A central pattern generator underlies crawling in the medicinal leech

Abstract  Crawling in the medicinal leech has previously been thought to require sensory feedback because the intact behavior is strongly modulated by sensory feedback and because semi-intact preparations will only crawl if they can move freely. Here we show that an isolated leech nerve cord can produce a crawling motor pattern similar to the one seen in semi-intact preparations, which consists of an anterior-to-posterior wave of alternating excitatory circular and longitudinal motor neuron bursts in each segment. The isolated cord also reproduces the patterns of activity seen in semi-intact preparations for several other kinds of cells: the dorsal inhibitor cell 1, the ventral excitor cell 4, and the annulus erector motor neuron. Because this correspondence is so strong, there must be a central pattern generator in the isolated cord that can produce the basic motor pattern for crawling without sensory feedback. A quantitative analysis of the isolated motor pattern, however, reveals that isolated and semi-intact preparations have longer periods than the intact behavior and that there are deficiencies in the timing of motor neuron bursts in the isolated pattern. These results suggest that sensory feedback modulates the isolated central pattern generator to help produce the normal motor pattern.

Key words  Leech · Sensory feedback · Central pattern generator · Locomotion · Electrophysiology

Abbreviations  $AE$ annulus erector (motor neuron) · $CPG$ central pattern generator · $C$ contraction · $CV$ ventrolateral circular excitator motor neuron · $DE$ dorsal excitor (motor neuron) · $DI$ dorsal inhibitor (motor neuron) · $E$ elongation · $DP$ dorsal posterior (nerve) · $ISTT$-E intersegmental travel time for elongation · $MA$ medial anterior (nerve) · $MA$:$B3$ medial anterior nerve branch 3 · $PCP$ post-contraction pause · $PEP$ post-elongation pause · $PP$ posterior posterior (nerve) · $VE$ ventral excitor (motor neuron) · $VI$ ventral inhibitor (motor neuron)

Introduction

The motor programs underlying adaptive behaviors are produced by neural circuits that are synchronized with the state of the outside world by feedback from peripheral sensory receptors (Barnes and Gladden 1985; Pearson 1993). Modern work in motor control has emphasized the potentially autonomous role of central pattern generators (CPGs; Delcomyn 1980; Grillner 1985), but recently there has been renewed interest in the contribution made by sensory feedback (Bush and Clarac 1985; Pearson 1987, 1993; Strausfeld 1997; Willis and Arbas 1997). In some systems, sensory feedback is necessary for the pattern generating circuitry to produce the correct motor pattern (Bässler 1993). In others, sensory feedback complements a CPG by triggering transitions between the phases of a motor pattern (Grillner and Rossignol 1978; Wolf and Pearson 1988), reinforcing centrally generated motor activity (Wilson 1961), or modulating postural reflexes (Capaday and Stein 1986; Skorupski and Sillar 1986).

In the medicinal leech, crawling is a locomotor behavior used to travel across all types of terrain. Each crawling step consists of alternating phases of elongation and contraction (Fig. 1) generated, respectively, by contractions of circular and longitudinal muscles. The phases of a step are delimited by the release and attachment of the leech’s front and rear suckers, which anchor it to the substrate. Crawling has previously been thought to require sensory feedback (Gray et al. 1938; Stern-Tomlinson et al. 1986; Baader and Kristan 1992),

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in part because sensory feedback strongly modulates the intact behavior. Leeches can adjust the kinematics of individual body regions during crawling, for example to bend around reeds in a pond (W.B. Kristan Jr, unpublished observations), and the anterior end of the animal often produces a searching behavior during elongation. In addition, crawling has been thought to require sensory feedback because abnormal feedback can stop or alter the crawling rhythm. For example, semi-intact preparations become stuck in mid-step if they are prevented from making normal crawling movements (Badger and Kristan 1992; M.P. Nusbaum, unpublished observations).

In conflict with these observations, Cacciatore et al. (2000) have recently shown that crawling can occur and that intersegmental coordination is apparently normal in animals with up to six denervated segments. This result proves that complete sensory feedback is not necessary for crawling, but it is possible that sensory feedback from unmanipulated segments is able to compensate for the missing feedback from denervated segments. To determine if at least some sensory feedback is necessary for crawling, we attempted to produce a crawling motor pattern in an isolated nerve cord preparation that was deprived of all sensory information. Gray et al. (1938)

previously reported that they could find no indication of a crawling rhythm in an isolated preparation, but here we show that the isolated cord can in fact produce the crawling motor pattern. This pattern includes the basic alternation between excitatory circular and longitudinal motor neuron activity that drives the crawling rhythm, as well as appropriately timed activity in other identified cells. We also analyze the isolated motor pattern quantitatively and compare it to results for semi-intact preparations and intact animals. This analysis identifies several differences between the patterns, suggesting that sensory feedback modulates the motor pattern produced by the crawling CPG.

Fig. 1 The four phases of a crawling step. The phases are delimited by motions of the leech’s front and rear suckers. Elongation begins when the fully-contracted leech (top schematic) lifts its front sucker and begins to extend forward. The body elongates from front-to-back in response to a wave of circular muscle activity that passes down the length of the animal. When the leech reaches maximal elongation, it puts its front sucker down and immediately begins to contract. Contraction is driven by a front-to-back wave of longitudinal muscle activity. The initial part of contraction is called the post-elongation pause (not shown) because the total length of the leech does not change until it lifts its rear sucker. Once this happens, the contraction phase proper begins. After a few seconds the leech reaches maximal contraction, and it puts its rear sucker back down, completing the step. There is then a post-contraction pause, after which the leech either begins another step or remains at rest in the maximally contracted position.

Portions of this work have appeared previously in abstract form (Eisenhart et al. 1995).

Materials and methods

Animals

Leeches were obtained from a commercial supplier (Leeches USA, Westbury, N.Y.) and stored in glass aquaria containing artificial pond water (Instant Ocean, Carolina Biological Supply) at 15 °C. Generally they were used within 3 months of their arrival. If they were stored for longer, they were fed with cow’s blood since Kristan and Calabrese (1976) found that feeding was necessary to get robust swimming from an isolated nerve cord. Fed animals were allowed to recover for at least 2 weeks before use. To further insure that animals were healthy, they were tested before experiments by allowing them to crawl freely on a dry surface. Only animals that crawled vigorously were used.

Preparations

For isolated nerve cord experiments, animals were anesthetized by placing them in chilled leech saline (composition after Nicholls and Purves 1970). The body was opened dorsally, and nerves were dissected free from the body tissue in two to four segments. In one to two of these segments, the ventral blood sinus surrounding the nerve cord was cut away to expose the ganglia for intracellular recording. The entire nerve cord was then removed with the head and tail brains still attached, and it was pinned out in a large Sylgard-coated tissue culture dish filled with saline. In some preparations, the front of the nerve cord produced spontaneous longitudinal contractions, presumably caused by the muscles in the lateral connectives. These never occurred in time with the crawling rhythm, but they were often large enough (100–300 μm) to disrupt intracellular recordings.

For semi-intact preparations, we anesthetized leeches by placing them in saline containing 8% ethanol for 20–30 min. Their front and back suckers were then sewn shut with surgical thread, and they were allowed to recover for at least 24 h. Animals were again tested before each experiment by letting them crawl on a dry surface. Most animals produced apparently normal vermiform crawling movements, even though they made little forward progress because they could not attach their suckers. Only those animals that produced robust crawling were used. The body wall was then removed in five to seven midbody segments to expose the nervous system for recording, and the preparation was pinned out in a tissue culture dish. The behavior of these preparations was monitored by recording the transitions between step phases (Fig. 1) using an electronic device with separate buttons for the experimenter to press at the beginning of each phase. Sucker release and attachment were apparent from movements of the head and tail, even though the suckers were inactivated.