

Neural Mechanisms of Reflex Reversal in Coxo-Basipodite Depressor Motor Neurons of the Crayfish

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Le Ray, Didier and Daniel Cattaert. Neural mechanisms of reflex reversal in coxo-basipodite depressor motor neurons of the crayfish. *J. Neurophysiol.* 77: 1963–1978, 1997. The *in vitro* preparation of the fifth thoracic ganglion of the crayfish was used to investigate the mechanisms underlying the reflex reversal in a sensory-motor pathway. Sensory afferent neurons from the coxo-basipodite chordotonal organ (CBCO), which senses vertical movements of the limb, connect monosynaptically with basal limb motor neurons (MNs). In tonically active preparation, stretching the CBCO (corresponding to downward movements of the leg) stimulates the levator MNs, whereas releasing the CBCO activates the depressor (Dep) MNs. These reflexes, opposed to the imposed movement, are termed resistance reflexes. By contrast, during fictive locomotion, the reflexes are reversed and termed assistance reflexes. Intracellular recordings from all 12 Dep MNs were performed in single experiments. It allowed us to characterize three types of Dep MNs according to their response to CBCO imposed step-and-ramp movements: 8 of the 12 Dep MNs are resistance MNs that are depolarized during release of the CBCO and are connected monosynaptically to release-sensitive CBCO neurons; 1 Dep MN is an assistance MN that is depolarized during stretching of the CBCO and is connected monosynaptically to exclusively velocity-coding stretch-sensitive CBCO neurons; in our experimental conditions, 3 Dep MNs do not display any response to CBCO stimulation. Assistance reflex interneurons (ARINs), involved in polysynaptic assistance reflexes recorded from depressor MNs, are presented. During low-velocity (0.05 mm/s) stretching ramps imposed on the CBCO, ARINs display compound excitatory postsynaptic potentials (EPSPs), whereas during high-velocity (0.25 mm/s) ramps, they display a mixed excitatory and inhibitory response. Whereas a single MN generally receives monosynaptic EPSPs from three to six CBCO neurons, ARINs receive monosynaptic EPSPs from up to eight velocity-coding stretch-sensitive CBCO neurons. In addition, ARINs receive disynaptic inhibitory phasic inputs from stretch-sensitive CBCO afferents. Injection of a depolarizing current pulse into ARINs elicits a fast transient voltage-dependent depolarization. Its time to peak decreases, and its peak amplitude increases with increasing current intensity. ARINs likely are to be connected directly to Dep MNs. The synaptic delay between these nonspiking ARINs and Dep MNs is short (<2 ms) and constant. The postsynaptic EPSP amplitude increases with increasing current pulse intensity injected into ARIN. The dual sensory control (excitatory and inhibitory) makes it likely that ARIN represents a key element in reflex reversal control.

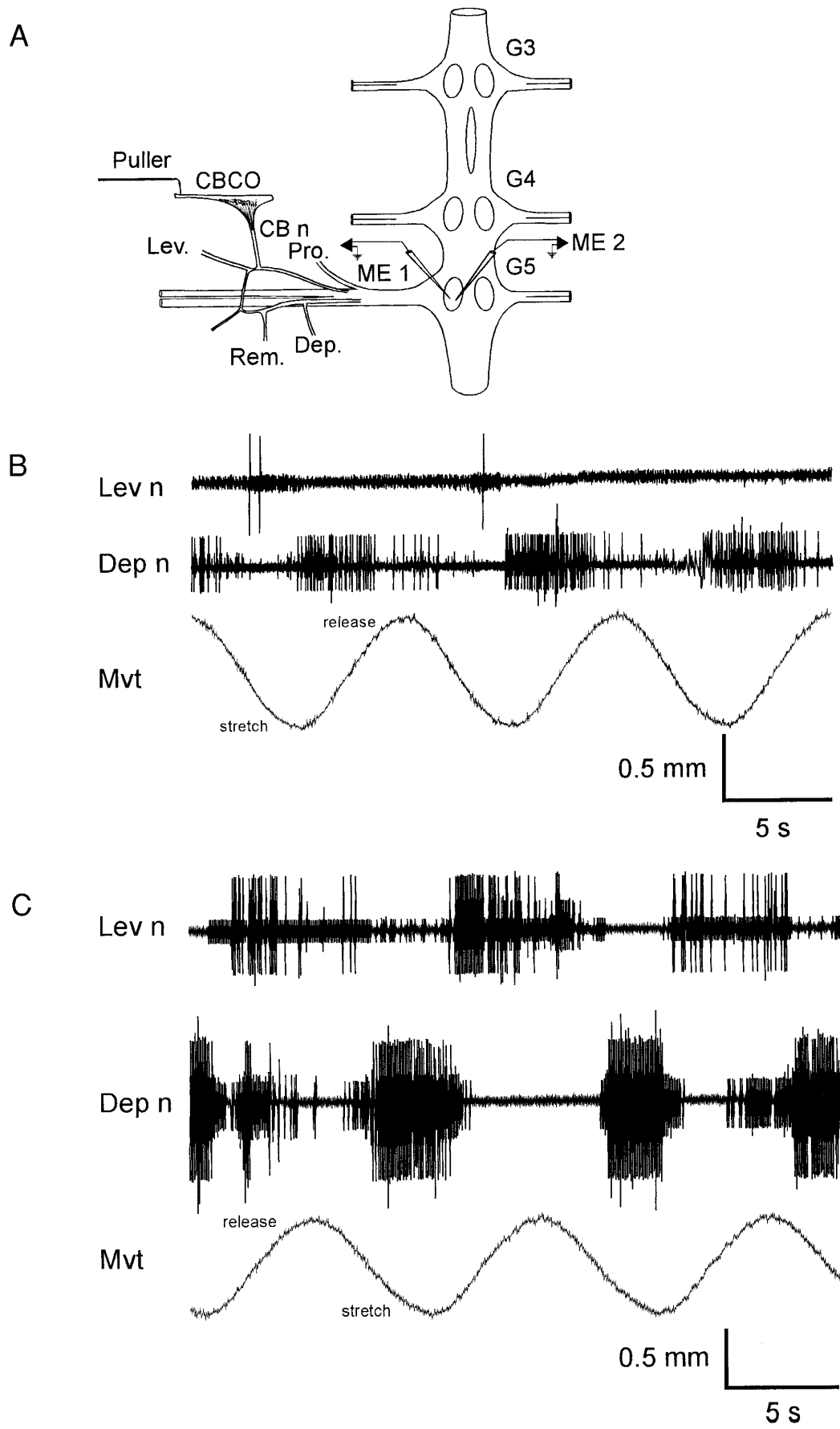
INTRODUCTION

In walking animals, sensory receptors are involved in the adaptation of posture and ongoing movements to external perturbations. In vertebrates, the neural circuitry underlying stretch reflex and more complex spinal reflexes has been studied extensively. Integration of sensory information is

subject to considerable modification when involved in centrally programmed movements such as locomotion in both vertebrates (Forssberg et al. 1976; Grillner 1975; Rossignol et al. 1981, 1988) and invertebrates (Bässler 1986).

However, compared with the complex organization of vertebrate sensory-motor pathways, arthropods are good models with which to study sensory-motor interactions, due to the reduced number of neurons involved. Therefore, many of studies on sensory-motor control have been carried out on insects (Bässler 1993; Burrows 1992) and on crustaceans (Bush 1962; Cannone and Bush 1980; Cattaert et al. 1992; El Manira et al. 1991a,b; Wiens and Gerstein 1976). In arthropods, chordotonal organs are a principal source of proprioceptive information from limb joints (Mill 1976). They mediate intrajoint resistance reflexes where stretch or release of the receptor excites the motor neurons (MNs) innervating muscles that resist the movement of the joint (Bush 1965). The resistance reflex is a negative feedback reflex, as is the vertebrate stretch reflex, mediated by direct connections between primary sensory afferents and MNs (El Manira et al. 1991a; Skorupski and Hustert 1991). Stretch receptors also are involved in the control of rhythmic motor patterns (Clarac 1991) by providing phasic inputs to the central motor network.

The *in vitro* preparation of the thoracic ganglia of the crayfish provides a good model to study sensory-motor interactions involved in motor control of leg movements. Generally, this preparation produces a spontaneous tonic motor activity, in which the “classical” resistance reflexes are elicited in response to stretch and release of the coxo-basipodite chordotonal organ (CBCO). The CBCO is an elastic strand in which ~40 sensory cells are inserted. The CBCO neurons can be divided into two groups: 20 stretch- and 20 release-sensitive cells, the axons of which compose the CBCO nerve and project to the ipsilateral thoracic hemiganglion. The CB joint allows upward and downward movements of the leg and is controlled by levator (Lev) and depressor (Dep) MNs (El Manira et al. 1991a). Within the hemiganglion, sensory-afferent terminals from the CBCO connect with Lev and Dep MNs (20 and 12 neurons, respectively) (Bévengut et al. 1996). These connections are responsible for reflex responses: stretching the CBCO activates Lev MNs whereas releasing the CBCO activates Dep MNs. However, as is the case for other receptors, these negative feedback reflexes vary in a phase-dependent way during centrally programmed rhythmic activities (El Manira et al. 1991b; Skorupski et al. 1992). The reflex may even reverse in sign, so that the reflex assists the ongoing movement. This “assistance reflex” has been described in many species (Bässler 1976; DiCaprio



and Clarac 1981; Forssberg et al. 1975; Rossignol and Drew 1986; Skorupski and Sillar 1986). The neural mechanisms underlying the phase-dependent modulation and reversal of reflexes during voluntary movements remain unclear in any species.

Nevertheless, this requires that the central network is able to select the suitable sensory inputs and direct them toward the appropriate MNs. At least three levels in the reflex pathway have to be considered in the analysis of this phenomenon. The first level concerns the sensory input that can be blocked by presynaptic inhibition (Baev and Kostyuk 1982; Boyan 1988; Cattaert et al. 1992; Eccles et al. 1962; Kennedy et al. 1974). This blockade of sensory inflow is essential to block the monosynaptic pathway from primary afferents to MNs. However, at least some sensory inputs may be not blocked by presynaptic inhibition to allow the polysynaptic assistance reflex. The blockade may be limited to some particular primary afferents or some branches of each. The inputs not subjected to presynaptic inhibition would use alternative interneuronal pathways, which represent the second level. This second level has been analyzed in different preparations (Jankowska et al. 1967; Reichert and Rowell 1985; Sillar and Roberts 1988). A third level concerns the MN pool itself where gating could take place (Lund et al. 1981).

In this study, we focused on the Dep MN pool, because of its accessibility and its small number of well-localized neurons, to investigate the last two levels. We have established a wiring diagram based on systematic intracellular recordings from all 12 Dep MNs. The Dep MN pool appears to be constituted of three types of MNs with respect to their direct connections with CBCO afferents: resistance unit, assistance unit, and not connected to CBCO. Assistance reflex interneurons (ARINs) also are described that are involved in the disynaptic assistance reflex pathway. These INs are recruited by velocity-sensitive CBCO afferents. Our results indicate that the recruitment of the disynaptic reflex pathway is controlled by inhibitory sensory inputs that prevent ARIN from activating assistance reflexes in quiescent central network. Finally, both pathways coexist and MN reflex responses observed during locomotion may be the result of a centrally modulated balance between assisting and resisting influences.

METHODS

Results are based on 83 intracellular recordings from MNs and INs (24 experiments) that were performed on adult male and female crayfish, *Procambarus clarkii* and *Pacifastacus leniusculus*. Animals were maintained in aquarium at 17–18°C and fed once a week.

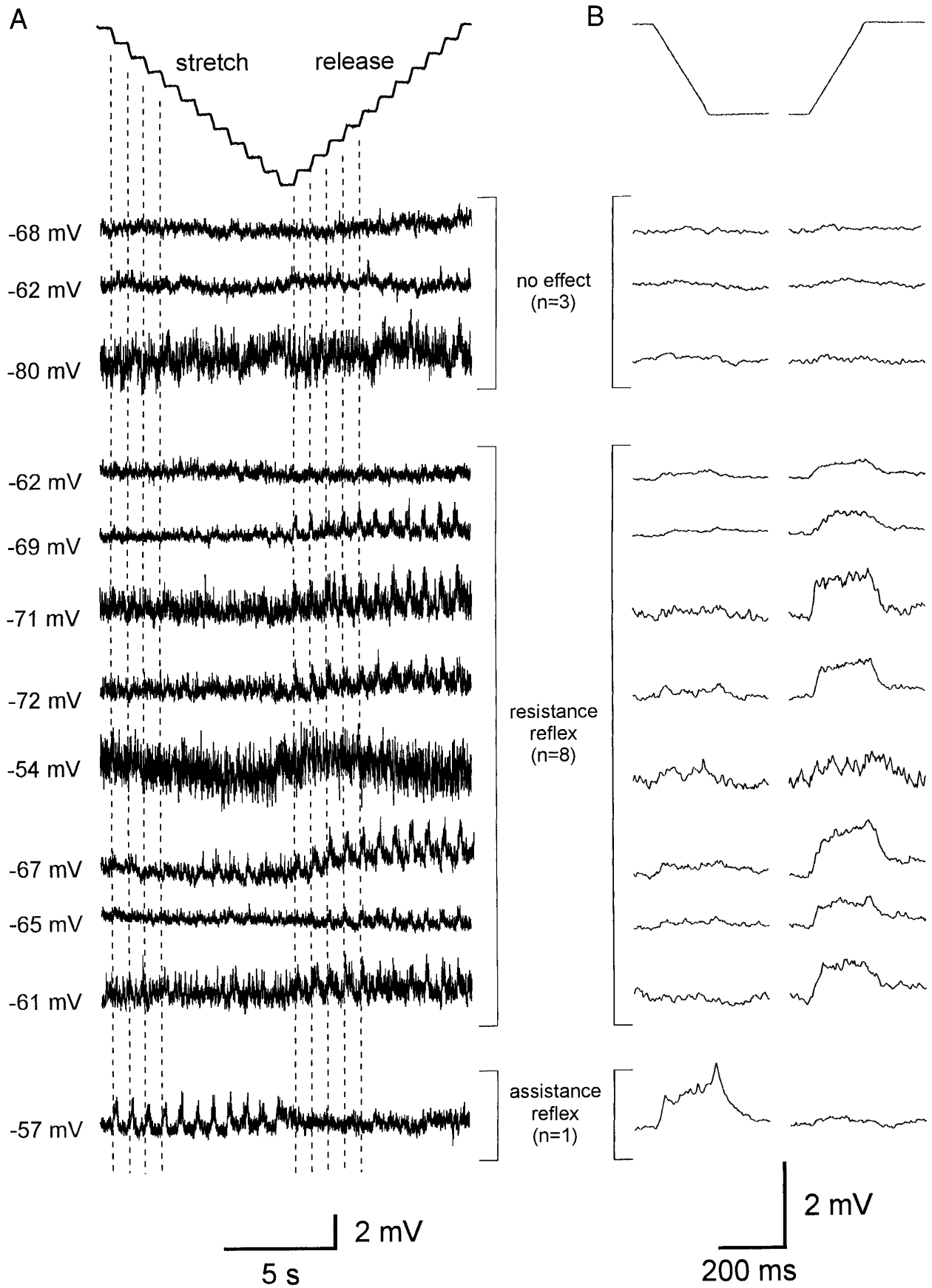
The in vitro preparation consisted of the last three thoracic gan-

glia, the two first abdominal ganglia, and the motor nerves innervating the two proximal joints of the fifth leg (the 2 sets of antagonistic motor nerves Promotor/Remotor and Levator/Depressor, Fig. 1A). The CBCO was dissected out together with its sensory nerve, which encodes the vertical movements of the leg. The preparation was pinned down dorsal side up in a Sylgard-covered petri dish and superfused with oxygenated crayfish saline [which contained (in mM) 195 NaCl, 5 KCl, 13 CaCl₂, and 2 MgCl₂]. In some experiments, divalent cation concentration was raised [which contained (in mM) 34 CaCl₂ and 6.4 MgCl₂] with the sodium concentration reduced accordingly. Saline solutions were buffered with 3 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) and pH adjusted at 7.7 at 15°C. In some experiments ($n = 5$), oxotremorine 10^{-5} M (oxo) was added in normal saline to induce fictive walking activity. However, because this study aimed to describe the wiring of the sensory-motor pathway and because of the great variability of neuronal discharge induced by oxo, all intracellular responses presented have been recorded in the absence of oxo.

Monopolar extracellular recordings and nerve stimulations were performed using platinum pin electrodes contacting the nerves, isolated from the bath with petroleum jelly (Vaseline) and directed to a four-channel differential AC amplifier (A-M System). Single and paired intracellular recordings from MNs and INs were made with thin-walled glass micro-electrodes filled with a potassium chloride solution (3 M) and having a 25- to 30-M Ω resistance. The signals were amplified by an Axoclamp 2B (Axon Instruments). Intracellular current pulses, delivered through the recording micro-electrodes, and nerve stimulations were controlled by an eight-channel digital stimulator (A.M.P.I.). A homemade electromechanical puller was coupled to the distal end of the CBCO strand attached to provide mechanical stimuli. These were characterized by cyclic stretch-release of the CBCO strand, according to a sinusoidal or a ramp protocol (i.e., succession of ramps and steps that permitted separation of movement- and position-related responses). Sinusoidal and ramp stimulations were performed from the most relaxed position of the CBCO strand. Total movement amplitude was one-third of the most relaxed CBCO strand length (i.e., 1–1.8 mm). All signals (physiological recordings and movement control voltage traces) were monitored on an eight-channel oscilloscope and a four-channel digital oscilloscope (Yokogawa DL 1200) and stored on DAT tapes (BioLogics digital tape recorder) and digitized on PC-based computer through an A/D interface (Cambridge Electronic Device, CED 1401PLUS). Intracellular and extracellular recordings were digitized at 5–10 kHz and written to disk. Signals were analyzed using the CED programs SPIKE2 for spike sorting and SIGAVG for spike-triggered averaging.

Dep MNs were identified after penetration with a micro-electrode by the following procedure: 1) the antidromic spike evoked by electrical stimulation of its axon in the identified nerve could be recorded by the micro-electrode. 2) There was a one-to-one correlation between MN intracellular spikes and motor nerve extracellular spikes during spontaneous activity. 3) Intracellular injection of depolarizing current into the MN-evoked orthodromic

FIG. 1. Intra-joint reflex patterns in quiescent and rhythmic preparations. A: schematic drawing of in vitro preparation, consisting in third, fourth, and fifth thoracic ganglia (G3, G4, and G5), motor nerves innervating proximal muscles (Pro, promotor; Rem, remotor; Lev, levator; Dep, depressor), and coxo-basipodite chordotonal organ (CBCO) sensory nerve (CB n). A mechanical puller controls movements (length changes) of CBCO strand. Fifth ganglion is desheathed to permit intracellular recordings from neurons (ME 1, ME 2). B: in a quiescent preparation, sinusoidal movements applied to CBCO strand induced relatively stable joint reflexes in levator (Lev) and depressor (Dep) motor nerves (Lev n, Dep n). This is a resistance reflex pattern in which Lev motor neurons (MNs) are activated during CBCO strand stretching (which mimics downward movements of leg), whereas Dep MNs are activated during CBCO release (which mimics upward movements of leg). C: when rhythmic activity (fictive locomotion) was induced by superfusing preparation with oxotremorine (10^{-5} M), application of sinusoidal movement to CBCO elicited an intra-joint reflex corresponding to an assistance reflex pattern: Lev MNs burst during CBCO release and Dep MNs during CBCO stretch.



spikes correlated one to one with extracellular spikes recorded in the corresponding motor nerve.

ARINs were identified by the following criteria: 1) electrical stimulation of any motor or sensory nerve of the leg never evoked any antidromic spikes in the intracellular recording; 2) injection of depolarizing current into the ARIN evoked an excitatory response of Dep MNs, recorded extracellularly from the Dep motor nerve and/or intracellularly from Dep MN; and 3) stretching movement applied to the CBCO strand evoked a dual depolarizing and hyperpolarizing response in the intracellularly recorded ARIN. Intracellular recordings from ARINs were performed in six experiments. However, paired recordings from ARIN and Dep MN were difficult to perform, and only two successful experiments were used in this study.

RESULTS

CBCO-induced reflex activities

In quiescent preparations superfused with normal saline, we observed that the motor nerves exhibited low-frequency tonic discharge. During sinusoidal stimulation of the CBCO strand (Fig. 1*B*), Dep and Lev nerves produced a resistance reflex response: stretching the CBCO strand (that is, analogous to the depression of the leg) elicited the activation of one to three distinct units in the Lev nerve in a given burst whereas releasing the CBCO strand (which mimics upward leg movements) induced the firing of two to four units in the Dep nerve.

In preparations superfused with oxo, the global activity of the preparation is increased, MNs and INs being more excitable (Cattaert et al. 1995). The antagonistic Lev and Dep nerves fire bursts of action potential in rhythmical alternation. In these conditions, a similar stimulation of the CBCO (Fig. 1*C*) elicited an assistance reflex, characterized by the Dep MNs being excited during stretching of the CBCO strand and Lev MNs being activated during releasing of the CBCO strand. On the Lev neurogram, two units (1 with a large extracellular spike and 1 with a smaller spike) were added to the three units observed in the tonic preparation and fired with a higher frequency during the releasing phase of the CBCO mechanical stimulation. In the Dep nerve, eight different units fired at high frequency during stretching of the CBCO strand (3 large amplitude units and 1 medium amplitude unit were added to the 4 units that were observed in the tonic preparation). The total number of MN units activated in assistance reflex is, however, variable (generally 3–5 Lev and 4–9 Dep MNs), depending on the state of the preparation. Thus oxo not only induced rhythmic activity but also caused the reversal of the reflex response. In the Dep MN pool, this consisted of a reversal of the reflex response of individual units that, in quiescent preparation,

were activated by movements in a resistance manner and the appearance of an assistance reflex response in units previously silent.

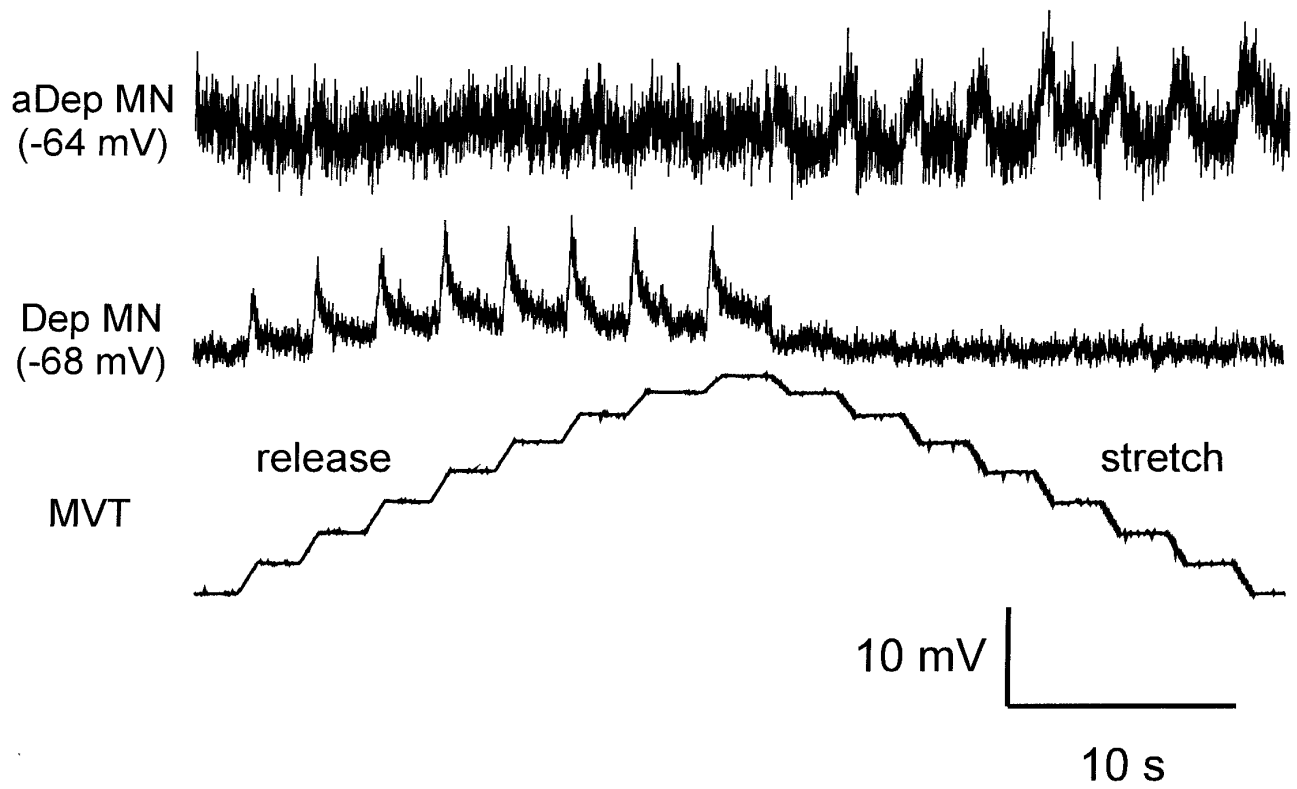
Evidence for a monosynaptic assistance reflex

Each hemiganglion has been reported previously to contain 12 distinct Dep MNs that can be identified according to the size and shape of their extracellular action potential and their conduction velocity (Bévençut et al. 1996). Figure 2 shows intracellular recordings of these 12 MNs, which were impaled successively in the same experiment. Mechanical stimulation of the CBCO elicited two types of responses from the Dep MNs in a quiescent preparation: either a resistance reflex ($n = 8$) or an assistance reflex ($n = 1$); 3 Dep MNs didn't respond to the mechanical stimulation applied to the CBCO. The resistance reflex observed in eight MNs was characterized by membrane potential depolarizations of 0.5–4 mV, resulting from the summation of excitatory postsynaptic potentials (EPSPs) during the release of the CBCO strand. Generally, the MNs that received the larger EPSPs were responsible for resistance responses recorded extracellularly. However, in some quiet preparations, most of the MNs were hyperpolarized, and their membrane potential kept under threshold for spiking (Fig. 2*A*). Even though each of these eight MNs displayed a different response to CBCO strand release, there was nevertheless a clear correlation between movement phases and membrane potential depolarizations (see vertical dotted lines in Fig. 2*A*). Traces in Fig. 4*B* were obtained by averaging the Dep MN responses to all stretching or releasing ramps from four cycles of CBCO mechanical stimulation. In each case, starting membrane potentials were offset. The eight resistance MNs exhibited depolarizations (mean values from 0.4 to 1.4 mV) during the release of the CBCO strand (Fig. 2*B*, *right*). Each compound EPSP could be related to release-sensitive CBCO afferent (according to the protocol used in Fig. 5*A*). Moreover, it is noticeable that most of the resistance Dep MNs received weak phasic depolarizing inputs (0.05–0.3 mV) during the stretching movements applied to the CBCO strand (Fig. 2*B*, *left*). However, these weak assistance responses disappeared in the presence of high-divalent cation concentration; therefore, these depolarizations had a polysynaptic origin (Berry and Pentreath 1976).

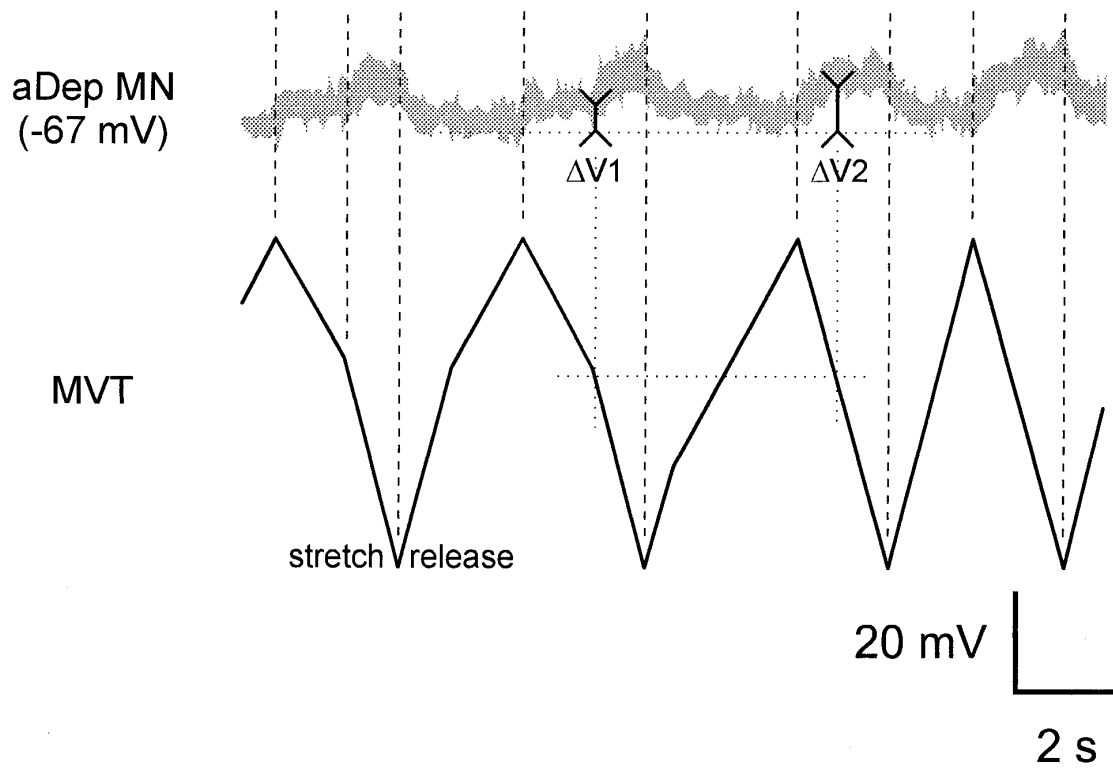
Finally, one of the Dep MN was characterized by an assistance reflex response to the stimulation of the chordotonal organ: depolarizations (from 1.2 to 3 mV) were observed during stretching of the CBCO strand (Fig. 2*A*, *bottom*). The averaged trace in Fig. 2*B* shows a mean value of 1.5 mV. When perfusing the preparation with high Ca^{2+} and

FIG. 2. Responses of each of 12 Dep MNs to ramp stimulation of CBCO in quiescent preparation. *A*: stimulation applied to CBCO (*top*) was composed of stretch (downward) and release (upward) movements, each made of a succession of small ramps: following traces show intracellular recordings of all 12 Dep MNs of left fifth hemiganglion (all 12 recordings were performed in same experiment, though not in order in which they are presented). Three first neurons did not show any response to CBCO stimulation. Eight Dep MNs presented a resistance reflex characterized by depolarizations of their membrane potential (0.5–4 mV) during each release movements of CBCO (broken vertical lines: each compound excitatory postsynaptic potential (EPSP) could be correlated with release-sensitive sensory action potentials). Only 1 Dep MN (*bottom*) produced depolarizations (2 mV) during CBCO stretching, eliciting an assistance reflex. *B*: averaged traces from each Dep MN obtained from a total of 48 stepwise ramps, in 4 successive cycles of movement over full-length range. Resistance Dep MNs exhibited depolarizations during release phases (*right*). In contrast, assistance Dep MN only exhibited depolarization during stretch of CBCO (*left*).

A



B



high Mg^{2+} , the CBCO stimulation still was capable of producing the assistance reflex response in this Dep MN (Fig. 3A, *top*), whereas in the other Dep MNs, only the resistance response was maintained (Fig. 3A, *middle*). Therefore, the EPSPs observed in the "assistance" Dep MN (aDep MN) were elicited by monosynaptic connections from a mean of four stretch-sensitive CBCO afferents (number calculated according to the protocol used in Fig. 5A). This aDep MN displayed ≤ 10 -mV depolarizations in response to CBCO stretching movements whereas no significant variation of its membrane potential was observed during the other phases of imposed movement.

The response of the aDep MN was speed dependent, as shown on Fig. 3B. When continuous ramp stimuli (without any steps) were applied, the aDep MN exhibited membrane potential depolarizations (~ 4.5 mV) due to summations of EPSPs during stretching movements. A fivefold increase of the stretch velocity resulted in a twofold increase of the stretch-induced depolarization ($\Delta V_2 = 12$ mV vs. $\Delta V_1 = 4.7$ mV; ΔV_1 and ΔV_2 being measured for an equal amplitude of movements). It appeared then that the amplitude of the depolarizing response was related closely to the speed of the movement.

Interneuron-mediating polysynaptic assistance response

We have investigated a possible mechanism by which Dep MNs may switch from a resistance reflex to an assistance reflex in response to CBCO stimulation during fictive locomotion, namely an interneuronal stage fulfilling two criteria: it receives excitation from stretch-sensitive CBCO afferents and it is excitatory to Dep MNs.

Such an assistance reflex interneuron (ARIN) has been found in six preparations in the neuropilar region of Dep MNs, in a slightly more ventral position. Its main features and its relations with one postsynaptic Dep MN are illustrated in Figs. 4–8. To date, our results suggest that there may be only one such ARIN per MN pool in the hemigan-glion.

In a quiescent preparation perfused with normal saline, the ARIN responded in an opposite way to resistance Dep MNs. ARIN exhibited subthreshold depolarizing events during stretch movements applied to the CBCO (Fig. 4A). Figure 4B presents average traces ($n = 36$ ramps) of both neuron reflex responses. ARIN received weak hyperpolarizing influences during the release of the CBCO strand whereas the Dep MN responded by a large depolarization (*top traces*). In contrast, during the stretch of the CBCO strand (*bottom traces*), the Dep MN exhibited a very weak depolarization (< 0.1 mV) whereas ARIN exhibited a large depolarizing response (the mean amplitude of which decreased along the ramp). By increasing the velocity of the mechanical stimulation applied to the CBCO (Fig. 4C), the responses of both neurons were emphasized.

ARIN especially exhibited a compound hyperpolarization (summation of inhibitory postsynaptic potentials) on termination of each of the small successive high-velocity stretching movements. This observation suggests that the compound inhibitory postsynaptic potential (IPSP) recorded in ARIN originates from velocity-sensitive CBCO afferents, responding during and immediately after stretch movements. Figure 4D shows averaged traces obtained from 36 high-velocity ramps. It appears that the hyperpolarization of ARIN during release movements increased with the velocity of the release ramp as did the depolarizing resistance response of Dep MN (Fig. 4D, *top*; cf. B). The increase in stimulus velocity unmasked the hyperpolarizing influence that ARIN received during the stretching phase (Fig. 4, C and D, *bottom*); this may explain the declining depolarization recorded during slow stretch movements (Fig. 4B, *bottom*). The Dep MN also exhibited with this stretch velocity a greater depolarizing response (1 or 2 mV), which appeared with a greater latency than its release-related response, suggesting a polysynaptic origin. The presence of both responses suggests that the MN reflex response to CBCO stimulation is the result of the balance between monosynaptic and polysynaptic inputs.

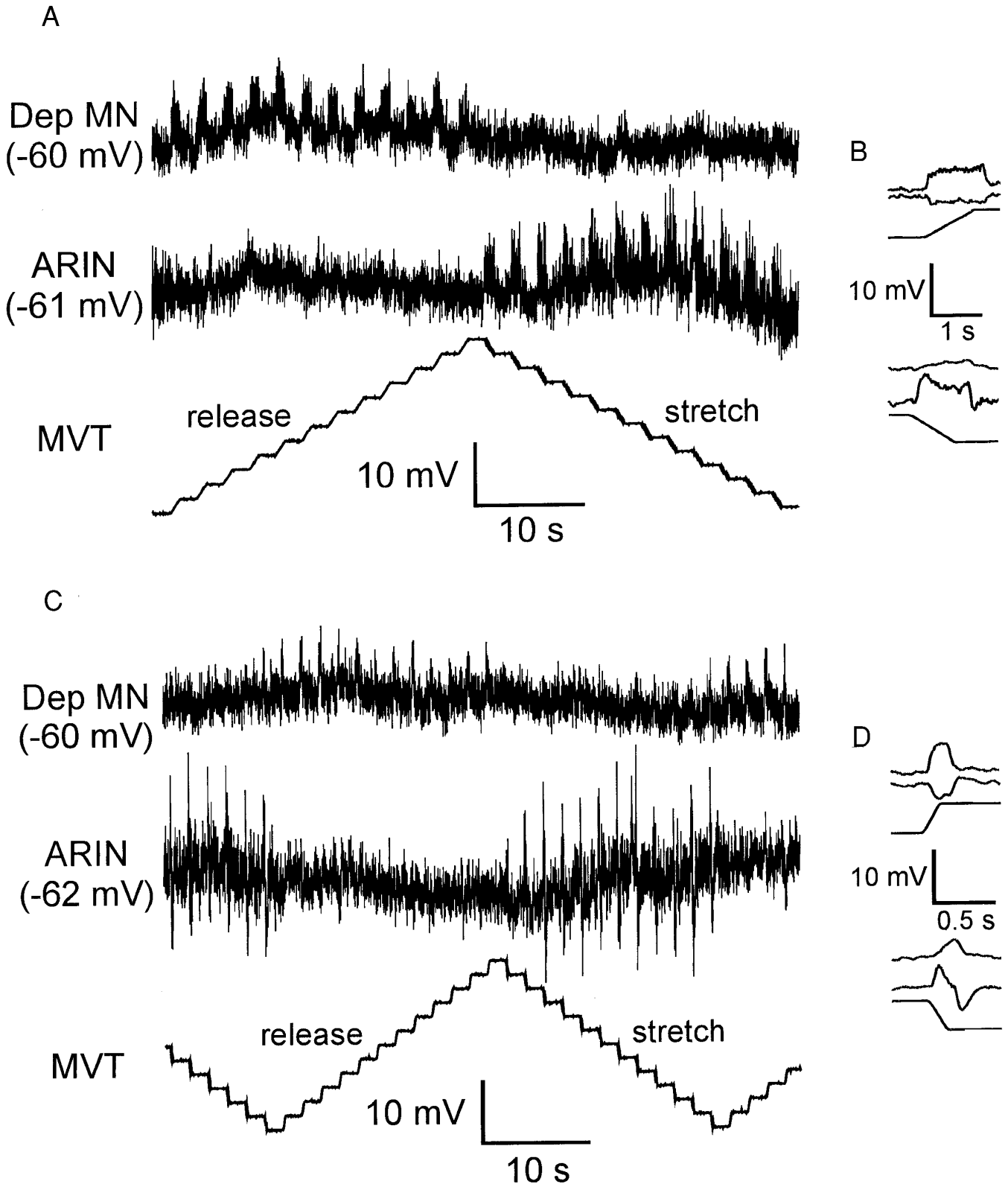
To characterize the sensory units connected to each intracellularly recorded neuron, we analyzed the extracellular action potentials recorded from the CBCO nerve during slow stepwise movements (presented in Fig. 4A) imposed on the CBCO strand (Fig. 5, A and B, from the same preparation). Three to six CBCO afferents (mean: 4; $n = 71$), active during CBCO release, evoked a time-locked EPSP (delays from 4–6 ms, amplitudes from 7 to 12 mV) in Dep MNs (Fig. 5A). The ARIN was found to receive excitatory inputs from about eight stretch-sensitive CBCO units that evoked EPSPs having a 6- to 14-mV amplitude with a 2.5- to 7-ms delay (Fig. 5B). The delays are consistent with conduction delays observed by El Manira et al. (1991a). The stability (amplitude and delay) of each EPSP evoked in ARIN and Dep MN makes it likely that they were monosynaptic. However, large differences exist among CBCO units that elicit an EPSP in the Dep MN or in the ARIN: some display large amplitude extracellular spikes, whereas others display very small extracellular spikes. Moreover, the large range of delays between sensory extracellular spikes and EPSPs recorded from the ARIN and Dep MN, which are not correlated to the amplitude of extracellular spikes, would indicate a large heterogeneity in diameters and hence conduction velocities of CBCO fibers that connect with the ARIN or Dep MNs.

The temporal occurrence of each of the EPSPs produced in both postsynaptic neurons was analyzed by triggering on all the CBCO nerve extracellular spikes eliciting a response

FIG. 3. Monosynaptic assistance reflex. A: in saline containing elevated levels ($2.5 \times$ normal) of divalent cations Ca^{2+} and Mg^{2+} , assistance reflex response persisted in single assistance Dep MN (*top*). This response consisted in a compound EPSP (3–10 mV) during each stretching movement of CBCO. Resistance reflex of 8 resistance Dep MNs (represented here by 1 only; *middle*) was not affected by high divalent cation saline. B: velocity-dependence of Dep MN assistance response. Release of CBCO produced a depolarization whose amplitude increased with increasing velocity of movement: ~ 12 mV (ΔV_2) for higher speed (0.25 mm/s) against ~ 5 mV (ΔV_1) for lower speed (0.05 mm/s); values were measured for same amplitude of movement. MVT: CBCO imposed movement.

in the impaled neurons, irrespective of spike size and EPSP latency. This procedure allowed us to measure the amplitude of individual EPSPs. As shown on Fig. 6, *A* and *B*, the EPSPs induced by the CBCO units were larger during the movement phases of the CBCO stimulation (respectively

releasing movements for the Dep MN and stretching movements for the ARIN). This corroborates the results illustrated in Fig. 4*A* and suggests that the larger EPSPs are locked more closely to movement-sensitive than to position-sensitive afferents.



Characterization of ARIN response to injection of depolarizing currents

Although nonspiking in the conventional sense, depolarization of ARIN nevertheless revealed active membrane properties: As shown on Fig. 7A, injection of increasing depolarizing current pulses (+1–22 nA by 1-nA steps, 35-ms duration) caused a biphasic variation of ARIN membrane potential. An early transient phase of depolarization developed for current intensities above +3 nA and peaked 15.1 ms (+4-nA stimulation) to 3.5 ms (stimulation above +15 nA) after the pulse onset. The time to peak of this transient phase decreased with increasing current (see *graph 1* on Fig. 7B). The second depolarizing phase, which developed from the lowest currents, is the commonly observed passive response due to capacitive charge of the membrane (no reliable value could be measured from +16 nA). The membrane potential of this second phase varied in nearly the same parabolic manner for positive currents as that of the transient phase (see *graph 2* of Fig. 7B).

Although the second depolarizing phase is commonly observed, the first, transient phase is unusual and is likely to be different from a simple Na⁺ spike because it is a graded depolarizing response and because its time to peak decreases with increasing currents.

Synaptic relation between ARIN and Dep MNs

Because ARINs are not spiking neurons (positive current injection never triggered any spike in the ARIN, Fig. 7A), the synaptic relation between ARINs and Dep MNs was studied with the following protocol (Fig. 8A): positive current pulses that were injected intracellularly into the ARIN elicited EPSPs in the postsynaptic Dep MN. Figure 8B1 presents an overdraw of the averaged EPSP recorded for each current pulse intensity. The postsynaptic EPSP developed gradually for intensities of current pulses above +4 nA and reached its maximum value (~5 mV) with current intensities above +15 nA. The amplitude of the MN EPSP as a function of the peak of the ARIN transient shows a sigmoidal relation (see *graph 2* on Fig. 8B). The EPSP appeared for transient amplitudes >2.8 mV and was maximum for transient amplitudes >26 mV. As the current pulse intensity increased, the latency of the MN EPSP decreased from 9 to 5.4 ms as shown in Fig. 8B1. The synaptic delay between the two cells is expressed in *graph 3* on Fig. 8B: the relation between the time to peak of the transient in ARIN and the latency of the EPSP development is linear [$y = ax + b$, with $a = 1.08 \pm 0.05$ (mean \pm SE), and $b = 1.67 \pm 0.03$ being the synaptic delay], indicating a constant

delay. The fitting of linear regression is excellent ($r = 0.99$) in the whole range of current intensities and gives a 1.67-ms synaptic delay.

DISCUSSION

Monosynaptic assistance reflex

In many different preparations, negative feedback responses (resistance reflex) have been demonstrated to be mediated largely by monosynaptic sensory-motor pathways. In vertebrates, the direct connection that Ia afferents have with homonymous MNs produces the “stretch reflex”, which opposes to the imposed movement that provoked it (Henneman and Mendell 1981). In invertebrates resistance reflexes also are mediated by monosynaptic connections between sensory afferents and MNs, as shown in the crayfish walking system (El Manira et al. 1991a) as well as in the locust flight network (Burrows 1975). Here we provide evidence for a monosynaptic sensory-motor assisting pathway to the Dep MN pool (Fig. 3) in the walking network of crayfish. Our experiments showed the existence of different MN reflex responses to the same CBCO stimulation. In the fifth thoracic ganglion of the crayfish, the characterization of all the monosynaptic reflex responses of a specific pool of MNs to a complex movement could be performed due to the small number of neurons (only 12 Dep MNs; Fig. 2). Generally, the response observed was the classical resistance reflex, but it was always possible to identify one Dep MN that presented a nontypical monosynaptic assisting response to the CBCO stimulation. Previous studies on the fourth thoracic ganglion have described such a positive feedback reflex involving the thoraco-coxal muscle receptor organ (TCMRO) and remotor MNs (Skorupski 1992). The T fiber of the TCMRO, an muscle receptor organ that controls the antero-posterior movements of the leg, connects monosynaptically with promotor MNs to elicit a resistance reflex response during the stretch of the organ. It has been shown that this stretch-sensitive fiber also was able to excite directly a specific pool of remotor MNs, then evoking a monosynaptic assistance response. The functional significance of this connection remains uncertain. Nevertheless, at least two nonexclusive hypotheses can be proposed: 1) MN pools are heterogeneous and could be considered as sets of more or less independently driven MNs. Within a pool, each set could be activated or inactivated by the central network, depending on the required behavioral situation. Such differential aminergic control of MN sets has been demonstrated recently to exist in the promotor and remotor MN pools of the crayfish (Skorupski 1996). In this view, assistance and resistance MNs would not be simultaneously active. 2) In rhythmic walking activity, the recruitment

FIG. 4. Assistance reflex interneuron (ARIN). A: paired intracellular recordings made from a Dep MN and an ARIN. In response to CBCO release, Dep MN (*top*) displayed a resistance reflex. During stretching movements, ARIN (*middle*) produced depolarizing responses (9–13 mV). B: average traces of both phases obtained from 36 ramps. *Top*: response of both neurons to CBCO release: Dep MN was depolarized whereas ARIN was hyperpolarized weakly. *Bottom*: response of both neurons to CBCO stretch: Dep MN was depolarized weakly whereas ARIN exhibited a large depolarizing response. C: when movement velocity was increased (5 times velocity in A), Dep MN reflex response remained unchanged, although each compound EPSP duration decreased as did ramp duration in releasing movements. By contrast, ARIN response changed: in addition to depolarizations (~14 mV), ARIN exhibited hyperpolarizations (~10 mV) at end of each stretching movement. D: average traces of response of both neurons obtained from 36 high-velocity ramps. Increase in velocity increased ARIN hyperpolarizing responses evoked by release of the CBCO (*top traces*) and unmasked hyperpolarizations at end of stretching ramps (*bottom traces*). Dep MN exhibited a large depolarizing response to CBCO release and a weak depolarization during CBCO stretch. This second response appeared with a greater latency than release-related one.

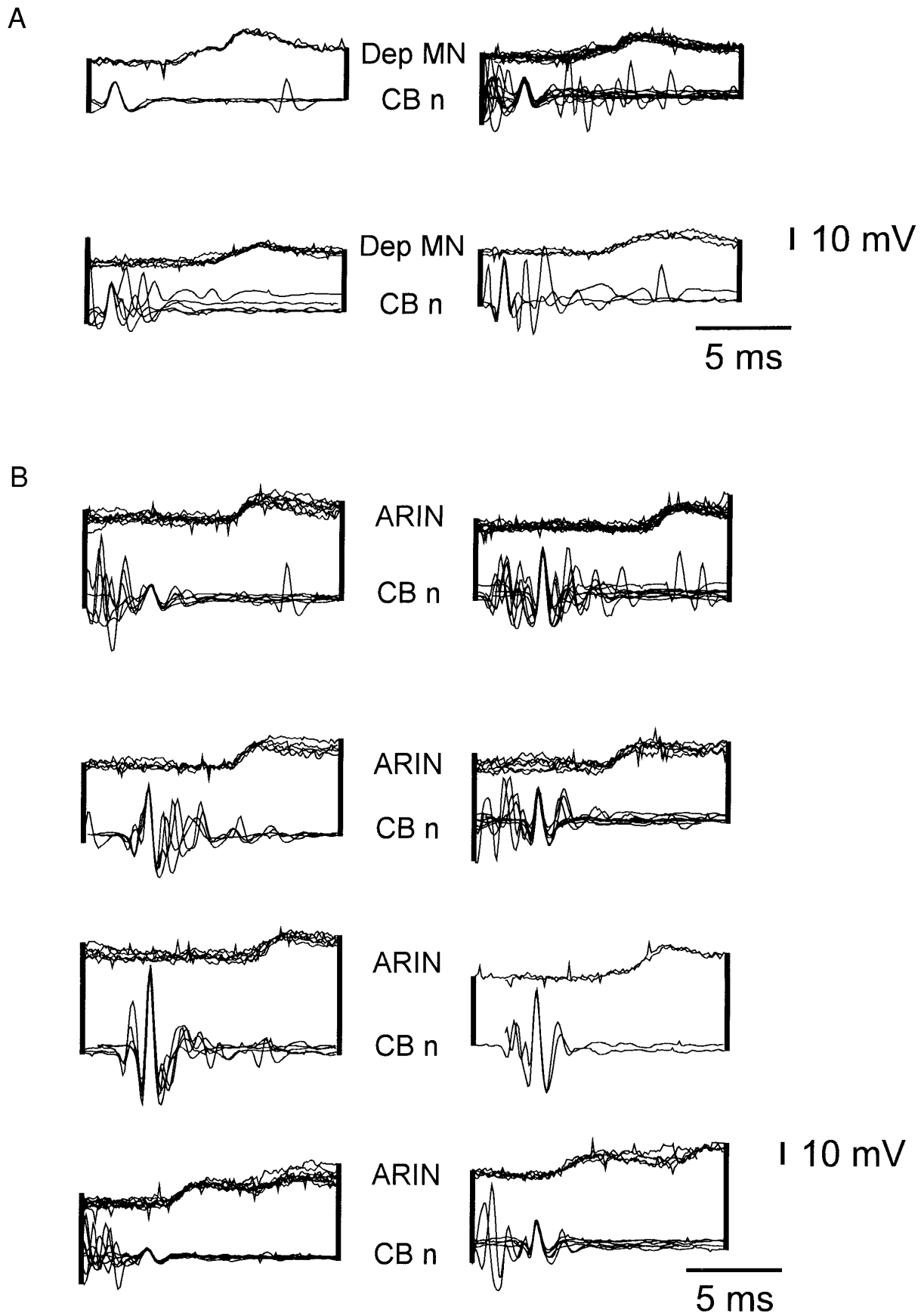


FIG. 5. Monosynaptic CBCO inputs to Dep MN and ARIN. *A*: four distinct release-sensitive CBCO units (extracellular recordings, *bottom*) were found to produce an invariant monosynaptic EPSP (intracellular recordings, *top*) in a Dep MN. *B*: during stretch, 8 different CBCO units connected to ARIN and produced EPSPs of constant amplitude and delay. In both cases, superimposed traces show constancy of each EPSP. Data for *A* and *B* from preparation with low-velocity movement of Fig. 4A.

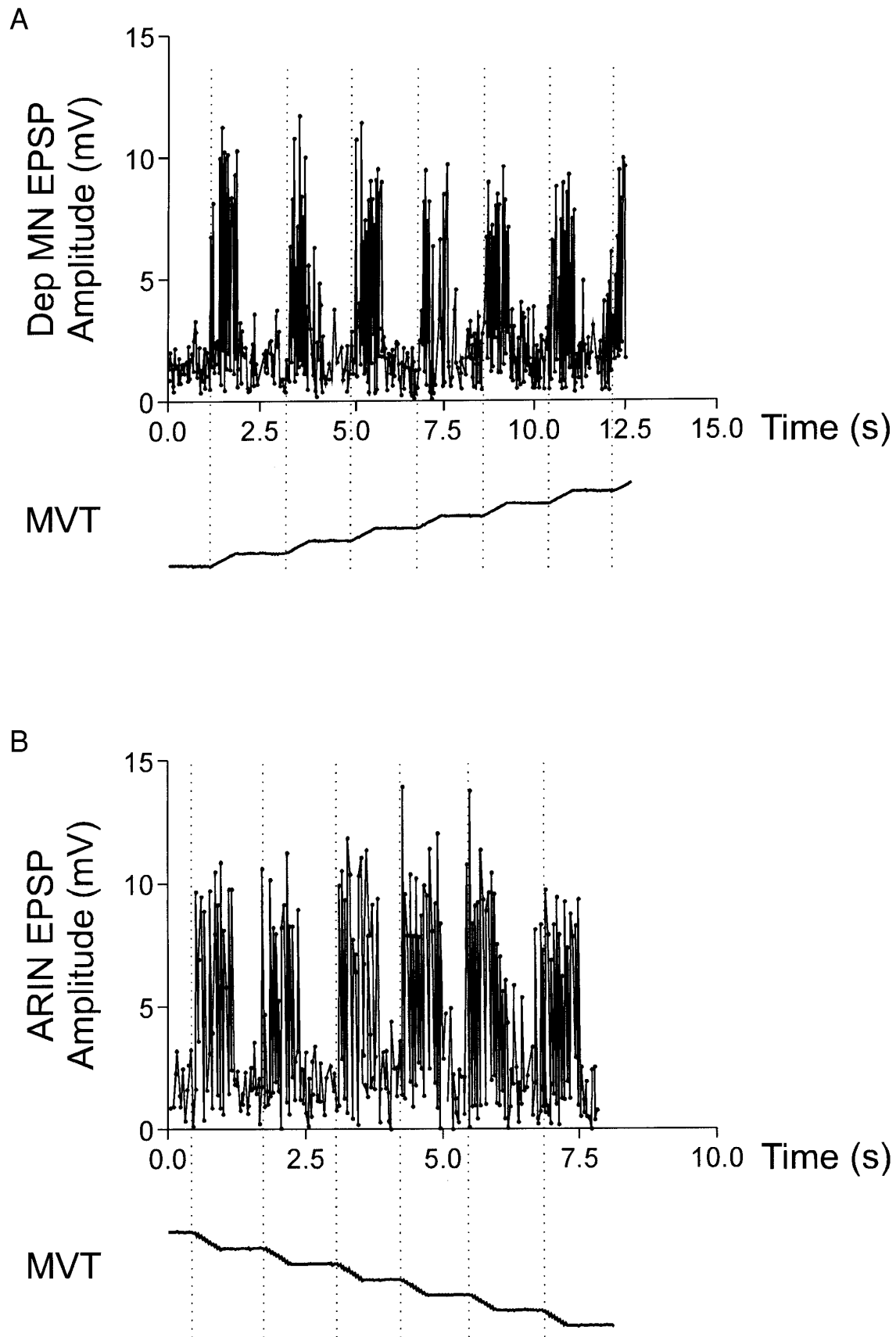
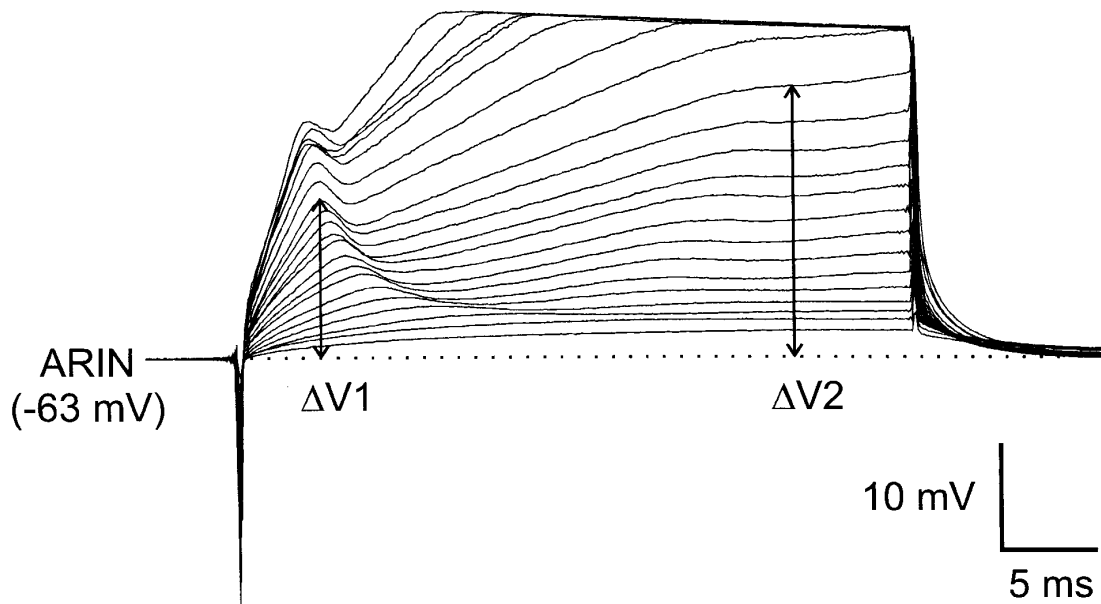


FIG. 6. Dep MN and ARIN sensory EPSPs occurred during movements of CBCO. *A*: amplitude of EPSPs recorded in a Dep MN and triggered by CBCO extracellular action potentials represented as a function of time. *B*: same analysis performed on ARIN. In both cases, bursts of large EPSPs started with onset of ramp movements (\cdots). MVT: CBCO mechanical stimulation. Data for *A* and *B* from same experiment as Figs. 4*A* and 5.

A



B

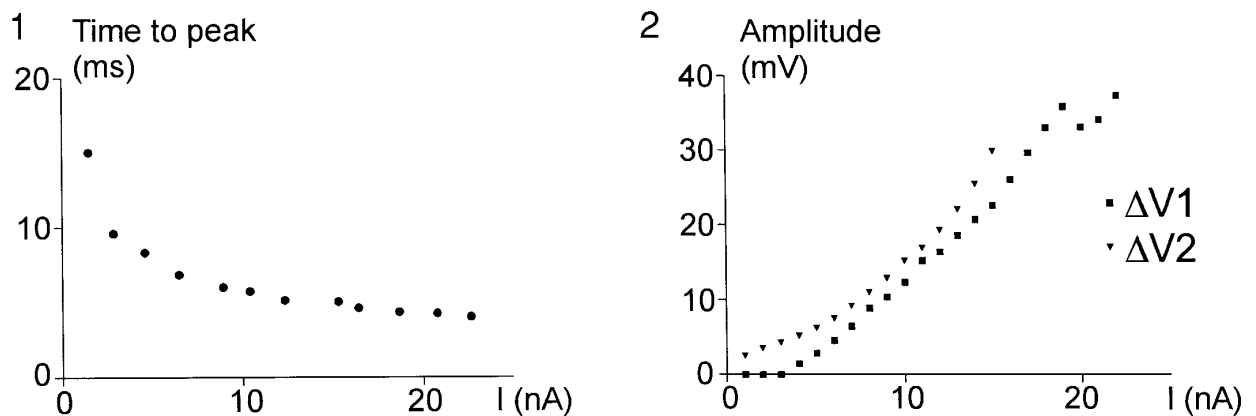


FIG. 7. Electrical properties of ARIN. *A*: injection of increasing depolarizing current pulses (35 ms at 2 Hz, in 1-nA steps from +1 to +22 nA) elicited a 2-phase electrical response in interneuron. First, a graded transient "spike-like" depolarization occurred ($\Delta V1$) for current intensities higher than +3 nA. Second, a classical electrical rectification developed ($\Delta V2$). *B*: time to peak (*B1*) and amplitude (*B2*) of transient depolarization varied in an intensity-dependent way.

of the different MNs in a given MN pool is generally progressive. The units of small spike amplitude being activated before the larger ones, the transition from resistance to assistance, or assistance to resistance, also could be considered as being a part of this pattern of activity of the MN pool. For example during stance, mainly resistance reflexes occur whereas during swing, assistance reflexes are involved. This phase-dependent reflex reversal has been described in many vertebrate and invertebrate preparations (Rossignol et al. 1981, 1988; Skorupski and Sillar 1986).

Active transient depolarization in ARIN

Injection of a depolarizing current in ARIN elicited an active transient depolarization that was graded with current intensity. Such an active transient response already has been described in the T-fiber of the TCMRO where, as in our model, this "spike-like transient" was sometimes sufficient to produce an EPSP in the promotor MNs (Skorupski 1992). In the same

way, active depolarizing transients have been shown in the lobster stomatogastric ganglion (Graubard 1978), in the crab T-fiber (Blight and Llinás 1981), and in the locust nonspiking INs (Laurent 1990). The nature of the conductances underlying the graded spikes described was clarified neither in these preparations nor in ours. According to Blight and Llinás (1981), it may be due to a calcium current, although Bush (1981), in the same preparation, suggested a tetrodotoxin-sensitive sodium current was involved. The physiological role of this graded active depolarization is unclear. We can assume it may operate in a "preintegration" of the sensory signals by the IN, functioning as an amplifier of the amplitude and/or the duration of the sensory EPSP.

Involvement of ARIN in the polysynaptic sensory-motor pathway

Our experiments aimed at a better characterization of the MN reflex responses to the CBCO mechanical stimulation.

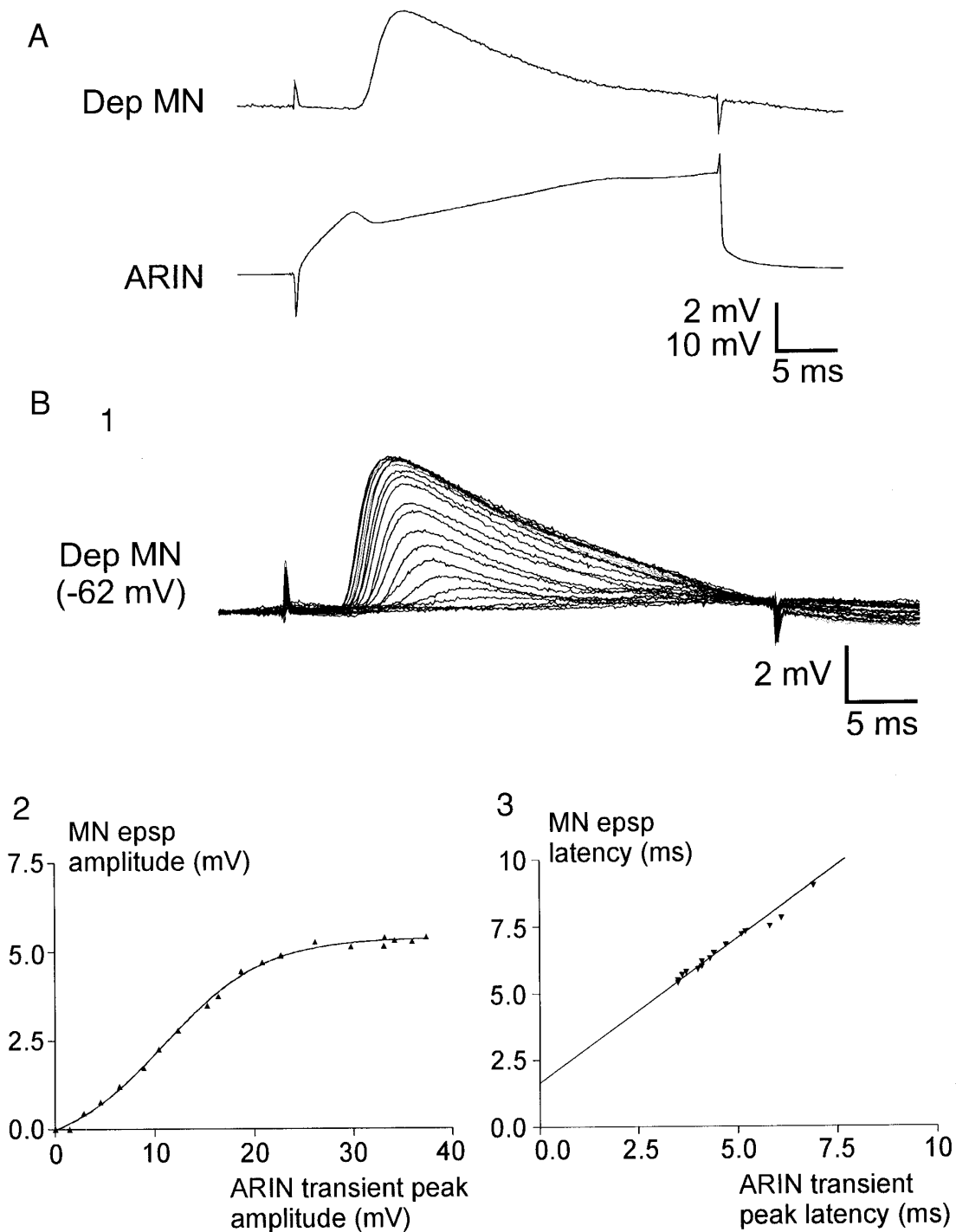


FIG. 8. Evidence for connection of ARIN with postsynaptic Dep MN. A: experimental protocol: a depolarizing current pulse injected into ARIN elicited an early transient that evoked an EPSP in Dep MN. B: quantitative study of synaptic relation. B1: MN EPSPs related to graded "spike-like" transients (similar to that in Fig. 7A) also were graded; B2: plot of EPSP amplitude as a function of transient peak amplitude (relation was of sigmoidal-type, maximum EPSP amplitude being reached with about a 25-mV transient peak); B3: EPSP latency as a function of transient peak latency; relation was linear ($R = 0.99$) and gave a synaptic delay (intercept) of 1.67 ± 0.03 ms.

We found three to six (average 4) sensory afferents connecting with one Dep MN ($n > 70$ Dep MNs) with some heterogeneity in the nature of those CBCO afferents (movement/position selectivity and conduction velocity). Concerning the INs, it appears that only movement-sensitive afferents connect to ARINs (Fig. 6B). It also appears that a greater

number of afferents control ARIN: eight monosynaptic excitatory CBCO afferents (Fig. 5B) and at least one inhibitory (probably disynaptic) influence from release-coding CBCO afferents responsible for compound IPSPs (Fig. 4, C and D). This finding is in agreement with El Manira et al. (1991a): these authors showed that electrical stimulation of

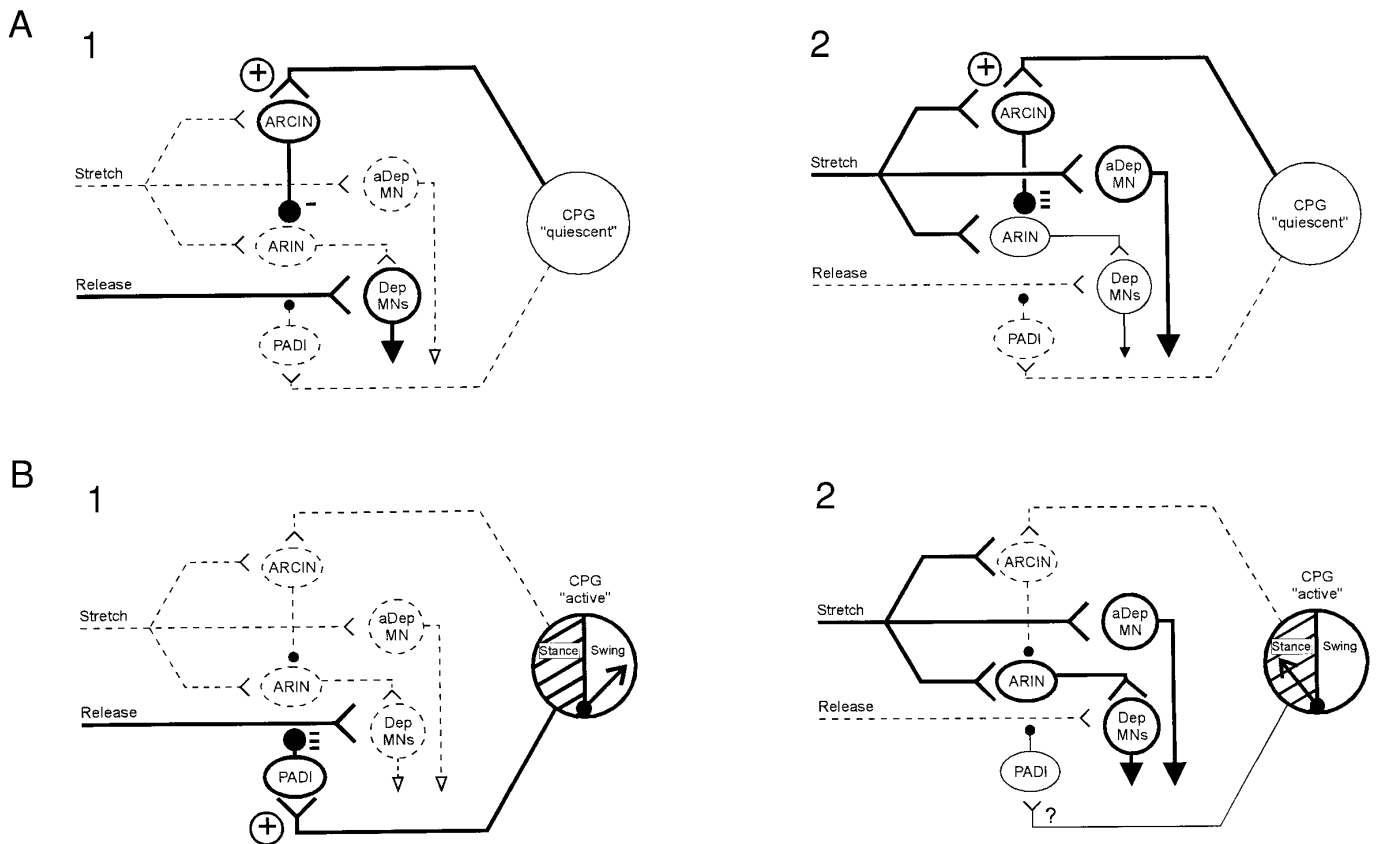


FIG. 9. Functional schema of sensory-motor network that controls the Dep MN reflexes. *A*: when preparation produces a tonic pattern, central pattern generator (CPG) activates tonically assistance reflex controlling INs (ARCIN), which inhibits the ARIN. During release of CBCO strand (*A1*), resistance reflex is produced normally. During CBCO stretch (*A2*), ARIN is inhibited by ARCIN activated by both CPG and stretch-sensitive CBCO afferents; ARIN inhibition prevents polysynaptic Dep assistance response. However, monosynaptic pathway (through aDep MN) still should result in a weak assisting influence. *B*: in contrast, when CPG is active (i.e., when preparation shows rhythmic activity), CPG tonic activation of ARCIN does not occur but CPG activates the IN producing primary afferent depolarizations (PADI, *B1*). In these conditions, during swing phase when CBCO is released, PADI strongly inhibits afferent signals and consequently Dep MN resistance reflex. During stance phase, stretch-sensitive CBCO afferents are activated (*B2*) and excite ARIN. A strong polysynaptic Dep MN assistance reflex therefore occurs in addition to monosynaptic one.

the CBCO nerve elicited a short-latency EPSP for weak intensities (1.3 V), followed by a long-latency EPSP (or IPSP) for higher intensities (4 V). The increase of intensity recruited more CBCO afferents, and we can suppose that this number (for 4 V) was sufficient to activate the polysynaptic sensory pathways. Then, there may be a convergence of the sensory inputs to a small number of ARINs (perhaps only one per group of MNs), and subsequently, a divergence to the MNs involved in the expected assistance reflex activity.

The real connectivity, especially the number of ARINs and related postsynaptic MNs, remains to be determined. According to Bässler (1993), a single nonspiking IN is able to excite one pool of MNs (for example the Dep pool) and inhibit another antagonistic pool (the Lev one). Another possibility would be the existence of two INs, one excitatory, the other inhibitory on the same pool of MNs (Burrows et al. 1988). The systematic investigation of the CBCO units connecting both kinds of neurons (Fig. 5) allowed us to measure the latency of appearance of the sensory EPSPs in Dep MNs and ARINs. It appeared that latencies in both neurons were quite similar and compatible with a monosynaptic connection in both cases. Moreover, the synaptic delay

(~2 ms) calculated between ARIN and the postsynaptic motor neuron (Fig. 8) is consistent with the timing of the polysynaptic part of the response observed by El Manira et al. (1991a). Therefore we may assume that ARIN can be the only link between the CBCO afferents and the motor neurons in the polysynaptic reflex pathway; it therefore may be a disynaptic pathway.

CBCO-inhibitory input to ARIN

We have demonstrated that ARINs were activated by movement coding CBCO neurons (Figs. 4 and 6). Moreover, the amplitude of the compound EPSP is velocity sensitive: the faster the movement, the larger the EPSP. However, the response observed during fast movement is also more variable and may be partly masked by IPSPs. These characteristics explain why the average traces (Fig. 4*D*, cf. *B*) do not reflect the velocity-dependence observed in raw data (Fig. 4*C*; cf. *A*). It is striking that the IPSP only became evident with the faster movements studied. This inhibitory connection limits the amount of depolarization induced by the summation of unitary EPSPs. It is noticeable that for

slow movements imposed to the CBCO strand, EPSPs do not summate much with each other, so that the amplitude of the response does not exceed 10 mV. By contrast, during faster movements, summation actually would occur and depolarization would reach >20 mV. In reality, due to the IPSP, this response is limited to 10–15 mV (see Fig. 4, *B* and *D*). This velocity-sensitive inhibition could play at least two roles: IPSPs could represent a gain control mechanism that limits the efficacy of the assistance (positive feedback) reflex that otherwise could result in an “explosive-like” reaction and IPSPs could be mediated by assistance reflex controlling INs (ARCINs) controlled by both the central locomotor network and velocity coding CBCO afferents. Such central control therefore could work in parallel with presynaptic control of primary afferents, in such a way that monosynaptic resistance reflexes or polysynaptic assistance reflexes are selected.

Phase-dependent modulation of reflex transmission

From all these data, and those from previous work on presynaptic inhibition of primary afferents in this preparation (Cattaert et al. 1992), we can propose a more complete organization for the sensory-motor reflex network controlling the coxo-basipodite joint of the leg. Figure 9 presents a schema of this organization concerning exclusively the Dep MN reflex activities. Both cases, quiescent and active preparation, are presented. In the first case (Fig. 9A), the central pattern generator (CPG) is not rhythmic and some of its outputs tonically excite (\oplus) the ARCIN responsible for the inhibition ($-$) of ARIN (Fig. 4, *A* and *C*). In that case, upward movement of the leg would activate release-sensitive CBCO fibers that stimulate monosynaptically most of the Dep MNs (Fig. 9A1); the Dep reflex expressed in the preparation would be a resistance response. However, downward movement of the leg would activate stretch-sensitive afferents that stimulate the aDep MN, resulting in a weak assisting influence in the Dep activity (Fig. 9A2); at the same time, the concomitant activation of ARCIN by stretch-sensitive CBCO fibers and tonic outputs from the CPG would result in a strong inhibition ($-$) of the ARIN and subsequently of the polysynaptic transmission of the stretch signal (Fig. 9A2). In contrast, when the CPG is rhythmically active (Fig. 9B), the central inhibition of ARIN through ARCIN is supposed to be removed. During the swing phase (Fig. 9B1), the CBCO strand is released by upward movement of the leg but Dep MNs stay silent, due to presynaptic inhibition (Cattaert et al. 1992). During the stance phase, Dep MNs are activated centrally. The CBCO strand is stretched during downward movement of the leg, and ARIN transmits efficiently the stretch inputs to the Dep MNs, which therefore respond in the same way as the aDep MN. The Dep response expressed in the preparation would be a strong assistance reflex.

Our model for the control of reflex activities in the crayfish walking system is comparable with those described in the locust (Burrows et al. 1988) or in the stick insect (Bässler 1993). Nevertheless, it is interesting to remark that in the crayfish, the interneuronal level seems to be specialized in the reversal of the reflex activities: the monosynaptic sensory-motor connections elicit resistance reflexes whereas the

interneuronal pathway, when permitted by the CPG, participates in the expression of assisting reflex responses in the motor nerves. It appears therefore that the reflexes expressed by the preparation are the result of a CPG-controlled balance between resistance and assistance patterns (as is suggested by Fig. 4). These results indicate that the CPG not only recruits sets of MNs, but also controls the sensory-motor pathways in such a way as to adapt them to the prevailing motor behavior.

We thank Dr. J. Y. Barthe, Dr. P. Skorupski, and Dr. L. Vinay for helpful discussions and improving the manuscript. We are grateful to Dr. F. Clarac for constructive comments and laboratory facilities.

This study was supported by the European Community Contract Number CT-930190 and the Centre National de la Recherche Scientifique.

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Received 3 July 1996; accepted in final form 19 December 1996.

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