Supplemental Methods

Electrophysiology during Intact Swimming

Electrode Assembly

Cuff electrodes were fabricated from PE-50 tubing (Intramedic; Becton, Dickinson) and 75-μm Teflon-coated silver wire (Supplemental Figure 1c), according to the methods of [1]. Briefly, the wire was inserted into a short length of the tubing through a small hole. A knot was made in the wire to keep it from being pulled back out through the hole. The Teflon coating was stripped off the end of the wire, which was then formed into a hook.

Electrode Placement

Larger leeches (3–6 g) were anesthetized in 8% ethanol, 60–90 minutes prior to surgery. A small ventral incision was made between the ventral midline and the most medial ventral sensillum in segment M8 or M10. Dissecting away muscle tissue exposed the DP nerve, which was then caught in the wire hook and gently pulled up into the tubing. The other end of the tubing was filled with a mixture of oil and Vaseline petroleum jelly to provide electrical insulation, and the tubing was cut to size. The electrode was placed parallel to the incision, which was sutured shut afterwards. The electrode was secured using a loop in the wire near the tubing, so the sutures could pull it snugly against the inside of the body wall, to prevent electrode movement during active swimming. Leeches recovered in cold normal saline for about 60 minutes after surgery.

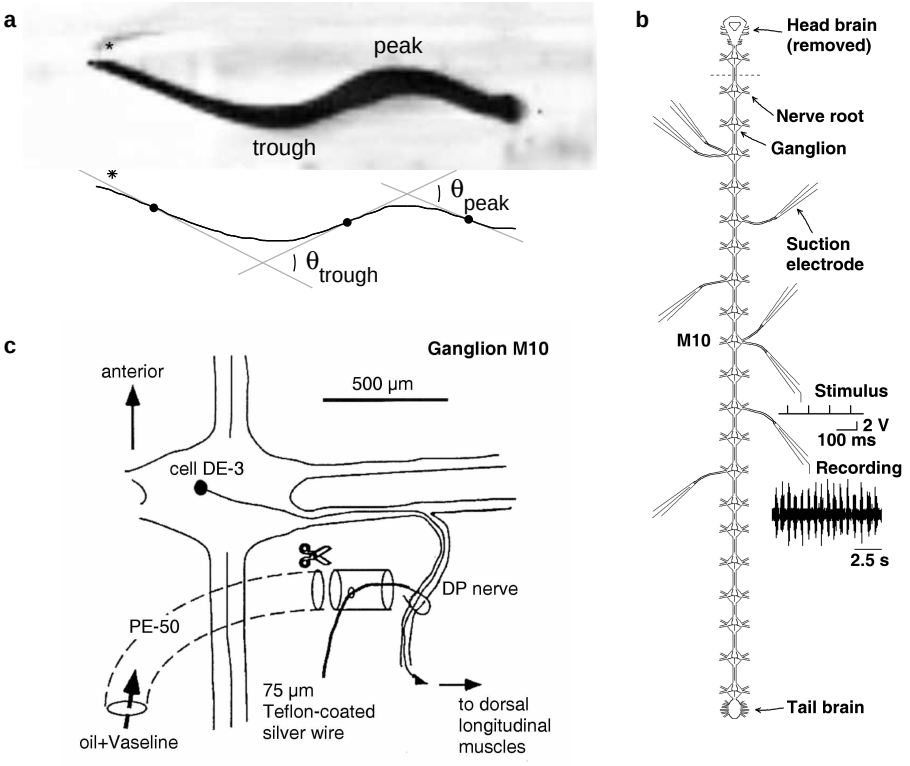
Data Acquisition and Analysis

Cuff electrode signals were amplified as for isolated cord recordings and digitized at 5 kHz using a National Instruments AT-MIO-16E-10 data acquisition system. Spikes from the dorsal longitudinal excitor motor neuron DE-3 could readily be identified in the resulting recordings. Electrophysiology and simultaneously acquired video recordings were synchronized using a flash of light. Spikes and bursts were defined and analyzed as described above.

References

[1] J. A. Murray, R. J. A. Wilson, W. B. Kristan. Motorneuron activity in freely swimming medicinal leeches. In *Society for Neuroscience 22nd annual meeting.*, p. abstract no 107.9 (1996).

Supplemental Figure



Supplemental Figure 1. Recording methods. **a.** Single frame from a video of a swimming leech (top) and skeletonization by the "Wormfinder" algorithm (bottom). Asterisks indicate the head and the dorsal side of the animal. The bending points (dots) of the skeleton curve were used to define the transition points between peaks and troughs; the angle (named θ_{peak} or θ_{trough}) between the tangential lines (gray lines) at adjacent transition points were used to define the magnitudes of peaks and troughs. **b.** Schematic drawing of an isolated nerve cord showing the sites of extracellular recordings and a typical motor neuronal burst pattern during swimming. **c.** Schematic of the cuff electrode used for recording from nerves in a behaving intact animal.