INTRODUCTION

The octopus arm is a highly flexible structure with a virtually unlimited number of degrees of freedom. The arm’s extraordinary motor capabilities are achieved despite the absence of a rigid skeleton and a composition of mainly incompressible muscle tissue (a muscular hydrostat, Kier & Smith 1985). These features pose a challenge for motor control in both biological and computational systems.

The octopus’s unique body plan - an active body with eight long and highly flexible arms – most likely evolved together with the special arrangement and function of its central and peripheral nervous systems (Zullo et al. 2009, Packard 1972). Motor control in the octopus nervous system is hierarchically organized through a division of labor between the central and peripheral nervous system (Wells 1978, Messenger 1983). Centrally, a relatively small brain (~50 million of a total of ~500 million neurons) integrates a huge amount of sensory information and projects to both intermediate and lower motor centers (Messenger & Woodhams 1976, Messenger 1967, Hobbs & Young 1973, Nixon & Young 2003). The higher motor centers lack a somatotopic motor representation and movements may be represented by a number of overlapping circuits (Zullo et al. 2009).

The lower motor centers consist of the highly autonomous neuromuscular system of the arms (~300 million neurons) (Budelmann & Young 1985, Budelmann 1995, Young 1971, Hochner 2008). This peripheral system consists of an arm nerve cord running from the base (proximal region) to the tip of the arm (distal region) and comprising both sensory and motor circuits (Young 1963, 1971, Rowell 1966, Graziadei 1971). Each arm nerve cord is composed of two dorsally located bundles of axon tracts and a ventral chain of ganglionic swellings (arm ganglia).

Motor commands are preferentially transmitted from the brain to the arms via the arm axon tracts (Graziadei 1971). Dorsal roots originating at the border of the axon tract send motor activity to the intrinsic arm musculature (Matzner et al. 2000, Gutfreund et al. 2006). The arm ganglia collect sensory and motor information from the suckers via the numerous ventral roots projecting out to the suckers. The nerve cord circuitry is not a simple relay station between the brain and the arms (Gutfreund et al. 1996, 1998, 2006), as many of the complex actions performed by octopus arms are organized within the arm nerve cord (Altman 1971, Wells 1978, Sumbre et al. 2001, 2005, 2006).

The local neural circuitry in the arm also plays a major role in controlling local reflexes involving chemical and touch sensation (Ten Cate 1928, Rowell 1966, Wells & Wells 1957, Wells 1978, Altman 1971). Sufficiently strong mechanical or chemical stimulation of a sucker elicits the arm arch reflex - the arm bends and adjacent suckers (both distal and proximal) serially protract toward the stimulus (Ten Cate 1928, Rowell 1966, Wells 1978, Altman 1971). Stimulation of one sucker has also been shown to generate a burst of neuronal activity in neighboring ganglia. This activity may subserve local reflexes whose pathways are confined to the ventral surface of the cord linking successive ganglia.
Here we investigated the transmission of information in the peripheral arm nervous system. We developed an \textit{in vitro} preparation of an isolated arm nerve cord and analyzed patterns of activity generated in the arm ganglia after electrical stimulation of neighboring ganglia. This allowed us to identify the possible sensory pathways in the arm nerve cord and suggest their involvement in arm movement.

**METHODS**

\textit{Animal treatment:} \textit{Octopus vulgaris} of both sexes were collected from the Ligurian coast and held in 120x50x45 cm marine aquaria. The tanks were filled with artificial sea water (ASW) and kept at a temperature of 18°C at 12 h light/dark cycle (ASW: NaCl 460 mmol/l, KCl 10 mmol/l, MgCl\textsubscript{2} 55 mmol/l, CaCl\textsubscript{2} 11 mmol/l, Hepes 10 mmol/l, glucose 10 mmol/l, pH = 7.6). The water was cleaned and oxygenated by a pump-filter and aeration system which continuously circulated the water through biological filters. All relevant chemical/physical water parameters were constantly checked to prevent unhealthy or stressful conditions for the animals. Octopuses were left to adapt to captivity for at least 10 days before experimentation. Six experimental animals were selected on the basis of the following criteria: healthy appearance (all arms and body parts intact), normal reflexes and voluntary movements (arm extension, walking, etc.), regular eating and motivation to attack prey.

\textit{Electrophysiology:} Once the animals were anesthetized (3.5 \% MgCl\textsubscript{2} in ASW, see Messenger \textit{et al.} 1985), an arm segment was cut from the middle of the arm and held in oxygenated ice-cooled ASW for up to 10 minutes. All segments were cut at approximately the same distance from the distal tip of arms and ranged from 35 to 38 cm in length.

The arm segment was micro-dissected to expose the arm nerve cord which was isolated and placed in the recording chamber. It was continuously perfused with oxygenated ASW (flow rate ~3.5 ml/min, ~0.5 bath volume/min at ~ 10°C.). Silver wire suction electrodes pulled from polyethylene tubing (opening of 50–150 μm) were used for extracellular recording and stimulation. A silver wire wrapped around the outside of the pipette served as a reference electrode. Recordings from a whole ganglion were made while stimulating another ganglion along the nerve cord. The stimulation electrode was then moved to another ganglion farther from or closer to the recording electrode.

Electrical stimulation was applied as two consecutive trains (200ms, 50Hz) of 1 ms negative current pulses. Stimulus intensity was set slightly above response threshold. All recordings were amplified (X 10,000) using a differential amplifier (A-M Systems), filtered (300 Hz-10 KHz), digitized with a Digidata\textsuperscript{®} 1440A Data Acquisition System, saved, and analyzed using pCLAMP 10 Electrophysiology Data Acquisition and Analysis Software (Axon).

\textit{Data Analysis:} Data was divided into two classes according to whether the stimulating electrode was positioned distal to the recording electrode (DTP, \(n = 12\); Fig. 1a) or proximal to it (PTD, \(n = 12\); Fig. 1b). In the DTP recordings the activity

Fig. 1. – Schema of the isolated arm segment preparation. (a) Example of a distal to proximal (DTP) experiment in which the recording electrode was positioned distal to the stimulation point (black trace). (b) Example of a proximal to distal (PTD) experiment in which the recording electrode was positioned upstream on the pathway of signal transmission from arm to brain. (c) Transverse structure of the octopus arm. The area where the suction electrodes were positioned is marked in red.

Fig. 2. – Measurement of the response latency and amplitude. Stimulus trains applied to the arm ganglia (red trace) induced a response in the ganglia located beyond the stimulation point (black trace). Inset: The horizontal arrow indicates the response latency, the vertical arrow the maximal amplitude of the burst response, which was used as the measure of amplitude.
recorded is directed from the arm to the brain, while the opposite occurs in PTD recordings. The threshold response along the arm was determined by stimulating each ganglion at various amplitudes. Response latency was measured as the interval between stimulus onset and the beginning of the first response (“delay” on inset Fig. 2) and the maximal amplitude of the burst response was taken as response amplitude (see inset Fig. 2). The distance from the recording and the stimulating electrode was represented by the number of ganglia located between them. Velocity of transmission of sensory signals was calculated from the slope of the delay – distance correlation.

Statistics: The program SigmaPlot 11.0 (Systat Software, Inc.) was used for statistical analysis, evaluating the distributions of the data with normality tests (Shapiro Wilk), t-tests and regression analyses.

RESULTS

As the arm segments were taken from approximately the same distance from the arm tip, the interganglionic distance was similar in all experiments. Stimulation of one arm ganglion evoked bursts of activity in both distal and proximal ganglia at various distances along the arm cord (Fig. 2).

In both distal (DPT) and proximal (PDT) response latencies were normally distributed (Shapiro Wilk; P > 0.05) and positively correlated with the distance (measured as number of ganglia, G) between the stimulation and the recording electrode (Fig. 3a; DTP, R² = 0.644; PDT, R² = 0.676). Transmission velocity was estimated from the slope of the regression lines to be ~81 cm/sec for DTP recordings and ~36 cm/sec for PDT recordings.
The average latencies measured at different distances were significantly different in DTP and PDT experiments, being lower for the same distance in DTP, where the natural arm-to-brain direction of sensory transmission was respected, (Fig. 3b-d; t test, $P < 0.05$).

We next examined the effect of stimulus amplitude on the ganglionic response. First the threshold stimulation, below which no response could be elicited, was determined. The ganglion was then stimulated with stimulus trains of increasing amplitude. To compare data across experiments we normalized the amplitude of the threshold response for each experiment, after which response amplitude was correlated with stimulus intensity (Fig. 4a). Response amplitudes did not rise with increasing stimulus strength. Average DTP responses were greater than average PDT responses (Fig. 4b; t test, $P < 0.05$).

**DISCUSSION**

The importance of sensory signals (mechanical and chemical) for the execution of correct and coordinated behaviors in the octopus has been addressed by several authors (Gutfreund et al. 2006, Sumbre et al. 2005, 2006). Yet, it remains unclear how this information is used peripherally and whether it is integrated peripherally with motor information to generate movements.

In this study we ‘emulated’ the generation of a sucker response by electrically stimulating an arm ganglion in an isolated arm cord and studied transmission of the evoked response along the arm by recording from arm ganglia at different distances along the cord. As mechanical and chemical inputs from the suckers entering via the ventral roots form the major part of the ganglion, stimulation of a ganglion may mimic the sensory signal from the sucker and the burst of activity recorded in the neighboring ganglia may reflect the result of the sensory processing during the sucker arch reflex.

Signals passed from ganglion to ganglion at velocities of 60-100 cm/sec. These values lie within the range of velocities of sensory transmission (6-100 cm/sec) measured by Gutfreund et al. (2006) by stimulating the arm ventral roots and recording the bursting activity induced in nearby dorsal roots. In contrast, transmission velocities along the axial nerve cord, through the axons of the axonal tract involved in motor control are three times higher (~300 cm/sec, Gutfreund et al. 2006). This difference in velocity suggests that sensory and motor pathways are separately organized within the arm nerve cord.

The transmission velocity in proximal recordings (DTP) was significantly higher than in distal recordings (PTD). This suggests that sensory information from suckers follows two paths; in the DTP direction signals may pass directly from ganglion to ganglion and also through the axonal tract. The slower velocity in the PTD direction suggests that this faster axonal transmission is either completely lacking or is only a minor component of the signal. That is, in this direction, signals appear to be mainly transmitted directly from ganglion to ganglion.

In conclusion, we suggest that once the sucker response is induced, the evoked information follows two different pathways along the arm. One is via slower, direct ganglion-to-ganglion connections and may transfer information to the neighboring sucker, as in the sucker arch reflex. The other, faster pathway may pass through the axial nerve cord.

![Fig. 4. – Analysis of response amplitude. (a) Mean and SD of the normalized response amplitude in DTP ($n=12$) and PTD ($n=12$) recordings plotted against stimulus intensity. (b) Box plot of the normalized response amplitudes in DTP and PTD experiments.](image-url)
nerve cord to the brain. This dual projection may ensure that information from effective stimuli is both conserved at the peripheral level for local processing and transported to a higher central level for more global computation.

Acknowledgments. — We thank Prof J. Kien for her editorial assistance and Dr G. Pruzzo for his technical assistance. This work was supported by the EU-EP7 project FP7-231608 OCTO-PUS IP.

REFERENCES


