

REFLEX MODULATION OF MOTONEURONE ACTIVITY IN THE CHELIPED OF THE CRAYFISH *ASTACUS LEPTODACTYLUS*

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SUMMARY

1. The reflex activity elicited by movement of the mero-carpopodite (M-C) joint in the cheliped of the crayfish *Astacus leptodactylus* is investigated and the role of the different proprioceptors (chordotonal and myo-chordotonal organs) separately studied.

2. The reflex discharge involves mainly the tonic motoneurons of the extensor (E), the flexor (F) and the accessory flexor (AF) muscles.

3. M-C joint posture is also regulated by the cuticular stress detector (CSD₂) afferents: they increase mainly the F discharge and secondarily the AF command.

4. The activity of the motor axons supplying the muscles of the mero-podite can be also influenced by a variety of natural stimuli applied to other appendages. The effect usually produced is a general flexion reaction which is characterized by a reciprocity between E and F involving both central and peripheral mechanisms.

5. The AF muscle is innervated by two antagonistic motoneurons, an excitatory neurone functionally linked in its discharge with one of the four exciters supplying F and an inhibitory motoneurone, common with E. The resulting competitive effect between these two neurones has been recorded intracellularly in AF muscle fibres.

6. The role of the myochordotonal organ (MCO) in the crayfish is discussed. In particular the modulation of the AF command in relation to the discharges of the motor nerves to the main muscle E and F is studied.

INTRODUCTION

Two mechanisms seem to interact in the generation of motor sequences: intrinsically central influences, and sensory input from the periphery. The relative importance of these two mechanisms is quite variable from one motor activity to another.

The swimmeret movement of the lobster (Davis & Davis, 1973) and the stomatogastric motoneurone discharge (Maynard, 1971; Mulloney & Selverston, 1974) provide examples of stereotyped rhythmic activities which can appear even in the absence of proprioceptive feedback. Nevertheless, recent work has emphasized the complexity of decapod crustacean proprioceptors. In the walking legs of the crab *Carcinus*, Whitear (1962) described the chordotonal organs. Alexandrowicz & Whitear (1957), Whitear (1965), Bush & Roberts (1971) gave information on a special

stretch receptor at the base of each appendage, the thoracic-coxal organ (TCO). Another sensory-muscular structure, previously anatomically defined by Barth (1934) as the myochordotonal organ (MCO), has been extensively investigated (Cohen, 1963, 1965; Clarac, 1968; Clarac & Masson, 1969; Evoy & Cohen, 1969; Clarac, 1970; Wales *et al.* 1970; Horch, 1971; Alexandrowicz, 1972; Fournier & Evoy, 1973). More recently, cuticular structures have been shown to be sensitive to the load supported by each leg and consequently named cuticular stress detectors CSD₁ and CSD₂ (Wales, Clarac & Laverack, 1971; Clarac, Wales & Laverack, 1971; Moulines & Clarac, 1972). Finally, rows of bipolar cells located on the muscle tendons seem specifically sensitive to the tension of the muscle fibres (Macmillan & Dando, 1972).

Proprioceptive control involving some of the above receptors has been studied. Excitatory and inhibitory influences can act reciprocally as they do in the regulation of abdominal posture (Kennedy, Evoy & Fields, 1966). In the claw of the crab the discharge frequency of both excitatory and inhibitory axons is enhanced, and the reflex is seen in a preferential increase of one of them (Bush, 1962*a*).

The present investigation is an attempt to answer some questions concerning the regulation of posture and co-ordination of movements of the mero-carpopodite (M-C) joint in the cheliped of the crayfish. The M-C joint of Crustacea is of special interest in that it possesses not only two chordotonal organs MC (MC₁ and MC₂) as do the other joints, but also the MCO. Bush (1965) and Cohen (1965) studying in the M-C joint in crabs suggested that MC and MCO were involved in different ways in complex reflex loops. The chordotonal organs located at each joint of the appendages participate in resistance reflexes analogous to the myotatic loop as described by Bush (1965). The MCO initiates a servo-system, driven directly by the CNS, which corrects and stabilizes the M-C joint in a rest position, according to Evoy & Cohen (1969).

This study is one of a series investigating how MC, and particularly MCO, are involved in other decapods in the regulation of the motor commands to the main muscles: the extensor (E), the flexor (F) and the accessory flexor (AF). The existence of peripheral inhibitory mechanisms is now well established in crustaceans and insects. Wiersma & Ripley (1952) described inhibitory fibres regulating the M-C joint motor activity. Nevertheless Bush (1965) and Evoy & Cohen (1969) did not mention the occurrence of any inhibitory regulation when studying M-C reflex movement in the crab. Using intracellular recording methods we have recently confirmed in the crayfish that a common inhibitory motoneurone supplies both E and AF muscles (Angaut-Petit, Clarac & Vedel, 1974). Therefore, another purpose of this study is to look for and compare the excitatory and inhibitory events occurring in the AF muscle fibres when the receptors are selectively activated.

MATERIALS AND METHODS

Experiments were carried out on intact crayfish, *Astacus leptodactylus*. The perfusion solution used was aerated Van Harreveld liquid (Van Harreveld, 1936), its temperature being maintained at 5–10 °C. The animal was strapped, by means of rubber bands, ventral-side up in a perspex dish (Fig. 1). The tendon attachment of

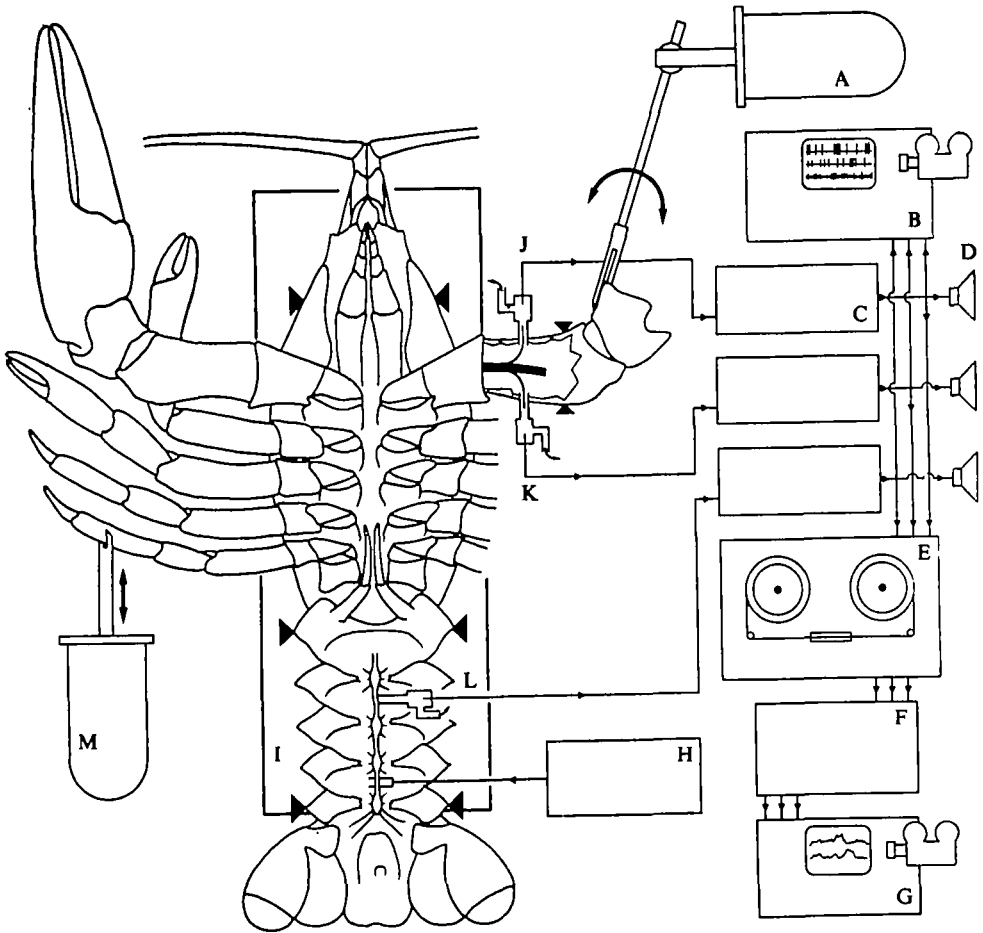


Fig. 1. Diagram of the experimental arrangement for the study of the motoneurone activity in the cheliped of the crayfish. *A*, Electromechanical apparatus moving M-C joint. *B*, Oscilloscope recording of spike activity. *C*, Amplifiers. *D*, Loudspeaker. *E*, Tape recorder. *F*, Integrator. *G*, Oscilloscope recording of integrated nervous activity. *H*, Ventral nerve cord stimulation. *I*, Cooling system. *J*, *K*, *L*, Suction electrodes. Records of motoneurone and ventral activities. *M*, Electromagnetic stimulator.

the basi-ischium levator muscle of the cheliped was cut to prevent autotomy. The cuticle of the meropodite and the ischiopodite was opened ventrally to isolate the motor nerves: at this level the limb nerve trunk consists of two bundles (Fig. 2). The motor axons to the flexor muscle are located in the small dorsal bundle and leave it close to the muscle fibres. The large ventral bundle includes most of the sensory afferent fibres and the motor axons to the extensor muscle. The motor nerve to AF lies close to this main trunk and leaves it at the level of the muscle fibres it supplies.

The motor commands to the muscles in the meropodite were recorded by means of suction electrodes. The activity of the motor axons to E and F was recorded on specific branches near the main artery of the leg. It was more difficult to record the motor activity to AF than the E and F commands because the specific bundles to F were too short in the proximal part of the meropodite, while distally it was

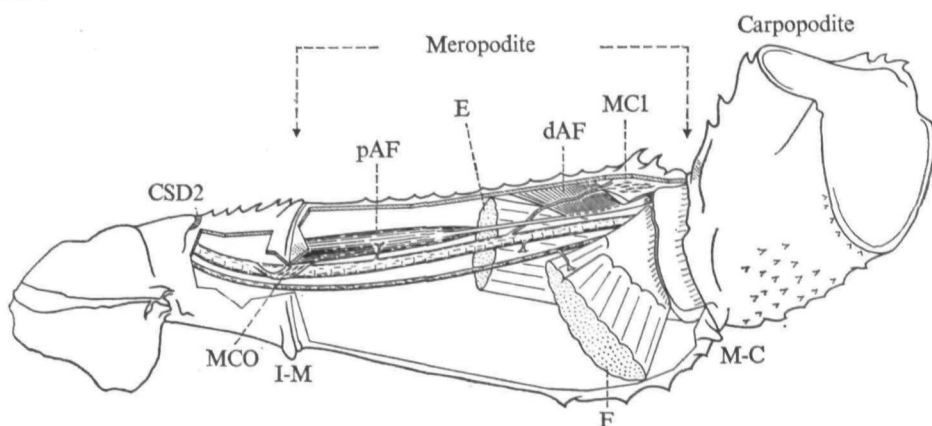


Fig. 2. Posterior view of the first right cheliped of the crayfish *Astacus leptodactylus*. The cuticle has been removed to expose the main nervous and muscular structures. The muscle fibres of the 2 main muscles (extensor E and flexor F) have been sectioned to clarify the picture. The accessory flexor muscle is composed of a proximal part (pAF) and a distal part (dAF), connected by a thin tendon. The myochordotonal organ, MCO, is close to the ischio-meropodite joint (I-M). The proprioceptive receptors also include two chordotonal organs: MC1, shown with its afferent nerve joining the thicker nerve trunk; and MC2 (not shown), hidden by the accessory flexor tendon.

difficult to expose them without damage to the mechanical organization of the M-C joint. Therefore the AF axon activities were recorded from the main nerve trunk itself where they run on its surface. It was always possible to record the activity of the excitatory motoneurone because of its very large diameter in comparison with the other fibres: the inhibitory axon was more difficult to find being smaller in diameter and sometimes more deeply situated.

Single muscle fibre activity was recorded intracellularly with 3 M-KCl-filled glass microelectrodes and conventional recording techniques, after having first removed the cuticle just above the AF muscle. A study of the 2 parts of the AF muscles was made. Starting at the distal edge of the distal head of AF and progressing across the muscle to the proximal edge the activity of muscle fibres was recorded. For the spindle-shaped proximal part of AF, the study was done in a dorso-ventral direction. MCO sensory discharges were also recorded, using suction electrodes. In order to apply controlled movement to the M-C joint, the distal segment of the cheliped was severed at the carpo-propodite (C-P) joint and a small forceps clamp was attached to the carpopodite. The clamp was mounted on a servo-controlled electromechanical apparatus giving sinusoidal movements of variable frequency and amplitude (see Clarac & Vedel, 1971). In some cases the AF muscle was moved separately: the tendon that joins the 2 parts of AF muscle was cut and its proximal end fixed to a similar device also providing sinusoidal displacement.

In the abdomen, stimulating electrodes were disposed on the isolated ventral chain between abdominal ganglia 5 and 6. Recording electrodes were located on the connective between ganglia 2 and 3. Mechanical stimuli were applied manually with a small probe or by means of an electromechanical vibrator to various parts of the body: thoracic or abdominal appendages, antennae, antennulae or telson.

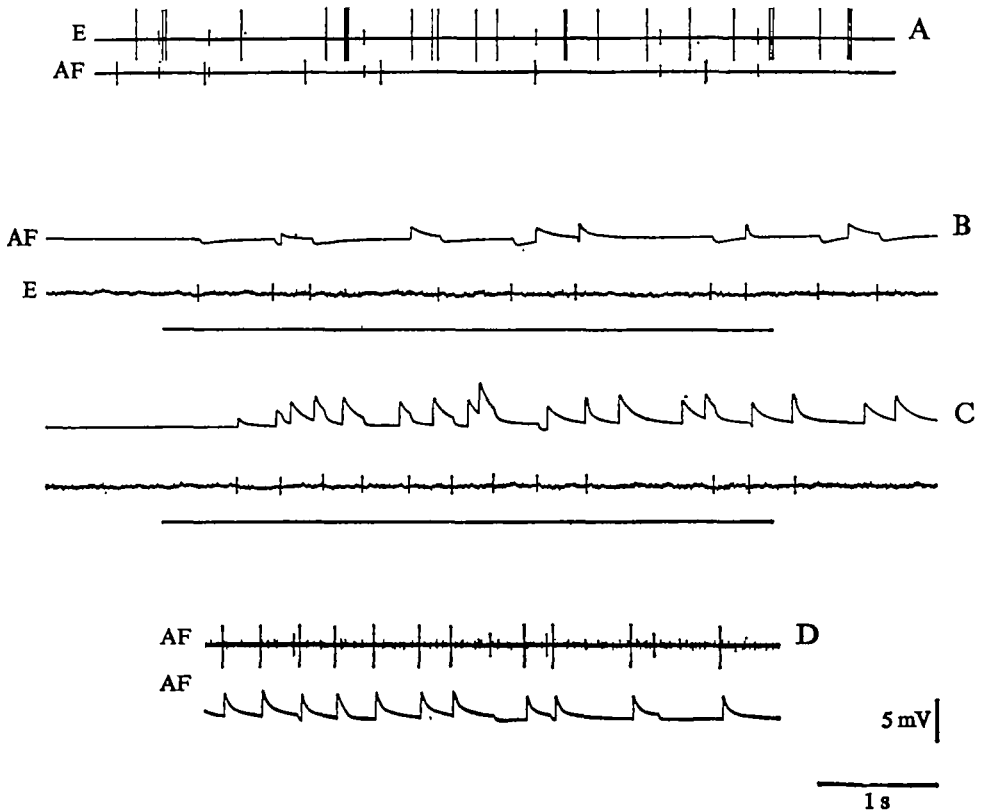


Fig. 3. Simultaneous recordings from motoneurone to E and AF and the resulting intracellular activity from a single AF muscle fibre. It illustrates the discharges of the common inhibitory unit to E and AF. A, Comparison between E and AF motor nerve activity; note the small amplitude impulses, synchronous in the two traces. B, C, D, Comparison between one motoneurone (the extensor in B and C, the accessory flexor in D) and the AF muscle fibre intracellular activity: the impulses of the extensor nerve are synchronous only with the IJP of the AF muscle fibre. The impulses of high amplitude of the AF motor nerve are synchronous with the EJP of the AF muscle fibre; the impulses of the smaller amplitude unit are synchronous with the IJP.

ANATOMICAL OBSERVATIONS

The general organization of the different segments (cheliped and thoracic limbs) in *Astacura* has been described previously (Clarac & Masson, 1969). The contraction of the muscles located in the meropodite moves the mero-carpopodite (M-C) joint, and consequently moves the big chelae perpendicularly to the plane of symmetry of the animal. Three different muscles are involved in these movements. The extensor is disposed dorsally and anteriorly, while the main flexor lies ventrally and posteriorly. The two muscular parts of the accessory flexor lie ventrally and are linked together by a very thin rod-like tendon; the larger distal part (dAF) is flattened and obliquely disposed, the proximal part (pAF) is spindle shaped and runs longitudinally within the meropodite (Fig. 2).

In the crayfish the proprioceptors are located near the two parts of the AF muscle. The two chordotonal receptors MC₁ and MC₂ are situated on each side of the AF muscle apodeme.

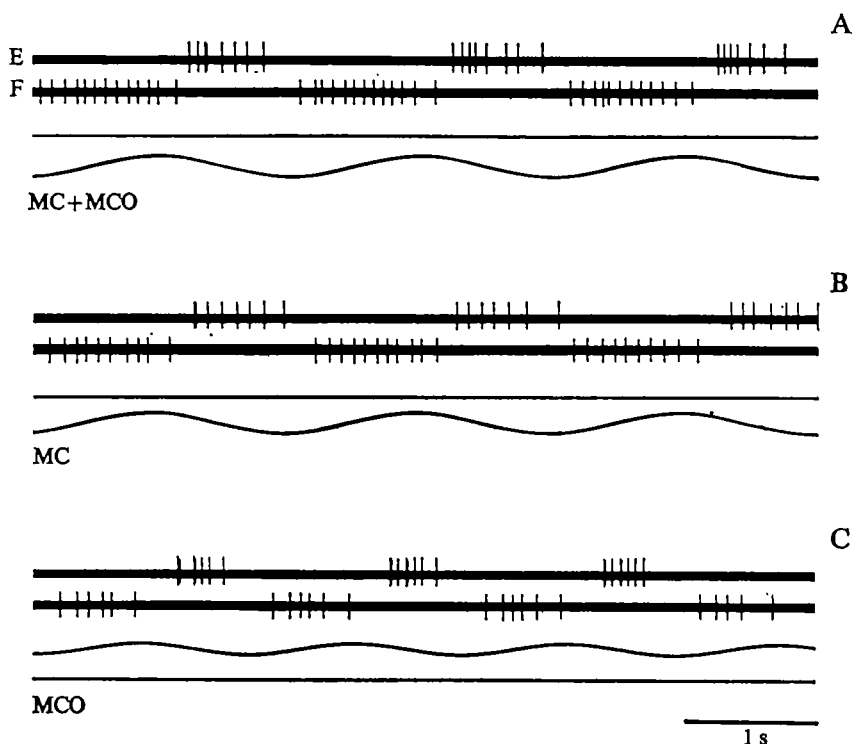


Fig. 4. Reflex modulation of tonic motoneurone activity of M-C joint extensor (E) and flexor (F) muscles by MC and MCO afferents. A, Resistance reflexes induced by M-C joint. MC and MCO organs are simultaneously stimulated by the movement. Both MC and MCO are intact. Upwards deflexion of the sinusoidal line indicates extension. B, Resistance reflexes induced by the same M-C movement after cutting the AF tendon, so that AF remains at a constant length. Thus only MC sensory cells discharge during M-C movements. C, Resistance reflexes induced by selective sinusoidal stretch and release of pAF while M-C joint is held stationary. Now only MCO is stimulated by pAF length variation. Upward deflexion of the sinusoidal record indicates stretch.

MCO is attached to the proximal end of pAF near the ischio-meropodite (I-M) joint. This proximal part of the muscle is divided into two different muscular bundles. The thinner dorsal one is elongated by connective tissue in which the thirty bipolar cells of the MCO organ are embedded, while the large central muscular bundle is attached directly to the cuticle.

The cuticular stress detectors (CSD, Wales *et al.* 1971) stimulated in some of our experiments are situated in the basi-ischiopodite. There are two different receptors visible at the surface of the segment through a small window of thin cuticle. One (CSD₁) is anteriorly disposed in the basipodite near the breakage plane. The other (CSD₂, Fig. 2) is located in the ischiopodite quite close to the ventral basi-ischiopodite (B-I) joint; it is inserted in a soft area of cuticle and comprises more than 50 bipolar sensory cells.

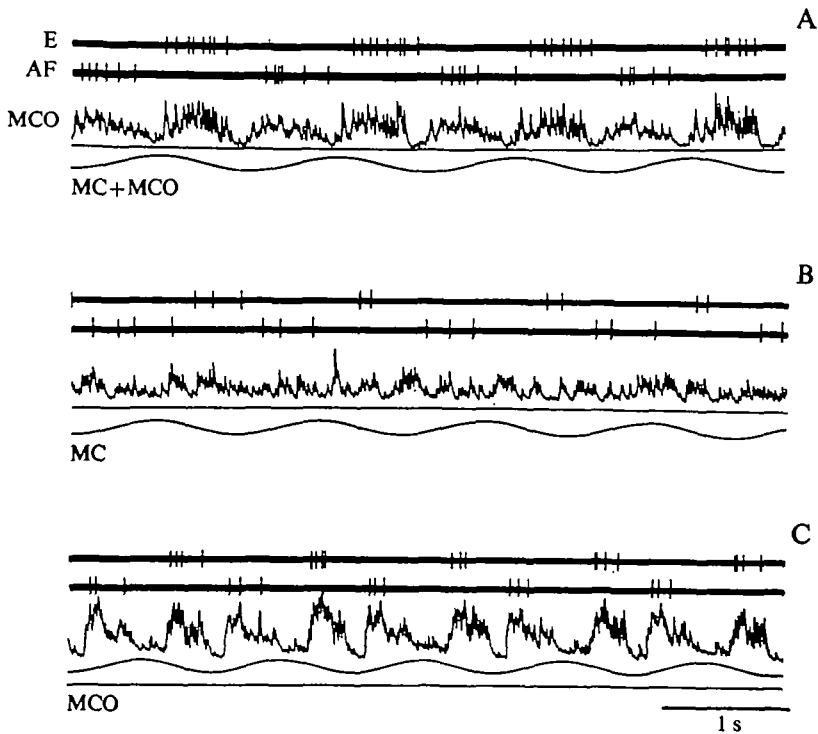


Fig. 5. Reflex modulation of tonic motoneurone activity of M-C joint extensor (E) and accessory flexor (AF) muscles by MC and MCO afferents. E and AF motoneurone discharges and MCO integrated activity are recorded simultaneously. A, Resistance reflexes induced by M-C joint sinusoidal movements. MC and MCO organs are simultaneously stimulated by the movement. Upward deflexion of the sinusoidal records indicates extension. B, Resistance reflexes induced by the same M-C movement after cutting AF tendon. Only MC sensory cells discharge during M-C movement, since AF is at a constant length. C, Resistance reflexes induced by selective sinusoidal stretch and release of pAF while M-C joint is held stationary. Only MCO is stimulated by pAF length variations. Upward deflexion of the sinusoidal record indicates stretch.

ELECTROPHYSIOLOGICAL RESULTS

(1) Motor innervation of M-C muscles

A small number of axons innervate each muscle. F is supplied by five motor axons, and E by three (Wiersma & Ripley, 1952). Each of these two muscles receives one inhibitory axon, the others are excitatory. The AF muscle is supplied by two motor fibres. We simultaneously recorded its motor output and the intracellular activity produced in AF muscle fibres by the efferent impulses (Fig. 3). When a large amplitude impulse is present in the motor nerve an excitatory junctional potential (EJP) appears in the muscle fibres. A small amplitude impulse, on the other hand, elicits an inhibitory junctional potential (IJP). The two motor axons transmit antagonistic effects. One of them depolarizes the membrane and provokes the contraction of the fibre, the other is inhibitory and hyperpolarizes the muscle membrane (see Angaut-Petit *et al.* 1974). We have confirmed the early anatomo-

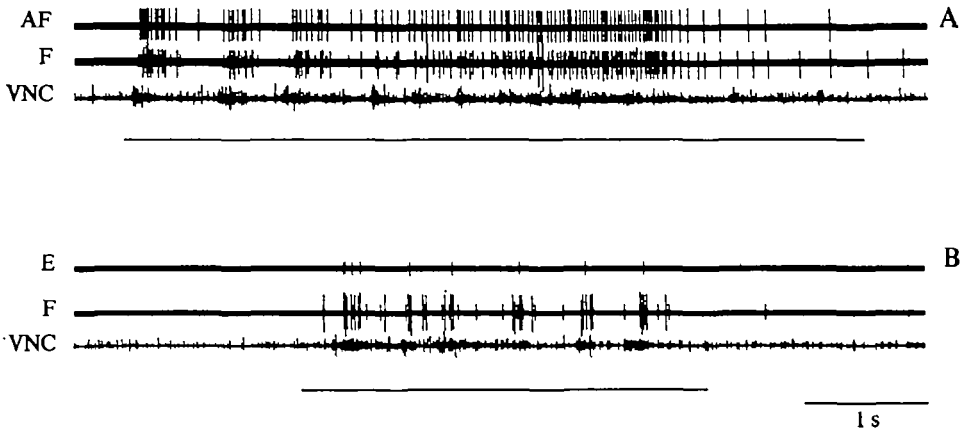


Fig. 6. Flexor effect on M-C joint induced by abdominal appendage stimulation (indicated on the bottom trace in A and B). A, the activities of accessory flexor (AF) and flexor (F) muscle nerves are recorded simultaneously with those of the ventral nervous chain (VNC). The abdominal stimulation produces an activation of the excitatory motoneurons to the two flexor muscles, correlated with a bursting activity in the VNC. B, In the same condition, activity of flexor (F) and extensor (E) nerves are contrasted. As described above the abdominal stimulation activates different flexor motoneurons, while the inhibitory motoneurone is the only one to fire in the extensor group.

physiological work of Wiersma & Ripley (1952) and shown by means of electrophysiological methods that the same inhibitory motoneurone innervates the extensor and the AF muscles (see Fig. 3). When the carpopodite is held in a steady position a background activity is usually present in the excitatory motoneurone to AF and in one of those to F (low frequency discharge: 1–2/s). In contrast the extensor motor fibre remains inactive or bursts very rarely; although one of the E excitatory motoneurons responds with a tonic discharge to M-C movement. Therefore in our experimental conditions it appears that the tonic activity is conveyed to each muscle by only one motoneurone. This may seem reasonable for AF and E muscles that are poorly innervated (2 or 3 motoneurons), but the result was unexpected for F which receives 4 excitatory motoneurons: among the other units 2 are phasic and can be activated by a strong stimulation; the third axon appears to be occasionally active and was usually unresponsive to the stimuli we used.

(2) *Reflex activity elicited by M-C joint movement*

Carpodite movements evoke the usual resistance reflexes in the motoneurone to F, AF and E muscles. No conspicuous changes in the reflex activity were observed whatever the movement velocity (15°/s, 30°/s, 45°/s), or whatever the fraction of the entire flexion-extension range, of a sinusoidal movement passively imposed on the M-C joint.

In order to determine the specific reflex activity of each M-C joint proprioceptor, we stimulated selectively MC1, MC2 and MCO, as did Bush (1965) and Evoy & Cohen (1969).

In these conditions the two kinds of receptors apparently produce the same reflex activities in F, AF and E motoneurons as those previously described, the only

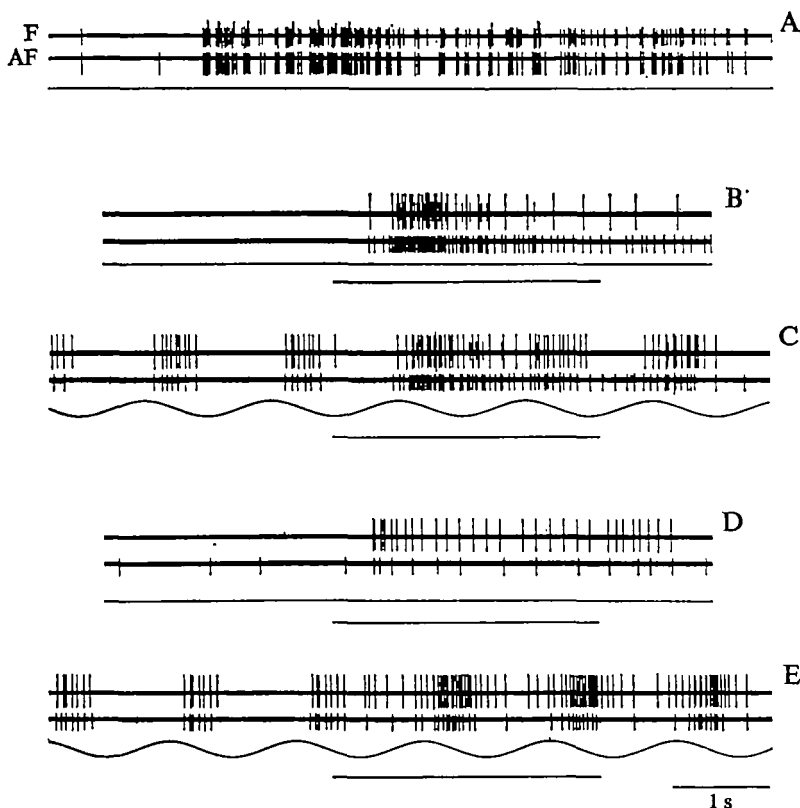


Fig. 7. Simultaneous modulation of F and AF motoneurone activities by different stimulations. A, Increase of F and AF motoneurone activities during a spontaneous flexor movement. B-C, Effects of sexual pleopod stimulation (bottom trace) on the tonic discharge (B) of F and AF motor fibres, and on their reflex activation (C) by M-C joint movement. Downward deflexion of the sinusoidal trace indicates extension. D-E, Effects of CSD stimulation; on the tonic discharges (D), and on reflex activity induced by M-C joint movement (E). In A, B and D the M-C joint is held stationary. B, C, D, E, recordings from the same experiment.

difference being a decrease in the number of spikes (Figs. 4B, C; 5B, C). In *Astacus*, cutting the AF tendon disrupts MCO input but does not abolish it; tonic units signalling the position of the proximal AF are continuously firing as it is illustrated in Fig. 5B. MC and MCO activities appear to have an additive excitatory effect on the motor fibres during flexion or extension reflexes. The superimposed activity of MCO enhances flexor as well as extensor motor responses elicited by MC afferents: depending on whether pAF is maintained at its maximal or minimal length, differences in the flexor and extensor MC reflex induced by M-C movements can be observed.

(3) Reflexes elicited by stimulation of other parts of the body

The motoneurons supplying the muscles of the cheliped meropodite can also be reflexly activated by mechanical stimuli applied to other segments or other appendages.

Roughly the same pattern of activity was elicited whichever appendage was stimulated. Application of the stimulation provoked the firing of some units of the ventral nerve cord (VNC) (Fig. 6) and a corresponding activation of several motor axons.

The result is an antagonistic action between the flexor and extensor muscles: it facilitates the contraction of the former and inhibits the latter. 3 or 4 axons fire in the F motor nerve; two of them seem tonic, one or two phasic. They stop firing as soon as the stimulus stops. The discharge frequency in the excitatory AF motor axon is modulated in the same way (see also Fig. 7B, D); in addition the inhibitory impulse to E starts firing.

Electrical stimulation directly applied to the VNC elicits a response of the excitatory units to F and AF muscles at the same stimulation voltage. Increasing the intensity of the stimulus evokes a response of the common AF-E inhibitory unit. On the other hand it is very difficult to obtain a response of the E excitatory motoneurone.

In a few cases (5 out of 25) a somewhat different pattern of activation is produced in the extensor motor nerve. Both the inhibitory and the excitatory units are facilitated. Pressure applied to the soft cuticle of the cuticular stress detector (CSD) evokes a response in both tonic motoneurons of F and AF. The discharge frequency to F is always higher than that to AF (Fig. 7D). In contrast to the unspecific mechanical stimulation, only the spontaneously active F motoneurone is activated by the CSD2. If temporarily it does not spontaneously discharge, it can be fired. The active extensor unit does not seem to be affected, but the result is inconsistent.

The modulation of E, F and AF motor commands, by passive M-C joint movement, by mechanical stimulation of an appendage, or by simultaneous application of these stimuli, shows the relations that exist between the efferent discharges to E and F on the one hand, and to F and AF on the other.

(a) Antagonism between E and F motor commands

When an electrical stimulation of the VNC or a mechanical stimulation of an appendage are delivered simultaneously with a passive M-C joint movement, the two mechanisms responsible for the reciprocity between the E and F motor commands become obvious. Indeed the intrinsic reflex activity induced by M-C movement may be completely masked (Fig. 8A). In the flexor motoneurone group, not only does the tonic motoneurone that was previously active increase its discharge frequency, but several other units (usually 3) become active, and their discharges are still present one or two seconds after the end of the electrical stimulation. By contrast, the activity of the E excitatory unit disappears during several imposed M-C movements, and the inhibitory motoneurone common to E and AF is activated. This type of modulation is the one that is most commonly observed. When the effect of the stimulus eliciting the general flexor response is weak (Fig. 8B), the reflex responses to M-C movement, although modulated, are still present. In this case the F motoneurons which are driven by the mechanical stimulation of the antennae (but not the M-C movement when it is delivered alone), fire in bursts corresponding to each extension movement of the carpopodite. Further, the inhibitory unit of the extensor nerve is also modulated by the M-C movement.

From these examples it appears that the intensity of the remote stimulus determines the interactions that occur between the two responses. A light stimulus blocks the E responses to M-C movement by simultaneously increasing the F excitatory and E inhibitory responses. A strong stimulus induces a general flexor reaction in the leg, characterized by the disappearance of the E excitatory responses, whereas the F

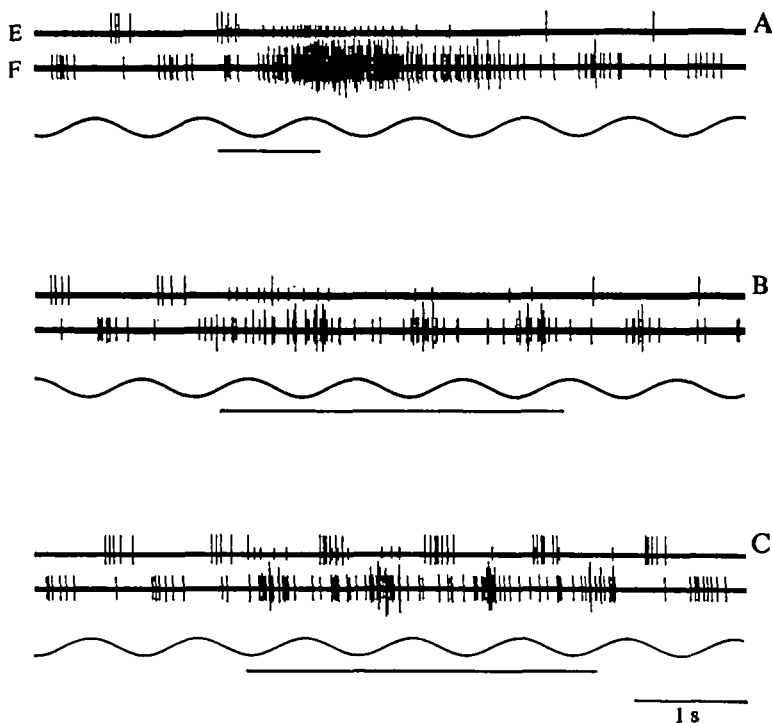


Fig. 8. Simultaneous recordings from motor nerves to E and F during reflex activation by passive M-C joint movement (extension indicated by upward deflexion of the sinusoidal trace), with various stimuli (indicated on the bottom trace): A, Electrical stimulation of the ventral nerve cord. B, Mechanical stimulation of both antennae. C, Mechanical stimulation of the contralateral appendages.

excitatory and the E inhibitory discharges are considerably enhanced, so that the responses to M-C movement are completely masked. Another type of interaction that occasionally appeared in our experiments is shown in Fig. 8C, in which the response elicited by stimulation of a contralateral walking leg enhances the flexor effect of M-C extension by increasing the F excitatory and the E inhibitory discharges as described above; however, the extensor effect of the M-C flexion is also somewhat enhanced. Thus both flexor and extensor effects of M-C joint movement are increased in this latter case.

(b) *Synchronous responses in F and AF excitatory motoneurons*

Our data indicate that F and AF tonic excitatory motoneurons fire during the same sequences (Fig. 7), and a parallel modulation of their frequency discharge is elicited by various stimuli. This is true whichever excitatory motoneurone (F or AF) exhibits the higher discharge frequency. Their response to stimulation of the CSD (Fig. 7D, E) provides an example in which these two discharges seem the most independent in terms of their general frequency: the CSD afferents enhance the excitatory F discharge considerably more than the AF discharge (Fig. 7D, E).

These F and AF excitatory motoneurons appear to fire concomitantly in that most of the spikes of the neurone discharging with the lower frequency are approximately simultaneous with spikes of the other motoneurone. This particular relation-

ship can be observed during spontaneous activity, as well as during reflex activity, elicited by M-C joint movement. When F and AF are activated by the afferent discharges from either the MC or the MCO sense organs this relation still exists. A study of the temporal linkage between F and AF has shown that it occurs mainly within a delay of 20 ms (in low frequency discharges: 5/s) (D. Angaut-Petit & F. Clarac, in preparation). A striking feature of this linkage is that an impulse in the AF motor axon could precede as well as follow a spike in the F axon. It seems that the relation between the two motor commands can be either loose or tight since an F impulse can be either coupled or not coupled with an AF impulse.

(4) *Neuromuscular activity of the accessory flexor muscle*

It appears that the excitatory and the inhibitory motor axons to AF fire during the same sequences. What happens to a single AF muscular fibre? In order to answer this question, intracellular records of AF muscle fibre activity were obtained. An effective contraction of AF should occur as the result of the competition between the two influences. The interaction between the IJPs and the EJPs depends on their relative time of occurrence. If it is clear that the depolarizing effect of an EJP can be reduced or shortened by the occurrence of an IJP, it is also obvious that some IJPs occur completely independently of an EJP (Fig. 3). Though only one excitator motoneurone supplies the entire muscle, the EJPs are different from one muscle fibre to another. They can be roughly classified in terms of their amplitude: some are considered as small and the depolarization produced by individual EJPs does not exceed 1 or 2 mV; others are large and can reach 10 mV. When the motor discharge frequency increases, the response of some muscle fibres shows an increasing amplitude of successive EJPs which may reach a 'plateau' (tonic fibres). In other muscle fibres, even at a 10 Hz frequency of stimulation the EJPs are only increased by $\times 1.5$ (phasic fibres). It appears that the fibres with the small amplitude EJPs are also characterized by a marked increase, whereas little increase occurs in muscle fibres with large EJPs (see also Lang & Atwood, 1973). Many fibres can be clearly classified thus, but others may be considered as intermediate.

IJPs were recorded in nearly all the fibres investigated; their amplitude is usually small (0.5–1.5 mV). Though an extensive study of these muscle fibres has been made, it is difficult to categorize them in order to compare the physiological properties of the two parts of the AF muscle with the anatomical results. It was established by Atwood & Dorai Raj (1964) that long sarcomere and short sarcomere fibres are present in both parts of AF. Though no clear localization of fibre types was possible from our study, it appears that in the proximal part of the AF, the muscle fibres connected directly to the connective tissue in which MCO is inserted tend to be phasic. In the distal part of AF there is a tendency for the more distal fibres lying closest to the M-C joint to be phasic, while those situated near the proximal edge of this part of AF muscle are mainly tonic. The muscle fibres rarely exhibit all-or-none spikes; nevertheless spontaneous spikes (70–80 mV) have been observed in 3 distal fibres of the dAF in which unitary EJPs reached 10–15 mV amplitude.

DISCUSSION

Reflex modulation of motoneurone discharges has previously been studied in the walking legs (Bush, 1965; Cohen, 1965; Field, 1974) or in the abdomen (Kennedy *et al.* 1966) of Crustacea. The present results enable us to determine the relative importance of the different peripheral feedback loops in an inhibitory and excitatory motor command.

(1) Technical consideration

The 'en passant' recording with suction electrodes leaves the reflex loops intact. As the muscles are still connected with their motor nerves, their tension depends on the level of activity of the CNS. According to the initial tension of the muscles the mechanical conditions in which a joint movement is accomplished will be very variable and could involve the proprioceptors in very different ways. To avoid these variations, the previous authors cut the motor axons distally to open the feedback loops.

The various forms of 'natural stimuli' applied to the appendages and abdominal segments induce roughly the same pattern of responses in the motoneurons that innervate the muscles of the meropodite. This kind of stimulation has previously been used by Wilson & Davis (1965), Kennedy *et al.* (1966) and Spirito (1970). Wilson & Davis (1965) remark that a high level of activity can be obtained by 'touching, pinching or scratching various parts of the body'. Spirito used a 'tactile stimulation'. We have shown that the location of the stimulus was not significant; its intensity seemed a much more important factor in determining the response. These natural stimulations are of course very imprecise and can roughly correspond to an alert signal ('alerting stimuli') eliciting a defensive reaction in which the first periopods are raised high, flexed at the M-C level and with the claws open (Wiersma, 1961*b*).

*(2) Inhibitory and excitatory motoneurone activity**(a) The common inhibitory motoneurone*

A common inhibitory unit in the crustacean legs was activated by a particular stimulation eliciting a defence reflex. Since they have been discovered, there have been numerous hypotheses about common inhibitory motoneurons (see Wiersma, 1961*a*; Pearson & Bergman, 1969; Evoy & Cohen, 1971; Atwood, 1973).

The common inhibitory neurone that we have studied innervates two muscles in the meropodite, the extensor and the accessory flexor (Angaut-Petit *et al.* 1974); and one in the carpopodite, the bender (Wiersma & Ripley, 1952). The roles of the impulses conveyed by E and AF branches of this axon are different.

The inhibitory impulses to E muscle usually occur when the activity of the excitatory axon to E decreases or stops. The stimulation which elicits a general flexion, induces at the same time a twofold inhibition in the extensor muscle: one inhibition is central and blocks the activity of the excitatory tonic fibre to E, the other one appears at the periphery and modulates or prevents the muscular contraction through the activation of the inhibitory motoneurone to E. Thus, reflex reciprocity in the action of the 2 antagonistic muscles E and F is achieved by central and peripheral mechanisms. Such is also the case for reflex activity of the abdominal segment (Kennedy *et al.* 1966).

The inhibitory impulses to AF muscle in the common inhibitory motor axon fire during the same sequences as the excitatory axon of AF. It does not seem that the inhibitory and excitatory discharges are coupled as they are reported to be in the stretcher or opener muscles of the crayfish cheliped (Spirito, 1970).

(b) *Excitatory motoneurones*

As Atwood (1973) recently defined them, we have to consider tonic units which are active most of the time 'for normal movements and postural control' and phasic motor axons discharging in bursts during 'vigorous activity'.

The reflex discharges elicited by M-C joint movement influence mainly the tonic motoneurone of E, F and AF; the F and AF motoneurone present the same linkage as during spontaneous activity. Bush (1962*b*, 1963) noted that reflex activity of the C-P and P-D joints differed between *Brachyura* and *Anomura* in several respects. It was weaker and less specific in the former than in the latter species. Our results support this finding; though the general reactivity seems good in our experimental conditions, the frequency discharges of the motoneurones were lower than in analogous reflex responses studied in other species (Bush, 1965; Field, 1974; F. Clarac & J. P. Vedel, in preparation).

The M-C joint posture is regulated through loops involving receptors of other joints and segments of the same appendage. The action of the CSD2 afferents on M-C joint posture that we describe provides an example of that sort of interaction.

The ventral location of the CSD2 in the ischiopodite, in a piece of cuticle which is distorted by the load supported by each leg, could make this receptor sensitive to the weight of the animal. Through this reflex loop, the regulation of the posture involving the weight of the body or pressure supported by the animal can occur. The frequency difference that occurs in AF and F motoneurones in response to CSD stimulation, suggest that the MCO-AF loop is not as concerned as the F motor output by the CSD2 afferents. It appears in this case that the 2 flexor muscles can almost be influenced independently. The results of the simultaneous application of two stimuli confirm on the one hand the linkage between the two excitatory motoneurones, and on the other hand their independence.

The general flexion produced by the alerting stimuli is characterized most of the time by a reciprocity between E and F. When the general flexor reaction is superimposed on a M-C passive joint movement, the reflex activity induced by this movement is less conspicuous and can be completely masked or stopped.

This sort of defence reflex does not involve giant fibre activity (Wiersma, 1961*b*). The responses recorded in the ventral nerve cord (Fig. 6) show no discharge of giant fibres, only numerous small amplitude units. Among these latter, it is reasonable to think that there are some command interneurones which activate simultaneously the flexor motoneurones and inhibit the extensor ones – that the influence of the 'alerting stimuli' on E and F motor commands was not always reciprocal has previously been observed, (Larimer & Eggleston, 1971; Sokolove, 1973).

(3) *The AF-MCO system*

In the AF muscle the presence of an excitatory motoneurone (Frank, 1973) functionally linked with another supplying F, and an inhibitory motoneurone common with E, raises the question of how this muscle responds to their patterns of activity and whether their responses are meaningful in terms of behaviour (Spirito, Evoy & Fournier, 1973).

According to Atwood (1973), AF muscle is a 'mixed system' with a wide range of muscle fibres (phasic, intermediate and tonic) innervated by a single motor axon, the activity of which can be considered as tonic. The arrangement of the AF muscle fibres in the crayfish seems analogous to the disposition described by Atwood & Dorai-Raj (1964) in *Cancer magister*; the inhibitory potentials have been recorded in all the fibres investigated in the proximal and distal parts of AF muscle. Clarac & Vedel (1971) have demonstrated in the rock lobster that the contraction of the distal part could provide a mechanical force powerful enough to move the M-C joint; whereas the proximal part exerted a traction on the attachment of the myochordotonal organ. The inhibitory command has different effects on these two parts. In the distal head, it decreases the tension of the muscle fibres and prevents the flexion, whereas in the proximal head it modifies the MCO activity.

Our results also give some information on the possible action of MCO. Cohen (1965), Evoy & Cohen (1969) described some regulatory loops in the crab showing that they were organized according to a central reference. They showed that the influence of MCO activity on the AF motor command depended on the initial length of AF muscles fibres: the excitatory motoneurone response was enhanced when the tendon of the muscle was pulled or released away from a rest position, whereas it was reduced when the tendon returned back to this rest position. We did not confirm this result in the crayfish. Stretching the AF tendon increased the AF excitatory motoneurone discharge whatever the initial length of the system. During the release of the tendon the same activity decreased or became silent. Therefore the MCO afferents elicit in the crayfish a classical resistance reflex on AF motor command.

The reflex activity elicited by M-C passive movement originates from the two chordotonal organs MC1 and MC2 and from the MCO (Bush, 1965; Evoy & Cohen, 1969; Clarac, 1970). The presence of apodeme tension receptors analogous to those discovered in the crab by Macmillan & Dando (1972) seems doubtful here. Our findings suggest that the change in AF tension produced by its own motor command, independently of M-C joint movements, can modify not only the reflex actions of MCO afferents on E and F motoneurons but also those of MC organ sensory discharges, i.e. MC1 and MC2 (see Fig. 4). Although the effects of the sensory discharges from MC and MCO on E, F and AF motor commands are cumulative, there is no redundancy between them since MCO – the activity of which can be modulated by the central nervous system – can modulate reflex activity elicited from other proprioceptors.

Considering the interaction between the motor commands to the 3 muscles E, F and AF, three cases are theoretically possible and can be considered to try to ascertain the role of MCO.

(1) The AF command is inactive. The AF follows the movement provoked by the

extensor and the flexor muscles. When the extensor muscle is active, the extensor units of MCO respond (Clarac & Vedel, 1971). When the flexor contracts the AF follows passively the movements of its synergic, the MCO presents the same 'behaviour' as the chordotonal organs (MC1-MC2). In fact this case never occurs because the F command is linked with the AF excitatory motoneurone activity.

(2) The AF excitatory motoneurone discharges. F contraction is simultaneous with an active AF contraction: in this case the chordotonal organs give the same information elicited by the M-C movement as in case I. On the contrary, the sensory information from MCO is much more complex; the movement cells indicating the extension are activated by pAF contraction as shown by Clarac & Vedel (1971) while some units sensitive to flexion still respond to M-C flexion. Our results suggest the complexity of the MCO discharge because a functional linkage exists between F and AF.

(3) A third alternative is that the excitatory command of AF is completed by an inhibitory one (which is common to E). The muscular contractions of F and AF is dissociated. The AF contracts slightly and is moved passively by F. MCO discharge, as in the first case, is induced by the flexion movement or is influenced by competitive action between excitatory and inhibitory influences that impinge on the different AF muscle fibres.

In conclusion, Cohen (1965), describing the MCO functional loops, showed the peculiarity of its discharges in the crab where they modify the AF command so as to maintain a centrally determined referential position. In the crayfish it appears that the main significance of the MCO system has to be sought in the modulation of AF command in relation to the discharges of the motor nerves to the two main muscles E and F.

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