Sensory modification of leech swimming: interactions between ventral stretch receptors and swim-related neurons

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Abstract The neuronal circuits that generate the leech swimming rhythm comprise oscillatory interneurons that provide appropriately phased output to drive swim-related motoneurons. Within ganglia, these interneurons express three phases; between ganglia there exists a phase delay between homologs. Our earlier experiments revealed that stretch receptors embedded in the body wall participate in intersegmental coordination and setting intersegmental phases. To identify the basis for these sensory effects, we mapped interactions between a ventral stretch receptor and swim-related neurons. Connections between this receptor and motoneurons are weak and variable in quiescent preparations, but during fictive swimming stretch receptor activation modulates motoneuron oscillations; hence, these effects are polysynaptic, mediated by interneurons. We identified a strong, nonrectifying, and apparently direct electrical connection between the stretch receptor and oscillator neuron 33. The ventral stretch receptor also interacts with most of the other oscillatory interneurons, including inhibitory inputs to cells 28 and 208, excitatory input to the contralateral cell 115, and mixed input to the ipsilateral cell 115. These direct and indirect interactions can account for previously described effects of body-wall stretch on motoneuron activity. They also could mediate the previously described modification of intersegmental phase relationships by appropriately phased stretch receptor activation.

Keywords Leech · Locomotion · Central pattern generator · Sensory feedback · Stretch receptor

Abbreviations CNS central nervous system · CPG central pattern generator · DE dorsal excitor · DP dorsal posterior nerve · IN interneuron · MN motoneuron · VE ventral excitor · VSR ventral stretch receptor

Introduction

The activity patterns underlying animal locomotion are generated by the neuronal oscillators located within the central nervous system (Deleomy 1980; Mark and Calabrese 1996). Proprioceptive feedback, however, is essential for the animal to produce efficient, normal movement. For example, the afferent feedback in locust flight mediated by tegulae and stretch receptors reconfigures the functioning of the central oscillator network to increase frequency and duration of the flight (Pearson and Ramirez 1997). Similarly in the crayfish swimmeret system, current injection into a single non-spiking stretch receptor modulates the frequency of spontaneously generated rhythms (Heitler 1986). It is also known that intraspinal stretch receptors (edge cells) in lamprey have synaptic connections with locomotor neurons (Viana di Prisco et al. 1990), and that the fictive swimming rhythm of lamprey can be entrained by lateral body undulations (Grillner et al. 1981).

As in other systems, sensory input from the leech body wall contributes greatly to the generation and modification of its swimming rhythm. Kristan and Stent (1976) demonstrated that stretching the body wall alters motoneuron (MN) activity, and that if the body movement is not properly realized, proprioceptive feedback causes an immediate reinitiation of a new body wave and thus resets the perturbed rhythm. When fictive motor patterns are generated in leech nerve cords isolated from sensory feedback, they display smaller intersegmental phase lags than those in intact animals.
(Kristan and Calabrese 1976; Pearce and Friesen 1984). Alternatively, in leeches with severed nerve cords, where sensory feedback alone generates intersegmental coordination, the intersegmental phase lags of the neuronal activity are larger than those of intact animals (Yu et al. 1999). It was recently observed that caudal half-leeches swim well even though isolated chains of caudal ganglia cannot generate the rhythm (Hocker et al. 2000).

The stretch receptors that innervate longitudinal muscles in the leech body wall are good candidates for mediating phasic sensory input to modulate the swimming rhythm. A pair of identified ventral stretch receptors (VSRs) hyperpolarizes in response to stretch of the body wall (Blackshaw and Thompson 1988). Hyperpolarization of the VSRs, in turn, changes the activity of swim-related MNs (Blackshaw and Kristan 1990). We recently demonstrated that these VSRs convey sensory information via membrane potential oscillations and that rhythmic activity of the VSRs modifies intersegmental phase lags in a phase-dependent manner (Cang and Friesen 2000). The imposed rhythmic activity of VSRs can also entrain the swim pattern generated by a short chain of nerve cord ganglia (Yu 2001).

The neuronal network that generates the leech swimming rhythm is largely known, consisting of segmentally iterated oscillatory interneurons (INs) that are interconnected via intrasegmental and intersegmental synapses (Friesen 1989a; and Fig. 1). Consequently, we were able to study the neuronal pathway for sensory feedback by surveying the interactions of VSRs with identified swim-related MNs and INs. The results obtained from this study describe a missing link from sensors in the body wall to the motoneurons. A preliminary report of these results was presented in an abstract (Cang et al. 1999).

![Fig. 1 Schematic diagram of interactions between oscillator interneurons. The known intra- and intersegmental interactions between oscillator interneurons (INs) in adjacent ganglia are shown. The INs in each ganglion are arranged into three columns according to their activity phases (indicated at the top of each column), with only one member of bilateral homologues shown. Some of the oscillator circuit is still unknown, including the intersegmental targets of cells 115 and 60, neurons presynaptic to cells 33 and 60, and intrasegmental connections between cell 208 and INs located on the dorsal aspect of the ganglion. Interaction symbols: filled circle chemical inhibition; “T” excitation; diode – rectifying electronic connection](image)

**Materials and methods**

**Preparations**

Adult medicinal leeches, *Hirudo medicinalis*, were obtained from Biopharm (Charleston, N.C.) and Leeches USA (Westbury, N.Y.), and maintained in artificial pond water in a controlled room on a 12-hour light/dark cycle at 18–20°C. The central nervous system (CNS) of leeches consists of the ventral nerve cord, which includes supraoesophageal and suboesophageal ganglia (the head ganglia), a chain of 21 metameric midbody ganglia, labeled as M1 through M21, and a large posterior tail ganglion (denoted by ‘T’). Each midbody ganglion of the leech nerve cord contains cell bodies of bilaterally symmetrical neurons; we use “L” and “R” to indicate the left and right sides of ganglia, respectively. For example, VSR(R,10) denotes the ventral stretch receptor on the right side of midbody ganglion 10. Similarly, 115(L,12) denotes the cell 115 on the left side of M12.

During dissections animals were anesthetised at 4°C with leech saline containing (mmol 1·L⁻¹): 115 NaCl, 4 KCl, 1.8 CaCl₂, 2 MgCl₂, 10 HEPES. Two types of preparations, isolated nerve cords and nerve cord-body wall preparations, were used. Both preparations consisted of the nerve cord from midbody ganglion 2 through the tail ganglion (M2–T). For nerve cord-body wall preparations, we removed a flap of the ventral body wall extending three segments; however, only the middle body segment of the three was innervated by the nerve roots of one midbody ganglion – the two end segments were denervated. The body-wall flap was cut along the lateral midline and ventral midline so that only the VSRs on one side of the body were included. The preparations were pinned out in a glass-bottom dish to visualize the cells with dark-field illumination, and superfused with leech saline.

**Physiology**

Swim episodes were evoked by extracellular stimulation of dorsal posterior (DP) nerves from posterior ganglia, usually DP(16). The impulse bursts of a MN, dorsal excitor (DE) cell 3, were recorded by extracellular recordings from DP nerves to monitor the fictive swimming activity of the isolated nervous system.

We penetrated the VSR axon at the anterior margin of the anterior root (Blackshaw and Thompson 1988; Cang and Friesen 2000; and Fig. 2) with a sharp electrode (filled with 2 mol 1·L⁻¹ KAc, resistance: 40–50 MΩ). In some experiments, the morphology of VSR was revealed by intracellular lucifer yellow injection. Briefly, the lucifer yellow was iontophoresed with rhythmic or pulsed hyperpolarizing currents (2–4 nA, 0.5–1 Hz, 30 min). The ganglion was then viewed and photographed in Vectashield mounting medium for fluorescence (Vector, Burlingame, Calif.) under a microscope.

To examine the interaction of the VSRs with swim-related neurons, we also recorded intracellularly from swim-related MNs or oscillatory INs (Fig. 2A). The identity of swim-related neurons was determined by the size and position of their somata, the cycle phase and shape of their membrane potential oscillation during swimming, and interactions with other swim-related neurons. An Axoclamp2A amplifier (Axon Instruments) was used to amplify the signals and inject currents. Electrical signals recorded from the nerve cord were amplified and stored on magnetic tape for later analysis.

Mapping of synaptic interactions between VSRs and swim-related neurons was carried out using the method described previously (Ort et al. 1974; Friesen 1989a). In brief, short depolarizing and hyperpolarizing current pulses were injected into VSR axons and the responses in putative postsynaptic neurons were examined. The amplitude of the injected current was 2 nA unless otherwise stated; recordings were in bridge mode with the electrode approximately balanced, or sometimes overbalanced to compress apparent membrane potential excursions during current injection. The results of synaptic mapping experiments presented in this paper are