In Vivo Analysis of Proprioceptive Coding and Its Antidromic Modulation in the Freely Behaving Crayfish

Didier Le Ray, Denis Combes, Cyril Déjean, and Daniel Cattaert
Laboratoire de Neurobiologie des Réseaux, Centre National de la Recherche Scientifique–Unité Mixte de Recherche, Université Bordeaux 1, Talence, France

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Le Ray, Didier, Denis Combes, Cyril Déjean, and Daniel Cattaert. In vivo analysis of proprioceptive coding and its antidromic modulation in the freely behaving crayfish. J Neurophysiol 94: 1013–1027, 2005. First published April 13, 2005; doi:10.1152/jn.01255.2004. Although sensory nerves in vitro are known to convey both orthodromic (sensory) and antidromic (putatively modulating) action potentials, in most cases very little is known about their bidirectional characteristics in intact animals. Here, we have investigated both the sensory coding properties and antidromic discharges that occur during real walking in the freely behaving crayfish. The activity of the sensory nerve innervating the proprioeceptor CBCO, a chordotonal organ that monitors both angular movement and position of the coxo-basipodite (CB) joint, which is implicated in vertical leg movements, was recorded chronically along with the electromyographic activity of the muscles that control CB joint movements. Two wire electrodes placed on the sensory nerve were used to discriminate orthodromic from antidromic action potentials and thus allowed for analysis of both sensory coding and antidromic discharges. A distinction is proposed between 3 main classes of sensory neuron, according to their firing in relation to levator muscle activity during free walking. In parallel, we describe 2 types of antidromic activity: one produced exclusively during motor activity and a second produced to their firing in relation to levator muscle activity during free walking. We report that in vivo, sensory coding by the CBCO nerve activity has been found by which the distribution of sensory orthodromic and antidromic activity changes with the physiological state of the biomechanical apparatus.

INTRODUCTION

During walking, various sensory receptors continually supply central locomotor networks with information about changes in the external environment and position and movement of limbs. In particular, proprioceptors play a crucial role in the direct adjustment of motor commands to various constraints applied to the biomechanical system (Cattaert and Le Ray 2001; Duysens et al. 2000; Hasan and Stuart 1988; Loeb 1987). However, depending on the activity in which the motor system is engaged, sensory information is subject to considerable presynaptic modulation before reaching its postsynaptic targets (for recent reviews, see McCrea 2001; Rudomin 2002). In crayfish for example, both long-lasting enhancement (Le Ray and Cattaert 1999) and rapid, short-lasting presynaptic inhibition (Cattaert and Le Ray 1998; Cattaert et al. 1992; Sillar and Skorupski 1986) were described in sensorimotor loops implicated in locomotor control. Bursts of antidromic action potentials, which sometimes accompany presynaptic inhibitory regulation (Cattaert et al. 2001; Dubuc et al. 1985, 1988; Gossard et al. 1991) and are conveyed from the CNS toward the peripheral sensory organ, were found to alter the coding properties of limbs proprioceptors in crayfish in vitro (Bévenegut et al. 1997; Cattaert and Bévenegut 2002) as well as in vertebrates (Dueñas et al. 1990; Gossard et al. 1999; Slesinger and Bell 1985). However, whether such antidromic control has functional significance in intact animals and whether it is subjected to behavioral constraints remain to be investigated.

To address these points, the electroneurographic activity of the sensory nerve innervating the coxo-basipodite chordotonal organ (CBCO), a proprioceptor that monitors upward and downward movements of the leg, was recorded in freely behaving crayfish, together with electromyograms from the levator muscle commanding this same joint. The orthodromic sensory action potentials as well as antidromic impulses conveyed in the sensory nerve were analyzed in relation to levator muscle activity. We report that in vivo, sensory coding by the CBCO appears much less specific than previously described in vitro (Le Ray et al. 1997a). In addition, we show that some antidromic action potentials in the CBCO nerve are generated exclusively during locomotion, whereas others may be linked to postural functions. Furthermore, our data suggest that antidromic discharges have an inhibitory effect on sensory coding associated with both locomotor and postural motor programs and that plasticity in both sensory coding and antidromic firing may occur as a function of the behavioral state of the sensory-motor apparatus.

METHODS

Experimental animals

Experiments were performed on 15 crayfishes, Procambarus clarkii (12–15 cm long), maintained in an aquarium at 17–18°C and fed once a wk. Animals were anesthetized on ice and remained immobilized during dissection and electrode placement. After surgery, they were kept in isolated compartments where they behaved freely while recordings were made over several consecutive days (≤8 days).

Innervation of the coxo-basipodite joint and disposition of recording electrodes

The present work was done on the sensorimotor system of the 2nd coxo-basipodite (CB) joint of the 4th leg. The 4th leg was chosen

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because of its major role in walking (Domenici et al. 1998; Jamon and Clarac 1995, 1997). Leg levation is mainly controlled by the 2nd proximal joint, the CB joint (Fig. 1, A and B), which allows exclusively vertical movements of the basipodite (in the following, no distinction will be made between basipodite displacement and CB joint angular movement). The sensorimotor system of the CB joint consists of a proprioceptor, the chordotonal organ CBCO that monitors upward and downward movements of the leg, and a pair of antagonistic levator (Lev) and depressor (Dep) muscles that control, respectively, upward and downward movements of the leg. The choice of this sensorimotor system was also dictated by the large amount of anatomical and physiological knowledge accumulated from extensive in vitro studies. The organization of the sensorimotor innervation of the joint is particularly convenient because sensory and motor nerves originating from the central ganglion diverge proximally, allowing us to make differential recordings of sensory and motor discharge patterns. Moreover, the CBCO sensory nerve is long enough to allow the placement of 2 electrodes, permitting the discrimination of orthodromically and antidromically traveling action potentials.

The procedure used for chronic recording from nerves was adapted from the technique developed by Böhm (1996) for the crayfish stomatogastric nervous system. Four thin monopolar wires, traveling in a grounded cable fixed with wax on the back of the animals were used and, usually, 3 of them were attached to 50-μm insulated wire electrodes to record muscular and nerves activity (see above), whereas a 4th electrode was placed under the carapace and used as a reference. Two wire electrodes were implanted through the cuticle and placed either both on the sensory nerve (7 experiments) innervating the CBCO (Fig. 1, B–D) or one on the CBCO sensory nerve and the other on the anterior motor root (AMR) that includes CBCO axons before their entry into the ganglion (4 experiments). This latter recording configuration presented the advantage of increasing the distance between the recording electrodes to improve the precision of conduction velocity measurement, but also decreased the signal/noise ratio (in the AMR neurogram), which made identification of the CBCO units in the AMR more difficult (e.g., Fig. 9C). Impulses were accepted for analysis only when a constant shape was found in the AMR neurogram and a constant delay was found with a unit identified in the CBCO neurogram. Electrodes were isolated from the hemolymph with a flexible silicone elastomer (World Precision Instruments, Sarasota, FL; Fig. 1D). The 3rd recording electrode was implanted directly into the anterior levator muscle through the cuticle and used to monitor muscle EMG activity (Fig. 1B) to monitor active movement of the CB joint (see Fig. 2 and METHODS below). Each recording electrode was fixed separately on the leg cuticle with wax before it reached its attachment to the grounded cable and connection to 3 homemade extracellular amplifiers. Amplified neurograms and EMG signals (e.g., Fig. 1G) were directed through a CED 1401 interface (Cambridge Electronic Design [CED], Cambridge, UK) to a computer for storage and analysis.

In addition, in 4 other animals, the CBCO sensory nerve was cut distally, close to the proprioceptor, to avoid orthodromic sensory spikes. In this condition, only a single electrode was needed to record antidromic action potentials. Finally, in 2 animals the recorded leg was blocked temporarily in an elevated position by sticking the basipodite (4th segment) against the thorax with wax, thereby maintaining the elastic strand of the CBCO in an almost completely released state. In both conditions, recordings of Lev EMG and CBCO nerve activity were performed during natural, free locomotor behaviors. Except in these last experimental conditions, animals performed normal displacements of the 4th leg during free walking. In particular, CB joint angular movements were not visibly perturbed and, although not measured during our experiments, they seemed to correspond to the standard angular ranges during locomotion (between about 15 and 40°, 0 degree corresponding to the full levation, i.e., leg in contact with the thorax) reported previously (Jamon and Clarac 1997).

Signal processing and data analysis

Sensory neurograms were analyzed using the “wave-marker” procedure of the Spike2 program (CED). This procedure identifies most of the action potentials that are conveyed by a given nerve, according to their shape and amplitude, and groups similar action potentials into distinct classes. Each class was then considered as a unique CBCO unit (i.e., a single CBCO neuron). Thereafter, a homemade Spike2 script program was used to compare the 2 recordings of the CBCO nerve. With this procedure, each impulse of a CBCO unit was used as a trigger to average (Fig. 1E) or superimpose (Fig. 1F) action potentials within the 2 neurograms to define the direction of their propagation and thus to identify sensory from antidromic units (although incorrect, the term “antidromic unit” is used in this report to describe a CBCO sensory neuron that also conveys antidromic action potentials). Because most action potentials were of small amplitude (especially several hours after the implantation of the electrodes) and
could occur simultaneously in the neurograms, the shape of their average trace may appear deformed (e.g., Figs. 6 and 7). To eliminate identification problems, only the CBCO action potentials that were readily recorded with a constant delay between both electrodes were kept for subsequent analysis. For each spike shape recorded with an electrode, the occurrence (within a given time window) of a corresponding spike with a regular shape was sought in the recording from the other electrode. Although this procedure dramatically reduced the number of CBCO units considered in this study (and for each unit the number of orthodromic or antidromic action potentials, which consequently causes a strong underestimation of unit firing frequency), it also ensured a reliable description of unit properties.

In some experiments, the angular movements of the CB joint were continuously monitored using a movement detector adapted from Marrelli and Hsiao (1976) and previously used to record swimmeret movements in lobster (Cattaert and Clarac 1983) and tail fan movements in rock lobster (Newland et al. 1992). This movement transducer is based on the propagation of electric fields in water. Two fixed electrodes are glued onto the proximal edge of the CB joint and produce an electric field that is measured by a 3rd electrode, which is glued to the distal edge of the CB joint and moves with the joint (Fig. 2A). This mobile electrode gives a linear measurement of the joint angle. The electric field is generated by a high-frequency (10- to 20-kHz) voltage instead of continuous current to avoid electrode polarization and measurement errors. Electrodes were made with silver wire, the tip of which was melted by flame to form a small silver sphere. This system has several advantages: 1) easy installation; 2) reliability; and 3) small size that does not perturb the movements of the animal. Nevertheless, when associated with the recording of nerve activity described above, this movement-recording apparatus substantially complicated the experiment and slightly altered the locomotor performance of the animal. For this reason therefore we developed an
then stopped and produced complex movements of the leg (Fig. 2). In this example, after a series of irregular movements, EMG activities were produced during active movements of the CB joint, position changes induced by muscle contraction. Therefore, in a series of experiments we analyzed the correlation between CB joint movements and integrals from Lev and Dep muscles EMGs. Figure 2B exemplifies such recordings in which a large variety of Lev and Dep EMG activities were produced during active movements of the CB joint (CB Mov). In this example, after a series of irregular movements, the animal performed 4 walking steps (arrowheads in Fig. 2, B and C), then stopped and produced complex movements of the leg (Fig. 2B). In the following analysis, only actual walking episodes were considered. Using a homemade Spike2 script program, EMG recordings from Lev and Dep muscles were rectified and integrated using several integration times: 0.01, 0.05, 0.5, and 1 s. The actual angular movement of the CB joint was then correlated to both the Lev (Fig. 2C) and Dep (not shown because no clear correlation was found) integrals. This analysis demonstrated that the t = 1 s integration of the Lev EMG (Fig. 2C) gives an excellent prediction of CB angular movements (compare the bottom 2 traces in Fig. 2C), especially concerning the ascending phase of the leg movement (see details in Fig. 2D).

This was confirmed by a linear regression analysis performed on the complete walking episode (Fig. 2E), which indicated that the relationship between integrated Lev EMG and angular position of the CB joint could be line fitted with a correlation coefficient of r = 0.91 (normalization of both the EMG integral and CB joint movement was performed because of the large difference between both sets of values). The slope of the curve was significantly different from 0 (P < 0.0001). Similar observations were made from 5 experiments, which supported the hypothesis that the Lev EMG integral could reflect reliably the angular displacements of the CB joint and, consequently, vertical movements of the leg. Thus an integral positive slope indicated a rising leg movement, whereas a negative slope putatively corresponded to a leg downward movement. Of course, this method introduces a certain insensitivity in the detection of the movements used for the latter analysis. For example, small-amplitude movements may be produced, whereas the EMG integration does not reflect them (e.g., Fig. 2B, movement trace between the last 2 arrowheads). However, in this report we focused on large steps during free walking without considering such small-amplitude movements that were rather involved in posture correction. In addition, our analysis being performed on several walking steps and data thus representing an accumulation of spikes from identified CBCO units, taking into account such small-amplitude movements, would not sensibly modify the results. Therefore to simplify the protocol and avoid implantation of too many wires when the CBCO neurograms were recorded, we used the integrated Lev EMG as an indicator of CB joint movements and the onset/offset of the Lev EMG as a reference for the analysis of CBCO neurons during walking (see Fig. 2F).

The activity of CBCO units were presented in raster displays using the onset/offset of lever EMG activity as a trigger. Also, for each CBCO sensory unit the spike occurrence was presented in an event histogram (Figs. 3–5). Changes in the spike occurrence distribution, which define the most efficient joint position for each unit, were determined by means of the cumulative sum (Ellaway 1978) of the changes in the bin count with respect to the mean count in a baseline (see also Mattei et al. 2003). The baseline was 1 s before either the onset (left part of panel A in Figs. 3–5) or the offset (right part of panel A in Figs. 3–5) of the Lev EMG burst. A similar analysis was performed on antidromic firing (Figs. 6 and 7), as well as on orthodromic–antidromic discharge correlations (Fig. 8).

RESULTS

The CBCO nerve is purely sensory and contains the axons of 40 sensory neurons (see Cattaert and Le Ray 2001), with cell bodies located in the receptor organ itself (Fig. 1C). In the present study, results were obtained from 31 distinct locomotor episodes performed by 11 freely behaving intact crayfishes. Only clearly identified orthodromic spikes were considered to be generated by CBCO sensory units. This usually corresponded to a small number of unit profiles (with a mean number of 15, over a range from 3 to 28; n = 31 recordings analyzed in intact animals). Similarly, few CBCO antidromic profiles were clearly identified in intact animals (from 0 to 8, with a mean number of 2; n = 31 recordings). However, in certain conditions, for example with the CBCO nerve cut distally (see following text), the number of identified antidromic profiles increased significantly ≤18 (with a mean number of 8; n = 10 recordings from 4 operated animals).

Sensory coding in freely locomoting animals

During free walking episodes, sensory units of the CBCO were analyzed according to the phase of movement (levation or depression, estimated with the Lev EMG integral; see methods) in which they were most active. Almost half of the identified sensory units were more active during the initial part of the Lev EMG burst and probably coded for upward movement or the elevated position of the leg, whereas only a few units showed a higher firing probability around the offset of the Lev EMG burst or between 2 consecutive bursts (Table 1). These latter units probably coded for downward movement and/or the depressed positions of the leg. Although every CBCO sensory neuron showed a specific pattern of discharge during free walking, it was possible to classify these activities (i.e., these coding properties) in 3 main groups according to the locomotor phase in which they were most active.

During locomotion, most of the identified CBCO sensory neurons showed a higher spike occurrence during the initial part of Lev EMG bursts (Fig. 3A, left, and Table 1), which
corresponded to the levation phase of a step, and a decreased spiking activity during the decaying part of the Lev EMG integral (Fig. 3A, right). However, fine analysis revealed that they presented distinct peaks of activity, indicating specific sensitivities. Three types of sensory coding of levation are exemplified in Fig. 3A. Some CBCO neurons showed increased firing during the whole Lev EMG burst (e.g., unit 1 in Fig. 3A, top left) and a progressive reduction of spiking activity when the Lev EMG ceased (Fig. 3A, top right). However, the majority of CBCO neurons fired preferentially during specific components of the EMG burst: neurons could have higher probabilities of firing that showed a symmetry related to the

**FIG. 3.** Sensory units coding for leg levation movements during free walking. A: raster, event histograms, and cumulative sum curves of the spiking activity of 3 different CBCO sensory neurons during 18 walking steps, with the onset \((t = 0\) on left panel) and the offset \((t = 0\) on right panel) of the Lev EMG used as the event trigger. Specific time points used as intervals for statistical calculations are indicated on the x-axis of histograms (−1 to 0 s for baseline, 0–0.4, 0–1, 1–2, and 2–3 s). Distinctly different sensitivities of the 3 units are illustrated: unit 1 codes for both positional and dynamic parameters, whereas unit 2 codes only for position and unit 3 for velocity. Shape of the corresponding action potentials on both CBCO recording electrodes is illustrated by the average traces between left and right panels. \(* P < 0.05; \** P < 0.01; \*** P < 0.001. B: instantaneous firing frequency of the 3 same CBCO sensory units during a locomotor episode (represents a part of the total locomotor episode used to generate raster and event histograms in A).
peak of the EMG integral (unit 2 in Fig. 3A, middle left), which suggested a specific sensitivity to the elevated position of the leg rather than to movement velocity. This assumption is corroborated by the fact that no coding (i.e., no significant change in spike occurrence) was found at the end of and after the Lev EMG burst (Fig. 3A, middle right). In contrast, other sensory neurons increased their firing only at the onset and during the first 100 ms of the Lev EMG burst during the maximum velocity phase (unit 3 in Fig. 3A, bottom left), suggesting that they selectively sensed levation movement rather than joint position (again, the absence of a change in activity at the end of the EMG burst corroborated this possibility; Fig. 3A, bottom right). Considering the overall activity during walking, levation-sensitive CBCO units had a low mean firing frequency of 6.72 ± 0.23 Hz (calculated from a sample of 26 identified units during 8 walking episodes; e.g., Fig. 3B). However, single-unit instantaneous frequency could reach very high values at the beginning of the levator muscle activity (192 ± 20 Hz; n = 520 action potentials from 26 identified neurons).

Besides levation-sensitive CBCO neurons, some sensory units were found to fire preferentially at the end of the Lev EMG burst or between 2 consecutive Lev EMG bursts (Table 1), i.e., when the leg depressed or was already in the down position (Fig. 4). As observed for the CBCO units coding for levation parameters, sensory neurons sensitive to leg depression also presented variable coding properties. Figure 4 illustrates 2 types of depression-sensitive CBCO neurons. Sensory unit 1 increased its firing around the trough of the Lev EMG integral (putative downward position; Fig. 4A, top right), whereas it expressed no significant change in firing around the Lev EMG onset (Fig. 4A, top left). Sensory unit 2 showed both a decreased firing during the initial part of the Lev EMG integral (i.e., leg levation; Fig. 4A, bottom left) and an increased discharge when the Lev EMG integral decreased (i.e., probable leg depression to lowest position; Fig. 4A, bottom right). In contrast to sensory unit 1, sensory unit 2 did not seem to have a clear position sensitivity because no significant change of firing occurred in the latter part of the Lev EMG integral (i.e., leg levation; Fig. 4A, bottom left) and an increased firing when the Lev EMG integral decreased (i.e., probable leg depression to lowest position; Fig. 4A, bottom right).
this work, and most CBCO neurons that were more active during this part of the locomotor cycle showed increased firing when the Lev EMG integral decayed or between consecutive Lev EMG bursts (Table 1). So, for a given amplitude of Lev integral (i.e., a given position; see METHODS), the neuron had 2 distinct firing behaviors: an increased firing while the integral decayed (i.e., putative downward movement of the leg) and no change when integral increased (upward movement), perhaps thus indicating a sensitivity to velocity in the downward direction. As described above for levation-sensitive sensory neurons, the latter, putatively depression sensitive CBCO units fired during the whole locomotor cycle during free walking (Fig. 4B), from a low resting firing rate (3.06 ± 0.17 Hz; n = 764) to an increased frequency of 150 ± 84 Hz (n = 764) during their preferred phase of the locomotor cycle (n = 20 identified neurons during 8 walking episodes).

Finally, during free walking episodes, a large majority of CBCO sensory neurons were found to produce a tonic discharge that never increased significantly (Fig. 5). In contrast, if any change was observed it consisted of a decrease in the
occurrence of spikes at a given phase of the locomotor cycle. Indeed, most of these tonically active sensory neurons never showed any significant changes related to the locomotor cycle (unit 1 in Fig. 5A, top), whereas other tonic sensory neurons exhibited a small but significant reduction in spike occurrence during rising (unit 2 in Fig. 5A, middle) or falling (unit 3 in Fig. 5A, bottom) phases of the Lev EMG integral (Table 1). Overall, tonic CBCO sensory units fired with a low frequency, ranging from 0.04 to 81 Hz during normal locomotor or postural activities (mean: 3.22 ± 0.68 Hz, calculated from a sample of 5,441 action potentials corresponding to 38 neurons identified during 8 walking episodes; e.g., Fig. 5B).

Aside from the above classification based only on direction coding, the analysis of spike distribution histograms and cumulative sum curves highlighted that CBCO sensory neurons may present distinct movement-parameter sensitivities. Although the coding characteristics showed variation among the neurons, and it seemed that each neuron possessed its own properties, 2 types of firings showed clear increases in relation to Lev EMG activity, whereas another seemed to provide tonic information that might decrease during given phases of the locomotor cycle. Thus some sensory neurons (about 45%) specifically increased their firing probability in correlation to the steepest slopes of the Lev EMG integrals (e.g., Fig. 3A, bottom), i.e., at the onset of the leg levation (positive integral slope) or putative depression (negative integral slope), which indicated a sensitivity specific to the velocity associated with either movement. Other CBCO sensory neurons (about 24%) changed their firing probability without any clear peak of discharge at the onset or offset of Lev EMG (e.g., Fig. 4, bottom right), which could indicate a lower sensitivity to velocity and a higher sensitivity to position. Finally, about 31% of CBCO sensory neurons were characterized by a discharge that could not be clearly correlated to either part of the Lev EMG integral (e.g., Fig. 5, top).

Antidromic action potentials

In vitro, CBCO sensory neurons were found to propagate action potentials in both directions. Although the aim of
orthodromic action potentials is to convey sensory information, the antidromic spikes seem to exert an inhibitory effect on sensory activity (Bévengut et al. 1997). In the freely behaving crayfish, antidromic action potentials generated in the CNS and conveyed toward the peripheral sensory organ were recorded from the CBCO nerve during both walking and postural activities (see following text). No antidromic activity was found that was specific to tail flips or defensive reactions (not illustrated; see DISCUSSION). Therefore we did not investigate further the 2 identified antidromic units, whereas 2 other units (AU 2 and AU 3) showed a large increase in activity preferentially in the absence of motor activity and AU 2 and AU 3 exclusively during motor episodes (AU 2 and AU 3 in Fig. 7B): whereas the antidromic firing of AU 1 seemed to be little affected during locomotion, AU 2 was strongly depressed during free walking (note that in this figure, locomotor movements were mixed with complex Lev muscle contractions that did not alter the observed walking).

In intact animals, the number of distinct antidromic profiles clearly identified from the CBCO neurogram was always rather small (with a maximum of 8 distinct profiles of antidromic action potential). This might be explained by the fact that the larger number of orthodromic (sensory) action potentials mask antidromic activity. We therefore recorded CBCO neurograms in animals (n = 4) with the CBCO sensory nerve cut close to the peripheral organ, and therefore in the absence of orthodromic activity. A larger number of antidromic units were now identifiable that were classified into 2 distinct groups. The 1st group occurred both during, and in the absence of motor activity (e.g., AU 1 in Fig. 7; see also Fig. 6 and text above), with an increased probability a few tens or hundreds of milliseconds before the onset of levator EMG activity (see Fig. 6). In contrast, the 2nd group of antidromic units was found exclusively during motor episodes (AU 2 and AU 3 in Fig. 7A, middle and bottom). Both AU 2 and AU 3 displayed a large increase in firing probability during the first 100 ms that followed the onset of levator EMG bursts, but only AU 3 maintained a higher level of discharge during the whole levator muscle burst (Fig. 7A). In contrast, these 2 identified antidromic activities showed little or no discharge between successive locomotor cycles (Fig. 7B; in this figure, the locomotor episode occurred after a complex Lev EMG burst during which the animal changed its orientation before walking). Thus this latter group may be defined as a mainly “locomotor” antidromic group, whereas the first one may represent a “postural” antidromic group (Table 2). Indeed, it is noticeable that the probability of firing “postural” antidromic action potentials was strongly reduced during the first tens of milliseconds after...
the onset of levator EMG bursts during locomotor bouts (which corresponded to active movements; Fig. 7A, top), whereas that of “locomotor” antidromic action potentials was maximal (Fig. 7A, middle and bottom). Overall, the mean frequency of “locomotor” antidromic discharge was 1.11 ± 0.17 Hz (calculated from 10,475 antidromic action potentials corresponding to 57 profiles identified from 25 locomotor episodes), whereas “postural” antidromic discharges had a significantly higher (P < 0.05) mean frequency of 2.07 ± 0.40 Hz (calculated from 39,327 antidromic action potentials corresponding to 68 profiles identified from 25 locomotor episodes). Other than the higher mean number of antidromic profiles that were identified in the cut CBCO nerve (6.8 ± 1.4) compared with the intact CBCO nerve (3.6 ± 0.7), no qualitative differences were observed. The mean frequency of antidromic action potentials was not significantly different in intact and cut CBCO nerves (respectively 2.32 ± 0.53 Hz, for 18,107 action potentials from 44 profiles, and 1.24 ± 0.22 Hz, for 31,695 action potentials from 81 profiles). Moreover, “locomotor” and “postural” antidromic activities were identified in similar proportions (about 40 and 60%, respectively) in the 2 experimental conditions.

**TABLE 1.** Summary of sensory CBCO units identified from 11 freely behaving animals

<table>
<thead>
<tr>
<th>Main Phase of Firing</th>
<th>Total Number</th>
<th>Mean %</th>
<th>“Velocity” (only)</th>
<th>“Position”</th>
<th>Decrease of Tonic Activity During</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levation</td>
<td>156</td>
<td>43</td>
<td>16%</td>
<td>84%</td>
<td>Levation</td>
</tr>
<tr>
<td>Presumed depression</td>
<td>17</td>
<td>5</td>
<td>0%</td>
<td>100%</td>
<td>Presumed Depression</td>
</tr>
<tr>
<td>Tonic units</td>
<td>184</td>
<td>52</td>
<td></td>
<td></td>
<td>None</td>
</tr>
</tbody>
</table>

Total number of units in either levation-sensitive or putatively depression sensitive groups and in tonic neurons, as well as the mean percentage of these units compared to the total number of sensory neurons identified. Similar proportions were found in single animals. For nontonic neurons, a classification in probable “movement” and “position” sensitivity is given, as well as for tonic neurons the locomotor phase causing a decrease in their firing.
TABLE 2. Summary of antidromic discharges analyzed in 11 freely behaving animals

<table>
<thead>
<tr>
<th>Main Type</th>
<th>Total Number</th>
<th>Mean %</th>
<th>Increase of Firing at the Onset of</th>
<th>Major Decrease of Firing at the Onset of</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locomotor</td>
<td>62</td>
<td>40</td>
<td>Levation</td>
<td>Presumed depression</td>
</tr>
<tr>
<td>Postural</td>
<td>93</td>
<td>60</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Same organization as Table 1, with the locomotor phase associated with the peak of “locomotor” antidromic discharge and the major decrease of “postural” antidromic activity given.

Inhibition of sensory coding by antidromic discharges

Recent studies on in vitro crayfish (Bévengut et al. 1997; Cattaert and Bévengut 2002) and acute cat (Gossard et al. 1999) have suggested an inhibitory role for antidromic discharges in primary afferent sensory nerves. We assessed this possible function in the freely behaving crayfish by performing cross-correlations between sensory and putatively suppressive antidromic discharges (Fig. 8). Because it is impossible to associate individual antidromic and orthodromic spikes in vivo (arising from dramatic differences in shape, resulting from opposite directions of conduction), correlations were tested between every single CBCO orthodromic and antidromic discharge during all walking episodes. Figure 8 shows examples of successful cross-correlations, centered on the antidromic spikes ($t = 0$), with their curves of cumulative sum (in this case, control baseline was calculated between 1 and 0.1 s before the occurrence of the antidromic action potential, and statistical tests were performed on different time intervals after the antidromic spike). Two examples of such an analysis are presented in Fig. 8, A and B: after the occurrence of antidromic spikes ($t = 0$), the discharge of the corresponding orthodromic (sensory) unit was significantly decreased. This is indicated by the negative slope of the cumulative sum curve and was observed in all 3 classes of CBCO sensory neurons described above (i.e., in “velocity-” and “position-coding” neurons, and in tonically active neurons). These results suggest that every CBCO sensory neuron may convey antidromic action potentials that would exert an inhibitory regulation on its sensory coding. Although inhibitory modulation of tonic sensory neurons was easier to detect, clear depression of the discharge of “velocity-sensitive” and “position-sensitive” sensory neurons could also be observed after antidromic action potentials (Fig. 8). Note that in some cases (e.g., Fig. 8B, bottom) an increased orthodromic discharge could occur several hundreds of milliseconds (here, >500 ms) after antidromic spikes. However, this increased sensory firing could result from either a property of the sensory neuron, similar to a postinhibitory rebound effect, or to the specific coding of an ongoing leg movement.

“Locomotor” antidromic discharges [indicated by “(L)” near $t = 0$ in Fig. 8] affected only the firing of phasic sensory neurons (i.e., neurons probably coding for dynamic parameters of leg upward movement), and no suppressive effects could be found on tonic sensory neurons. In contrast, “postural” antidromic discharges [indicated by “(P)” near $t = 0$ in Fig. 8] were found to depress the activity of all 3 classes of CBCO sensory neurons. These results suggest that “locomotor” antidromic discharges may exclusively be conveyed in “velocity-sensitive” sensory neurons, whereas any kind of CBCO sensory neurons may convey “postural” antidromic activities. However, the extracellular shapes of antidromic and orthodromic spikes are so different that such clear correlations would require further experiments using intracellular recordings from identified CBCO sensory neurons. Surprisingly, any inhibitory function could not be found for a substantial proportion (about 50%) of the antidromic activities identified from CBCO neurograms. In some cases, as a result of our experimental conditions this could stem from a substantially too low basal discharge frequency of the sensory unit to show such modulation. In other cases, we cannot exclude the possibility that some of the identified antidromic action potentials were conveyed in the axon of sensory units that were not active in the angular range covered during free walking. When detected, depression of the sensory discharge lasted from 100 ms (e.g., Fig. 8A, bottom) to $\pm 1$ s (e.g., Fig. 8A, middle) with a mean duration of inhibitory effect of $192 \pm 44$ ms ($n = 19$ correlations). Although it was impossible to test in our experimental conditions, the duration of the inhibitory effect might depend on the frequency of the antidromic discharge as previously suggested by in vitro experiments (Cattaert and Bévengut 2002).

Plasticity of sensory coding and antidromic discharges

Our in vivo experiments, in which the CBCO nerve was cut distally, suggested that the probability of firing of antidromic action potentials in sensory neurons might rely closely on the state of the proprioceptive apparatus itself. To investigate this possibility, recordings from the intact CBCO nerve were performed in 2 animals in control and after the 4th leg was blocked in a position where the CBCO strand was maintained in a released state (see METHODS). Both sensory and antidromic discharges were then analyzed without discrimination of walking from nonwalking activities (e.g., Fig. 9). In the control condition, several orthodromic but no antidromic profiles were identified from the CBCO neurogram. In Fig. 9A, for example, 14 distinct orthodromic sensory units were identified within the 3 main classes defined above. After keeping the leg blocked for several days, several profiles of antidromic firing were detected, whereas the number of orthodromic units decreased dramatically. In fact, after 5 days none of the 14 orthodromic profiles detected in control conditions (Fig. 9A) was still encountered, but 12 distinct antidromic profiles were identified (Fig. 9B). So, when the leg was kept immobile the orthodromic/antidromic ratio of profiles (OAR) was inverted, suggesting that the direction in which action potentials are conveyed in CBCO sensory neurons is subject to plasticity according to the state of the biomechanical apparatus.

In another animal the time course of the OAR inversion was analyzed in 3 conditions in which the sensory feedback generated was different: during free walking episodes, in the absence of locomotion, and when the animal was held above ground and produced walkinglike leg movements. In the latter condition the weight constraints and therefore a part of postural control were suppressed and therefore probably did not participate in the process of antidromic discharge generation. We found that whatever the behavioral context the OAR was subjected to similar changes (Fig. 9C). During free walking ($\delta$) in the control condition, a large majority of orthodromic units were detected (OAR = 85.9%). As soon as 30 min after the leg was immobilized, the...
in this study to be active during walking episodes. In contrast to earlier in vitro experiments (Le Ray et al. 1997a) identified sensory units were active in every phase of the locomotor cycle. For example, a CBCO sensory unit that fired during levator muscle activity also fired, albeit to a lesser extent, between consecutive levator EMG bursts and even in the absence of movement. Sinusoidal mechanical stimulation of the CBCO strand in vitro also caused some sensory units to fire during the full extent of the stimulation cycle. The main coding of these neurons therefore resulted from the sum vector of all single action potentials as determined from circular statistics (Le Ray et al. 1997a), and as also demonstrated more recently in humans (Roll et al. 2000). Although the irregularity of the locomotor cycle impedes such circular analysis, similar properties may also apply to in vivo sensory coding during free walking. Our present in vivo results show that most CBCO sensory neurons exhibit a low discharge rate throughout the locomotor cycle and, surprisingly, that the coding of a movement may reside in a decrease in neuronal discharge (e.g., Fig. 5A). Taken together with our earlier in vitro results, these data suggest that the CBCO monitoring of leg movements consists of a “collegial” coding of the various biomechanical parameters of movements, and information consists of the “summation” of all the slight discharge changes that occur in the whole population of sensory neurons. This also suggests that small modifications in this sensory information, which will be integrated by motoneurons through many direct and polysynaptic connections (Le Bon-Jego and Cattaert 2002; Le Bon-Jego et al. 2004; Le Ray et al. 1997a,b), are sufficient to trigger adaptive locomotor responses. In fact, such a system would prevent sudden and abrupt motor responses that would invariably disturb equilibrium and result in locomotor deficiencies.

In insects, leg chordotonal organs are sensitive enough to detect very small vibrations (Cokl and Virant-Doberlet 2003; Gopfert et al. 2002; Stein and Sauer 1999). Similarly, because of its location between the anterior and posterior levator muscles in the coxopodite (Cattaert and Le Ray 2001) the CBCO elastic strand may detect muscle contractions and relaxations that occur during all leg movements. In addition, leg movements may not be as regular as they seem to be and some up and down micromovements of the joint may be encoded by the CBCO. This would explain why the CBCO sensory neurons that were found to be unidirectional in responsiveness in vitro (Cattaert and Le Ray 2001) seem to turn into bidirectional neurons in vivo. Thus further investigation combining electromyogram recordings and micromovement analysis is required to fully understand the encoding properties of CBCO proprioceptive neurons in freely behaving crayfish.

**Antidromic activities in the freely behaving crayfish**

Antidromic discharges have been described in sensory nerves during central motor activity in both vertebrates (Beloozerova and Rossignol 1999; Dubuc et al. 1985, 1988; Gossard et al. 1989, 1991; Westberg et al. 2000) and invertebrates (Cattaert et al. 1992; Marchand and Leibrock 1994; Marchand et al. 1997; Wildman and Cannone 1996). They have been related to the locomotor central pattern generator (CPG) activity (Cattaert et al. 1992; Fellippa-Marques et al. 2000), as well as to sensory influences and descending commands (Vinay and Clarac 1999; for review, see Rudomin et al.

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**DISCUSSION**

**In vivo sensory coding**

Based on correlations between neuronal discharge and Lev EMG activity, 3 classes of sensory neurons were distinguished

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**FIG. 9.** Behavioral plasticity of sensory and antidromic activities recorded in vivo from the CBCO nerve during free walking. A and B: in a crayfish with the 4th leg free, 14 orthodromic sensory units were identified (A), whereas after 5 days of leg restraint in elevated position no sensory coding persisted but 12 antidromic profiles appeared (B). In this study, no distinction was made between Lev EMG activities associated or not with walking (arrowheads). C: changes in the ratio of identified orthodromic (empty bars) and antidromic units (filled bars) in control and in 3 conditions at various times after the leg had been immobilized in the up position. §: during free locomotion; €: when the animal was held above the ground; ¥: in the absence of locomotion. Averaged action potentials on the right part of the figure exemplify orthodromic (OU) and antidromic units (AU) recorded from both the CBCO nerve (CBn) and the anterior motor root (AMR).

OAR became inverted (OAR = 37.6%) and the number of identified antidromic profiles represented >50% of the profiles recorded from the CBCO/AMR neurograms (see METHODS). Thereafter, the OAR was calculated for 2 consecutive days in the 3 behavioral conditions. In all cases, the OAR decreased with time because of both a reduced number of orthodromic units and an increase in the number of both “locomotor” and “postural” antidromic profiles. However, although OAR changes were similar in suspended (€) and freely walking animals (OAR = 20 and 17.1%, respectively, after about 40 h) it was noticeable that sensory activities disappeared only in the absence of locomotion (¥), suggesting that some sensory neurons might be directly sensitive to muscle contraction and remain excitable (see DISCUSSION).
was analyzed in freely behaving intact animals in this study. Antidromic activities. Consequently, the sensory coding that type. Together with the fact that antidromic discharges are the former on the sensory discharge of CBCO neurons of any 2002), correlations between antidromic and orthodromic discharges in vivo might also result from CPG activity (Coˆte´ and Gossard 2003). In contrast, “postural” antidromic discharges were observed during the whole locomotor cycle, although they also presented peaks of activity around the onset and the offset of Lev burst (i.e., probably during levation–depression transitions), and their origin remains unclear. Because they were never recorded from in vitro preparations that lacked both sensory and descending influences, one may exclude a CPG source and suggest an origin in either sensory feedback or descending commands, or both. Nevertheless, “postural” antidromic spikes in vivo might also result from CPG activity induced by a specific neurohormonal environment that is removed in isolated preparations.

In this study, we analyzed the occurrence of antidromic CBCO spikes during different motor behaviors. Whereas some antidromic activity could be related to locomotion and posture, no antidromic action potentials were found to be specifically correlated to the defense reaction (i.e., when the crayfish faces a danger with its claws open) or escape behavior (tail flips). This is especially surprising because during tail flIp a powerful depolarizing presynaptic inhibition of the CBCO sensory afferents is produced (El Manira and Clarac 1994) and thus antidromic spikes might also be expected to be generated (Cattaert et al. 2001). The absence of antidromic spikes associated with tail flips could result from our inability to extract them from the powerful sensory activity that also occurs during a tail flip or it might also result from synaptic mechanisms incompatible with antidromic spike generation (see Cattaert et al. 2001). However, the absence of antidromic discharge during specific locomotor functions may also reflect distinct levels of presynaptic control, as was recently demonstrated in the cat (Côté and Gossard 2003).

Modulation of CBCO sensory coding

As suggested by in vitro analysis (Cattaert and Bévengut 2002), correlations between antidromic and orthodromic discharges during free walking indicated an inhibitory effect of the former on the sensory discharge of CBCO neurons of any type. Together with the fact that antidromic discharges are always present in intact animals, this latter negative correlation suggests that sensory coding is permanently regulated by antidromic activities. Consequently, the sensory coding that was analyzed in freely behaving intact animals in this study already reflected this regulation and thus did not correspond to the basic coding properties of the CBCO. Future comparison of sensory coding in intact CBCO nerve and proximally cut CBCO nerve, which would convey only orthodromic (sensory) action potentials, will be required to fully appreciate the extent of the inhibitory control exerted by antidromic discharges in freely behaving animals.

Antidromic spikes seem to depress a sensory discharge for several hundreds of milliseconds (see Fig. 8), which may be sufficient to filter specifically the sensory discharges associated with the dynamic component of leg movement (e.g., Fig. 3). Because the resistance reflex is mainly based on dynamic sensitivity (Le Ray et al. 1997b), the removal of movement-evoked sensory information would facilitate the expression of active movements. Indeed, preliminary experiments suggest that about 20% of the CBCO sensory neurons may lose their dynamic sensitivity during free walking when compared with imposed levation of the leg (unpublished observation). Although future experiments will be necessary to investigate this possibility further, these findings support our hypothesis that presynaptic inhibition, and thereafter antidromic discharges, are preferentially directed against the dynamic component of the resistance reflex (Le Ray et al. 1997a,b). Nevertheless, several sensory neurons may convey antidromic activity with distinct patterns of discharge during a given locomotor episode (e.g., Fig. 7), which suggests distinct levels of control exerted among the population of CBCO sensory neurons during locomotion. In addition, any negative correlation could be found for about half of the identified antidromically active units, because either the orthodromic firing rate of these neurons was too low or antidromic activity may support other possible functions.

Plasticity of CBCO nerve activity

Our in vivo results indicate that the state of the proprioceptor may be a source of plasticity in CBCO nerve activity. In experiments in which the leg was constrained, sensory discharges slowly faded with time, and almost no coding persisted after several days. Nevertheless, some CBCO units that were active during locomotion remained active for longer than other sensory neurons. We hypothesize that, despite limb immobilization, the preservation of this specific coding was a consequence of the continual activation of CBCO sensory units during limb muscle contraction, which further suggests that the long-term maintenance of CBCO coding properties is an activity-dependent phenomenon. Similar activity dependency has already been described in vitro in the sensory terminal-to-motor neuron synapses that control the movement of the CB joint in crayfish (Le Ray and Cattaert 1999). Taken together, our data strongly suggest that the strength of the sensorimotor loop is highly dependent on the expression of recurrent locomotor movements.

Beside this state dependency of sensory coding, a large increase in antidromic firing was observed in the CBCO nerve of immobilized legs. Previous experiments in crayfish in vitro (Cattaert and Bévengut 2002) and the acute cat (Gossard et al. 1999) suggested that the function of the antidromic action potentials is to reduce the sensitivity of the peripheral proprioceptor. Although our results obtained with blocked legs also support this hypothesis, the observation of antidromic spikes without clear inhibitory effects on any CBCO sensory neuron
may suggest another role for the antidromic activity. Beloozerova and Rossignol (1999) reported that the number of antidromic action potentials recorded from cat dorsal root filaments increased after peripheral anesthesia or transection (i.e., in the absence of sensory activity in those filaments) and suggested that antidromic activity may create a link between central and peripheral processes. Taken together with our present results, we propose that antidromic discharges represent a form of central “command” toward the peripheral proprioceptor to set its sensitivity into a dynamic range compatible with the state of both the locomotor nervous system and the sensorimotor apparatus.

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Present address of D. Le Ray and D. Combes: Laboratoire de Physiologie et Physiopathologie de la Signaux Cellulaire, CNRS-UMR 5543, Université Victor Ségalen, BP 22, 146 rue Léo Saignat, 33076 Bordeaux cedex, France.

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