Functional Analysis of the Sensory Motor Pathway of Resistance Reflex in Crayfish. II. Integration of Sensory Inputs in Motor Neurons

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Le Ray, Didier, François Clarac, and Daniel Cattaert. Functional analysis of the sensory motor pathway of resistance reflex in crayfish. II. Integration of sensory inputs in motor neurons. J. Neurophysiol. 78: 3144–3153, 1997. The in vitro preparation of the fifth thoracic ganglion of the crayfish was used to analyze the connections supporting the monosynaptic reflex responses recorded from the depressor motor neurons (Dep MNs). Dep MNs are directly connected by the release-sensitive afferents from a proprioceptor, the coxo-basipodite chordotonal organ (CBCO), which is released by upward movements of the leg. Sine-wave movements, applied to the CBCO strand from the most released position, allowed us to stimulate the greatest part of release-sensitive CBCO fibers. Systematic intracellular recordings from all Dep MNs performed in high divalent cation saline allowed us to determine the connections between CBCO afferents and their postsynaptic Dep MNs: it highlighted the sequential activation of the different Dep MNs involved in the monosynaptic reflex. The convergence of different sensory afferents onto a given Dep MN, and the divergence of a given sensory afferent onto several Dep MNs illustrates the complexity of the sensory-motor reflex loops involved in the control of locomotion and posture. Electrophysiological experiments and simulations were performed to analyze the mechanisms by which Dep MNs integrate the large amount of sensory input that they receive. Paired intracellular recording experiments demonstrated that postsynaptic response shapes characteristic of both phasic and phasotonic afferents could be induced by varying the presynaptic firing frequency, whatever the postsynaptic Dep MN. Compartment model simulations were used to analyze the role of the sensory-motor synapse characteristics in the summation properties of postsynaptic MN. They demonstrated the importance of the postsynaptic compartment geometry, because large postsynaptic compartments allowed to generate greater excitatory postsynaptic potential (EPSP) summations than small ones. The results presented show that velocity information is the most effective to elicit large compound EPSPs in MNs. We therefore suggest that the negative feedback reflex is mainly based on the detection of leg movements.

INTRODUCTION

The role of sensory information, especially proprioceptive feedback (Sherrington 1910), in the organization of motor output has been studied extensively in a variety of different preparations, invertebrate and vertebrate alike (Barnes and Gladden 1985). Proprioceptive inputs may be integrated at different levels of the motor command. The simplest interactions consist of direct contacts between proprioceptive sensory afferents and motor neurons (MNs). More complex integrations involve one or several interneurons. For example, in the cat spinal cord, Ia afferents evoke monosynaptic excitatory postsynaptic potentials (EPSPs) and Ib afferents evoke disynaptic inhibitory postsynaptic potentials (IPSPs) in homonymous MNs (Eccles 1967). Polysynaptic connections also exist both in insects (Bässler 1993; Burrows and Pflüger 1988; Büschges and Wolf 1995) and in crustaceans (Clarac et al. 1991; Le Ray and Cattaert 1997). Recent intracellular studies have shown that the properties of simple neuronal networks depend on both the connectivity and the endogenous properties of the component neurons (Harris-Warrick et al. 1992). Therefore sensory-motor integration involves complex dynamic processes.

However, studies of sensory-motor interactions often concern simple reflexes. Among these, a large amount of data concerns the vertebrate stretch reflex: it is a negative feedback loop in which stretching the muscle spindle evokes activation of homonymous MNs (Eccles 1957; Granit 1955; Matthews 1972). For the last 40 years, physiological studies of this reflex have demonstrated that most of the excitatory connections between sensory afferents and MNs are monosynaptic (Burke and Rudomin 1977; Redman 1979). In arthropods too, monosynaptic connections between primary afferents from proprioceptors and MNs are widespread (Blight and Llinás 1980; Burrows 1987). In all cases, these connections support resistance reflexes, comparable with the vertebrate stretch reflex, in which MNs respond to counteract an imposed movement. More recently, in crayfish walking legs, afferents from the coxo-basipodite chordotonal organ (CBCO) have been shown to make direct excitatory synapses onto levator (Lev) and depressor (Dep) MNs: stretch-sensitive sensory fibers connect Lev MNs, and release-sensitive fibers connect Dep MNs (El Manira et al. 1991).

Although systematic analysis of the coding of movement parameters have been extensively performed (Bush 1965a,b; Le Ray et al. 1997; Matheson 1990), the organization of the reflex responses, and particularly the role of different types of sensory information in this organization, remains unclear. The sensory-motor loop involving the CBCO in the crayfish thoracic in vitro preparation gives us the opportunity of answering this question, for only ~20 sensory afferents connect to <10 Dep MNs (Le Ray and Cattaert 1997). The different Dep MN monosynaptic reflexes elicited by ramp movements imposed to the CBCO strand were described, and three types of Dep MN response were demonstrated: three Dep MNs did not present any monosynaptic response, one displayed an assistance reflex response (i.e., depolarizations during the stretch of the CBCO), and eight Dep MNs produced a resistance reflex response (i.e., depolarizations during the CBCO release).
In an associated work, we did an extensive analysis of the release sensory coding and demonstrated the existence of two distinct resistance responses: one characterized only by phasic EPSP bursts related to release ramps, the other characterized by both phasic bursts of EPSPs and a graded membrane depolarization during the release phase of the movement (Le Ray et al. 1997). The present study aims at analyzing the transfer of information from CBCO fibers to monosynaptic output Dep MNs. We analyze what type of information each identified Dep MN receives from the CBCO during imposed movements. A functional wiring diagram is established, and the role of EPSP characteristics in the integration of the MN response is analyzed using a compartment model simulation.

**METHODS**

**Preparation**

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

The in vitro preparation consisted in the last three thoracic ganglia and the two couples of antagonistic motor nerves innervating the two proximal joints of the 5th leg (Promotor/Remotor and Depressor/Leverators). The CBCO, which encodes the vertical movements of the leg, was dissected out together with its sensory nerve. The preparation was pinned down dorsal side up in a silicone elastomer (Sylgard)–covered Petri dish and superfused with oxygenated crayfish saline.

**Stimulations/recordings**

Extracellular recordings were performed using pin platinum electrodes contacting the nerves, isolated from bath with petroleum jelly (Vaseline), and directed to a four-channel differential AC amplifier (A-M Systems). Single and paired intracellular recordings from CBTs and Dep MNs (Fig. 1, A and B) were realized 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 (Axon Instruments). Intracellular current pulses were controlled by an eight-channel digital stimulator (A.M.P.I.). All signals were monitored on an eight-channel oscilloscope, a four-channel digital oscilloscope (Yokogawa DL 1200) and 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 20 kHz and written to disk.

A homemade puller controlled mechanical stimuli characterized by cyclic stretch and release of the CBCO strand, according to a sinusoidal (Fig. 1C) or a ramp protocol (Fig. 4). Movement 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). The movement control voltage traces were visualized on the oscilloscopes and stored on both tape and computer.

**Salines**

Vaseline wall was used to superfuse separately the CBCO and the thoracic ganglia. The CBCO was superfused with saline, which contains (in mM) 195 NaCl, 5 KCl, 13 CaCl₂, and 2 MgCl₂. The thoracic ganglia was superfused with saline where divalent cation concentration was increased (in mM: 34 CaCl₂ and 6.4 MgCl₂, with the sodium concentration reduced accordingly), to raise the activation threshold of all central neurons without affecting the CBCO sensory inputs. It allowed to mask the monosynaptic reflex response from MNs (Berry and Pentreath 1976). Moreover, in experiments where intracellular current pulses were delivered into the CBTs, the high divalent cation concentration saline did not affect the monosynaptic EPSP produced in the post synaptic Dep MN (Fig. 5B). 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**

Physiological signals were analyzed with the CED SPIKE2 program. Data and traces were acquired using scripts running with the SPIKE2 program. The SPIKE2 “Wave Marker” tool allowed to identify extracellular CBCO units from the neurogram. Subsequently, it was possible to determine which sensory unit connected the given intracellularly recorded assistance (A MN) or resistance (R MN1 and 2) Dep MN (Fig. 1, C1 and C2).

Circular statistics were used to analyze the distribution of identified CBCO EPSPs recorded from a given MN during imposed sine-wave movements. The phase occurrence of each sensory unit over several movement cycles (starting of the release phase being chosen as zero) was figured by a unit vector \( \hat{u}_i \). The resulting mean vector \( \bar{R} \) was calculated as the vectorial sum of all (n) unit vectors \( \hat{u}_i \) divided by n (Fig. 1, C3, left), for each identified CBCO unit.
with \( R_c \) and \( R_x \) representing the \( x \) and \( y \) coordinates of the resulting mean vector \( \mathbf{R} \). The resulting mean vector length (\( ||\mathbf{R}|| \)) has a value between 0 and 1 and is a measure for the mean distribution of spikes within a stimulus cycle: a value of zero would indicate that the spikes were evenly distributed; a value of one would indicate that all spikes occurred at the same phase. In Fig. 1C3, the right circular diagram illustrates distribution of the resulting mean vectors of the three different CBCO units (1, 2, and 4) that make direct EPSP in R MN1 (illustrated in Fig. 1C2).

Statistical analysis and fittings were performed with the GraphPad Prism statistic programs.

Simulations

Spatial and temporal integration of EPSPs produced at different locations of MN dendrites was simulated with a compartment model (program SWIM) (Ekeberg et al. 1991). The model allows construction of arbitrarily complex dendritic trees (Fig. 9A). The simulation model used 60 compartments. The main neurite, from which intracellular recordings are usually performed in real MNs, was composed of 20 compartments each 20 \( \mu \)m long and 10 \( \mu \)m diam. In addition, two primary branches, one composed of 15 compartments (length, 20 \( \mu \)m; diameter, 2 \( \mu \)m), the other composed of 7 compartments (length, 20 \( \mu \)m; diameter, 10 \( \mu \)m), and two secondary branches composed of 8 compartments (length, 20 \( \mu \)m; diameter, 2 \( \mu \)m) were simulated too. In real crayfish MNs, input synapses are likely to be located on primary and secondary neurite branches. Because no active spike propagation occurs in crayfish MN neurites, only passive electrical properties were simulated.

The intracompartment potential \( E \) is described by the differential equation

\[
\frac{dE}{dt} = \frac{I_{leak} + I_{leak} + I_{leak} + I_{syn}}{cm}
\]  

(1)

\( I_{leak} \) models passive leakage through the cell membrane. It is \( I_{leak} = (E_{leak} - E)G_{leak} \), where the parameters \( E_{leak} \) and \( G_{leak} \) are the equilibrium potential and the leak conductance, respectively. \( I_{leak} \) is the electrical coupling to neighboring compartments summed over all neighbors

\[
I_{core} = \sum_{i \in \text{neighbors}} (E_i - E)G_{core}
\]

The parameter \( G_{core} \) denotes the core conductance from the compartment in question to the neighboring compartment.

\( I_{syn} \), in Eq. 1, model the flow of ions through active channels and synapses, respectively. The parameter \( cm \) describes the capacity of the compartment

\[
cm = C_m \times \text{area}
\]

(area is the membrane surface of the compartment, \( C_m \) is the specific capacitance).

All compartments had the same membrane resistivity \( R_m \) that was adjusted to 4,000 or 8,000 \( \Omega \cdot \text{cm}^2 \). All computations were carried out assuming a specific capacitance \( C_m \) of 1 \( \mu F/cm^2 \) and a cytoplasmic resistivity \( R_c \) of 75 \( \Omega \cdot \text{cm} \).

Synaptic excitatory input was modeled with a conductance in the postsynaptic compartment, and an activation level of the postsynaptic channel, \( s \); which is 0 if the synapse is “closed” and 1 if it is fully “opened.” The maximum conductance \( G_{syn} \) is fixed, but the value of the actual conductance \( G_{syn} \) varies with \( s \); the kinetics of \( s \) are controlled by two parameters: the duration and the decay time. The synaptically induced current that enters the postsynaptic compartment is calculated by

\[
I_{syn} = \sum_{i \in \text{synapses}} (E_{syn} - E)G_{syn}s
\]

in which \( E_{syn} \) is the equilibrium potential for EPSPs.

RESULTS

Projections of CBCO units onto the Dep MNs: extracellular and intracellular studies

To find out the type and number of CBCO afferents that project onto a given MN, we performed systematic intracellular recordings from all Dep MNs in single experiments and used the procedure described in Fig. 1C to identify the different CBCO units from the sensory nerve. Figure 2 shows the results obtained in each of the nine Dep MNs that received monosynaptic CBCO inputs, impaled successively in the same experiment. The figure presents the resulting vector
of each of the CBCO afferents that connect the MNs (thin lines). From three to seven (mean = 5) afferents contacted each resistance Dep MN, and four afferents contacted the assistance Dep MN. This representation confirmed that some resistance Dep MNs received monosynaptic information even during the stretch of the CBCO. To represent the activity of all the CBCO afferents that connect a given MN, the corresponding resulting vectors were averaged (thick line, outside each circle). Although five resistance Dep MNs received one stretch-sensitive input, it was obvious that the greatest number of CBCO inputs were received during the CBCO release (white part of the cycle). The sectors of imposed movement in which Dep MNs received the greatest number of monosynaptic EPSPs are represented on the right of each circular diagram (dark part of the sine waves). It appeared that not all the resistance Dep MNs received the same information concerning the imposed movement and the position of the leg. It seems that each of the resistance Dep MNs was excited preferentially during a limited part of the movement. The same kind of temporal organization has been found in all these experiments (n = 4).

Table 1 summarizes the results obtained in several experiments. In our conditions, the assistance Dep MNs received about five exclusively stretch-sensitive sensory units. The resistance Dep MNs received an averaged number of six CBCO units (from 3 to 8) in sine-wave movement conditions. Resistance Dep MNs seemed to receive inputs from a larger variety of CBCO units than the assistance Dep MN; the organization of CBCO afferent projections onto the Dep MNs is given in Table 1. Although all of the impaled resistance Dep MNs received release-sensitive CBTs, some (17 Dep MNs) also received inputs from nonspecific CBCO units, and in some cases (4 Dep MNs) from stretch-sensitive CBCO units.

In some experiments, we determined the number of postsynaptic Dep MNs that received monosynaptic EPSPs from a given sensory unit. We focused exclusively on resistance Dep MNs, using both extracellular and intracellular approaches. Figures 3 and 4 show two examples of the results obtained with both methods. In experiments where sinusoidal movements were applied to the CBCO strand, we impaled successively the different postsynaptic Dep MNs and extracellularly recorded the CBCO units. Figure 3 presents one of these experiments in which 14 different CBCO units were identified and the 8 postsynaptic resistance Dep MNs were impaled 2 by 2 successively. Most of the recorded CBCO units elicited EPSPs in only a few number of postsynaptic Dep MNs (from 3 to 8 distinct postsynaptic Dep MNs); there exists one CBCO afferent that produce EPSPs in each of the eight resistance Dep MNs. It is noticeable that the same CBCO afferent is able to produce different shape and amplitude EPSPs in the different postsynaptic Dep MNs (see the averaged EPSPs in Fig. 3). Thus there exist both a divergence and a convergence of the sensory informations onto the different Dep MNs.

In some experiments, ramp movements were applied to

![FIG. 3. CBCO-related EPSPs in the resistance Dep MNs. In an experiment in which all resistance Dep MNs were successively impaled, we identified from the CBCO neurogram 14 sensory units that produced an EPSP in the postsynaptic Dep MNs. Each trace represent an average over >20 occurrences (except traces marked with a superior 1, which were averaged over 6 occurrences). Single CBCO unit-related EPSPs present different shapes and amplitudes in the different postsynaptic Dep MNs. Vertical bars for all recordings were 0.4 mV, except traces marked with an asterisk (0.8 mV).](http://jn.physiology.org/)

| TABLE 1. Connections between sensory fibers and Dep MNs |
|-----------------------------------------------|-----------------|-----------------|-----------------|
| Type of |
| Number of CBCO Units | CBCO Unit Type |
| Response | Release | Nonspecific | Stretch |
| MN 1 | Ø | 0 | 0 | 0 | 0 |
| MN 2 | Ø | 0 | 0 | 0 | 0 |
| MN 3 | A | 5 | 0 | 0 | 5 |
| MN 4 | A | 4 | 0 | 0 | 4 |
| MN 5 | A | 4 | 0 | 0 | 4 |
| MN 6 | R | 5 | 5 | 0 | 0 |
| MN 7 | R | 7 | 6 | 1 | 0 |
| MN 8 | R | 7 | 4 | 3 | 0 |
| MN 9 | R | 5 | 5 | 0 | 0 |
| MN 10 | R | 4 | 4 | 0 | 0 |
| MN 11 | R | 5 | 5 | 0 | 0 |
| MN 12 | R | 4 | 4 | 0 | 0 |
| MN 13 | R | 7 | 6 | 1 | 0 |
| MN 14 | R | 6 | 6 | 0 | 0 |
| MN 15 | R | 6 | 4 | 2 | 0 |
| MN 16 | R | 5 | 3 | 2 | 0 |
| MN 17 | R | 6 | 5 | 0 | 1 |
| MN 18 | R | 6 | 5 | 1 | 0 |
| MN 19 | R | 3 | 3 | 0 | 0 |
| MN 20 | R | 7 | 5 | 2 | 0 |
| MN 21 | R | 4 | 3 | 1 | 0 |
| MN 22 | R | 7 | 5 | 1 | 1 |
| MN 23 | R | 6 | 5 | 0 | 1 |
| MN 24 | R | 4 | 4 | 0 | 1 |
| MN 25 | R | 6 | 4 | 2 | 0 |
| MN 26 | R | 6 | 5 | 1 | 0 |
| MN 27 | R | 5 | 2 | 3 | 0 |
| MN 28 | R | 4 | 3 | 1 | 0 |
| MN 29 | R | 3 | 2 | 1 | 0 |
| MN 30 | R | 7 | 4 | 2 | 1 |
| MN 31 | R | 7 | 7 | 0 | 0 |

On the basis of the results obtained with circular statistics from several experiments, we classified the coxobasipodite chordotonal organ (CBCO) units that connected the depressor motor neurons (Dep MNs) according to their reflex response. The number of afferents and their specificity to movement (release, nonspecific, and stretch) are indicated for each Dep MN. Ø, nonresponding; A, assistance; R, resistance.
the CBCO strand, and successive paired intracellular recordings were performed from a given CBT and several resistance Dep MNs. In Fig. 4, while a phaso-tonic release-sensitive CBT was intracellularly recorded, we impaled successively all 12 Dep MNs. The phaso-tonic release-sensitive CBT connected monosynaptically five different resistance Dep MNs (distinguished with their different extracellular spikes), and produced EPSPs of different shape and size (see averaged EPSPs on right part of Fig. 3). These results confirmed the divergence of sensory information from single afferents onto different Dep MNs.

‘‘Manipulation’’ of the signal transmission

To confirm that the MN response to ramp stimulation of the CBCO strand was due exclusively to the temporal characteristics of the sensory inflow (since the associated paper demonstrated that MN properties were not involved), we analyzed the effect of a single sensory input on the MN reflex response. We therefore performed paired intracellular recordings from both a CBT and a Dep MN. Injection of depolarizing current pulses at high-frequency rates (30–200 Hz) into the CBT was then used to study the properties of sensory-motor synapses in high-Ca\(^{2+}\)/high-Mg\(^{2+}\) saline. In such experiments, high-frequency stimulations evoked sustained depolarizations in Dep MNs (Fig. 5A), and each presynaptic spike was capable of producing an EPSP in the postsynaptic Dep MN (Fig. 5B). As shown in Fig. 3, CBCO-related EPSPs displayed different time constants. The graph in Fig. 5C presents the plot versus time of the relative exponential decay of repolarizing phase from six representative EPSPs recorded in different MNs. Each one-phase exponential decay fitting allowed us to calculate the time constant (\(\tau\)) for each CBCO-related EPSP: the extreme \(\tau\) values ranged from 2.5 ms up to 12.5 ms, but most of the Dep MN EPSPs had a \(\tau\) value between 6 and 7 ms.

MODIFICATION OF THE DEP MN RESPONSES BY CONTROLLING THE CBT FIRINGS. An associated paper showed the existence of several kinds of CBCO unit firing patterns and two types of resistance reflex responses in Dep MNs (Le Ray et al. 1997). By using various combinations of stimulation frequencies, it was possible to mimic the different kinds of CBT activity recorded during ramp movements and, consequently, analyze the different Dep MN monosynaptic responses (results presented from 6 experiments). Figures 6 and 7 present the normal and modified reflex responses of both phasic and phaso-tonic Dep MNs in high-Ca\(^{2+}\)/high-Mg\(^{2+}\) saline.

Figure 6A shows the normal resistance reflex of a phasic
to reproduce a phasic CBT discharge (Fig. 7B2) by intracellular injection of depolarizing current pulses (+12 nA, 3 ms, 100 Hz) delivered in trains (0.5-s duration, 2-s interval). Consequently, the postsynaptic Dep MN response was transformed in a purely phasic reflex response.

HIGH-FREQUENCY EPSP SUMMATIONS IN PHASIC AND PHASO-TONIC DEP MNS. In paired intracellular recording experiments (n = 9), we compared the different postsynaptic Dep MN responses produced by high-frequency stimulation of a single CBT. During high-frequency CBT stimulations, summation of EPSPs evoked in the Dep MN resulted in a sustained depolarization (Fig. 5A). This effect was progressive; the rate of depolarization depended on the stimulation frequency and the stimulated CBT. Graphs representing the rates of depolarization for both phasic and phaso-tonic Dep MNS are shown in Fig. 8.

The responses obtained in a phasic Dep MN with the same CBT for 50- and 200-Hz stimulations are presented in Fig. 6A: phasic Dep MN (resting potential: −71 mV) response to times higher and was reached four times faster for 200-Hz CBCO strand imposed ramp movement is characterized by phasic bursts stimulation frequency than for 50-Hz stimulation frequency. of EPSPs occurring exclusively during releasing movements.

B: in the absence of movement, phasically applied high-frequency intracellular stimulation of a single presynaptic CBT is able to reproduce a phasiclike response in the Dep MN (1). A phaso-tonic—like response could be elicited in the Dep MN by applying tonic stimulation between the 40-Hz stimulations (2). Dep MN resting potential: −70 mV.

Dep MN in response to the application of a ramp stimulation of the CBCO strand (only the release phase of the stimulation is presented). This Dep MN reflex response was characterized by bursts of EPSPs occurring only during the release ramps. When the CBCO stimulation was stopped (Fig. 6B), it was possible to reproduce sensory phasic firing by intracellular injection of depolarizing current pulses (+8 nA, 3 ms, 40 Hz) delivered in trains (0.2-s duration, 1-s interval) into a presynaptic CBT. Under these conditions, the reflex response of the postsynaptic Dep MN was comparable (although the EPSP amplitudes were lower) with its normal resistance response (Fig. 6B1). Another CBT intracellular stimulation protocol (+8 nA, [40 Hz, 0.5 s], [20 Hz, 2 s], [40 Hz, 0.5 s], [30 Hz, 2 s], [40 Hz, 0.5 s]) allowed us to transform the same CBT firing in such a way as to reproduce a phaso-tonic pattern (Fig. 6B2). In these conditions, the postsynaptic Dep MN response resembled the phaso-tonic type of reflex response, i.e., the membrane potential did not return to its resting value between each 40-Hz bursts, but was gradually depolarized in relation with the CBT tonic frequency. Thus the control of the firing of only one presynaptic CBT was sufficient to transform the Dep MN phasic reflex response into a phaso-tonic—like reflex response.

Figure 7A shows the normal resistance reflex of a phaso-tonic Dep MN in response to the application of a ramp stimulation to the CBCO strand (only the release phase of the stimulation is presented). In the absence of movement (Fig. 7B), to reproduce a phaso-tonic sensory firing, we injected depolarizing current pulses (+12 nA, [100 Hz, 0.5 s], [50 Hz, 2 s], [100 Hz, 0.5 s], [80 Hz, 2 s]) into a presynaptic CBT. The evoked Dep MN response resembled the natural one, i.e., large phasic EPSP bursts added onto a graded tonic depolarization of the membrane (Fig. 7B1). The firing pattern of the single presynaptic CBT was modified to reproduce a phasic CBT discharge (Fig. 7B1) by intracellular injection of depolarizing current pulses (+12 nA, 3 ms, 100 Hz) delivered in trains (0.5-s duration, 2-s interval). Consequently, the postsynaptic Dep MN response was transformed in a purely phasic reflex response.
quency dependent for that CBT. However, great differences were observed among the CBTs that connect a single Dep MN, because the same stimulation frequency (200 Hz) could produce motor neuronal responses of quite different amplitudes (1- to 4-fold, Fig. 8A, bottom). Nevertheless, each of these CBTs evoked frequency-dependent motor neuronal response comparable with that shown in Fig. 8A (top).

Similar results were found with a postsynaptic phas tonic Dep MN. The amplitude of the depolarization was frequency dependent (Fig. 8B, top). The maximum depolarization amplitude was three times higher for 200-Hz stimulation frequency than for 50-Hz stimulation frequency, but was reached nearly at the same time (50 ms). Here again, great differences were observed among the CBTs that connect a single Dep MN, because the same stimulation frequency (100 Hz) could produce motor neuronal responses of quite different amplitudes (1- to 3-fold, Fig. 8B, bottom).

Simulation of the sensory-motor synapse

Simulation experiments were performed to analyze the integration processes of the afferent signal in the postsynaptic MN. The simulated MN is restricted to a main neurite, two primary and two secondary branches in which the conduction is passive. The input synapses can be located in different parts of the postsynaptic MN (to match more to reality, synapses were only located on primary and secondary branches). Then, it is possible to simulate the spatial and temporal integration of one or several sensory inputs.

A first set of simulations was performed to analyze the role of the spatial arrangement of synapses, and of geometry of the postsynaptic compartments on the shape of the centrally recorded EPSP (Fig. 9). One possibility could be that the shape of the EPSP is the same (possibly with various amplitudes) whatever the compartment in which it is produced, but because of passive propagation in dendrites, its temporal characteristics change and could give rise to the whole range of EPSP shapes recorded from the main neurite (see Fig. 3). Moreover, during a high-frequency train of EPSPs, the amplitude, time-to-peak, and duration of each EPSP should be responsible for dissimilarities in the amount of depolarization (Fig. 8). The shape of EPSPs depends on the amount of membrane that has to be charged in the postsynaptic compartment. Therefore we simulated large (20 μm diam) and small (1 μm diam) postsynaptic compartments (Fig. 9A). On the other hand, the amplitude of EPSPs recorded in the main neurite depends on the diameter of compartments that are in between the synapse and the recording electrode. To maximize the low filtering effects of passive propagation in dendrites, compared with reality, 1-μm-diam and 300-μm-long branches were used. Because membrane resistivity ($R_m$) is a parameter difficult to measure in real dendrites, four $R_m$ values were tested (4,000, 8,000, 12,000, and 20,000 $\Omega \cdot \text{cm}^2$) in both large and small postsynaptic compartment configurations. The results are presented in the graphs of Fig. 9B: in each case, measurements were made in the main neurite. Peak amplitude, time-to-peak, and time constant of the propagated EPSP were increased with increasing $R_m$. Simulated synaptic inputs in large postsynaptic compartments (filled squares) induced an EPSP of smaller amplitude, longer time-to-peak, and larger time constant than in small postsynaptic compartments (filled circles). Also, whatever the postsynaptic compartment configuration, more proximally generated EPSPs (open symbols) showed smaller differences than distally generated EPSPs (filled symbols). Thus proximally generated EPSPs had a shorter time-to-peak and a smaller time constant than distally generated ones.

The parameters involved in the evolution of trains of EPSPs were studied using the same models. We studied effects of $R_m$ values of 4, 8, 12, and 20 $k\Omega \cdot \text{cm}^2$, but Fig. 10 only presents the results obtained for $R_m$ values of 4 $k\Omega \cdot \text{cm}^2$ (right) and 20 $k\Omega \cdot \text{cm}^2$ (left), for clarity. To compare in each case the evolution of membrane potential during...
EPSP trains, all single simulated EPSPs (distal and proximal) were normalized to the same amplitude (100%). Three presynaptic spike frequencies (50, 80, and 140 Hz) were tested on each postsynaptic configuration at distal and proximal synapse locations. For both $R_m$ examples, fittings of summations are presented on graphs for 50-, 80-, and 140-Hz stimulation frequencies (left: distal synapses; right: proximal synapse); the corresponding simulated raw data are presented for 50- and 140-Hz stimulation frequencies (left: distal; right: proximal). With increasing frequencies, the depolarization amplitude increased, and the rise time of the depolarization decreased. In each case, the distal synapse–induced EPSP summation was always of greater amplitude than the proximal one. Moreover in the large postsynaptic compartment configuration (20 μm diam, Fig. 10, B and D), differences between proximally and distally generated compound EPSPs were more marked than in the small postsynaptic compartment configuration (1 μm diam, Fig. 10, A and C). The proximally generated compound EPSP changed very little (<1% of the normalized single EPSP) with diameter (compare Fig. 10, A right with B right, and Fig. 10, C right with D right). The distally generated compound EPSP showed larger differences related to the compartment diameter: the amplitude of the compound EPSP varied from 2% (when $R_m$ was 4 kΩ·cm²; compare Fig. 10, C left with D left) to >50% (when $R_m$ was 20 kΩ·cm²; compare Fig. 10, A left with B left) of the normalized single EPSP.

Although the geometry of the postsynaptic neuron seems to be able to determine the shape of the response recorded from the main neurite, the small variability observed between the two extreme postsynaptic compartment configurations are unable to explain the great differences observed among the EPSPs that can be recorded from real MNs (see Fig. 3), or observed among the Dep MN reflex responses elicited by CBCO release (see Le Ray et al. 1997). Some other simulations were performed to study the importance of the synapse temporal characteristics. It appeared that long-lasting synapses, i.e., synapses with a long decay time, produced long time constant EPSPs that were able to summate much more than short time constant EPSPs (not shown). Although these EPSPs with long time constants are rarer than short ones (see graph in Fig. 5C), it seems that properties of synapses between sensory afferents and MN are also important for the development of phasotonic reflex responses in some Dep MNs.

**DISCUSSION**

**Functional consequences of sensory-motor wiring**

Contrary to the cat spinal cord, in which la afferent fibers have been demonstrated to project onto most (65–80%) of the homonymous MNs (Nelson and Mendell 1978; Scott and Mendell 1976), in crayfish 1/2 of the 14 release-sensitive CBCO fibers activated by sinusoidal movements that approximately mimic locomotor movements project onto only one Dep MN (Fig. 3). This might indicate some specific input-output connections. However, this does not seem to be the case because each of the nine Dep MNs involved receives several (from 2 to 5) CBCO afferents coding different angular sectors, and therefore integrates a compound sensory signal. Moreover, none of the MNs realizes the same proprioceptive integration. These integration processes result in the different Dep MNs being sequentially activated during passive levation of the leg (Fig. 2). By comparison of the results obtained in other preparations, convergence of proprioceptive inputs is a widespread phenomenon also described in the crayfish (Leiboek et al. 1996; Newland and Nagayama 1993) as well as in the stick insect (Sauer et al. 1996) as in the locust (Burrows 1987) and as in the leech (Lockery and Kristan 1990). However, in these cases, proprioceptive afferents mainly project onto spiking or nonspiking interneurons that define the motor response (shape, strength, and direction). Surprisingly, convergence of direct connections from proprioceptive afferents onto MNs are a shared feature of the resistance reflex in crayfish and the stretch reflex in vertebrates (Eccles et al. 1957). This common characteristic would lead to a very rigid reflex behavior that is avoided by the means of presynaptic inhibition of central origin in both cases (Cattael et al. 1992; Eccles et al. 1962).

In opposition to the homogeneity of the convergence of CBCO inputs onto resistance Dep MNs (~5 CBCO units per MN), divergence is quite heterogeneous (from 1 to 8 MNs connected by a single CBCO fiber; see Fig. 3). This characteristic would allow differential regulation of the sensory-motor connections: presynaptic inhibition of sensory afferent that connect all resistance reflex Dep MNs would affect all Dep MN responses, whereas presynaptic inhibition of sensory fiber connecting only one Dep MN would only affect this MN response. Such differential reflex responses have been demonstrated in the thoraco-coxal joint (Skorupski et al. 1992) and could be supported by specific sensory-motor projections such as the one presented here. The functional analysis of divergence of sensory inputs remains a difficult question to unravel. So far, the crayfish thoracic in
vitro preparation is one of the rare models that appears to be adapted to solve this problem. Nevertheless, we must keep in mind that results obtained in vitro are oversimplified compared with intact animals in which more neurons would be active, and modulation from central or peripheral origin would be capable of rebuilding neuronal networks (Meyrand et al. 1991).

Role and interpretation of EPSP shape

This study has demonstrated that EPSPs from a given CBCO afferent recorded from different Dep MNs displayed different shapes (time-to-peak from 0.7 to 1.3 ms, decay time constant from 2.14 to 12.44 ms, amplitude from 0.07 to 0.5 mV). Because of the geometry of the MN dendritic trees, EPSP generated on small distant branches would elicit smaller and slower centrally recorded events than the same EPSP generated on proximal branches (Rall 1967; Rall et al. 1967). To test this assertion, we have simulated propagation of EPSPs in a compartment model (Fig. 9A). It appeared that temporal characteristics of the propagated EPSP also depends on the geometric characteristics of the postsynaptic compartment in which it has been produced: the EPSP duration is directly related to the membrane resistivity and the diameter of the postsynaptic compartment (Fig. 9B). Therefore, in electrophysiological recordings, the variability in the shape of the EPSPs recorded from distinct MNs could be due to MN morphological differences. However, recording different EPSP shapes from the same MN would indicate different synaptic locations (see differences between proximally and distally generated EPSPs in Fig. 9B). Thus the summation properties of EPSPs in synapses located at various places within the MN dendritic tree were studied in different conditions of membrane resistivity for both small and large postsynaptic compartment configurations (Fig. 10). Here again, the difference between proximal and distal synapses was more pronounced in the large postsynaptic compartment configuration (compare Fig. 10, B with C). Except in the case of low membrane resistivity ($R_m = 4 \, k\Omega \cdot cm^2$), the simulated frequency stimulation affected the postsynaptic depolarization amplitude in a very similar way to reality (cf. Fig. 8). On the basis of these simulation studies, we propose a functional interpretation of synapse location that matches with physiological observations: proximal synapses would produce fast, large-amplitude EPSPs involved in MN phasic firing; in contrast, distal synapses would not produce enough depolarization to give rise to an immediate MN discharge, but would rather contribute to a slow graded depolarization that would increase the neuron excitability (see complete fusion of distally generated EPSPs in Fig. 10B). However, synapse location is not the only way to account for the variety of EPSP shapes: time-to-peak, duration, and decay time are characteristics that could be specified at the synapse itself. Such parameters would thus depend on neurotransmitter release probabilities, diffusion coefficient, and uptake/degradation functions that can vary from one synapse to the other (Laurent and Sivaramakrishnan 1992; Redman and Walmsey 1983).

Functional implications of the resistance reflex design

In an associated paper, we described that, in the angular sector studied, movements imposed to the CBCO strand activated the release-sensitive sensory units in a greater number than stretch-sensitive ones. Moreover, although their coding was not so specific, these sensory units were activated in a sequential way (Le Ray et al. 1997). Here, we demonstrated that Dep MNs were also activated sequentially (Fig. 2). In both sensory coding and MN response, the angular sector eliciting the strongest neuronal activation lies between the point of full release (corresponding to the leg contacting the dorsal thoracic carapace) and $\sim 60^\circ$ in the stretching direction (corresponding to the basipodite at around the horizontal position). Although these results were obtained from an isolated nervous system in high-$Cu^{2+}$ and high-$Mg^{2+}$ saline, it is likely that the same sensory-motor pathways are involved in the intact animal. The monosynaptic connections from CBCO afferents onto Dep MNs would then operate to counteract gravity and hold the body up in the most commonly observed posture.

In this study, all intracellularly recorded Dep MNs displayed phasic or phas-tonic responses to CBCO strand ramp stimulation (Figs. 6 and 7). The absence of purely tonic MN response is the consequence of the absence of purely position-coding CBCO afferent (Le Ray et al. 1997). Because they receive several release-sensitive CBCO afferents (Fig. 3), all coding for release movements, all resistance Dep MNs showed larger reflex responses during movement than during maintained position (Figs. 4, 6, and 7). This mainly dynamic feedback would therefore operate as a zero-velocity servo control: it is not the position but the movement that is detected to maintain a constant position. We could have imagined that the resistance reflex, mainly involved in posture, would have been based on position coding. In contrast, the crayfish walking network adopted a “simpler” system that does not need to be continually compared with precise position-coding references. Moreover, a system only based on position coding would require an incompressible integration time to measure, and counteract, changes in position. The strategy adopted in the crayfish sensory-motor system allows faster dynamically adapted responses. Therefore, in the resting animal, the resistance reflex pathway would only be activated if a perturbation occurs, such as leg slipping during treadmill walking (Barnes 1977). How this neuronal pathway, here involved in purely negative feedback, would work during more complex motor behaviors in intact animal remains to be determined.

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