neuromuscular properties and thresholds to inputs (e.g. Atwood, 1973). Furthermore, the terms tonic and phasic have also been utilized to describe firing patterns in many systems (e.g. Kennedy and Davis, 1977). In view of such semantical ambiguities, we have restricted our usage of the terms phasic and tonic to naming different identified motor neurons (e.g. tonic extensor, phasic flexor etc.). This terminology is not intended to indicate the neuromuscular properties of these elements or their firing patterns. But merely to adhere to the conventional nomenclature for these elements (Atwood and Walcott, 1965; Clarac, 1977; Vedel et al., 1975).

Results

Rock lobsters only occasionally exhibit spontaneous locomotion when confined in an aquarium as in the present experiments. They are typically rather sedentary, but readily orient to movements in their visual field. By cautious stimulation with either a mechanical probe or with weak electrical fields it was often possible to evoke short sequences (i.e. 6–10 steps) of walking in a given direction, for the length of the aquarium (ca. 150 cm). In favorable animals, it was often possible to also control the velocity of walking within such a bout. Most animals, however, change walking direction within a bout, i.e. walking backwards then turning and walking laterally etc. Some animals preferentially walk in a particular direction (i.e. forwards or backwards etc.) and can only be induced to walk in other directions with difficulty.

Several types of behavioral variability can be observed during walking in a given direction. For example, animals will often walk at different heights above the substrate or vary their height systematically within a walking bout. Some animals will exhibit defensive maneuvers with their antennae, other superimposed postural contortions or occasional tail flips. Our analysis is confined to walking bouts where such extraneous activities or obvious height variations did not occur.

1. Joint Movements During Walking

Several previous investigations have indicated that positional proprioceptors may play a role in the patterning of the motor output in arthropod systems (Wendler, 1966; Pearson et al., 1976; Evoy and Fourtner, 1973), by initiating or terminating motor neuron bursts at a fixed joint position. In the present system, the power stroke or return stroke can be terminated during either extension or flexion and inputs from positional proprioceptors may be therefore functionally ambiguous. Furthermore experiments on

Table 1. Mean joint angles (deg.) of maximum extension, maximum flexion and angular excursion of merocarpopodite joint power stroke movements during walking in the four directions. The joint angles were determined using a capacitive movement transducer (Fig. 1, Sandeman, 1968). Measurements were taken only from steps selected from the middle of longer sequences of walking in a given direction. The data were pooled from several experiments with a single animal. *n* is the number of steps used in the computation of averages. See Figure 2 for reference coordinates

	Maximum extension	Maximum flexion	Angular excursion	<i>n</i>
Forward	121.1	94.8	26.3	56
Backward	125.8	96.2	29.6	45
Leading	133.4	66.1	67.3	65
Trailing	136.2	74.3	62.0	63

treadmill induced walking (Ayers and Davis, 1977a), have indicated that movements of the M-C joint are larger in amplitude during lateral walking than during forward and backward walking. These angular constraints of joint movement have not previously been examined in freely behaving animals.

To ascertain the angular constraints of movement of the M-C joint in unrestrained animals, we have determined the angles of maximum extension and flexion of this joint during cyclic walking in the four directions (Table 1). During forward and backward walking, the movements of the M-C joint occur over a similar restricted angular range (Table 1). In contrast, the total amplitude of joint movement observed during lateral walking is more than twice that of forward and backward walking (Table 1). This increase in the amplitude of the power stroke movement is realized largely by an increase in the degree of maximum flexion of the joint when compared to the situation during forward and backward walking (Table 1). Notice that the angle of termination of the power stroke during lateral trailing is nearly identical with the angle of termination of the return stroke movement during lateral leading.

2. General Organization of Muscle Activity

The walking movements in all four directions can be divided into two phases: the swing phase or return stroke and the stance phase or power stroke when the limb actually provides propulsive force. When compared to the motor output of the THC and CB joint muscles (Ayers and Davis, 1977a), the output of the M-C joint muscles is much more variable. Examples of electromyograms from the elevator of the CB joint and the three M-C joint muscles are presented in Figure 2. Walking in all directions is

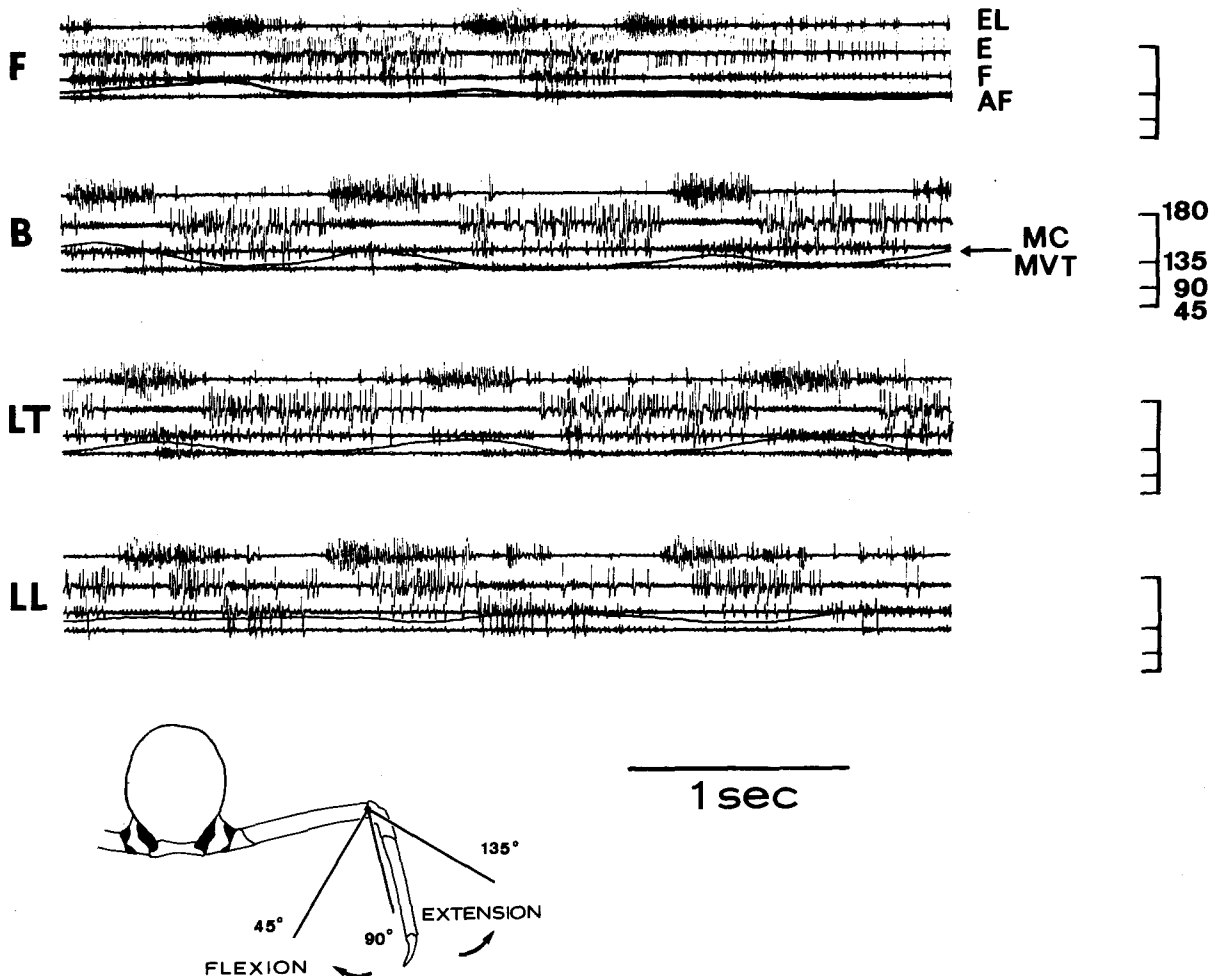


Fig. 2. Electromyographic recordings during walking in the four directions. The four records consist of simultaneous recordings from the anterior basipodite elevator of the CB joint (*EL*), the extensor of the M-C joint (*E*), the main flexor of the M-C joint (*F*) and the accessory flexor (*AF*) during forward (*F*), backward (*B*), lateral leading (*LL*) and lateral trailing (*LT*) walking. M-C MVT is a DC voltage which indicates the angle of the M-C joint. Reference coordinates for the joint angle are shown in the diagram at the bottom. Note in the interpretation of this figure that some of the activity in a given trace may represent cross talk from other muscles which is unresolvable at such slow recording speeds (see Materials and Methods)

characterized by rhythmic bursting in the elevator which is accompanied by either bursting or less patterned discharge in the M-C joint muscles. Four characteristic modes of M-C muscle discharge are apparent. During forward walking, both the extensor and flexor muscles discharge synergistically and tend to alternate with the elevator. During backward walking, the flexor discharges randomly, and the extensor bursts in alternation with the elevator. During both forward and backward walking, the accessory flexor exhibits unpatterned discharge.

The motor output which underlies lateral trailing walking in the extensor and flexor muscles is generally similar to that which underlies backward walking. The major difference is that the accessory flexor now exhibits a strong burst during the end of the return stroke. In contrast, during lateral leading walking,

rhythmic bursts in the extensor and flexor muscles alternate, with the extensor discharging synergistically with the basipodite elevators.

3. Motor Programs in Identified Motor Neurons

In order to extend the above qualitative description to the level of a quantitative description of the activity of individual identified motor neurons, we have performed a statistical analysis of the activity of each identifiable unit which can be recognized from electromyograms. In the following analysis, we determine the circular statistical distribution (Batschelet, 1965), within the step cycle of muscle potentials in identified units from data pooled from several steps. In *Homarus*, the limb elevators of the coxo-basal joint have

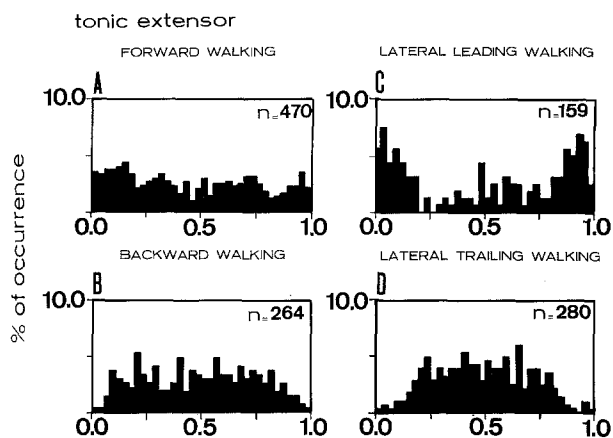


Fig. 3. Timing (phase) of the tonic extensor motor unit activity during walking in the four directions. Each histogram expresses the phase position of occurrences of tonic extensor impulses during several (5–11) cycles of ongoing walking in a given direction. All histograms are referenced (i.e. phase 0.0 and 1.0) to the mid-point of the burst of spikes in the anterior basipodite elevator muscle (Fig. 2). n is the number of muscle potentials

Table 2. Mean phase angles and angular deviations of muscle spikes in different identified motor neurons. All values referenced to the mid-point of the anterior basipodite elevator burst. Data averaged from 5–11 steps

	Forward	Backward	Leading	Trailing
t-EXT	86.8 (122.3)	103.7 (97.3)	289.9 (83.0)	108.6 (86.3)
p-EXT	97.6 (112.6)	105.6 (92.7)	323.6 (37.4)	111.1 (64.6)
t-FLX	102.7 (71.4)	94.0 (106.1)	110.9 (79.5)	83.7 (105.1)
p-FLX	237.5 (65.3)	99.8 (106.7)	103.6 (69.6)	119.7 (59.3)
AF	93.3 (111.9)	90.0 (105.1)	246.8 (77.5)	79.5 (93.7)

been considered the pacemakers of the walking system (Ayers and Davis, 1977a). We have therefore referenced the discharge of the M-C joint muscles to the mid-point of the anterior basipodite elevator burst in the present investigation.

Tonic Extensor. The statistical analysis of the activity of the tonic extensor motor neuron (t-E) is shown in Figure 3 and Table 2. This neuron exhibits three characteristic types of activity relative to the basilar leg muscles. During forward walking, its activity is unpatterned, with the mean phase position of spikes near the termination of the elevator burst (Table 2). During lateral leading walking, the tonic extensor burst occurs synergistically with the elevator burst

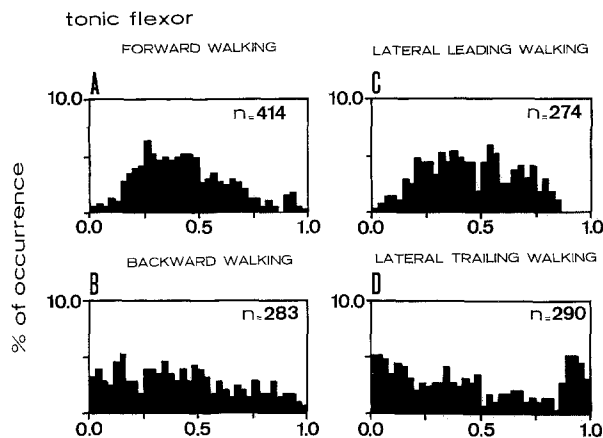


Fig. 4. Timing (phase) of the tonic flexor motor neuron pool activity during walking in the four directions. These histograms represent the summed activity of the 3 tonic flexor (*t-MCF*) motor neurons. All conventions as in Figure 3

(Table 2) and is much less diffuse (Fig. 3c, Table 2). The discharge of the tonic extensor is very similar during both backward and lateral trailing walking. In both cases, the unit exhibits fairly discrete bursts (Figs. 3b, d; Table 2) with similar mean angles.

Tonic Flexors. The main flexor muscle is innervated by three tonic excitatory motor neurons. Electromyograms from this muscle, therefore, present considerable ambiguity in the identification of individual tonic motor units. Due to this potential misinterpretation, we have therefore treated these tonic units as a pool. The statistical analysis of the activity of this pool is presented in Figure 4 and Table 2. The tonic flexors exhibit, in general, two characteristic patterns of activity. During both forward (Fig. 4A) and lateral leading (Fig. 4C) walking, the tonic flexors exhibit discrete bursts which alternate with the elevator activity. During both backward and lateral trailing walking, the discharge of the tonic flexors is much more diffuse. Here, these units exhibit rather unpatterned activity. During lateral trailing, however, there is a tendency for the flexors to burst synergistically with the elevators.

Accessory Flexor. The statistical analysis of the locomotory activity of the excitatory accessory flexor unit is shown in Figure 5 and Table 2. Like the tonic extensor, the accessory flexor exhibits three characteristic patterns of activity. During both forward and backward walking, the accessory flexor discharges rather tonically with the mean spike phase position corre-

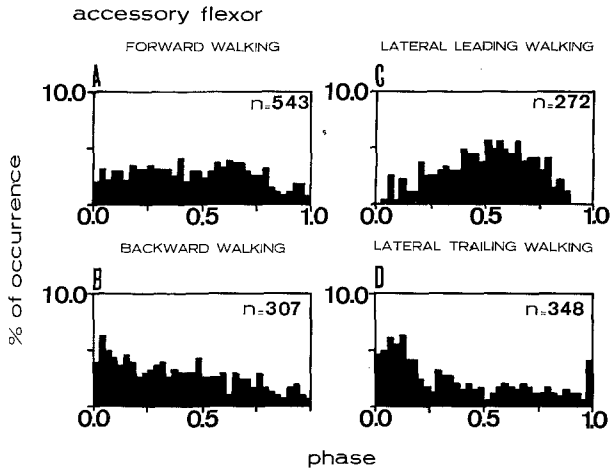


Fig. 5. Timing (phase) of the excitatory accessory flexor motor neuron activity during walking in the four directions. All conventions as in Figure 3

sponding to the end of the elevator burst. In contrast, during lateral leading (Fig. 5C) and lateral trailing (Fig. 5D), the accessory flexor bursts antagonistically or synergistically respectively with the coxo-basal elevator. Notice, however, that the AF burst during lateral trailing is superimposed upon a background discharge which is absent during lateral leading.

Phasic Extensor. The discharge of the phasic motor units is highly variable. The phasic extensor discharges during walking in all four directions and the statistical analysis of its activity is presented in Figure 6 and Table 2. The discharge of this unit occurs largely synergistically with that of the tonic extensor. For example, compare the mean angle and angular deviations (Table 2) of activity in the tonic and phasic extensors during backward and lateral trailing walking. During forward and lateral leading walking the activity of the phasic unit is slightly phase delayed relative to that of the tonic unit.

Phasic Flexor. In contrast to the phasic extensor, the phasic flexor normally discharges only during backward and lateral leading walking (Fig. 6, Table 2). In both cases, the phasic units exhibit nearly identical mean angles and angular deviations to those of the tonic flexors. Phasic flexor activity is rarely observed during forward or lateral trailing walking and never exceeds 1 or 2 spikes per cycle.

4. Sources of Variation in the Timing of Discharge of Identified Motor Neurons Within Motor Programs

Transitions Between Coordination Modes. Lobsters only rarely walk for long sequences in a given direc-

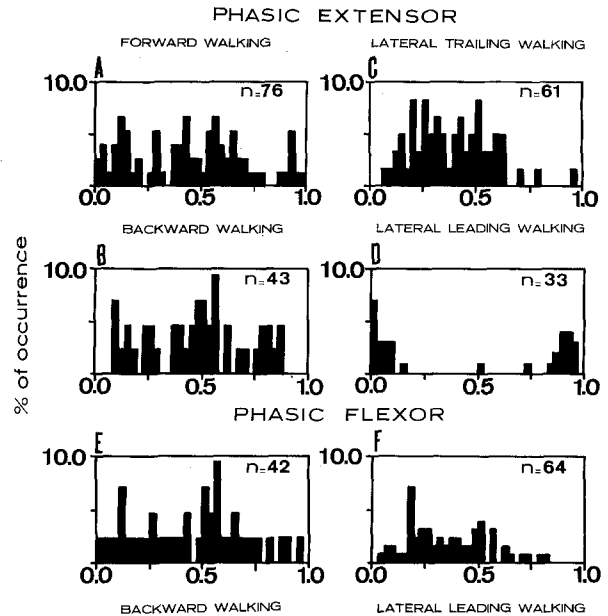


Fig. 6A-F. Timing (phase) of phasic motor neuron activity during walking in the four directions A-D Locomotory discharge of the phasic extensor (p-E) motor neuron. E-F Locomotory discharge of the phasic flexor (p-F) motor neuron. All conventions as in Figure 3

tion in the experimental situation we have employed. In the previous analysis we have attempted to restrict our description to ongoing locomotion in a given direction, but it must be recognised that step by step transitions between modes or even hybrid modes (i.e. walking diagonally) are common in most walking sequences. Examples of electromyograms from the M-C joint muscles during such transitions between coordination modes are shown in Figure 7. The most common transitions are from lateral walking to either forward or backward or the converse from forward or backward to lateral walking. Reversals of walking direction (i.e. from forward to backward etc.) are less common, but nonetheless occur. Such transitions in walking direction are typically brought about by one or both antagonists changing from bursting to unpatterned discharge or the converse (Fig. 7).

Hybrid Coordination Modes. Lobsters are not restricted to walking in the four perpendicular directions, but may often employ both the thoraco-coxal and merocarpopodite joints to walk diagonally. The electrophysiological correlates of such behavior are unfortunately rather complicated and are not sufficiently stereotyped for quantitative characterization and have therefore been omitted in our analysis.

Variability in Timing Within a Coordination Mode. In the previous analysis (Table 2, Figs. 3-6), we presented timing data averaged from several (5-11)

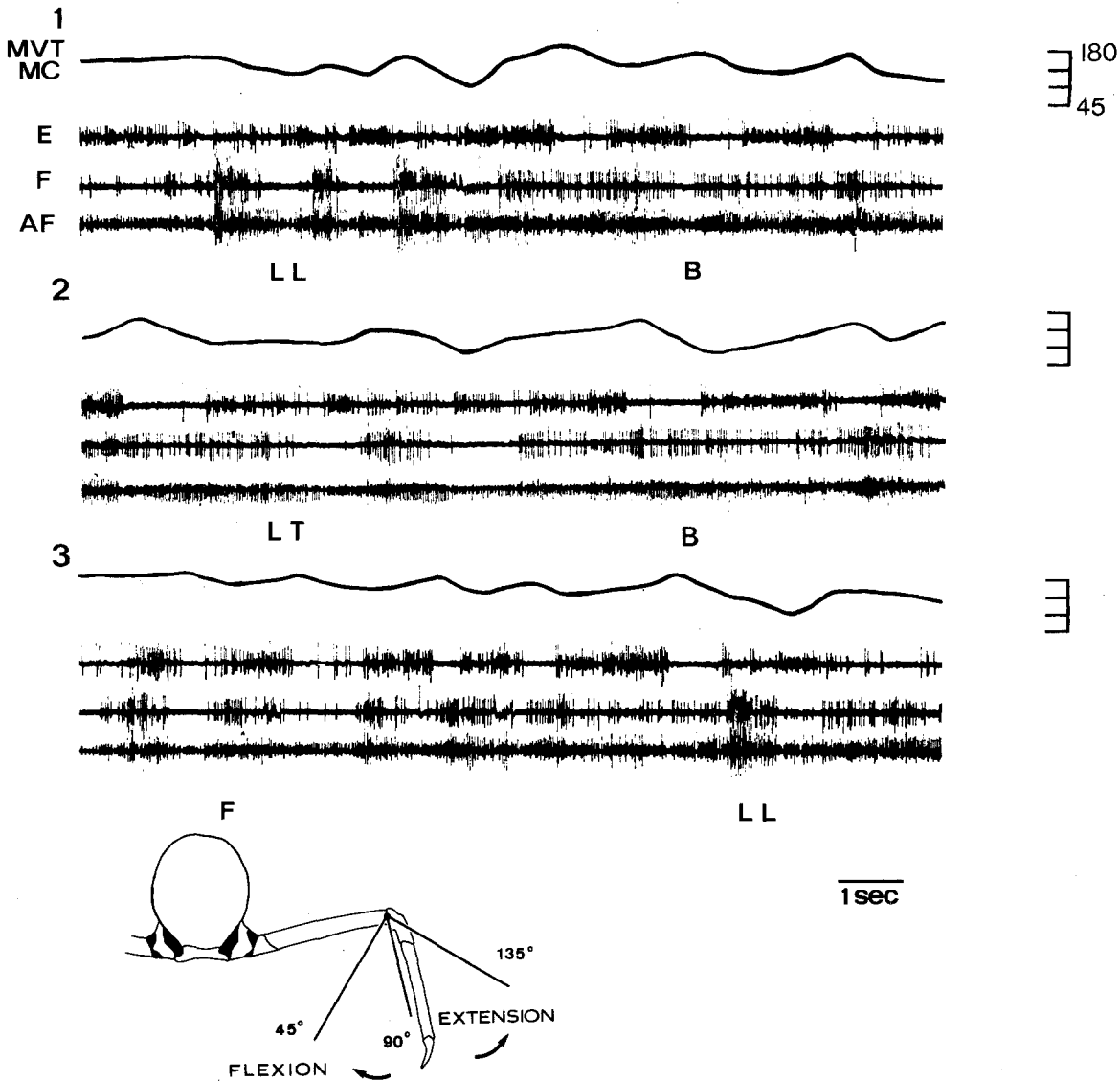


Fig. 7. Electromyographic recordings during transitions between coordination modes. Each of the three panels is a continuous recording from the three M-C joint muscles: extensor (*E*), flexor (*F*) and accessory flexor (*AF*). (1) Transition from lateral leading (*LL*) to backward (*B*) walking. (2) Transition from lateral trailing (*LT*) to backward (*B*) walking. (3) Transition from forward (*F*) to lateral leading (*LL*) walking. All other conventions as in Figure 2

steps in order to establish the general organization of the motor programs in these identified motor neurons. Such analysis, unfortunately, obscures the fact that in some coordination modes the timing of discharge of several of the motor neurons is extremely variable. To further illustrate this variability, we have compiled a table of the mean phase angle and angular deviation of the discharge of two identified motor neurons (tonic extensor, accessory flexor) in several individual steps (Table 3).

Several important conclusions obtain from such analysis. First, depending upon the walking direction, the motor output can be extremely variable in timing and this variability is not directly related to step

period. For example, during forward walking the tonic extensor can discharge at mean phase angles ranging from 39 to 108 degrees (Table 3). Similarly, the angular deviation can range from discrete bursts (dev. = 38 deg) to unpatterned discharge (note in this context, that the apparent tonic discharge which obtains in averaged timing data may actually represent bursting which occurs at random phase positions). In contrast, during backward and leading walking, the timing becomes more consistent, although divergent bursts occasionally occur. Secondly, the step by step variations in timing in corresponding bursts in the two motor neurons are not related but appear to be independent. Thirdly, in many cases, the motor

Table 3. Mean phase angle and angular deviations (dev.) of muscle potentials in simultaneous single steps of the tonic extensor and accessory flexor motor neurons. All computations are referenced to the mid-point of the anterior basipodite elevator burst

Burst period (s)	Tonic extensor		Acc. flexor	
	Mean angle	Dev.	Mean angle	Dev.
Forward				
1.50	100.8	99.2	94.0	125.9
1.64	39.4	38.5	261.8	78.5
1.71	46.4	48.1	245.7	71.3
1.89	107.6	87.2	97.0	92.0
2.05	71.7	82.6	99.2	78.1
2.35	102.4	45.6	87.6	91.5
2.49	65.1	75.1	253.3	90.4
Backward				
1.15	257.7	97.2	85.1	124.1
1.19	237.5	63.9	89.6	81.2
2.06	262.4	113.4	89.6	109.0
2.36	88.9	132.1	96.1	110.3
3.52	269.6	144.1	89.1	92.2
Lateral leading				
1.54	301.3	65.5	112.1	80.1
1.63	288.3	82.2	231.2	40.4
1.76	275.0	90.2	104.2	92.1
1.91	308.1	56.2	233.3	57.2
2.27	76.4	96.5	243.1	63.2
Lateral trailing				
1.42	295.1	74.9	263.3	96.8
1.52	248.7	81.4	107.0	82.8
1.73	118.5	70.3	77.4	84.0
2.13	112.5	77.1	71.9	79.7
2.22	242.8	71.7	59.4	52.9

neurons appear to have two stable phase positions at which they may discharge. For example, during forward walking the accessory flexor discharges either around 95 deg. or around 250 deg. This finding also obtains for the discharge of the tonic extensor during lateral trailing.

5. Gradation of the Intensity of Motor Output in Identified Neurons

Tonic Motor Neurons. In the present system, different motor neurons can subserve different functional roles depending upon the direction of walking. For example, the discharge of the tonic extensor underlies the return stroke during lateral leading, the power stroke during lateral trailing and functions more in a postural role during forward and backward walking. In order to determine whether these changes in functional role are accompanied by a corresponding alteration in the discharge frequency, we have examined the average inter-impulse interval during walking in the four directions in the tonic extensor and accessory

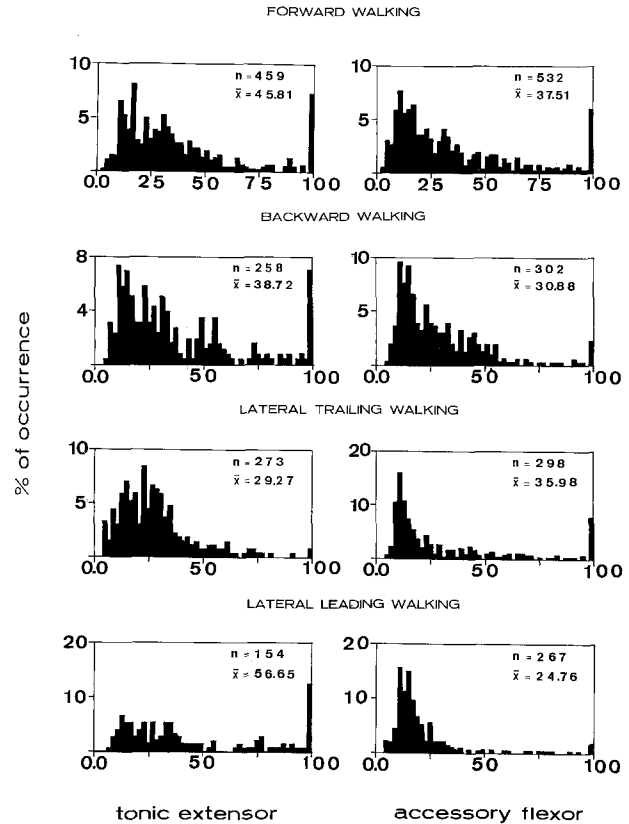


Fig. 8. Histograms of the inter-impulse interval between impulses in the tonic extensor and accessory flexor motor neurons during several (5–11) steps of walking in the four directions. \bar{x} is the mean inter-impulse interval. n is the number of intervals. The x axis is in ms

flexor motor neurons. The data are presented as interval histograms in Figure 8.

The tonic extensor exhibits clear differences in discharge intensity in the three behavioral roles. During lateral leading walking when the extensor muscle underlies the return stroke, the discharge frequency in the tonic extensor is the lowest of all four walking modes (ca. 17.6/s). The highest discharge frequency is reached during lateral trailing walking (ca. 34.2/s) when the extensor functions as a power stroke muscle. During forward and backward walking, the discharge frequencies are intermediate (21.8–25.8/s).

In contrast, in the accessory flexor motor neuron, the highest discharge frequencies (40.4/s) are reached during lateral leading walking, when the accessory flexor functions in part in the production of the power stroke (Fig. 8). In this motor neuron, the lowest discharge frequencies occur during forward (ca. 26.6/s) and lateral trailing walking (ca. 27.8/s). During backward walking, the discharge frequencies are intermediate (ca. 32.4/s).

A further question of the gradation of motor output which is not addressed by the above analysis,

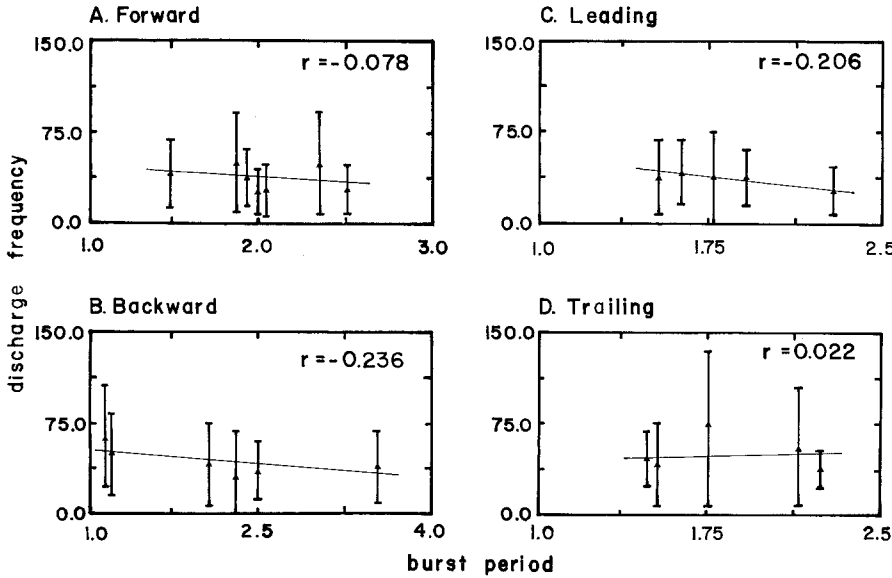


Fig. 9. Relationship of average instantaneous discharge frequency to stepping frequency in the tonic extensor motor neuron during walking in the four directions. Burst period (the inverse of stepping frequency) is the time interval between the mid-point of successive anterior basipodite elevator bursts and is in seconds. The vertical bars represent two standard deviations. The horizontal line is the linear regression line between the two variables. r is the Pearson rank-order correlation coefficient

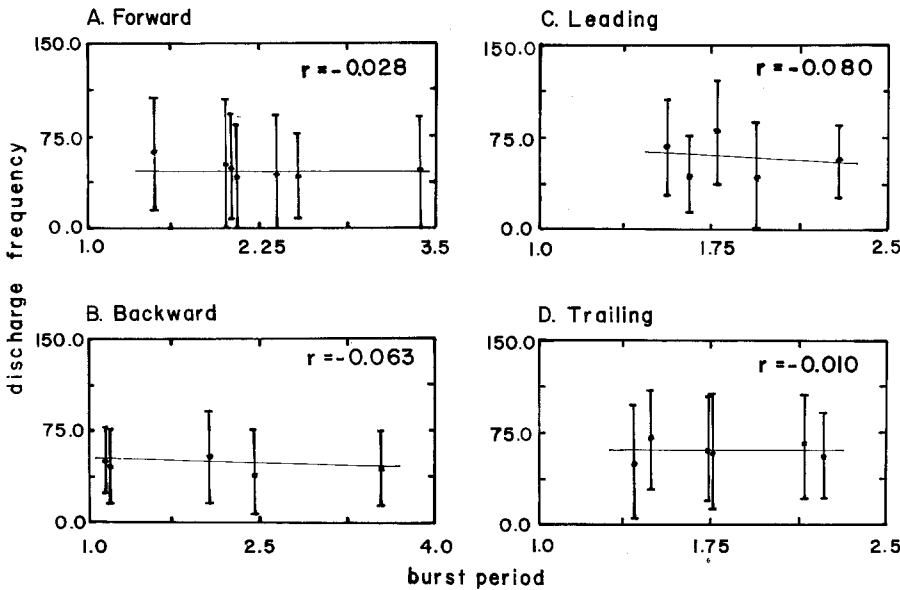


Fig. 10. Relationship of discharge frequency to stepping frequency in the accessory flexor motor neuron during walking in the four directions. All conventions as in Figure 9

is whether the discharge frequencies in these motor neurons vary when the organism walks at different stepping frequencies. To address this issue we have examined the relationship of instantaneous discharge frequency to step period (a parameter which is directly related to walking velocity, Ayers and Davis, 1977 a). The data are presented in Figure 9 for the tonic extensor and Figure 10 for the accessory flexor. There is a weak tendency for the discharge of the tonic extensor motor neuron to increase with stepping frequency

during backward (Fig. 9B) and lateral leading (Fig. 9C) walking, but in all other cases (Figs. 9 and 10), the correlation coefficient between these two variables and the regression slope is not significantly different from zero.

Phasic Motor Neurons. The intensity of discharge in both the phasic extensor and phasic flexor motor neurons is extremely variable during walking in all directions. The statistical analysis of the activity of these

Table 4. Mean inter-impulse interval between muscle potentials in simultaneous single steps of the phasic extensor and phasic flexor motor neurons, *n* is the number of intervals in the step

Burst period	Phasic extensor		Phasic flexor	
	Mean interval	<i>n</i>	Mean interval	<i>n</i>
Forward				
1.50	67.1	12		
1.71	97.5	17		
1.88	152.0	7		
2.43	69.3	22		
3.40	250.3	8		
Average	(106.8)			
Backward				
1.15	47.6	8	93.3	3
1.19	50.3	6	53.3	15
2.06	192.5	6	93.3	3
2.40	334.2	4	250.6	5
3.50	178.7	15		
Average	(150.1)		(100.4)	
Lateral leading				
1.54	238.8	6	62.2	17
1.62	194.3	8	16.0	7
1.76	337.4	5	59.3	23
1.91	60.0	2	144.8	5
2.27	308.0	7	141.7	7
Average	(248.2)		(72.0)	
Lateral trailing				
1.11	40.9	13		
1.41	177.6	7		
1.47	78.1	8		
2.13	178.7	7		
2.22	48.4	18		
Average	(85.3)			

neurons is presented in Table 4. Considerable differences are observed in the average discharge frequency between coordination modes (Table 4), and these differences parallel those observed in the tonic motor units (compare Table 4 with Fig. 8). For example, the discharge frequency in the phasic extensor is maximal (11.2/s) during lateral trailing when the extensor subserves a power stroke function, lowest (4.0/s) when the extensor subserves a return stroke function and intermediate (6.6–9.3/s) during forward and backward walking when the extensor subserves a postural function. In contrast to the situation in the tonic extensor, there is considerable step by step variation in phasic extensor discharge frequency between steps, which is not related to step frequency (Table 4).

Discussion

1. Perspective

A complete understanding of the segmental organization of a locomotory pattern generator requires analysis at at least four levels. First, an *in vivo* character-

ization must be performed in intact behaving animals in order to determine the degrees of freedom with regard to the behavioral output, the timing and intensity of discharge in identifiable motor units and the role of proprioceptive perturbations. Secondly, an *in vitro* analysis must be performed on the isolated nervous system to determine the central components of the motor program. Ideally, such an analysis should specify the timing and intensity of the centrally generated output, relevant cellular properties of the participating neurons and the underlying synaptic connectivity between identified motor neurons and interneurons. Thirdly, an analysis must be performed of the peripheral sensory components, preferably specifying monosynaptic connectivity between discrete sensory and central elements and the response dynamics of the peripheral sensory structures. Lastly, an analysis must be made of the properties and segmental connectivity of intersegmental command (Bowerman and Larimer, 1974) and coordinating (Stein, 1971, 1977) systems.

The decapod walking system has received considerable attention on several of these levels. Investigations on the *in vivo* organization have focused on forward walking in macrurans (MacMillan, 1975; Barnes, 1977) or lateral walking in crabs (Clarac and Coulmance, 1971; Evoy and Fournier, 1973; Atwood and Walcott, 1965; Burrows and Hoyle, 1973; Barnes, 1974). Furthermore, the ability of macrurans to walk in all directions has received increasing attention (Ayers, 1976; Ayers and Davis, 1977a, b, 1978; Clarac and Ayers, 1977). Such investigations have demonstrated that the coordination in the macruran walking system is metastable; i.e., lobsters can change their walking direction and underlying unit coordination on a cycle by cycle basis. Thus the macruran walking system exhibits a level of complexity beyond that described for most other invertebrate (see Kennedy and Davis, 1977; Bowerman, 1977; Stein, 1977), and vertebrate (Grillner, 1975; Orlovsky and Shik, 1976; Wetzell and Stuart, 1976) locomotory systems. Moreover, the peripheral sensory components (Ayers and Davis, 1977b, 1978; Vedel et al., 1975; Clarac, 1977) of the walking legs and their central connections with limb motor neurons (Lindsley and Gerstein, 1977; Wiens, 1976; Evoy, 1977) are beginning to be understood, as well as the effects of proprioceptive perturbations *in vivo* (Clarac and Coulmance, 1971; Fournier and Evoy, 1973; Evoy and Fournier, 1973; Barnes et al., 1972; MacMillan, 1975; Clarac, 1977; Ayers and Davis, 1977a; Barnes, 1977). Lastly, both forward and backward walking in macrurans can be elicited by single command neurons which descend from the brain (Bowerman and Larimer, 1974).

In the present investigation, we have described the strategies employed during metastable coordina-

tion at a single joint at the level of identifiable neurons. Our analysis characterizes the *in vivo* timing and intensity organization of the walking oscillator for future comparison with the probable central motor programs which may be generated by isolated central nervous systems. Furthermore, the analysis demonstrates that locomotory systems may operate in several functionally different coordination modes on a step by step basis.

2. Limitations of Electromyographic Recording

Although electromyographic recording provides a convenient technique for the examination of motor neuron activity in intact behaving animals, it provides an incomplete description, for it is incapable of specifying the activity of peripheral inhibitors. In the M-C joint system, two such peripheral inhibitors are present. One inhibitor innervates only the main flexor muscle, whereas the common inhibitor is shared by both the main extensor, the accessory flexor and several more peripheral muscles (Wiersma, 1961; Clarac, 1977).

What are the potential roles of peripheral inhibition in the walking system? Most evidence indicates that postsynaptic inhibition in Crustacea may decrease the tension produced by tonic excitatory motor units but has little effect on the tension produced by phasic units (see Florey, 1977, for review). Thus we are incapable of determining whether the peripheral inhibitors are utilized to modulate the tension evoked by the tonic motor units. Furthermore, it is possible that the inhibitors can be utilized to differentiate between coordination modes. For example, the excitatory motor output during backward walking is superficially similar to that observed during lateral trailing walking. Perhaps the difference in the two behaviors results from selective peripheral inhibition of extensor activity during lateral trailing, thus permitting the greater degree of M-C flexion which is characteristic of this behavior (Table 1). These speculations can only be resolved by neuronal recordings which are capable of monitoring the activity of the peripheral inhibitors.

A further ambiguity which is not resolvable by electromyographic recording is organization of activity in the tonic flexor motor units. The unpatterned discharge which is observed during some behaviors may potentially represent alternating discharge or random bursts in different tonic flexor units.

3. Behavioral Modes of the M-C Joint

The present findings demonstrate that there are three behavioral models of M-C joint activation: (1) postural mode (forward and backward), (2) flexion power stroke (lateral leading) and (3) extension power stroke (lateral trailing). The major difference between the propulsive modes and the postural modes is the degree of flexion of the M-C joint. During lateral walking the M-C joint is flexed at least 30 degrees more than during the postural modes of forward and backward walking. This regulation is based more upon anatomy than on function, however, for the power stroke can be terminated during either extension or flexion during lateral walking. Thus if positional proprioceptors are involved in terminating motor neuron bursts, they must be able to terminate flexor bursts at the end of either the power stroke

or return stroke of lateral walking. It is tempting to speculate, however, that positional proprioceptors are utilized during forward and backward walking to regulate the degree of flexion of the M-C joint perhaps by inhibition of flexor units or excitation of extensor units (see Clarac, 1977).

4. Neuromuscular Strategies in the Timing of Motor Output

Three characteristic patterns of motor discharge timing are observed in the M-C joint motor neurons. Some neurons discharge in large part randomly. Such random discharge may be either tonic or consist of short bursts which occur at random phase positions, as occurs in the tonic extensor during forward walking (Fig. 3, Table 3). Secondly, the muscles which are controlled by all neurons may exhibit discrete bursts which are either synergistic or antagonistic with that of the elevator of the CB joint (Figs. 2; 3C, D; 4A, B; 5C). The third type of discharge pattern is unpatterned background discharge with a superimposed burst. This type of patterning is exemplified by the discharge of the accessory flexor unit during lateral trailing walking (Fig. 5D).

Perhaps the most striking feature of the M-C joint motor output is the high degree of variability in the timing of motor neuron discharge. For example, during forward walking the tonic extensor typically discharges in short bursts which occur at highly variable phase positions (Table 3). This finding suggests that in some walking modes the timing of discharge is more dependent on proprioceptive contingencies than specific timing cues provided by a central generator. By this interpretation, specific motor neurons of some joints might be activated preferentially by a central generator during walking in some directions but by proprioceptive inputs during walking in other directions. This possibility can only be directly tested by recordings of potential central motor programs from isolated nervous systems during command fiber stimulation. In any event, assuming a common central pattern generator, it is rather clear that the motor units of the M-C joint are not intimately involved in the generation of rhythmic power stroke/return stroke discharge but function more as followers of a more fundamental pattern generator.

5. Neuromuscular Strategies in the Gradation of Motor Output

The present investigation demonstrates that one of the strategies utilized in differentiating among the alternate behaviors produced by the M-C joint is the employment of different characteristic discharge frequencies in the different coordination modes. For

example, the tonic extensor discharges at 34.2/s during lateral trailing when it functions as a power stroke muscle, but at only 17.6/s during lateral leading when the extensor functions as a return stroke muscle. Atwood (1973, 1977) has identified two mechanisms by which variation in tonic motor neuron discharge frequency may control muscle tension. The first is due to the excitation-contraction properties of the muscle fibers. In most long sarcomere (slow) fibers, the resultant tension is a function of the summated level of depolarization or EPSP input. The second mechanism is a property of the neuromuscular synapse. Some synapses release substantial transmitter at low discharge frequencies whereas other synapses only release substantial amounts of transmitter after facilitation by a high frequency train of input EPSP's. Hence, high frequency input can, in principle, recruit additional muscle fibers which are innervated only by such facilitating synaptic input. By such non-linear mechanisms, one might expect that a two fold difference in motor neuron discharge frequency as detailed above for the tonic extensor unit during lateral walking in the two directions would produce a considerable difference in the evoked tension in the two opposing coordination modes and hence in the resulting behaviors.

The second mechanism of gradation of motor output between coordination modes is the recruitment of phasic axons. For example, the phasic flexor motor neuron is recruited in synergism with the tonic flexors only during backward and lateral trailing walking. Similarly, during lateral leading walking, the phasic extensor unit (which normally discharges only during the power stroke), is recruited with the elevators and tonic extensors to produce the return stroke. When they are utilized, the phasic motor units exhibit the same general tendency to discharge at different characteristic discharge frequencies which are related to their functional role (Table 4).

Although there are substantial differences in the intensity of motor output between coordination modes (Fig. 8), our findings do not indicate that gradation in the discharge frequency of identified motor neurons is utilized within a coordination mode (Figs. 9, 10). These findings suggest that the overall strategy for the gradation of motor output in the M-C joint system during walking in a given direction is substantially similar to that utilized by vertebrates during locomotion in that motor neurons discharge at relatively constant frequency and variations in intensity are produced by recruitment of additional units (see Grillner, 1975; Wetzel and Stuart, 1976; Orlovsky and Shik, 1976). The model proposed for the gradation of motor output in the lobster system during alternate coordination modes may be sum-

marized as follows. First, when animals walk in different directions, identified neurons discharge at different frequencies which are characteristic of their functions. For example, the discharge in the tonic extensor is highest (34/s) when it subserves a power stroke function, intermediate (22–26/s) when this unit subserves a postural function and lowest (18/s) when it subserves a return stroke function. Furthermore, the recruitment of phasic motor units is utilized in part as a mechanism of differentiation between coordination modes. Moreover, the discharge frequency is invariant within a coordination mode and gradations in muscular force are apparently produced by temporal facilitation of muscular tension (see Spirito et al., 1973, for a review).

We thank R. Calabrese and W.B. Kristan for criticism of the manuscript and G. Wendler for making possible use of his magnetic tablet-digitizing system.

References

- Angaut-Petit, D., Clarac, F., Vedel, J.P.: Excitatory and inhibitory innervation of a crustacean muscle associated with a sensory organ. *Brain Res.* **70**, 148–152 (1974)
- Atwood, H.L.: Crustacean motor units. In: Control of posture and locomotion. Stein, R.B., Pearson, K.G., Smith, R.S., Redford, J.B. (eds.). New York: Plenum Press 1973
- Atwood, H.L.: Crustacean neuromuscular systems. In: Identified neurons and the behavior of arthropods. Hoyle, G. (ed.). New York: Plenum Press 1977
- Atwood, H.L., Walcott, B.: Recording of electrical activity and movement from legs of walking crabs. *Can. J. Zool.* **43**, 657–665 (1965)
- Ayers, J.L.: Programmes locomoteurs et organization reflexe chez le Homard. *J. Physiol. (Paris)* **72**, 18a (1976)
- Ayers, J.L., Davis, W.J.: Neuronal control of locomotion in the lobster. I. Motor programs for forward and backward walking. *J. comp. Physiol.* **115**, 1–27 (1977a)
- Ayers, J.L., Davis, W.J.: Neuronal control of locomotion in the lobster. II. Types of walking leg reflexes. *J. comp. Physiol.* **115**, 29–46 (1977b)
- Ayers, J.L., Davis, W.J.: Neuronal control of locomotion in the lobster. III. Dynamic organization of walking leg reflexes. *J. comp. Physiol.* **123**, 289–298 (1978)
- Barnes, W.J.P.: Nervous control of locomotion in crustacea. In: Simple nervous systems. Usherwood, P.N.R., Newth, D.R. (eds.). London: Arnold 1974
- Barnes, W.J.P.: Proprioceptive influences on motor output during walking in the crayfish. *J. Physiol. (Paris)* **73**, 543–564 (1977)
- Barnes, W.J.P., Spirito, C.P., Evoy, W.H.: Nervous control of walking in the crab, *Cardisoma guanhumi*. II. Role of resistance reflexes in walking. *Z. vergl. Physiol.* **76**, 16–31 (1972)
- Batschelet, E.: Statistical methods for the analysis of problems in animal orientation and certain biological rhythms. Washington, DC: American Institute of Biological Sciences 1965
- Bowerman, R.F.: The control of arthropod walking. *Comp. Biochem. Physiol.* **56A**, 231–247 (1977)
- Bowerman, R.F., Larimer, J.L.: Command fibers in the circumesophageal connectives of the crayfish. II. Phasic fibers. *J. Exp. Biol.* **60**, 119–134 (1974)

- Burrows, M., Hoyle, G.: The mechanism of rapid running in the ghost crab, *Ocypode ceratophthalma*. *J. Exp. Biol.* **58**, 327-350 (1973)
- Clarac, F.: Motor Coordination in Crustacean Limbs. In: Identified neurons and behavior of arthropods. Hoyle, G. (ed.). New York: Plenum Press 1977
- Clarac, F., Ayers, J.L.: La marche chez les crustacés: Activité motrice programée et régulation périphérique. *J. Physiol. (Paris)* **73**, 523-544 (1977)
- Clarac, F., Ayers, J.: Proprioceptive regulation during unrestrained locomotion of the rock lobster, *Panulirus vulgaris*. (in preparation) (1978)
- Clarac, F., Coulmance, M.: La marche latéral du crabe (*Carcinus*): Coordination des mouvements articulaires et régulation proprioceptive. *Z. vergl. Physiol.* **73**, 408-438 (1971)
- Evoy, W.H.: Crustacean motor neurons. In: Identified neurons and the behavior of arthropods. Hoyle, G. (ed.). New York: Plenum Press 1977
- Evoy, W.H., Fournier, C.R.: Nervous control of walking in the crab *Cardisoma guanhumi*. III. Proprioceptive influences on intra- and inter-segmental coordination. *J. comp. Physiol.* **83**, 303-318 (1973)
- Florey, E.: Role of peripheral inhibition in the control of arthropod muscle. In: Identified neurons and the behavior of arthropods. Hoyle, G. (ed.). New York: Plenum Press 1977
- Fournier, C.R., Evoy, W.H.: Nervous control of walking in the crab *Cardisoma guanhumi*. IV. Effects of myochordotonal organ ablation. *J. comp. Physiol.* **83**, 319-329 (1973)
- Grillner, S.: Locomotion in vertebrates: Central mechanisms and reflex interaction. *Physiol. Rev.* **55**, 247-304 (1975)
- Kennedy, D., Davis, W.J.: Organization of invertebrate motor systems. In: Handbook of physiology, Sect. I, Vol. I, Part 2. Geiger, S.R., Kandel, E.R., Brookhart, J.M., Montcastle, V.B. (eds.), pp. 1023-1087. Bethesda, MD: American Physiological Society (1977)
- Lindsey, B.G., Gerstein, G.L.: Reflex control of a crayfish claw motor neuron during imposed dactylopodite movements. *Brain Res.* **130**, 348-353 (1977)
- MacMillan, D.F.: A physiological analysis of walking in the American lobster (*Homarus americanus*). *Phil. Trans. R. Soc. Lond. B* **270**, 1-59 (1975)
- Orlovsky, G.N., Shik, M.L.: Control of locomotion: A neurophysiological analysis of the cat locomotor system. In: International review of physiology, neurophysiology II, Vol. 10. Potter, R. (ed.), pp. 281-317. Baltimore: University Park Press 1976
- Pearson, K.G., Wong, R.S.K., Fournier, C.R.: Connections between hair plate afferents and motoneurons in the cockroach leg. *J. Exp. Biol.* **64**, 251-266 (1976)
- Sandeman, D.C.: A sensitive position measuring device for biological systems. *Comp. Biochem. Physiol.* **24**, 635-638 (1968)
- Spirito, C.P., Evoy, W.H., Fournier, C.R.: Consideration of proprioception and neuromuscular integration in crustacean locomotion. *Am. Zool.* **13**, 427-434 (1973)
- Stein, P.S.G.: Intersegmental coordination of swimmeret motor neuron activity in crayfish. *J. Neurophysiol.* **34**, 310-318 (1971)
- Stein, P.S.G.: A comparative approach to the neural control of locomotion. In: Identified neurons and the behavior of arthropods. Hoyle, G. (ed.). New York: Plenum Press 1977
- Vedel, J.P., Angaut-Petit, D., Clarac, F.: Reflex modulation of motoneurone activity in the leg of the crayfish. *J. Exp. Biol.* **63**, 551-567 (1975)
- Wendler, G.: The coordination of walking movements in arthropods. *Symp. Soc. Exp. Biol.* **20**, 229-249 (1966)
- Wetzel, M.C., Stuart, D.G.: Ensemble characteristics of cat locomotion and its neural control. *Prog. Neurobiol.* **7**, 1-98 (1976)
- Wiens, T.J.: Electrical and structural properties of crayfish claw motor neurons in an isolated claw ganglion preparation. *J. comp. Physiol.* **112**, 213-233 (1976)
- Wiersma, C.A.G.: The neuromuscular system. In: The physiology of Crustacea, Vol. 2. Waterman, T.H. (ed), pp. 191-240. New York: Academic Press 1961
- Wiersma, C.A.G., Ripley, S.H.: Innervation patterns of crustacean limbs. *Physiol. Comp. Oecol.* **2**, 391-405 (1952)
- Zar, J.H.: Biostatistical analysis. Englewood Cliffs, NJ.: Prentice-Hall 1974