Functional Analysis of the Sensory Motor Pathway of Resistance Reflex in Crayfish. I. Multisensory Coding and Motor Neuron Monosynaptic Responses

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Le Ray, Didier, François Clarac, and Daniel Cattaert. Functional analysis of the sensory motor pathway of resistance reflex in crayfish. I. Multisensory coding and motor neuron monosynaptic responses. J. Neurophysiol. 78: 3133–3143, 1997. An in vitro preparation of the fifth thoracic ganglion of the crayfish was used to study in detail the negative feedback loop involved in the control of passive movements of the leg. Release-sensitive primary afferents of from the coxo-basipodite chordotonal organ (CBCO), a proprioceptor whose strand is released by upward movement of the leg, monosynaptically connect to depressor motor neurons (Dep MNs). Extracellular identification of sensory units from the CBCO neurogram allowed us to determine the global coding of a sinusoidal movement, imposed from the most released position of the CBCO strand. Intracellular recordings from sensory terminals (CBTs) and ramp movement stimulations applied to the CBCO strand allowed us to characterize two groups of release-sensitive CBCO fibers. The first group, divided into two subgroups (phasic and phasotonic), is characterized by discontinuous firing patterns: phasic CBTs fired exclusively during release movements; phasotonic CBTs displayed both a phasic firing and a tonic discharge during the more released plateaus. The second group was continuously firing whatever the movement, with higher frequencies during the release phase of the movement stimulation. All CBTs displayed a marked sensitivity for release movements while only the phasotonic ones showed a clear sensitivity to maintained positions. Surprisingly, no pure tonic sensory fibers were encountered. Systematic intracellular recordings from all resistant Dep MNs, performed in high divalent cation saline, allowed us to describe two shapes of monosynaptic resistance reflex responses. A phasic response was characterized by bursts of excitatory postsynaptic potentials (EPSPs) occurring exclusively during CBCO strand release movements. A phasotonic response was characterized by a progressive depolarization occurring all along the release phase of the stimulation: during maintained released positions, the amplitude of the sustained depolarization was position dependent; in addition, each release movement produced a phasic burst of EPSPs in the MN. The parallel study of the Dep MN properties failed to point out any correlation between the type of reflex response recorded from the MN and the MN intrinsic properties, which would indicate that the type of MN response is entirely determined by the afferent messages it receives.

INTRODUCTION

The stretch reflex is a very widespread phenomenon described in all groups of vertebrates and invertebrates (Granit 1955; Pringle 1961). At first considered rigid and stereotyped, the concept of the reflex as being capable of extensive modulation has evolved in parallel with the data accumulated on the central pattern generator (Barnes and Gladden 1985; Clarac 1991; Pearson 1993).

In crustacean walking legs, each joint possesses one or two mechanoreceptors, the chordotonal organs, able to record the different parameters of position and movements (Bush and Laverack 1982; Whitear 1962; Wiersma 1959). They are composed of an elastic strand in which 10–100 bipolar cells are inserted. Mill (1976) defined four different classes of sensory afferents in the dactyl chordotonal organ. Two are sensitive to changes of the chordotonal organ strand length; they operate in opposite direction, one being stretch sensitive, the other release sensitive, whatever the position. The two others code the strand length (stretched or released states of the strand) and are mainly activated for extreme angular positions and minimally activated for midramp positions. Bush (1962, 1965b) demonstrated that chordotonal organs are able to induce a stretch reflex, called a resistance reflex, which consists in the activation of the passively stretched muscle. The stretch reflex is not limited to its proper joint but can spread out on other joints or even on other legs (Clarac 1977; Vedel and Clarac 1979). Intensity and gain of this reflex appear to be very variable and may lead to complete reversal. For example, in crayfish, no resistance reflexes seem to exist during treadmill walking, while they could be generated during unintended movements (Barnes 1977). In stick insects (Bässler 1976), in crabs (Di Caprio and Clarac 1981), and in crayfish (Skorupski and Sillar 1986), it has been demonstrated that the same mechanoreceptor is able, depending on “the central state” of the CNS, to change a resistance reflex into an assistance reflex. Such variability has been similarly found in mammals (Pearson 1993), including humans (Duyens and Tax 1994).

In the arthropods, intracellular studies have demonstrated that resistance reflexes involve both monosynaptic and polysynaptic connections (Bässler 1993; Burrows 1975). The variability of the reflex has often been associated with interneurons that are inserted within the reflex pathway, between sensory terminals and motor neurons (MN) (Burrows 1992; Büschges and Schmitz 1991; Büschges and Wolf 1995; Le Ray and Cattaert 1997). However, in the crayfish, monosynaptic connections between the coxo-basal chordotonal organ (CBCO) and the levator (Lev) and depressor (Dep) MNs that command the coxo-basal joint have been demonstrated to be responsible for efficient resistance reflexes (El Manir et al. 1991).
METHODS

Preparation

Results are based on >130 intracellular recordings from CBTs and Dep MNs performed on adult male and female crayfish, Pacifastacus leniusculus and Procambarus clarkii. Animals were maintained in aquaria at 18°C and fed once a week.

The in vitro preparation (Fig. 1A) consisted of the last three thoracic ganglia along with the two couples of antagonistic motor nerves innervating the two proximal joints of the fifth leg (promotor/remotor and depressor/levators). The CBCO, which encodes the vertical movements of the leg, was dissected out together with its sensory nerve (CB nerve). The preparation was pinned down dorsal side up in a silicone elastomer (Sylgard)–lined Petri dish and superfused with oxygenated crayfish saline.

Stimulations/recordings

Extracellular recordings and nerve stimulations were performed using platinum electrodes contacting the nerves, isolated from the bath with petroleum jelly (Vaseline), and directed to a 4-channel differential AC amplifier (A-M Systems). Single and paired intracellular recordings from CBTs and MNs (Fig. 1B) were made with thin-walled glass microelectrodes filled with a potassium chloride solution (3 M) and having a 25- to 30-MΩ resistance. The signals were amplified by an Axoclamp 2B amplifier (Axon Instruments). Intracellular current pulses and nerve stimulations were controlled by an eight-channel digital stimulator (A.M.P.I.). All signals were monitored on an eight-channel oscilloscope and a four-channel digital oscilloscope (Yokogawa DL 1200), stored on D.A.T. tapes (BioLogic digital tape recorder), and digitized on a PC-based computer through an A/D interface (from Cambridge Electronic Device, CED 1401PLUS). Intracellular and extracellular recordings were digitized at 5–10 kHz and written to disk.

An electromechanical puller (Ling Dynamic systems) driven by a homemade controller was used to stretch and release the CBCO strand, according to sinusoidal (Fig. 1, A, C, and D) or ramp (Fig. 4) protocols. Ramp stimulations are very suitable for separating position from movement sensitivities in intracellular recordings from one CBT. However, this procedure tends to synchronize the firing at the onset of each ramp movement, which makes it very difficult to separate the different units within the neuron. Therefore sine-wave movements were used during experiments in which CBCO neurograms were analyzed. Stretching stimulations were performed from the most released position of the CBCO strand, and total movement amplitude was one-third of the released CBCO strand length (1–1.8 mm). It almost corresponds to the angular range in which the leg moves during locomotion. Imposed movements elicit stretch and release of the CBCO strand, as real leg movements do. The movement control voltage traces were visualized on the oscilloscopes and stored on both tape and computer.

Salines

A normal saline (in mM: 195 NaCl, 5 KCl, 13 CaCl₂, and 2 MgCl₂) was used to perform the sensory coding analysis. Because strong central synaptic inputs masked the monosynaptic sensory inputs from the CBCO, the Dep MN responses were analyzed with saline in which divalent cation concentrations were increased (in mM: 34 CaCl₂ and 6.4 MgCl₂, with the sodium concentration reduced accordingly) to raise the activation threshold of all central neurons; a Vaseline wall was used to restrict the perfusion of this high divalent cation saline exclusively on thoracic ganglia, in order not to affect the CBCO sensory inputs (Fig. 8). Saline solutions were buffered with 3 mM N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES) and pH adjusted at 7.7 at 15°C.

Analysis

Signals were analyzed using the CED programs SPIKE2 and SIGAVG. The “Wave Marker” SPIKE2 program was used to classify the different profiles of extracellular spikes recorded from the CBCO nerve. Circular statistics (Batchelet 1981) were used to study the sensory coding of the cyclic imposed movement. Each sensory unit was identified over several movement cycles. Each cycle is defined from the starting of the release phase (chosen as zero) to the next release phase (chosen as 360°). Each spike is figured by a unit vector \( \vec{v} \), with a phase angle according to its occurrence in the stimulus cycle. The resulting mean vector \( \overline{R} \) is the vectorial sum of all unit vectors, divided by the number (n) of spikes (Fig. 2A).

\[
\overline{R} = \frac{\sum \vec{v}}{n}
\]

\[
|R| = \sqrt{R_x^2 + R_y^2}
\]

\[
\alpha = \arctan \left( \frac{R_y}{R_x} \right)
\]
with $R_x$ and $R_y$ representing the $x$ and $y$ coordinates of the resulting vector $\bar{R}$.

The resulting vector length ($|\bar{R}|$) has a value between 0 and 1, and is a measure of the dispersion of the spikes within a stimulus cycle. A value of zero means that the spikes are evenly distributed; a value of one would indicate that all spikes occurred at the same phase. The frequency distribution of $|\bar{R}|$ over all experiments (Fig. 2B) is bimodal, the separation between the two peaks being 0.3. Therefore units with $|\bar{R}|$ value below 0.3 were considered as nonspecific units (Fig. 2C), whereas the others were classified as release or stretch-sensitive units.

SPIKE2 scripts were used to analyze, for each part of the mechanical stimulation, the instantaneous and mean firing frequencies from CBTs, and the Dep MN responses. SIGAVG program was used to record MN response to various intracellular current pulses, to realize the voltage-current ($V-I$) curves from Dep MNs. Statistical analysis and regressions were performed with the GraphPad Prism statistic programs.

**RESULTS**

As shown in Fig. 1, C and D, the mechanical stimulation of the CBCO strand is able to produce a reflex activity in the in vitro preparation. This is characterized by the discharge of the sensory units that can be recorded extracellularly in the CBCO neurogram and intracellularly in the CBTs (Fig. 1C). This sensory firing induces, in the postsynaptic Dep MN, a depolarization of its membrane potential (Fig. 1D) because of the summation of each compound excitatory postsynaptic potential (EPSP).

**Analysis of the CBCO sensory coding of the leg movements**

Two complementary studies have been performed to describe the coding of the CBCO sensory information. First, from the neurogram, an extracellular approach allowed us to characterize in a general manner the different CBCO units. Because of the limitations of this method, we carried out a second analysis, the intracellular characterization of the CBCO fibers. To facilitate our work, we focused the intracellular study on the release-sensitive afferents, impaled in their terminals.

**EXTRACELLULAR STUDY FROM THE CBCO NEUROGRAM.** The extracellular study has been performed from a sinusoidal movement stimulation of the CBCO strand. In the angular range of the imposed sinusoidal movement, and depending on the preparation, about one-half of the CBCO units were activated as shown on Figs. 2C and 3. In our conditions, release-sensitive afferents were predominantly recruited (11/20 ± 2 release-sensitive and 7/20 ± 3 stretch-sensitive CBCO fibers, mean ± SE). The table of Fig. 2C gives the number of the sensory afferents activated by sinusoidal movements imposed to 10 CBCOs. A total number of 195 extracellular spikes has been studied to determine their sensitivity to CBCO strand movement; 55.5% of the recorded units were release-sensitive fibers ($n = 108$) against 28% of stretch-sensitive units ($n = 55$). In both cases, most of the CBCO units coded one direction of the imposed movement, although their discharge frequency persisted during a part of the opposite movement. In all experiments, some CBCO fibers (16.5%, $n = 32$) were classified in the "Nonspecific" group because their response was not clearly related to either phase of the imposed movement ($R \leq 0.3$; see METHODS). Figure 3 presents a typical experiment in which different types of CBCO units were characterized from the CBCO neurogram, using the "Wave Marker" Spike2 program. The different identified extracellular spike templates are presented with the corresponding event histograms, which represent the occurrence of each extracellular spike in the stimulation cycle (bin size: 3 ms). In this experiment, 14 distinct release-coding and 5 stretch-sensitive CBCO units could be differentiated. Nevertheless, three sensory units did not code preferentially one direction of the imposed movement ($R \leq 0.3$). All the release-sensitive fibers responded during the whole range of the release movement. Therefore it appears that the CBCO coding is distributed, i.e., sensory fibers do not code an extremely specific range of the movement. The phase ($\alpha$) of the mean of each release-sensitive unit varied between 92.3° and 152.5°, which indicates some differences...
in the coding of the various sensory units. Moreover, although some interunit differences exist (event histogram peaks occurred between 65 and 125°), most of the units fired when the velocity of the imposed movement was maximum (90°).

Thus sinusoidal movement stimulation and extracellular studies of sensory neurograms allowed us to determine how many CBCO afferents were excited in the angular range of the stimulation applied to the CBCO strand. Ramp movement stimulation during intracellular recordings from identi-
from 0.25 to 1.25 mm/s (i.e., multiplied by 5) the firing frequency of the movement-sensitive component of the phasotonic fiber was multiplied by 3 (white histogram). By contrast, the firing frequency of the tonic component appeared to be nonsignificantly affected by the change in velocity (dark histogram).

**FIG. 4.** Phasic release-sensitive CBTs. A: phasic CBTs were characterized by spike bursts occurring only during release ramps (1.25 mm/s). As shown in the inset, their firing stopped since the plateaus started. The mean firing frequency calculated over 9 stimulation cycles for each ramp is presented below and demonstrates that the position of the leg did not affect the firing frequency of this phasic CBT (resting potential: −77 mV). B: mean firing frequencies from all individual release ramps over the 9 cycles were calculated for 0.25 and 1.25 mm/s ramp velocities. The phasic coding of this CBT was highly velocity dependent, because a 5-fold ramp velocity increase led to a 3-fold firing mean frequency increase (P < 0.001). Error bars: SE. Mvt, imposed ramp movement.

**FIG. 5.** Phasotonic release-sensitive CBTs. Phasotonic CBTs fire phasically during release ramps and develop a tonic discharge for the most released position of the CBCO strand. A: 1st type of phasotonic CBTs continued to produce spikes even after the beginning of the CBCO stretch (resting potential of CBT1: −73 mV). B: 2nd type stopped once the stretch started (resting potential of CBT2: −70 mV). A and B: mean firing frequency histograms (same disposition as in Fig. 4A): both phasic (white bars) and tonic (filled bars) codings were dependent on the position of the leg. Regression lines are drawn for each histogram during the release phase (correlation coefficient are in A: 0.99 for the phasic component, 0.96 for the tonic component; in B: 0.97 for the phasic component, 0.93 for the tonic component). C: same calculation as in Fig. 4B, for both phasic and tonic components. Only the phasic pattern (white histogram) was velocity dependent (P < 0.001), whereas the tonic discharge (filled histogram) was not significantly affected by the ramp speed. Error bars: SE. Mvt, imposed ramp movement.
correlated to the position itself. Nevertheless, this activity was greater during the release than the stretch phase of the imposed movement. During the CBCO stretch, there was no clear difference between both types of firing (ramps or plateaus). Although these fibers were activated by release movements, they did not code the velocity, neither for the movement-related firing (Fig. 7B, white histogram) nor for the maintained position activity (Fig. 7B, dark histogram).

Dep MN monosynaptic reflex responses

To analyze the MN monosynaptic response, we performed intracellular recordings from the Dep MNs in high-Ca\(^{2+}\) and high-Mg\(^{2+}\) concentration saline. As shown in Fig. 8 (left), in a normal saline the Dep MN monosynaptic reflex response induced by CBCO mechanical stimulation was strongly masked by all the central inputs that reach the Dep MN. Perfusing the divalent cation-enriched saline exclusively onto the ganglion allowed us to reduce dramatically the central activities without affecting the sensory afferent information (Fig. 8, right). In these conditions, the Dep MN monosynaptic responses were unmasked, and the functional relationship between the type of response of the Dep MNs and their intrinsic properties could be analyzed.

As Fig. 6 shows, the phaso-tonic CBCO units showed adaptation of the tonic firing when the mechanical stimulation was stopped in a released position for a long time (>10 s, Fig. 6A). At the end of the last ramp, the tonic firing began with a frequency of 18 ± 3 Hz that was reduced to a basal value of 4 ± 2 Hz within 5 s. Figure 6B shows the mean firing frequency calculated over 5-s intervals (histogram) and instantaneous frequency (●) plotted as a function of time, after the mechanical stimulation was stopped in the most released position (time 0), for one phaso-tonic fiber. The basal instantaneous frequency value was obtained after only 6 s in the released position.

Continuous discharge CBCO units. Never described in detail in previous reports, this kind of fiber was characterized by continuous firing (at least 10 ± 4 Hz) that was modulated by movements imposed to the CBCO strand. Nevertheless, release-sensitive units showed a specificity for the release phase of the mechanical stimulation (where firing frequency could raise to a mean value of 65 ± 15 Hz during ramps). As shown on Fig. 7A, continuous discharge fibers fired with a higher frequency during the CBCO release but were still spiking during the stretch of the sensory organ. In this type of unit, movement sensitivity was also evident. The mean frequency histograms (Fig. 7A) show a marked difference between the movement response (white histogram) and the position response (dark histogram), as was the case for phaso-tonic units, during the CBCO release. The movement coding discharge displayed a clear-cut position sensitivity: the response to the same ramp movement in the most released position reached 65 Hz, whereas in more stretched position it was <25 Hz. However, the position-related movement sensitivity was not linear but hyperbolic. In contrast, the activity during maintained positions did not seem to be correlated to the position itself. Nevertheless, this activity was greater during the release than the stretch phase of the imposed movement. During the CBCO stretch, there was no clear difference between both types of firing (ramps or plateaus). Although these fibers were activated by release movements, they did not code the velocity, neither for the movement-related firing (Fig. 7B, white histogram) nor for the maintained position activity (Fig. 7B, dark histogram).
The reflex responses displayed by the Dep MNs are variable (Le Ray and Cattaert 1997); there exist one Dep MN involved in assistance reflex (i.e., activated during stretch of the CBCO), and eight Dep MNs involved in the resistance reflex (i.e., activated by CBCO release). If the response of the assistance Dep MN is stereotyped, i.e., EPSP bursts occurring exclusively during stretching ramps of the CBCO stimulation, the eight resistance Dep MNs are able to display responses of different shape. Ramp movements (with the same plateau duration, 2 s) imposed to the CBCO strand were able to produce in the resistance Dep MNs two different types of resistance responses (Fig. 9). Some Dep MNs showed a reflex response characterized by bursts of EPSPs (~1-4 mV amplitude) during the releasing ramps (Fig. 9A). These bursts of EPSPs were almost constant from the first to the last releasing movement of a stimulation cycle. This kind of response, where EPSP bursts stopped at the beginning of the plateau (see detail in inset of Fig. 9A), was defined as a “phasic response.” The second type of reflex response (Fig. 9B) was characterized by the same phasic bursts of EPSPs, which summed on a general depolarization of the MN that occurred during the whole release of the CBCO. In this case, the amplitude of the EPSP burst weakly increased from the first to the last releasing ramp (1 ± 0.4 mV during the 1st ramp, and 3 ± 0.5 mV during the last). In parallel, the Dep MN depolarization was increasing from the beginning until the end of the CBCO release (from 0 to 1.7 ± 1 mV). This kind of response (see detail in Fig. 9B, inset) defined a “phasic-tonic” Dep MN response. As shown in Fig. 9C, although the phasic bursts of EPSPs swiftly disappeared, the tonic depolarization of the phasic-tonic Dep MNs persisted (with an almost constant value) when the stimulation was stopped in a released position. The Dep MN completely repolarized only when the CBCO stretch started.

Tonic/Phasic Properties of MN and Monosynaptic Response to Movement. Because it has often been claimed that phasic MN have larger diameters and higher conduction velocities, we used these parameters to classify the Dep MNs. Figure 10A shows the conduction delays of 59 Dep MNs recorded from 12 experiments. The different MNs are grouped according to the monosynaptic reflex response they displayed, i.e., assistance, phasic resistance, phaso-tonic resistance, or no response. In the four cases, no correlation appeared between conduction delays (which were measured as the time required for spikes to travel from the intracellular recording sites to the extracellular en passant electrode on the depressor motor nerve) and the kind of monosynaptic response. The dispersion observed in the delays in each group is quite large: from 2.35 to 10 ms for the no responding Dep MNs (∅); from 2.5 to 8.75 ms for the phaso-tonic resistance MNs (■); from 2.19 to 8 ms for the phasic resistance MNs (∆); and from 3.44 to 5.39 ms for the assistance Dep MN (∗). This dispersion of conduction delay values for the unique assistance Dep MN would indicate some variability from one animal to the other. Nevertheless, in a single experiment where all Dep MNs were recorded, the distribution of conduction delays was similar and did not show any relationship with the reflex response displayed by the different Dep MNs.

To classify MNs on the basis of their firing properties,
we used injection of long-lasting depolarizing current pulses (2-s duration, 0.3 Hz). Some MNs fired continuously all along the depolarizing pulse, with a discharge frequency depending on the amount of injected current; they are defined as tonic MNs. Other MNs fired only few spikes at the beginning of the current pulse and rapidly decreased their firing frequency. Their discharge stopped before the end of the pulse; they are defined as phasic MNs. It appeared that the firing pattern of the Dep MN and its reflex response were not correlated because both patterns could be recorded in Dep MNs displaying different monosynaptic reflex response (data not shown).

**ACTIVE MEMBRANE PROPERTIES OF MN AND MONOSYNAPTIC RESPONSE TO MOVEMENT.** The Dep MN \( V-I \) curves were performed in a 5-mV range around the membrane potential to estimate the role of the membrane properties in the reflex response, because CBT-related EPSPs generally may reach up to 5 mV in MNs. \( V-I \) curves, resulting from injection of depolarizing and hyperpolarizing current pulses, revealed four types of electrical behavior in high divalent cation saline (Fig. 10B). Some Dep MNs (35%) displayed almost a linear response to current injection. The majority (51%) presented a negative rectification for negative current pulses (\( \Delta V_1 \)) and a weak positive rectification for positive current pulses (\( \Delta V_2 \)). A smaller number of Dep MNs (8.5%) showed a double negative rectification (\( \Delta V_1 \) and \( \Delta V_2 \)). The last type of electrical behavior, a positive rectification \( \Delta V_1 \) and a negative rectification \( \Delta V_2 \), was detected in few cases (only 5.5% of the impaled Dep MNs). \( \Delta V_1 \) and \( \Delta V_2 \) values were calculated as the differences between the linear regression (around 0) and the real values for \(-5\) and \(+5\) nA injected current amplitudes.

To represent the rectification properties of all recorded Dep MNs in a single diagram, we plotted \( \Delta V_2 \) as a function of \( \Delta V_1 \) for each MN (Fig. 10C). This diagram revealed clusters of Dep MNs that would indicate distinct behavioral groups of MNs. However, each cluster contained phasic (□) and phaso-tonic (■) resistance Dep MNs, except for cluster 5 (positive \( \Delta V_1 \) and negative \( \Delta V_2 \)), which contained exclusively the assistance Dep MN (*). Nonresponding Dep MNs (○) were also found in clusters 1 and 2 (moderate negative \( \Delta V_1 \)), but not in clusters 3 and 4 (larger negative \( \Delta V_1 \)).

**DISCUSSION**

In this paper, we have characterized the properties of both elements involved in the “stretch reflex” in crustacean walking legs: the CBCO sensory afferents and the connected MNs. We will discuss successively the functional aspects of the present results, the types of sensory coding and MN responses.

**Crayfish thoracic ganglion in vitro preparation**

The crayfish thoracic walking system is dissected out in such a way as to eliminate most of the sensory inputs that can excite the MNs. Only the CBCO is kept intact in the Petri dish with its sensory nerve that innervates the fifth thoracic ganglion. Perfusing the ganglion with high-Ca\(^{2+}\) and high-Mg\(^{2+}\) saline raised the threshold for spiking and we used injection of long-lasting depolarizing current pulses thus strongly ... and reproducible responses before performing the recordings 

\(~2\) h after the onset of the dissection. The CBCO was dissected out and arranged in the Petri dish in such a position that the puller imposed movements to the strand in exactly the same way as if it were in the intact leg. The CBCO strand length was measured in the fully released position, to apply stretches that were one-third of the original length. All stimulations were applied in this angular sector that corresponds to the angular range covered during the locomotor movements of the leg: the CB joint allows movements within the angular sector from the maximum of levation when the leg contacts the thorax, to the maximum of depression when the leg contacts the ventral carapace. Accidentally the CB joint can reach these extreme positions, for example when the animal is upside down. Nevertheless, movements involved in locomotion or in posture are restricted to \(\pm60^\circ\), between the horizontal plane and the complete levation. During levation the CBCO strand is completely relaxed, and during depression it is slightly stretched; we focused our study on this functional part of CB joint movements.
Sensory unit coding

In the past, the crustacean walking leg chordotonal organs have been classified into two types: the first type presents a symmetrical discharge, and comprises the pro-dactylopodite (PD), the carpo-propodite (CP₁), the mero-carpododite (MC₁), and the CB chordotonal organs, i.e., they have fairly the same number of stretch-sensitive and release-sensitive sensory units. The second type only possesses release-sensitive sensory fibers and comprises the CP₂, the MC₂ and the basi-ischiopodite chordotonal organs (Mill 1976; Mill and Lowe 1973). Other chordotonal organs are not disposed at a joint: the cuticular stress detectors (CSD₁ and CSD₂), located near the CB joint, are linked to small windows of soft cuticle and respond to mechanical stretching and releasing of their strand when the cuticle is stimulated (Marchand et al. 1991). In 1991, El Manira et al. described the CBCO: it is specifically for a small joint angular sector.

The sinusoidal movements were used because it resembles the real movement performed by the CB joint during walking activity. Ramp movements were used to finely distinguish between position and velocity-sensitive components of the responses. However, ramp movements did not allow analysis from the neurogram because of the large number of units synchronized during each ramp. Therefore ramp stimulations were exclusively performed during intracellular recordings from CBTs. By contrast, sine-wave movements allowed the analysis of the neurogram because no synchronization occurred. From one experiment to the other, we were always very careful to perform ramps that had the same speed and amplitude characteristics. However, because the released CBCO length varied from 1 to 1.8 mm, the number of ramps imposed to the strand was modified accordingly (Figs. 4–7). This procedure results in ramp amplitudes being different relatively to the released CBCO length. However, the results were very comparable whatever the imposed ramp amplitudes.

Our results confirm previous observations that described the unidirectional responses of the sensory fibers (Bush 1965a,b; Mendelson 1963; Mill 1976) and, from more sophisticated tools, give new insights concerning chordotonal organ fine sensory coding. In the past, most of the studies were performed from extracellular recordings (Bush 1965a,b; Clarac 1970), the analysis being based on the amplitude of the action potentials, a technique that makes discrimination of units of similar size uncertain. Our extracellular analysis, based on the extracellular action-potential shape recognition, thoroughly studied the discharge of almost all the sensory units excited during movements imposed over a physiological range. Sensory terminal intracellular recordings allowed us to identify specifically different types of sensory units. They can be divided into three subtypes that are the phasic, the phaso-tonic and the continuously firing sensory fibers. This classification is mainly based on the position sensitivity because all of them are strongly sensitive to movement. The phasic units only responded to movement, their response being unchanged whatever the angular sector where it was measured. By contrast, in both phasotonic and continuously firing units, the response to movement was considerably affected by the angular sector where it was measured. Therefore, among movement-sensitive responses (dynamic responses), we can distinguish the position-unaffected dynamic (phasic units) from the position-modulated dynamic responses (phaso-tonic and continuously firing units). In addition, the dynamic response may be velocity sensitive (phasic and phaso-tonic units) or not (continuously firing units). Surprisingly, we did not find purely position-coding units as are usually described (Bush 1965a; Clarac 1970; Matheson 1990), for all units coding positions were also activated during movements (phasotonic CBTs). Whatever the imposed movement (ramp or sine wave), it appeared that each sensory fiber is activated in a wide angular sector. We never recorded any fiber coding specifically for a small joint angular sector.

As a conclusion of this study, it seems that the coding of movement is shared by all the sensory fibers, i.e., on the whole angular sector of the CB joint, upward movements should activate ~20 release-sensitive fibers and downward movements should activate ~20 stretch-sensitive fibers. Moreover, during movements, the firing frequency of any CBT is always 3–10 times higher than during maintained positions. Consequently, the major information conveyed by this sensory system is dynamic, and whatever the postsynaptic neurons they should be more excited during movements than during maintained positions. The CBCO movement sensitivity may therefore be responsible for the resistance reflex being a dynamic reflex, activated by any velocity variation rather than by static positions. Concerning the coding of position, CBTs seemed to present a low selectivity because during sine-wave movements all release-sensitive sensory afferents were activated on the whole range of the releasing movement. However, their maximum of discharge occurred at angular positions that were different from one CBT to the other (Fig. 3).

In the angular sector where movements were imposed to the CBCO strand, the release-sensitive sensory units were activated in a greater number than stretch-sensitive ones (almost twice the number of stretch-sensitive units). Thus, in the angular sector involved in locomotion and posture, the monosynaptic pathway excites more Dep MNs than Lev ones. Thus the resulting monosynaptic reflex effect is body upholding; i.e., it counteracts the force of gravity on the body.

Monosynaptic motor neuronal responses

Recently, different studies on the crustacean MNs have demonstrated that, in the thorax, the abdomen and the stomatogastric ganglia, motor neuronal pools are quite complex and not homogenous. Their intrinsic properties and their connections have been described to be responsible for this heterogeneity (Harris-Warrick et al. 1992; Le Ray and Cattaert 1997; Leibrock et al. 1996; Skorupski et al. 1992; Wine 1984). In the same MN pool, it seems that not all the MNs have the same roles. Skorupski et al. (1992) demonstrated that there were two groups of MNs in the promotor pool: one involved in the resistance reflex and the second in the assistant pathway. More recently, Le Ray and Cattaert
(1997) showed in the Dep MN pool the existence of one assistance-specialized Dep MN. In this study, we classified the Dep MNs according to their monosynaptic reflex response and their intrinsic properties. We did not take into account the electrotonic coupling that has been described as being very weak between Dep MNs (Chrachri and Clarac 1989). Classically, MNs have been classified into tonic and phasic depending on the type of muscle fibers they innervate and their activity (Kennedy and Takeda 1965a,b). Tonic MNs display continuous discharge, whereas phasic MNs are activated only during rapid movements (Kennedy and Davis 1977). Generally, phasic MNs have cell bodies and axon diameters larger than tonic ones, and they convey spikes more rapidly. Functionally, the resistance reflex is acting during posture and should involve preferentially postural MNs, i.e., tonic MNs. In the present work, we did not attempt to classify Dep MNs according to the muscle fibers they innervate or their activity in motor behavior. However, we did attempt to classify Dep MNs according to their conduction velocity (Fig. 10A) and their firing pattern. Surprisingly, this study demonstrates that this classification did not match the different types of reflex responses, because resistance reflex Dep MNs were found in the whole range of the conduction delays (Fig. 10A). It cannot, however, be excluded that conduction delays may not be related to the tonic or phasic behavior of a given MN. Concerning the three MNs that are not directly connected by the CBCO sensory afferents, they were also found in the whole range of Dep MN conduction delays (Fig. 10A). Nevertheless, this group may also be heterogeneous, some of them being excited through one or several interneurons and others not. Moreover, the properties of interneurons involved in polysynaptic pathways may be very different from one MN to the other. Such an organization has already been demonstrated in the case of the insect locomotor system (Burrows and Pfüger 1988; Sauer et al. 1995) and in the case of the crayfish reflex pathways (Clarac et al. 1991), and might be very important in reflex modulation.

In contrast, the classification of Dep MNs according to their membrane electrical properties shows that the assistance Dep MN has specific positive rectification for hyperpolarizing currents (Fig. 10, B and C). Because in these experiments sensory EPSP amplitudes were smaller than 5 mV (Fig. 9), we limited the exploration of the rectifying properties of MNs to a 10-mV range around the resting membrane potential. We thought that phaso-tonic responses might have involved slow active properties, whereas phasic responses might not. The results of the present analysis demonstrate that this is not the case. Nevertheless, this classification shows that Dep MNs are grouped into clusters, which reveals clear-cut heterogeneity in the Dep MN pool (Fig. 10C). However, this clear-cut classification of Dep MNs does not match the type of monosynaptic reflex response they exhibit.

In conclusion, Dep MN properties failed to explain the differences existing between Dep MN responses. The origin of such differences should therefore be searched for in the combination of sensory afferent types that innervate a given MN. Such an analysis is presented in an associate study, in which the properties of monosynaptic connections between the CBCO sensory afferents and the nine Dep MNs that express a reflex response to CBCO movements are considered.

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