Reference: *Biol. Bull.* **211:** 212–222. (December 2006)

Patterns of Motor Activity in the Isolated Nerve Cord of the Octopus Arm

YORAM GUTFREUND[1,*], HENRY MATZNER[1], TAMAR FLASH[2], AND BINYAMIN HOCHNER[1,†]

[1]*Department of Neurobiology and Center for Neuronal Computation, Institute of Life Sciences, Hebrew University, Jerusalem 91904, Israel; and* [2]*Department of Computer Science and Applied Mathematics, The Weizmann Institute of Sciences, Rehovot 76100, Israel*

Abstract. The extremely flexible octopus arm provides a unique opportunity for studying movement control in a highly redundant motor system. We describe a novel preparation that allows analysis of the peripheral nervous system of the octopus arm and its interaction with the muscular and mechanosensory elements of the arm's intrinsic muscular system. First we examined the synaptic responses in muscle fibers to identify the motor pathways from the axial nerve cord of the arm to the surrounding musculature. We show that the motor axons project to the muscles *via* nerve roots originating laterally from the arm nerve cord. The motor field of each nerve is limited to the region where the nerve enters the arm musculature. The same roots also carry afferent mechanosensory information from the intrinsic muscle to the axial nerve cord. Next, we characterized the pattern of activity generated in the dorsal roots by electrically stimulating the axial nerve cord. The evoked activity, although far reaching and long lasting, cannot alone account for the arm extension movements generated by similar electrical stimulation. The mismatch between patterns of activity in the isolated cord and in an intact arm may stem from the involvement of mechanosensory feedback in natural arm extension.

Introduction

In redundant systems, the number of degrees of freedom involved in movement generation is far greater than those needed to define the task, posing a great load on any motor control system. It is therefore a major challenge to understand movement control in such systems, be they robotic or biological (Chirikjian and Burdick, 1990; Hollerbach, 1990; Colbaugh and Glass, 1992; Bizzi, 1993; Walker, 2000; Flash and Hochner, 2005; Walker *et al.,* 2005). An extreme example of a redundant biological movement system is the octopus arm. The arm can bend at any point and in any direction and can elongate, shorten, and twist. Its extraordinary motor capabilities are achieved through the absence of a skeleton and its being composed almost entirely of muscle fibers (such a structure is called a muscular-hydrostat, Kier and Smith, 1985). The arm's capabilities and the variety and precision of its movements make it an ideal biological model system for studying the control of kinematically redundant movements (Gutfreund *et al.,* 1996; Sumbre *et al.,* 2005, 2006).

The octopus nervous system is divided into a central and a peripheral nervous system. The central nervous system contains the brain and the two optic lobes, while the peripheral nervous system includes the ganglia of the body and arms, the latter arranged as axial nerve cords projecting from the brain along the center of each arm (Young, 1963, 1971). Most of the peripheral neurons are located in the axial nerve cords, which are organized into an extensive nervous system comprising both sensory and motor circuits (Young, 1963; Rowell, 1966; Graziadei, 1971). Behavioral studies suggest that many of the complex actions performed by octopus arms are organized at the level of the peripheral nerve cord (Altman, 1971; Wells, 1978; Sumbre *et al.,* 2001, 2005, 2006). The nerve cord circuitry is thus not a simple relay station to and from the brain but plays a major role in the control of arm behavior.

Received 25 April 2006; accepted 9 August 2006.

* Present address: Department of Physiology and Biophysics, Technion, Haifa 31096, Israel.

†To whom correspondence should be addressed. E-mail: bennyh@lobster.ls.huji.ac.il

The actions of reaching toward a target or extending an arm are created by a stereotypical wave-like forward propagation of a bend along the arm (Gutfreund *et al.,* 1996). Bend propagation is accompanied by a wave of muscle activity traveling down the arm (Gutfreund *et al.,* 1998). Unique to the octopus arm, stereotypical bend propagation can be evoked in an isolated arm by electrically stimulating the axonal tract of the arm nerve cord or by applying tactile stimulation to the skin or suckers. Thus, the basic motor program for arm extension is embedded within the neuromuscular system of the arm itself (Sumbre *et al.,* 2001). The brain appears to activate and scale the output of a peripherally distributed control system, possibly demonstrating the importance of hierarchical organization in redundant motor systems.

To investigate this unique peripheral nervous system, we have developed an *in vitro* preparation of an isolated axial nerve cord. We physiologically identified the numerous nerve roots that arise laterally from the nerve cord as carrying both motor and sensory axons. Anatomical and lesion studies indicate that each root enervates muscle fibers within its vicinity (Martoja and May, 1956; Rowell, 1963). Identifying these roots as motor nerves to the intrinsic muscles of the arm allowed us to analyze patterns of activity generated by the isolated axial nerve cord and nerve roots after electrical stimulation of the axonal tract. We could then compare these outputs with the movements generated by such stimulation in isolated arms.

Materials and Methods

Experimental animals and physiological solution

Specimens of *Octopus vulgaris* were either collected from the Mediterranean shore by local fishermen or supplied by the Zoological Station in Naples, Italy. The animals were maintained in glass tanks, dimensions 50 × 50 × 40 cm, containing seawater at 17°C, and were kept on a 12-h light/dark cycle. The water, prepared from synthetic sea salt (Instant Ocean, Aquarium Systems), was continuously circulated in a closed system and filtered through coral dust and active charcoal. Artificial seawater (ASW) was used as the normal physiological solution during preparation and experiments. It contained (in mmol/l): NaCl, 460; KCl, 10; $MgCl_2$, 55; $CaCl_2$, 11; Hepes, 10; glucose, 10; pH 7.6.

Electrophysiological recordings

The octopuses were anesthetized in a 1:1 solution of 7.5% (weight/volume) $MgCl_2$ and seawater (Messenger *et al.,* 1985). Following anesthesia, a segment of an arm was quickly removed and placed in oxygenated ice-cooled seawater for up to 5 min. The length of the segment removed depended on the size of the octopus and the experiment to be performed. The arms from which the tip had been removed started regenerating a few days after lesion. We observed no visible effect of amputation on the octopus' feeding and foraging behavior and saw no changes in its food consumption, so the same octopus was used for several experiments.

The removed segment of the arm was pinned dorsal side up on a Sylgard-coated dish filled with cold ASW. A dorsal incision along the arm exposed the axial nerve cord, 2–3 cm of which was dissected away from the surrounding tissue and pinned lateral side up (Fig. 1A). Loose connective tissue was removed with fine forceps, and the clean isolated nerve cord was placed in the recording chamber and continuously perfused with oxygenated ASW at room temperature (flow rate ~3.5 ml/min ~ 0.5 bath volume/min). Glass suction electrodes with an opening of 50–150 μm were used for recording extracellularly from specific locations along the nerve cord, either by drawing a nerve root into the pipette or by placing the electrode over an axonal tract. A silver wire wrapped around the outside of the pipette served as a reference electrode. All recordings were amplified with a differential AC amplifier (Warner Instrument Corp. DP-304), filtered (300 Hz–10 KHz), and saved on a video-based system (Neurodata). Electrical stimulation consisted of 0.1-ms negative current pulses passed through similar glass suction electrodes or with bipolar silver electrodes. Stimulus intensity was generally set slightly above response threshold.

A similar preparation was used for intracellular recording from muscle fibers (for details, see Matzner *et al.,* 2000). After the suckers and skin were removed, a strand of longitudinal and transverse muscles from the lateral aspect of the arm was dissected out together with the axial nerve cord (about 20 mm long and 1 mm thick). Care was taken to keep the nerves between the axial nerve cord and the muscle strand intact. The preparation was pinned down on a Sylgard-coated dish and perfused with oxygenated seawater at room temperature. Intracellular recordings from muscle fibers were made with sharp glass microelectrodes (25–40 MΩ when filled with 3 mol/l K-acetate and 0.1 mol/l KCl) using the Axoclamp 2B in bridge mode. The nerves were stimulated with fine (75-μm) bipolar stainless steel electrodes. This preparation also served for identifying motor activity in the nerves by recording *en passant* from the nerve with a suction electrode while simultaneously intracellularly recording muscle cell postsynaptic potentials (PSPs). A similar preparation was used for identifying afferent mechanosensory activity in the dorsal roots. Here the muscles were stimulated mechanically with a fine rod driven either manually or with a loudspeaker activated by pulse generator (Master-8, AMPI).

Results

The anatomy of the nervous system of the octopus arm was first described by Martoja and May (1956) and Rossi

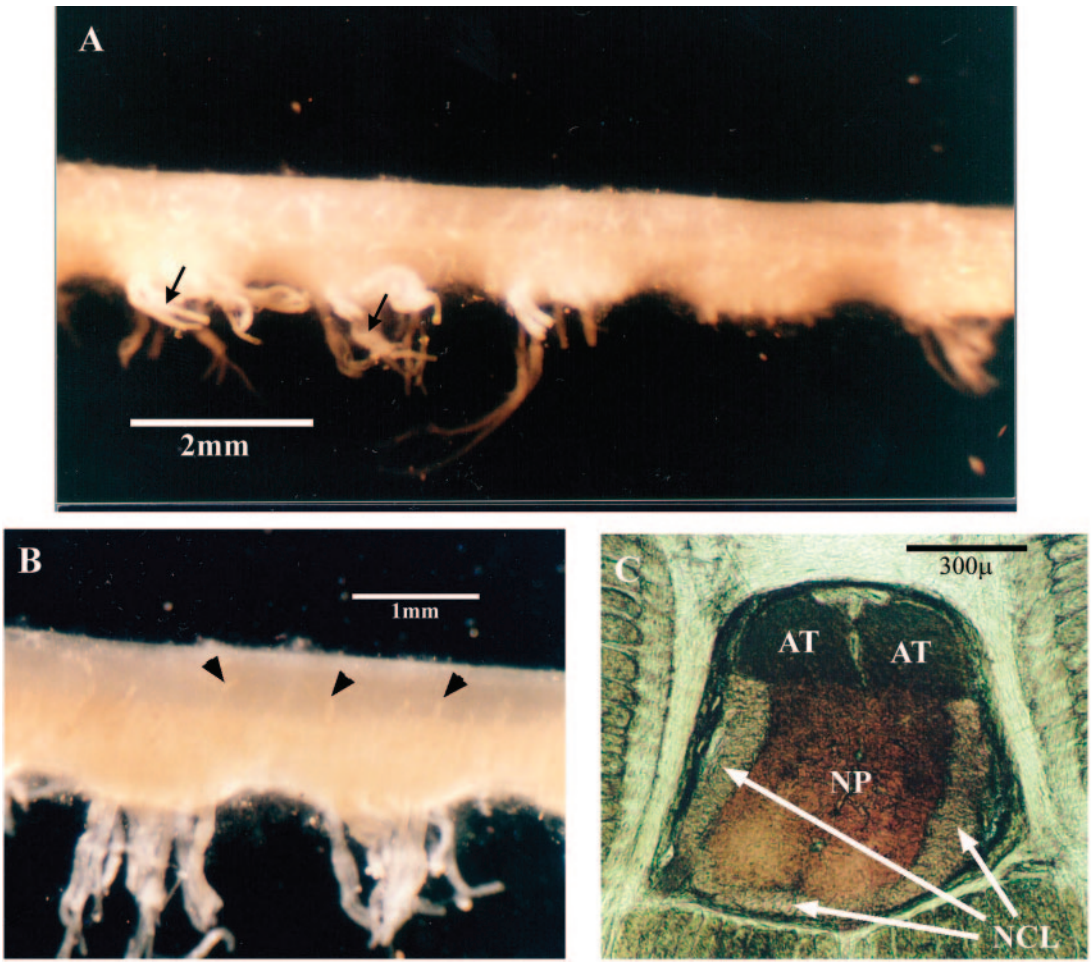

Figure 1. The *in vitro* preparation of the axial nerve cord. (A) A lateral view of the isolated nerve cord (dorsal side up). Each swelling is a single brachial ganglion. The numerous ventral roots are clearly seen projecting from each ganglion (arrows). (B) Dorsal roots originate from the borderline of the axonal tract (some marked by arrowheads). (C) Unstained transverse section of the axial nerve cord at the level of a brachial ganglion, showing the two dorsally located bundles of the axonal tract (AT), the cell body or perikaryal layer (NCL), and the internal neuropil (NP) of the ganglion.

and Graziadei (1954, 1956, 1958, summarized in Graziadei, 1971). The axial nerve cord, running along the center of each arm, consists of a chain of ventral ganglia and two dorsal axonal tracts (the cerebrobrachial tracts, AT in Fig. 1C). This division into the dorsal axonal tracts and ventral brachial ganglionic chain is clearly visible in the isolated axial nerve cord preparation (Fig. 1A, B). The axonal tracts form a more transparent band, contrasting with the opaque ganglionic chain. The latter consists of closely spaced ganglia, each of which can be seen as a swelling in the isolated preparation (Fig. 1A). Each ganglion is located opposite the alternating member of a pair of suckers of which about 300 are organized in two parallel rows along the ventral surface of the arm (Graziadei, 1954; Martoja and May, 1956). The brachial ganglia exhibit typical invertebrate organization (Fig. 1C), with an outer layer of cell bodies (perikaryal layer) enveloping an internal neuropil (Graziadei, 1971).

Many nerves project from each ganglion in different directions. Graziadei (1971) divided them into two groups: the nerves of the suckers (about 20 per ganglia) and the nerves of the intrinsic musculature. The nerves of the suckers arise ventrally (*i.e.,* oral side) from the ganglia and pass through the intrinsic musculature to innervate the suckers (Martoja and May, 1956; Graziadei, 1971). These relatively long and thick roots can be clearly seen in the ventral part of the preparation (Fig. 1A, B, arrows in A). We termed these the ventral roots. The nerves of the intrinsic musculature, which arise laterally from the ganglia, are thought to carry motor fibers to the intrinsic musculature and to the chromatophores, and sensory fibers from the arm periphery (Martoja and May, 1956; Graziadei, 1971). These nerves, which we term dorsal (*i.e.,* aboral) roots, mostly originate at the borderline between the axonal tract and the ganglionic chain (arrowheads in Fig. 1B). In the isolated preparation,

this latter group of nerves appears smaller and less regular than the ventral roots. In some areas they could not be observed at all, probably because they are embedded in the connective and muscle tissue and are frequently damaged during the isolation procedure.

Intracellular recordings from muscle fibers

To identify motor signals in the dorsal root activity, a suction electrode attached *en passant* recorded extracellular activity in the dorsal root, while the postsynaptic responses were recorded intracellularly from muscle fibers of the intrinsic musculature. Efferent activity was induced by a stimulus train (10–100 Hz) to the axonal tract. The activity of some of the neurons in the nerve root correlated with muscle excitatory postsynaptic potentials (EPSPs). Figure 2A shows a single large action potential preceding the internally recorded EPSP by a fixed delay (2.8 ms). Figure 2B shows the typical large triphasic (positive-negative-positive) waveform of a propagating action potential appearing 3.0 ms before the onset of a muscle EPSP. Figure 2C shows a train of action potentials corresponding one-to-one with the muscle EPSPs (the last EPSP in the train induced muscle contraction). These experiments confirmed that extracellular recording can detect motor activity in the dorsal nerve roots and that efferent activity from dorsal roots reflects at least part of the motor commands to the intrinsic musculature.

We next studied the spatial distribution of muscle innervation by dorsal roots. We stimulated single dorsal roots and recorded the responses evoked in muscle fibers located either adjacent to the stimulated root or adjacent to the next ganglion. In Figure 3A, six examples for muscle EPSPs recorded from a fiber in the vicinity of the stimulated root show a variety of rise times and amplitudes, allowing classification into three EPSP clusters (Fig. 3C) as found by Matzner *et al.* (2000). The probability of recording evoked EPSPs in muscle cells adjacent to the stimulated nerve were much higher (58%, $n = 45$) than from muscle cells adjacent to the next proximal or distal ganglia (7%, $n = 124$). In addition, as seen in Figure 3B, D, successful recording in the muscle adjacent to the next ganglion revealed fewer types of PSPs. These results suggest that the motor fields of the dorsal nerve roots are longitudinally restricted, each root mainly innervating the muscle area surrounding its ganglion.

Recordings of mechanosensory signals

Graziadei (1965) identified elements with morphological characteristics typical of stretch receptors in the intrinsic muscle of the octopus arms. To physiologically identify possible pathways for feedback signals to the axial nerve cord, we used the nerve cord–muscle preparation described above. The muscles were stimulated mechanically with a glass rod driven by a loudspeaker while extracellular neural activity was recorded from the dorsal roots.

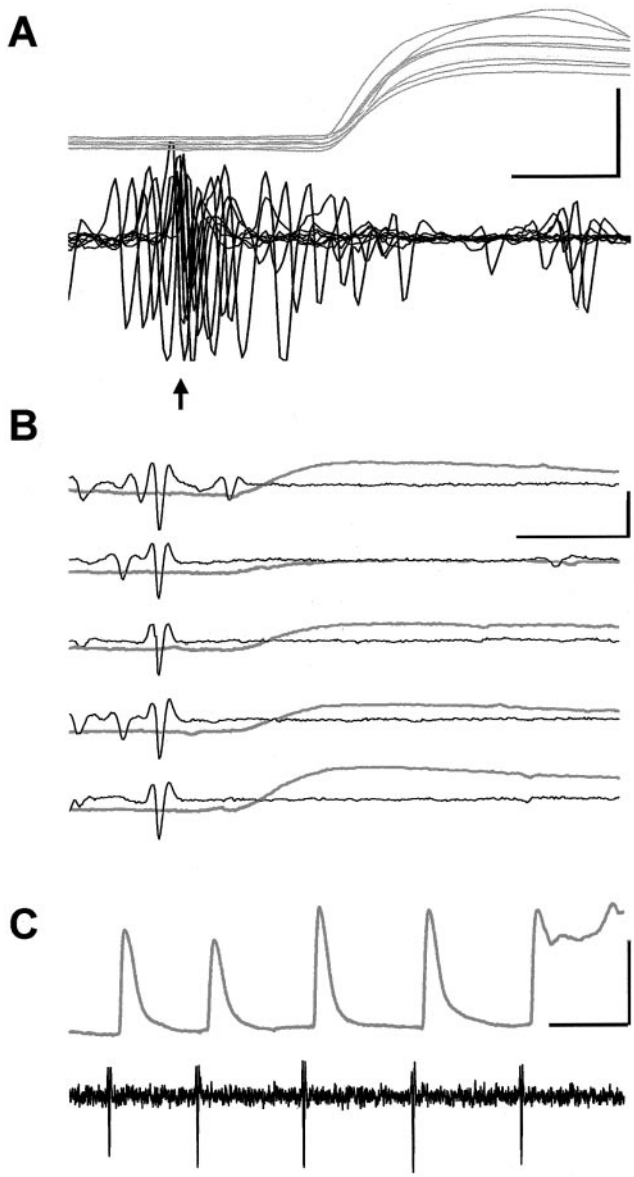

Figure 2. The dorsal nerve roots contain motor axons innervating the arm muscle fibers. (A–C) Three examples of EPSPs recorded intracellularly in the muscle that correspond with dorsal root activity recorded extracellularly by an *en passant* suction electrode. The EPSPs were recorded from a muscle fiber opposite the dorsal nerve root. (A) The traces are aligned according to the EPSP onset. A single unit (arrow) in the bursts evoked by axonal tract stimulation precedes the EPSP by a constant interval (2.8 ms). (B) The traces were aligned by the peak negativity of the root's action potentials. (C) The EPSPs in the muscle (upper trace) correspond with a spontaneous burst of action potentials in the root (lower trace). Calibrations: A, 20 mV/2 ms; B, 40 mV/4 ms; C, 10 mV/20 ms.

Figure 4 gives two examples of single units that were clearly responsive to the mechanical stimulation—a purely phasic ON-OFF response to the mechanical stimulation (4A), and mainly ON responses (4B). The spatial sensitivity of the mechanoreceptors was tested by using a micromanipulator to position the rod (Fig. 4). In the experiment in

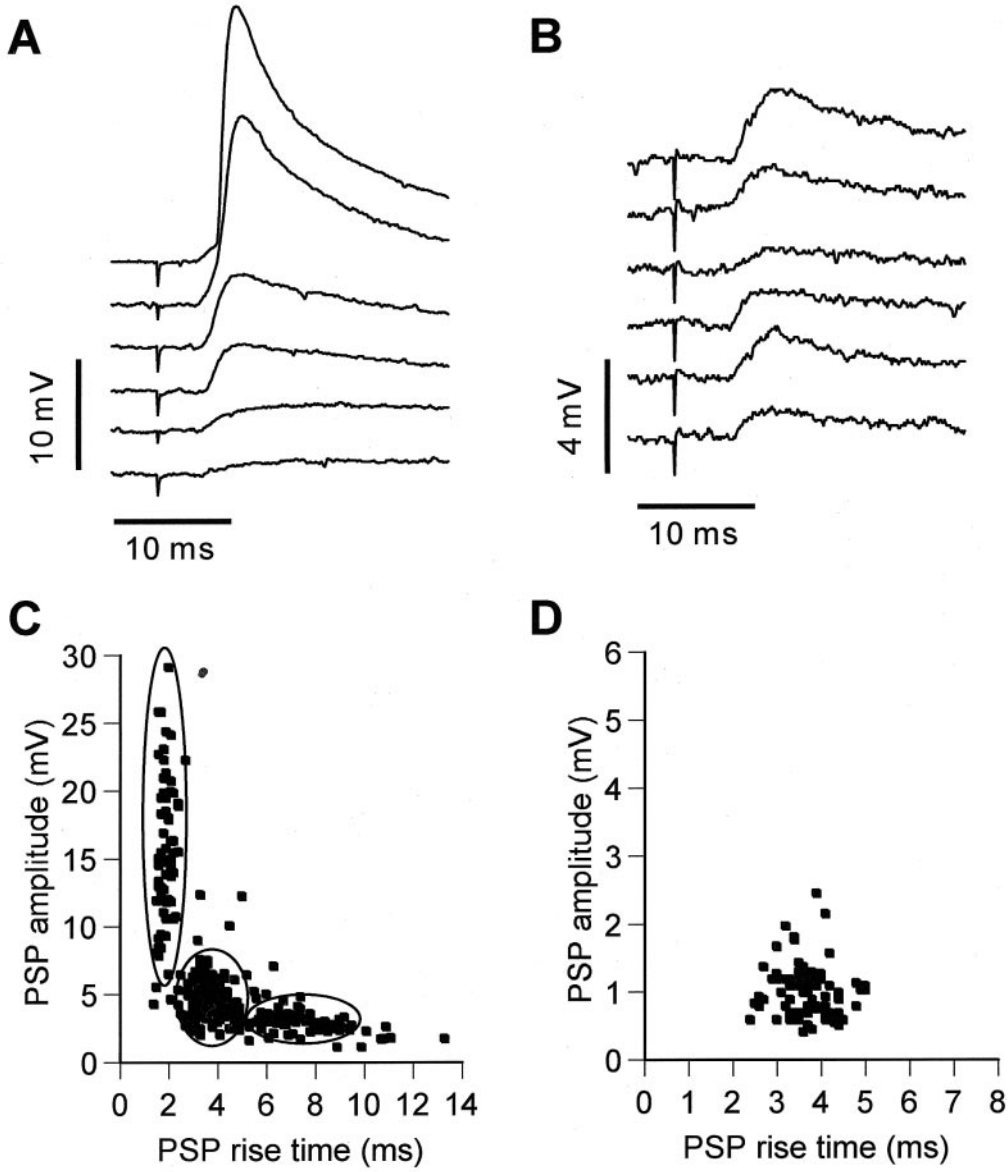

Figure 3. Motor neurons in the dorsal root innervate muscle fibers only in the close vicinity of the root. (A) Postsynaptic responses evoked by stimulating a dorsal root ipsilateral to the recorded muscle fiber. Three types of responses are recruited as stimulation strength increases from the lower to the upper traces. (B) Example of the one type of EPSP in a muscle fiber adjacent to the next ganglion evoked by the same stimulation as in A. (C) Plot of amplitude *vs.* rise-time of the EPSPs in A. Three distinct clusters, representing three types of neuronal inputs, can be recognized (marked by closed lines). (D) Amplitude *vs.* rise-time distribution of the EPSPs recorded in the muscle adjacent to the next ganglion shown in B. Only one class of PSPs can be distinguished.

Figure 4B, the intensity/response relationship was quantitatively measured. The lowest threshold intensity was obtained in the area marked 1, corresponding to the external oblique muscle group ipsilateral to the recorded root. We mapped spatial sensitivity in six preparations; the most sensitive area was always in the external margin of the arm musculature near the recorded root. Only single or very few units were ever activated by the mechanical stimulation (Fig. 4). In addition, afferent activity could be recorded in only about 1 out of 4 dorsal roots. These findings suggest that the mechanosensory system is sparsely distributed along the outer margin of the arm musculature.

Extracellular recordings in the isolated nerve preparation

Activity in the nerve roots of the isolated nerve cord could be maintained for 4–6 h, while action potentials in the axonal tract could be evoked up to 30 h after isolation of

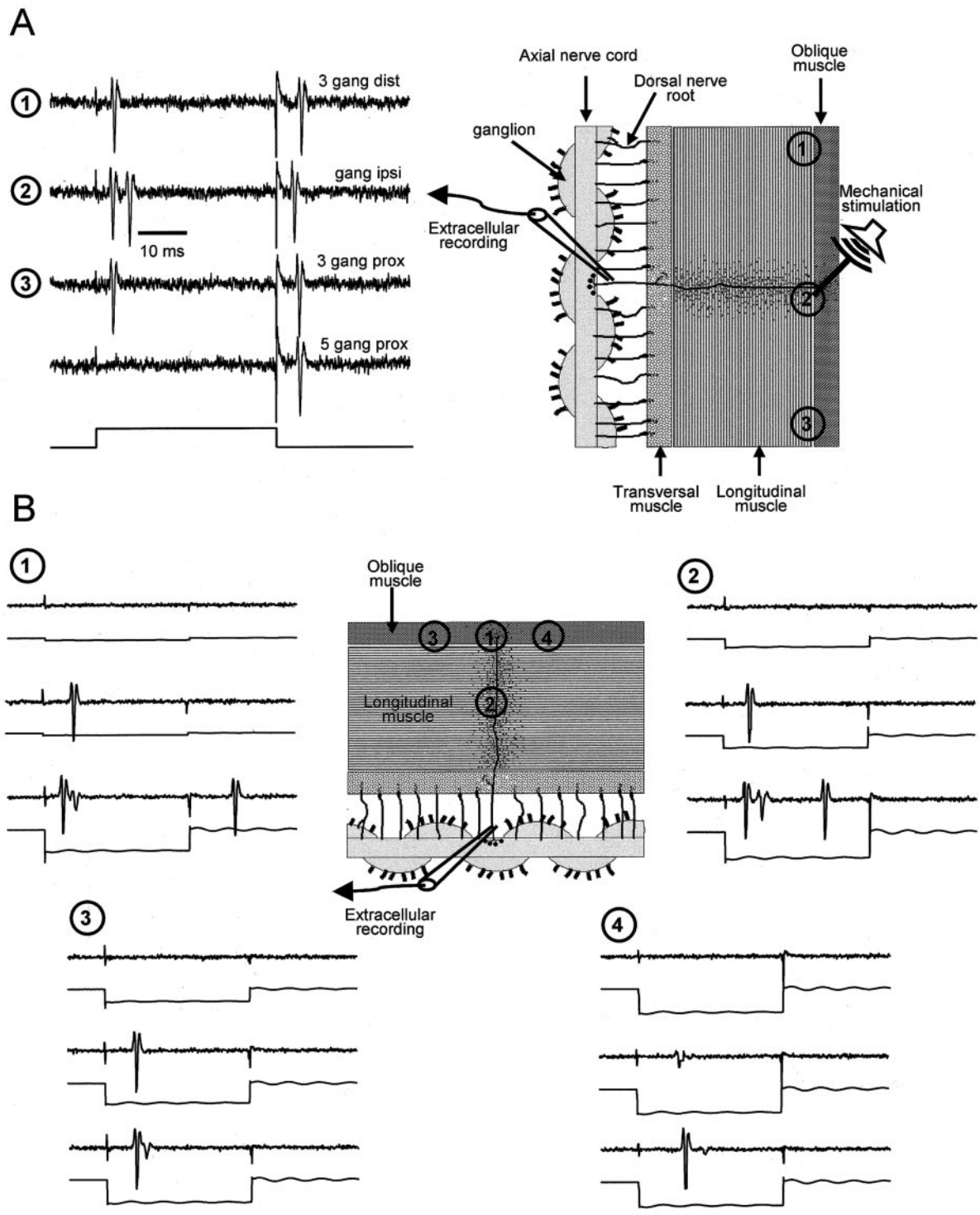

Figure 4. Mechanical stimulation of the muscles generates afferent activity in the dorsal nerve roots, recorded by a suction electrode (insets). (A) The spatial sensitivity was mapped with stimulus of constant force to the outer muscle groups. The highest response was obtained ipsilateral to the dorsal root. The circled numbers schematically show the locations and the corresponding responses. (B) An additional example of threshold mapping of the spatial sensitivity. In each site, responses to three different stimulus intensities are shown. The intensity of the mechanical stimulation is arbitrarily relative to the loudspeaker input voltage (lower traces). The circled numbers schematically show the locations and the corresponding responses. Note the lowest threshold at the outer ipsilateral side of the muscle (labeled 1). The dotted area shows schematically the area innervated by the ipsilateral root.

the cord. This allowed us to study the functional circuitry of the axial nerve cord *in vitro.* We focused on neural activity evoked in the dorsal roots that carry the motor axons to the intrinsic arm muscles (see above).

Activity generated by stimulating the ventral roots

Stimulation of ventral roots induced bursts of activity in nearby dorsal roots (Fig. 5). These bursts appeared to depend on synaptic transmission because they disappeared in low Ca^{++} ASW and showed a robust depression with repeated stimulation at rates higher than 1/min (not shown).

To ascertain whether there was some coordinated pattern among the different ganglia, we recorded simultaneously from two to three nerve roots at different locations along the nerve cord (Fig. 5A). The upper trace in Figure 5A shows the activity in the most proximal electrode; the lower trace shows the recording from the most distal electrode. The stimulus (10 pulses at 100 Hz) was applied through a fourth pipette to a more proximal ventral root. The neuronal response can be seen as a wave of activity propagating from the most proximal electrode to the most distal, with the number and frequency of spikes decreasing as it travels. The increase in delay and decrease of burst robustness with increasing distance between stimulating and recording electrodes was seen in most of the experiments (see Fig. 6).

To explore whether there is a preferred direction of propagation for this wave of activity, we stimulated a ventral root near the proximal end of the preparation and afterwards one near the distal end (schematically shown in the insets in Fig. 5). The recording electrodes were attached either to dorsal roots (Fig. 5B, D) or to ventral roots (Fig. 5C, E). In both cases, proximal stimulation caused the activity to appear first in the proximal electrode and later in the distal electrode. Distal stimulation reversed this order. No consistent difference was found between the velocities of propagation in the two directions. This shows that neuronal activity propagates away from the stimulus site equally in both directions.

We compared the speed at which the neuronal activity propagated from ganglion to ganglion to compare with the propagation velocities of electromyogram waves and with movement velocities measured in intact behaving animals (Gutfreund *et al.,* 1998, Sumbre *et al.,* 2001). To estimate propagation time, we used the lag of the peak in the cross-correlation function. The signals recorded simultaneously from two different nerve roots were rectified, shifted to zero, and cross-correlated. The period containing the stimulus artifacts was not included. Figure 6A shows the potential recorded from two dorsal roots 2 cm apart (inset Fig. 6). The stimulus was applied to a more proximal ventral root (5 pulses at 100 Hz). The cross-correlation function of these signals given in Figure 6B shows a phase lag of 26 ms between the two signals, giving a propagation velocity of 77 cm/s.

The same procedure was applied to 46 pairs of dorsal root recordings in different preparations. The histogram in Figure 6C summarizes the results, revealing a broad range of velocities, most of which lie within the range of velocities found in arm extensions (6–60 cm/s) in behaving animals (Gutfreund *et al.,* 1996) and in isolated arms (Sumbre *et al.,* 2001). Other velocities correspond either to very high speeds (small lag shifts in the cross-correlograms, $n = 12$, gray bar in Fig. 6C) or to bursts propagating back in the opposite direction (*i.e.,* negative lags, $n = 4$, empty bars in Fig. 6C). Note that propagation velocity can only be roughly estimated because of the burst variability (duration, frequency, envelope, etc.).

Activity generated by stimulating the axonal tracts

We next stimulated the axonal tracts with the protocol that successfully initiated arm extensions in isolated or amputated arms (Sumbre *et al.,* 2001) and recorded the evoked activity in the dorsal roots at two locations along the isolated nerve cord. In the examples in Figures 7A, B and 7C, D, the stimulus train triggered robust activity in the two dorsal roots, which declined gradually over several seconds. Cross-correlating the activities of the distal and proximal electrodes over a time span of 300 ms (bars) revealed a symmetrical distribution around zero delay, with no clear indication for a lag shift. In contrast to ventral root stimulation, axonal tract stimulation did not evoke propagating bursts in any of the 12 experiments.

Analysis of the activity generated by the first pulse in the train (Fig. 7B) revealed a positive phase shift of about 6 ms, corresponding to a propagation velocity of about 300 cm/s. This velocity reflects conduction in the axons of the axonal tract.

Detailed inspection of single units in the evoked activity revealed some correlations between neurons recorded at the two roots; for example, the evoked burst in Figure 7C contained a unit that appeared in the two roots at a fixed time interval of about 3 ms (Fig. 7D). This suggests that some neurons may have a bifurcating axon projecting in several roots.

Discussion

The experiments reported here are part of a long-term study of the unique, highly redundant motor system of the octopus arm. We have analyzed reaching and fetching movements, their dynamics and kinematics, and the underlying muscular activities. We are also examining the neuronal basis of the motor program for arm extension (Gutfreund *et al.,* 1996, 1998; Matzner *et al.,* 2000; Sumbre *et al.,* 2001; Rokni and Hochner, 2002), fetching (Sumbre *et al.,* 2005, 2006), and other stereotypical movements. The

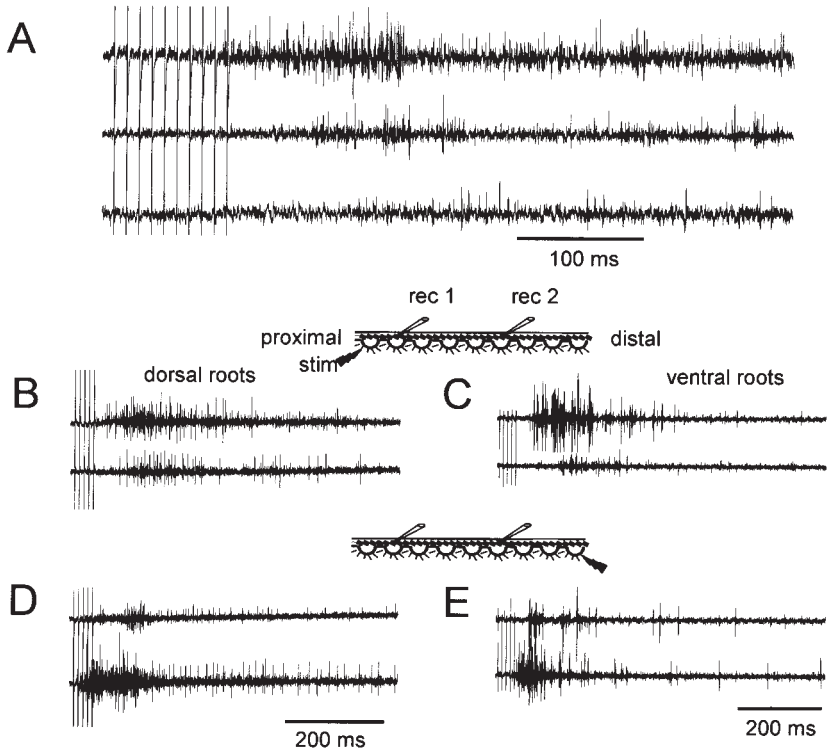

Figure 5. Simultaneous recordings from nerve roots distributed along the axial nerve cord. (A) Simultaneous recordings from three dorsal roots from proximal (upper trace) to distal (lower trace). The middle and distal electrodes were 5 and 11 mm from the most proximal electrode. A train of 10 pulses at 100 Hz was applied to a more proximal ventral root. The delay to the onset of activity increased and the strength of the burst decreased with distance from the site of stimulation. (B–E) There is no preferred direction of activity propagation. A stimulus applied to the proximal end of the preparation evoked activity in both dorsal (B) and ventral roots (C), which appeared first in the more proximal and later in the more distal electrode. Stimulation of the distal end of the preparation (D and E) reversed the order, so that activity appeared first in the distal and then in the proximal electrode.

newly developed *in vitro* preparations now allow us to probe the functional organization of the axial nerve cord in the octopus arm.

Intracellular EPSPs from longitudinal and transverse muscle fibers in the octopus arm were correlated with relatively large single units in the dorsal nerve roots of the axial nerve cord. We could thus confirm that the axons of the motor neurons to the arm muscles leave the axial nerve cord *via* its dorsal roots, with the field of innervation of each nerve restricted to an ipsilateral area near the ganglion. These findings agree with anatomical descriptions of radial connections from the axial nerve cord to the surrounding intrinsic musculature *via* the lateral and dorsal nerve roots (Martoja and May, 1956; Graziadei, 1971). In addition, we show that the dorsal roots also contain sensory afferents from mechanosensory elements in the intrinsic muscles of the arm.

We have previously reported three types of synaptic responses in octopus arm muscles, indicative of differential slow and fast motor innervation (Matzner *et al.,* 2000). Here we found that the core of both slow and fast muscle activation is limited to the region where the dorsal nerve root enters the muscles. The large number and close distribution of dorsal roots, all lying only about 0.2 mm apart, suggests that the motor innervation provides a local and continuous control of muscle contraction. Since some slow EPSP responses were observed farther from the site of stimulation, some slow motor neurons may have somewhat longer axons extending not only radially but also longitudinally. The function of these different motor neuron types is yet to be explored.

Octopus arm muscle fibers are only about 1 mm long, and they are also compact electrotonically. No significant electrical coupling between muscle cells has been revealed (Matzner *et al.,* 2000; Rokni and Hochner, 2002). Because of this, and as each motor nerve innervates only a small region, we would expect spatiotemporal patterns of muscle activation to be accompanied by similar patterns of activity in the motor neurons in the axial nerve cord; that is, the propagating wave of muscle activity observed during arm

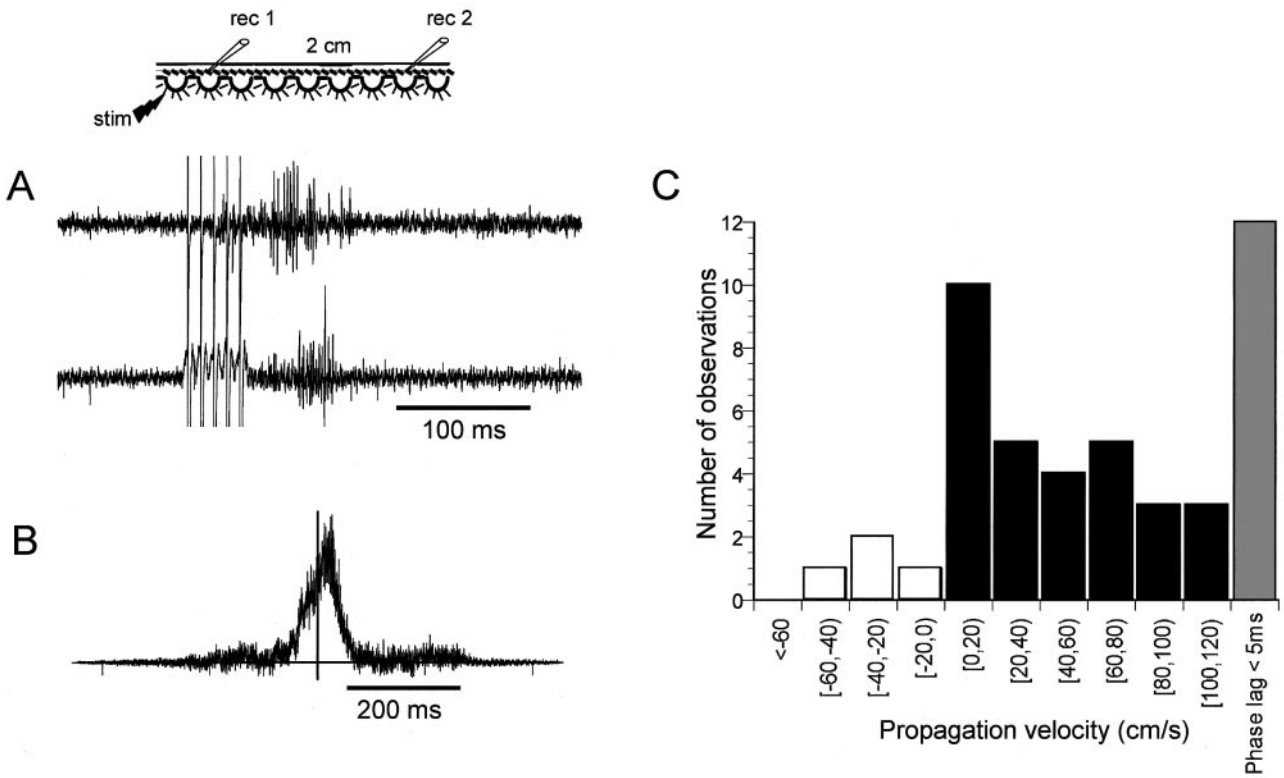

Figure 6. Estimation of propagation delay. (A) Simultaneous recordings from two dorsal roots 2 cm apart. A stimulus of 5 pulses at 100 Hz was applied to a ventral root located more proximally. (B) The cross-correlation function between the rectified signals in A over a span of 0.5 s. The axes intersect at zero phase lag. (C) Summary histogram of 46 experiments as in A. The bins represent the phase lags of the cross correlations (gray bar, less then 5 ms phase lag; black bars, positive delays; empty bars, negative delays).

extension (Gutfreund *et al.,* 1998; Sumbre *et al.,* 2001) should be accompanied by a wave of neuronal activity propagating along the axial nerve cord.

The extraordinary mobility of amputated octopus arms (Wells and Wells; 1957; Rowell, 1963; Wells, 1978; Altman, 1971; Sumbre *et al.,* 2001) indicates the extensive role of the axial nerve cord circuitry in controlling arm movements. We have shown here that the axial nerve cord contains networks that can generate a phase lag between the activity in adjacent ventral and dorsal nerve roots without requiring feedback (Figs. 5, 6). Stimuli delivered to the ventral root of the isolated nerve cord can evoke long-lasting neuronal discharges, which are synaptically mediated. However, the activity recorded in the isolated nerve cord was not sufficient to account for a complete whole-arm behavior such as arm extension: (1) the bursts generated by electrical stimulation tended to decrease as they traveled down the exposed nerve cord (Fig. 5); (2) in contrast to the very typical forward propagation in natural movements, the electrical activity did not show any clear directional preference (Fig. 5); and (3) it was impossible to distinguish between distal and proximal ends of the cord on the basis of the electrical response of nerve roots or axons.

Instead, this activity could be associated with the local reflexes. A common reflex of the octopus arm is the grip reflex (Rowell, 1963; Wells, 1978; Altman, 1971); sufficiently strong mechanical or chemical stimulation of a sucker causes the arm to bend and adjacent suckers (both distal and proximal) to serially protract toward the stimulus. Since ventral roots project directly to the sucker apparatus, stimulating a ventral root may mimic the sensory signal (mechanical and chemical) from a single sucker, and the burst of activity in ventral roots from neighboring ganglia may reflect the motor output to neighboring suckers. Activity in dorsal roots may reflect the motor signal to the intrinsic musculature for progressively bending the arm.

We were unable to initiate a propagating pattern of activity in the nerve roots by stimulating axonal tracts (Fig. 7) using the stimulation paradigm that evoked stereotypical arm extension in amputated or isolated arms (Sumbre *et al.,* 2001). Unlike the fictive rhythmical movements generated by central pattern generators in the spinal cord (see review

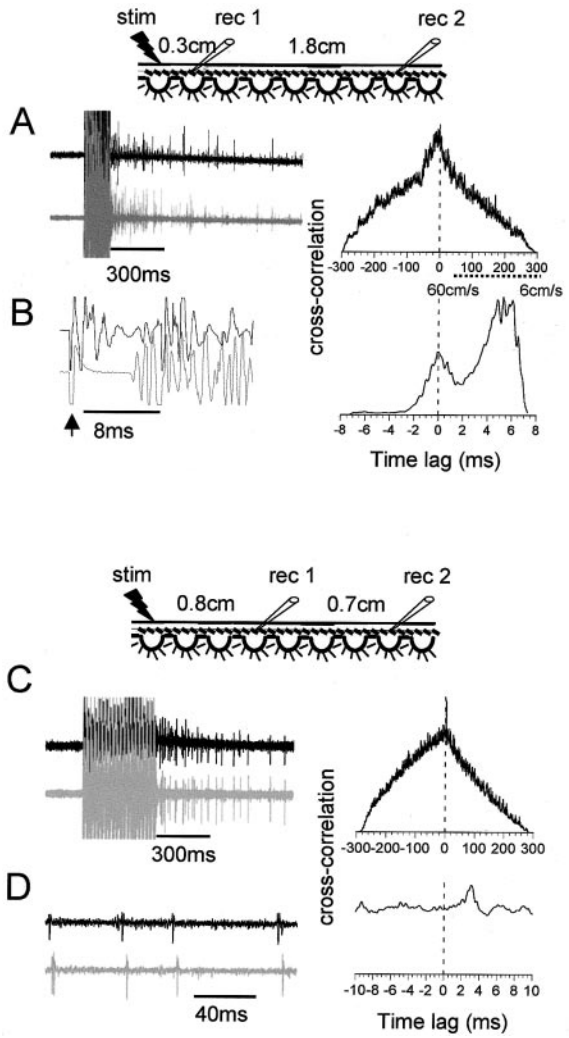

Figure 7. Two examples (A, C) of cross-correlation analysis of the activity in two dorsal nerve roots evoked by axonal tract stimulation (shown schematically). Cross-correlation functions between the activities of the proximal (black) and distal (gray) electrodes. No increase in correlation was seen within the range of time lags corresponding to the range of natural speeds of bend propagation (broken line below the correlogram). (B) The phase shift of the onset of activity evoked by the first stimulus in the train corresponds to a propagation velocity of about 300 cm/s. (D) In the example shown in C, there was a fixed delay of ~3 ms between units in the two roots.

by Grillner and Wallen, 2004), the axial nerve cord of the octopus arm cannot support such fictive arm extension. It therefore appears likely that feedback from muscles, for example from stretch receptors that we report here, is part of the feed-forward circuitry controlling arm extension.

Sensory feedback is important for the propagation of oscillatory activity in several systems generating rhythmic behavior, such as swimming in leech and lamprey (for review, see Hill *et al.,* 2003). Sensory feedback is especially important for coordination of the phase coupling along the body segments. In leeches, intersegmental coordination is maintained with severed nerve cords (Yu *et al.,* 1999), because feedback from stretch receptors in the body wall controls the segmental central pattern generators that produce the undulation rhythm (see Friesen and Cang, 2001). We suggest that some kind of feedback from the octopus arm musculature, skin, or suckers (or some combination of these) is similarly important for controlling the propagation of the activation wave during arm extension. Morphological studies (Graziadei, 1965) and the evoked responses to mechanical stimulation of the muscles (Fig. 4) suggest the presence of such a feedback system.

In addition to the axial nerve cord, four other, much smaller, nerve cords have been identified in the arms. These intramuscular nerve cords run along the periphery of the intrinsic musculature, connecting with the main axial nerve cord at regular intervals. Some neurons in these cords innervate local muscle fibers. However, more prominently, small multipolar nerves in the musculature, which may serve as stretch receptors, project to these nerve cords (Graziadei, 1965). Our finding that muscle mechanoreception is most sensitive in peripheral muscle fibers agrees with Graziadei's anatomical description. Moreover, it suggests an interesting organization in the context of the mechanics of the arm. The mechanosensory system of the intrinsic musculature is preferentially located in the periphery where muscle strain is expected to be stronger during bending of the arm. The peripheral location and the apparently serial local sensory organization make this afferent system ideally suited for sensing bending of the main arm trunk, in terms of bend position and direction (*cf.* Grillner *et al.,* 1984, for the lamprey). We suggest that this sensory information is crucial for organizing and executing the motor activity underlying bend propagation in arm extension and reaching movements, a hypothesis that requires further experimental testing.

Acknowledgments

We thank Drs. Graziano Fiorito and German Sumbre for their collaboration and advice, Hanoch Meiri and Naomi Feinstein for excellent technical assistance, and Drs. Jenny Kien and Mark Shik for advice and critical readings of this manuscript. This work was supported by the Office of Naval Research (N00014-94-1-0480), by the Israel Science Foundation (580/02-17), by the United States–Israel Binational Science Foundation (95-00170), and by DARPA (N66001-03-R-8043).

Literature Cited

Altman, J. S. 1971. Control of accept and reject reflexes in the octopus. *Nature* **229:** 204–206.

Bizzi, E. 1993. Intermediate representations in the formation of arm trajectories. *Curr. Opin. Neurobiol.* **3:** 925–931.

Chirikjian, G. S., and J. W. Burdick. 1990. Kinematics of hyper-

redundant manipulators. Pp. 391–396 in *Proceeding of the ASME Mechanisms Conference,* Vol. 25, Chicago, IL.

Colbaugh, R., and K. Glass. 1992. On controlling robots with redundancy. *Robot. Com.-Int. Manuf.* **9:** 121–135.

Flash, T., and B. Hochner. 2005. Motor primitives in vertebrates and invertebrates. *Curr. Opin. Neurobiol.* **15:** 660–666.

Friesen, W. O., and J. Cang. 2001. Sensory and central mechanisms control intersegmental coordination. *Curr. Opin. Neurobiol.* **11:** 678–683.

Graziadei, P. 1965. Muscle receptors in cephalopods. *Proc. R. Soc. Lond. B* **161:** 392–402.

Graziadei, P. 1971. The nervous system of the arms. Pp. 45–59 in *The Anatomy of the Nervous System of* Octopus vulgaris, J. Z. Young, ed. Clarendon Press, Oxford.

Grillner, S., and P. Wallen. 2004. Innate versus learned movements—a false dichotomy? *Prog. Brain Res.* **143:** 3–12.

Grillner, S., T. Williams, and P. A. Lagerback. 1984. The edge cell, a possible intraspinal mechanorereptor. *Science* **223:** 500–503.

Gutfreund, Y., T. Flash, Y. Yarom, G. Fiorito, I. Segev, and B. Hochner. 1996. Organization of octopus arm movements: a model system for studying the control of flexible arms. *J. Neurosci.* **16:** 7297–7307.

Gutfreund, Y., T. Flash, G. Fiorito, and B. Hochner. 1998. Patterns of arm muscle activation involved in octopus reaching movements. *J. Neurosci.* **18:** 5976–5987.

Hill, A. A. V., M. A. Masino, and R. L. Calabrese. 2003. Intersegmental coordination of rhythmic motor patterns. *J. Neurophysiol.* **90:** 531–538.

Hollerbach, J. M. 1990. Fundamentals of motor behavior. Pp. 151–182 in *Visual Cognition and Action,* Vol. 2, D. N. Osherson, S. M. Kosslyn, and J. M. Hollerbach, eds. MIT Press, Cambridge, MA.

Kier, W. M., and K. K. Smith. 1985. Tongues, tentacles and trunks: the biomechanics of movement in muscular-hydrostats. *Zool. J. Linn. Soc.* **83:** 307–324.

Martoja, R., and R. M. May. 1956. Comparaison de'l'innervation brachiale des céphalopodes *Octopus vulgaris* Lam. et *Sepiola rondeleti* Leach. *Arch. Zool. Exp. Gen.* **94:** 1–60.

Matzner, H., Y. Gutfreund, and B. Hochner. 2000. The neuromuscular system of the flexible arm of the octopus: physiological characterization. *J. Neurophysiol.* **83:** 1315–1324.

Messenger, J. B., M. Nixon, and K. P. Ryan. 1985. Magnesium chloride as an anaesthetic for cephalopods. *Comp. Biochem. Physiol. C* **82:** 203–205.

Rokni, D., and B. Hochner. 2002. Ionic currents underlying fast action potentials in the obliquely striated muscle cells of the octopus arm. *J. Neurophysiol.* **88:** 3386–3397.

Rossi, F., and P. Graziadei. 1954. Nouvelles recherches sur le système nerveux du bras des céphalopodes avec des méthodes spécifiques pour le tissu nerveux. *Acta Anat.* **22:** 202–215.

Rossi, F., and P. Graziadei. 1956. Nouvelles contributions à la connaissance du système nerveux du tentacule des céphalopodes. II. Cordons ganglionnaires ou moelles périphériques du tentacule d'octopus. *Acta Anat.* **26:** 165–174.

Rossi, F., and P. Graziadei. 1958. Nouvelles contributions à la connaissance du système nerveux du tentacule des céphalopodes. IV. Le patrimoine nerveux de la ventouse de l'*Octopus vulgaris. Acta Anat.* **34:** 3–79.

Rowell, C. H. F. 1963. Excitatory and inhibitory pathways in the arm of *Octopus. J. Exp. Biol.* **40:** 257–270.

Rowell, C. H. F. 1966. Activity of interneurons in the arm of *Octopus* in response to tactile stimulation. *J. Exp. Biol.* **44:** 589–605.

Sumbre, G., Y. Gutfreund, G. Fiorito, T. Flash, and B. Hochner. 2001. Control of octopus arm extension by a peripheral motor program. *Science* **293:** 1845–1848.

Sumbre, G., G. Fiorito, T. Flash, and B. Hochner. 2005. Neurobiology: motorcontrol of flexible octopus arms. *Nature* **433:** 595–596.

Sumbre, G., G. Fiorito, T. Flash, and B. Hochner. 2006. Octopuses use a human-like strategy to control precise point-to-point arm movements. *Curr. Biol.* **16:** 767–772.

Walker, I. D. 2000. Some issues in creating "invertebrate" robots. *Proceedings of the International Symposium on Adaptive Motion of Animals and Machines,* Montreal, Canada. [CD-ROM.]

Walker, I. D., D. M. Dawson, T. Flash, F. W. Grasso, R. T. Hanlon, B. Hochner, W. M. Kier, C. C. Pagano, C. D. Rahn, and Q. M. Zhang. 2005. Continuum robot arms inspired by cephalopods. *Proc. SPIE (Int. Soc. Optical Eng.).* **5804:** 303–314.

Wells, M. J. 1978. *Octopus.* Chapman and Hall, London.

Wells, M.J., and J. Wells. 1957. The function of the brain of octopus in tactile discrimination. *J. Exp. Biol.* **34:** 131–142.

Young, J. Z. 1963. The number and size of nerve cells in *Octopus. Proc. Zool. Soc. Lond.* **140:** 229–253.

Young, J. Z. 1971. *The Anatomy of the Nervous System of* Octopus vulgaris. Clarendon Press, Oxford.

Yu, X., B. Nguyen, and W. O. Friesen. 1999. Sensory feedback can coordinate the swimming activity of the leech. *J. Neurosci.* **19:** 4634–4643.