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Identified neurons and leech swimming behavior

Peter D. Brodfuehrer *, Maria Stella E. Thorogood

Department of Biology, Bryn Mawr College, Bryn Mawr, PA 19010, USA

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1. Introduction

How does the nervous system produce behavior? The desire to answer this question has led investigators to examine the biophysical properties of individual neurons and their synaptic connectivity during the production of specific behaviors (i.e. motor neuron impulse patterns). In order to accomplish this task 'simple' preparations were necessary where the activity of identified neurons could be directly correlated with behavior. Such preparations needed to be amenable to physiological recording techniques (primarily intracellular recording) while still producing a recognizable motor pattern. These requirements necessitated the use of reduced preparations: semi-intact and isolated nervous systems. The relative ease with which neuronal activity can be monitored in neuronal cell bodies, the simplicity of both the nervous system and the musculature, and its small repertoire of behaviors made the medicinal leech an ideal preparation to address how the nervous system produces behavior in terms of synaptic interactions between identified neurons. In this article, we will describe the organization of the leech nervous system, the experimental approach employed in characterizing identified neurons in the leech, and the extent to which the goal of understanding the neuronal basis of behavior has been accomplished with respect to leech swimming. We will also discuss how the initial focus of this research has expanded to take into account the role that neurotransmitters play in controlling swimming, how the decision to swim is processed and the behavioral specificity of identified neurons.

2. Leech neuroanatomy

Medicinal leeches, *Hirudo medicinalis*, belong to the phylum Annelida that comprises the segmented worms.

* Corresponding author. Tel.: +1-610-5265095.
E-mail address: pbrodfue@brynmawr.edu (P.D. Brodfuehrer).

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The most distinguishing characteristic of Annelida is body segmentation along the anterioposterior axis, which is clearly reflected in the organization of their central nervous system (CNS) (Barnes, 1980). The CNS of the medicinal leech is composed of 34 ganglia: six fused ganglia form the head ganglion, two of which form the supraesophageal ganglion and four form the subesophageal ganglion; seven fused ganglia form the tail ganglion and a chain of 21 ganglia, with one per mid-body segment, form the segmental nerve cord (Fig. 1a). The four fused ganglia comprising the subesophageal ganglion, the segmental ganglia and the seven fused ganglia of tail ganglion arise developmentally from the same neuroblasts, while the two fused ganglia comprising the supraesophageal ganglion have a different developmental origin (Weisblat et al., 1980). Each segmental ganglion and each of the four fused ganglia of the subesophageal ganglion contain approximately 200 pairs of neurons with unipolar cell bodies that range in diameter from 10–100μ and are located in a monolayer surrounding a central neuropile (Macagno, 1980 Fig. 2). All the synaptic connections between neurons occur in a central neuropile. Both impulse and synaptic activity can be monitored directly from the cell bodies of individual neurons with fine microelectrodes (Nicholls and Baylor, 1968). Over 50 pairs of segmental neurons have been identified and can be reliably located in the segmental ganglia based on their position within the ganglion, size, shape and physiological properties, while only 10–15 pairs of neurons have been identified in the subesophageal ganglion (Sawyer, 1986 and Brodfuehrer, pers. observ.). The segmental ganglia are joined by connectives composed of two large lateral bundles of axons and a thin, medial

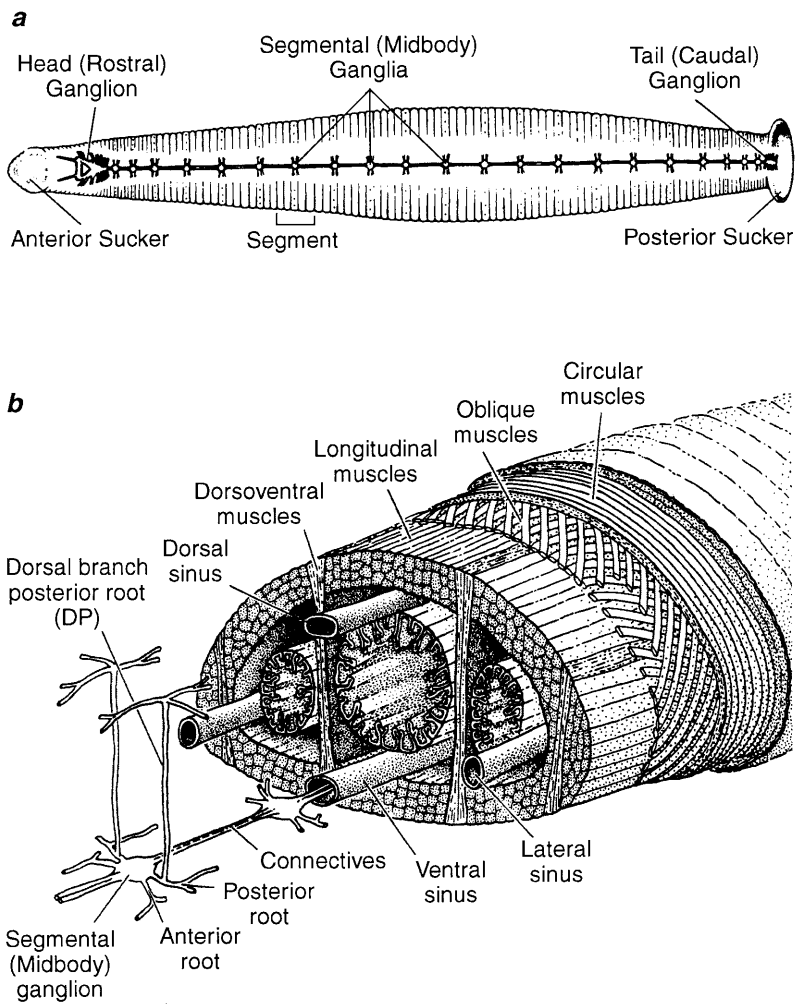

Fig. 1. Central nervous system of the medicinal leech. (a) The leech CNS is composed of 34 ganglia, six fused ganglia form the head (rostral) ganglion, seven fused ganglia form the tail (caudal) ganglion and a chain of 21 ganglia, with one per midbody segment, form the segmental nerve cord. Over most of the body, each segment is delineated by five circumferential annuli. (b) Cross-section through the leech. The ventral nerve cord lies within the ventral blood sinus. Each segmental ganglion is joined by connectives and innervates the body wall via paired nerve roots (from Kuffler et al., 1984 From Neuron to Brain: A Cellular Approach to the Function of the Nervous System, 2nd edn.© 1984, Sinauer Associates, Inc., p. 458 reprinted with permission).

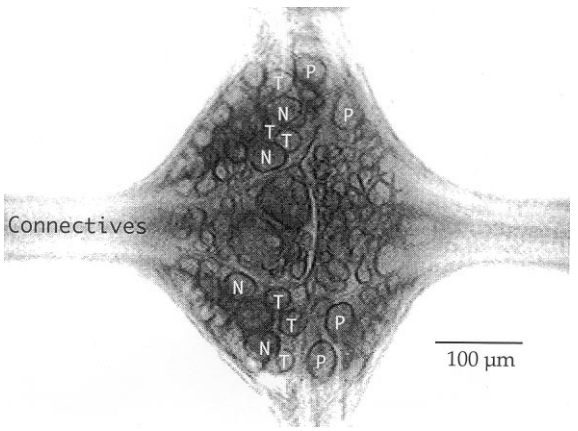

Fig. 2. Ventral aspect of a leech segmental ganglion showing the outline of neuronal cell bodies. Identified mechanosensory neurons, touch (T), pressure (P) and nociceptive (N), are labeled.

bundle called Faivre's nerve. A pair of nerve roots arises from each segmental ganglion to innervate the body wall musculature surrounding that segment. Three distinct muscle layers, circular, oblique and longitudinal, along with the skin form the tubular hydroskeleton (i.e. body wall) of the leech (Fig. 1b). These three muscle layers, together with the dorsoventral muscles are largely responsible for the relatively simple repertoire of movements in leeches (Rowlerson and Blackshaw, 1991).

3. 'Early' identified neurons in segmental ganglia

In the late 1800s it became apparent that neurons in the leech central nervous system (CNS) could be readily recognized from animal to animal based solely on anatomical characteristics: the size and position of neuronal cell bodies within a segmental ganglion. The first 'identified neurons' in the leech were a pair of giant nerve cells (ganglion balls, i.e. Leydig cells) and a pair of 'colossal cells' (i.e. Retzius cells) described by Leydig and Retzius, respectively (Kandel, 1976). Today, all the neuronal cell bodies on the dorsal and ventral surfaces of the segmental ganglia have been identified to some extent. They have all been given letter or number designation based primarily on position within the segmental ganglion, while the structure–function relationship or neuronal identity of almost half of these neurons is known (Muller et al., 1981).

Nicholls and Baylor (1968) performed the first extensive examination of neuronal identity on mechanosensory neurons, which are sensory neurons that respond to cutaneous stimuli. Mechanosensory neurons were well suited for this study because their somata lie within the segmental ganglion and their axons extend out the nerve roots where they terminate directly in the body wall. No peripheral synapses are involved in processing cutaneous stimuli.

How does one establish the structure–function relationship of neurons in a segmental ganglion? Nicholls and Baylor (1968) addressed this question using a preparation consisting of a flap of body wall that was several segments in length and connected to a segmental ganglion by the nerve roots. Using this preparation, Nicholls and Baylor (1968) found that each segmental ganglion contains three classes of mechanosensory neurons that respond specifically, and selectively, to various degrees of cutaneous stimulation: touch (T) cells (three pairs per ganglion) respond to light touch of the body wall; pressure (P) cells (two pairs per ganglion) respond following marked deformation of the body wall and nociceptive (N) cells (two pairs per ganglion) respond to noxious stimuli (Fig. 2). The size, shape, and position of the mechanosensory neurons within each segmental ganglion were remarkably stereotyped, as were the size, shape and position of their receptive fields on each body wall segment. Similarly, the physiological properties of each class of mechanosensory neurons were distinct from one another but consistent from one segmental ganglion to the next. Thus each class of mechanosensory neurons had a unique structure–function relationship. More importantly, the results of Nicholls and Baylor (1968) suggested that all the 200 pairs of segmental neurons could be identified and their functional role in leech behavior discerned. One of the first behaviors systematically studied at the level of identified neurons was leech swimming.

4. Swimming behavior

A leech swims by undulating its extended and flattened body in the dorsoventral plane producing a wave that travels rearward along the animal. The crests and troughs of the undulatory wave are produced by antiphasic contraction of dorsal and ventral longitudinal muscle in each body wall segment, while an intersegmental delay of this contractile cycle in posterior segments causes its rearward progression. The forces exerted against the water by the rearward traveling body wall wave propel the leech forward (Stent et al., 1978; Friesen and Pearce, 1993). The period of the segmental contractile cycle varies from about 400 to 2000 ms. A constant relationship between contractile cycle period and intersegmental delay time ensures that at all swimming speeds the body wall of intact leech maintains a waveform equal to approximately one wavelength (Kristan et al. 1974).

Two early studies of leech swimming movements provided the groundwork for further investigations into the neural basis of swimming. First, Uexküll (1905) showed that disconnecting the head and tail ganglia from the segmental nerve cord did not abolish leech swimming. Hence swimming movements are generated

within segmental ganglia. Second, Gray et al. (1938) demonstrated that the neural activity responsible for coordinating swimming occurred centrally in the intersegmental connectives and not peripherally through the mechanical movements of the body wall. A result of these two studies was the development of a semi-intact preparation, where several mid-body segmental ganglia were exposed for intracellular and extracellular recording, while leaving more anterior and posterior body wall regions intact (Kristan et al., 1974). Using semi-intact preparations, and later using isolated nerve cord preparations, the role of specific neurons in the segmental ganglia and subesophageal ganglion, with respect to swimming, was determined (Kristan et al., 1974; Kristan and Calabrese, 1976). Experiments were designed to identify how peripheral sensory information propagated, neuron-to-neuron, through the nervous system to generate the antiphasic contractions of the dorsal and ventral longitudinal muscles of a swimming leech.

4.1. Neuronal basis of swimming

The culmination of approximately 30 years of research has been the identification of five functional classes of neurons (mechanosensory, motor, oscillator, gating and trigger) that convert mechanosensory input into the swim motor program (Fig. 3a).

Body wall movements during swimming are controlled by four groups of segmental motor neurons, the ventral excitors (VEs) that excite the ventral longitudinal muscles; ventral inhibitors (VIs) that inhibit the ventral longitudinal muscles and the VEs; dorsal excitors (DEs) that excite the dorsal longitudinal muscles; and dorsal inhibitor (DIs) that inhibit the dorsal longitudinal muscles and the DEs (Ort et al., 1974). The membrane potential of each motor neuron oscillates and fires bursts of action potentials during their depolarized phase (Fig. 3b).

At the center of the swim-generating network is an ensemble of neurons that comprise the central pattern generator or oscillator which provides the appropriate intra- and intersegmental phasic input to segmental motor neurons that produce the undulatory body wall movements characteristic of swimming leeches. Three physiological criteria were used to determine if a given neuron was a member of the swim oscillator. First, the membrane potential of the neuron had to oscillate in phase with the swim motor pattern. Second, the neuron had to be synaptically connected to other members of the oscillator and lastly, perturbations of membrane potential oscillations in the putative oscillator neuron had to cause a transient phase-shift of the swim motor pattern (Friesen et al., 1978; Poon et al., 1978). To date, six paired (cells 115, 33, 28, 27, 123, and 60) and one unpaired interneuron (cell 208), and fours pairs of motor neurons (cells 1, 2, 102 and 119) meet these criteria (Friesen, 1989 Fig. 4a). The final composition and wiring diagram of the swim oscillator has yet to be determined. This is due in part to technical limitations of recording simultaneously from neuronal somata located on opposite sides of the ganglion.

With the identification of a central swim oscillatory network and its output connections to motor neurons, attention turned to understanding how the swim oscillatory network was 'turned-on'. Weeks and Kristan (1978) identified an unpaired intersegmental interneuron, cell 204, located in segmental ganglia 10–16 that

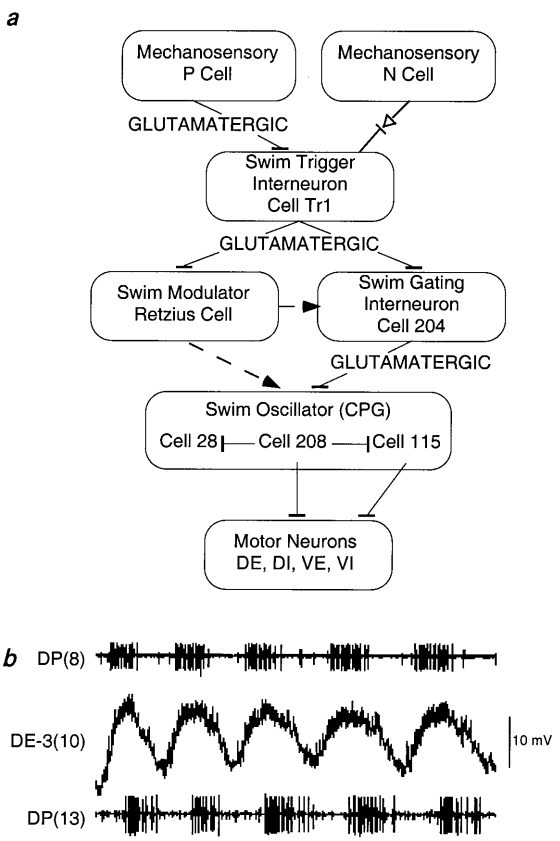

Fig. 3. (a) Schematic overview of the leech swim-generating network. Strong tactile stimulation of the body wall activates pressure-sensitive (P cell) and nocecptive (N cell) beginning a cascade of interactions between different functional classes of neurons that culminates in swimming. The monosynaptic connections from P cell to cell Tr1, cell Tr1 to cell 204, and cell 204 to cells 28, 208 and 115 are glutamatergic. Abbreviations: Motor neurons: DE, dorsal excitors, DI, dorsal inhibitors, VE, ventral excitors, VI, ventral inhibitors. Symbols: - - - ► direct, excitatory synapse, —| neuromodualtory effects, —▷| rectifying electrical connection (adapted from Thorogood and Brodfuehrer, Invertebrate Neurosci. 1:223–233© 1995 Sheffield Academic Press Ltd., reprinted with permission). (b) Motor neuron output pattern expressed during swimming. In isolated nerve cord preparations swimming is indicated by rhythmic bursts of impulses in the dorsal posterior (DP) nerves (top and bottom traces). The rhythmic bursts of impulses recorded extracellularly in the DP nerves are generated during the depolarized phases of cell DE-3 (middle trace), a motor neuron whose membrane potential oscillates during swimming. (From Brodfuehrer et al., J. Neurobiol. 27:403–418 © 1995, John Wiley & Sons, Inc., reprinted with permission.)

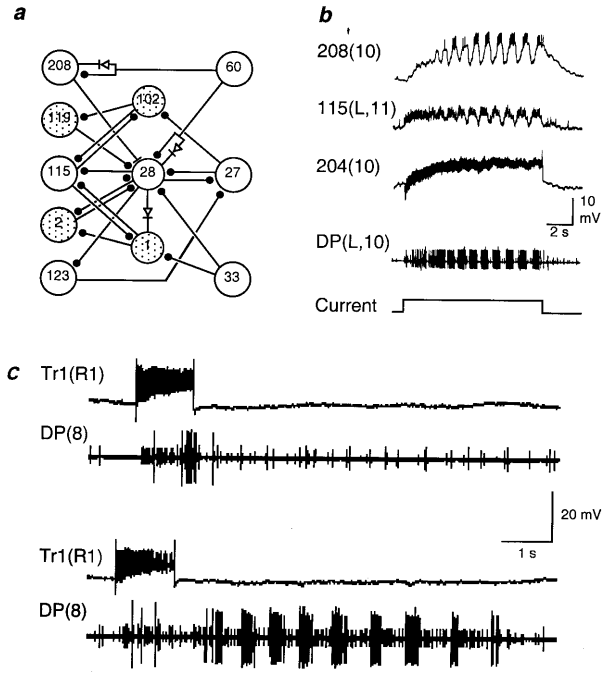

Fig. 4. (a) Intrasegmental oscillator network. Identified intrasegmental synaptic connections between oscillator interneurons. Note that not all the possible connections have been examined due to technical limitations of simultaneously recording from neuronal somata on opposite sides of the ganglion. Each of the circles represents a pair of neurons except for cell 208, which is unpaired. These neurons, found in most midbody ganglia, are identified by the number in the center of the circle. Motor neurons are indicated with stippling; the remainder are oscillator interneurons. Interganglionic connections are omitted. Symbols: —●inhibitory synapse, —|excitatory synapse, —▷ rectifying electrical synapse. (From Friesen J. Comp. Phys. A 166:205–215 © 1989 Springer-Verlag Inc., reprinted with permission). (b) Interactions between swim-gating and oscillator interneurons. Intracellular depolarization of swim-gating interneuron, cell 204, elicits swimming throughout the duration of the current pulse. Prior to swimming the membrane potential of cells 208 and 115 depolarize (from Nusbaum et al., J. Comp. Phys. A 161:355–366 © 1987, Springer-Verlag Inc., reprinted with permission). (c) Tr1 stimulation and swim variability. On one stimulus trial, brief intracellular stimulation of cell Tr1 does not lead to swimming (top traces), but does on the next trial (bottom traces).

had 'command-like' properties for the initiation of swimming. In isolated cords of segmental ganglia, depolarization of a single cell 204 such that the cell fires at an average frequency of 20–50 Hz, initiated swimming with swimming activity generally lasting as long as a suprathreshold firing frequency was maintained in cell 204 (Fig. 4b). Due to this latter property cells 204 were classified as swim-gating interneurons (Friesen, 1989). Cells 204 activate the swim oscillator, in part, via direct connections to three oscillator neurons, cells 28, 115 and 208 (Nusbaum et al., 1987). What was still lacking was an understanding of how the swim-gating interneurons were themselves driven, since cutaneous stimuli only indirectly excite cells 204 (Weeks and Kristan, 1978). A neuron or groups of neurons had to be interposed between the mechanosensory neurons and the swim-gating interneurons.

The identification of cells Tr1 provided, in part, the missing link between the mechanosensory input and the activation of swim-gating interneurons. Cells Tr1 are paired interneurons that have their somata in the subesophageal ganglion and extend their axons the length of the ventral nerve cord (Brodfuehrer and Friesen, 1986a,b). P and N cells located in the subesophageal ganglion and in the first segmental ganglion connect directly to cells Tr1 via a chemical synapse and a rectifying electrical junction, respectively (Thorogood and Brodfuehrer, 1995; Brodfuehrer and Friesen, 1986b). On the output side, cell Tr1 directly excites all segmental swim-gating interneurons (Brodfuehrer and Friesen, 1986b). Brief (approximately 1 s), high frequency (30–50 Hz) stimulation of cell Tr1 can elicit a swim episode (Fig. 4c), with the length of elicited swim episode being independent of cell Tr1 stimulation intensity. These physiological properties along with the fact that cells Tr1 are silent during swimming led to their classification as swim trigger neurons.

With the identification of cells Tr1 and their input–output connections, the goal of understanding the neuronal basis of leech swimming has been accomplished at one level. That is, at the level of knowing how specific mechanosensory inputs propagate through the leech CNS to activate the swim oscillator and produce swimming movements. Although significant, this connectionist model of the leech swim-generating network does not adequately explain the neuronal mechanism governing swimming nor does it explain the behavioral variability observed in the ability of a given input to initiate swimming. A complete understanding of the leech swim-generating network requires a thorough characterization of the biophysical (ionic currents and synaptic transfer functions) and the biochemical (neurotransmitter and receptor phenotypes) properties that govern the synaptic interactions between neurons, and must incorporate behavioral variability into the model. In the next two sections we will discuss recent observations that extend our connectionist understanding of leech swimming to include the roles that the neurotransmitter glutamate and the 'internal state' of the nervous system play in the initiation of swimming.

4.2. Glutamate and the initiation of swimming

The latency for swim initiation following stimulation of trigger neurons ranges from one to several seconds. During this latency period there is a gradual depolarization of the membrane potential and an increase in the firing frequency of swim-gating interneurons which are necessary for the initiation of swimming (Brodfuehrer and Friesen, 1986c). Insights into the mecha-

nism underlying this process occurred when it was shown that pressure ejection of L-glutamate, kainate or quisqualate onto the soma of cell 204 produced sustained excitation in cell 204 that closely mimicked cell 204's activity pattern following stimulation of cell Tr1 (Brodfuehrer and Cohen, 1990). Further analyses using antagonists to non-NMDA receptors (DNQX, CNQX, kynurenic acid and joro spider toxin) demonstrated that cells 204 possess non-NMDA receptors and that their activation by glutamate, which is most likely released by cell Tr1, leads to prolonged excitation of cells 204 (Thorogood and Brodfuehrer, 1995 Fig. 5a). Furthermore, extensive GluR 5/6/7-like immunoreactivity occurs throughout the neuropil of segmental ganglia and localizes onto processes of cell 204 (Thorogood et al., 1997, 1999). Experiments by Dierkes et al. (1996) demonstrated that the influx of both Na^+ and Ca^{2+} underlie glutamate-induced excitation of leech neurons and that increases in the intracellular free Ca^{2+} concentration occurs through voltage-sensitive ion channels and not through ionotropic glutamate receptors. A possible scenario for cell Tr1-induced prolonged excitation of cell 204 is that glutamate released by cell Tr1 binds to non-NMDA receptors on cell 204 causing an influx of Na^+. The Na^+ influx initially depolarizes cell 204 enough to open voltage-sensitive Ca^{2+} channels elevating intracellular free Ca^{2+} levels which, through a yet identified pathway, produces sustained membrane depolarization of cell 204.

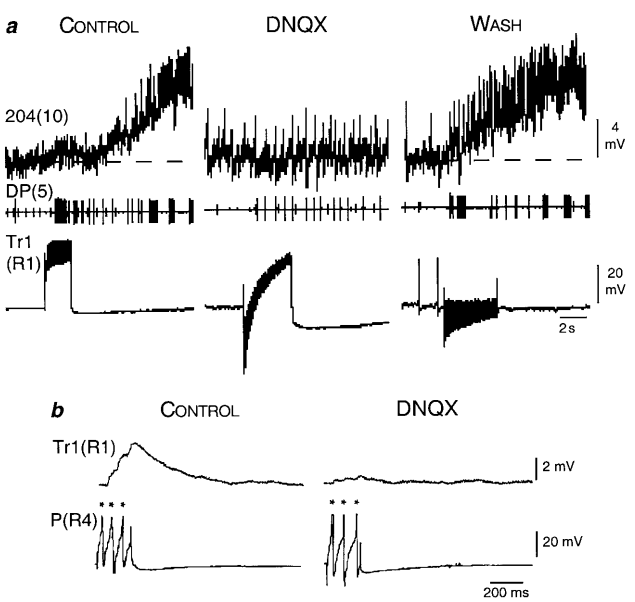

Fig. 5. Effect of non-NMDA antagonists on the input and output connections of cell Tr1. Control: in normal physiological saline intracellular cell Tr1 stimulation triggers prolonged membrane depolarization of cell 204 and swimming. DNQX: Cell Tr1 loses its ability to excite cell 204 and to trigger swimming in the presence of 100 μM DNQX (6,7-dinitroquinoxaline-2,3 dione). Wash: sustained excitation of cell 204 following cell Tr1 stimulation is restored when the nerve cord is returned to normal physiological saline (from Thorogood and Brodfuehrer, Invertebrate Neurosci. 1:223-233 © 1995, Sheffield Academic Press Ltd, reprinted with permission). (b) Pharmacology of the monosynaptic connection from mechanosensory to trigger neurons. Control: in high divalent saline, P cell action potentials (*) elicit summating EPSP's in cell Tr1. DNQX: 50 μM DNQX almost completely eliminates P cell-induced EPSPs in cell Tr1 (from Thorogood and Brodfuehrer, ibid, reprinted with permission).

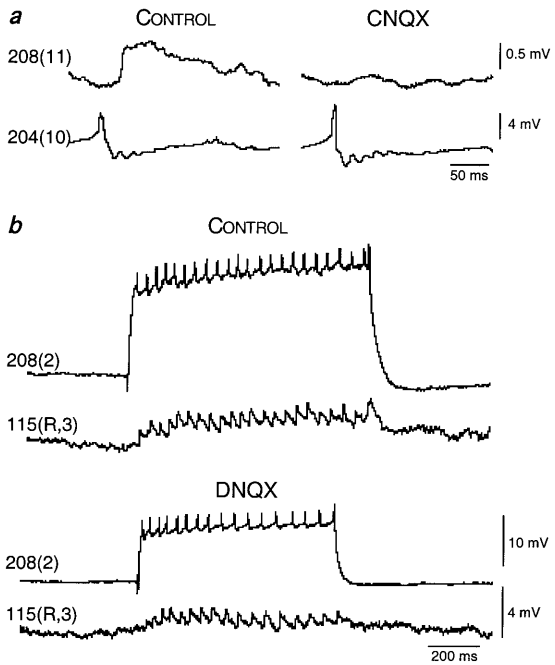

Fig. 6. Effect of non-NMDA antagonists on the monosynaptic connections of gating and oscillatory interneurons. (a). Connection from cell 204 to cell 208. Cell 204-induced EPSP in cell 208 (control: high divalent saline) is blocked in 50 μM CNQX (6-cyano-7-nitroquinoxaline-2,3 dione). Each trace represents ten computer averaged signals. (d) Connection from cell 208 to cell 115. Control: high divalent saline. Application of 100 μM DNQX does not block cell 208-induced EPSPs in cell 115.

Besides the connection from cell Tr1 to cell 204, glutamate and non-NMDA receptors mediate the interactions at several other synapses in the swim-generating network. The synaptic connections from P cells to cell Tr1 (Fig. 5b), from 204 to oscillator neurons–cells 208 (Fig. 6a), 28 and 115, and from cell SE1 to cells 204 and 115 are all blocked by non-NMDA antagonists (Thorogood and Brodfuehrer, 1995; Thorogood et al., 1996). Interestingly, all the known glutamatergic connections associated with swimming are part of the input pathway to the swim oscillator. Excitatory connections within the oscillator network (e.g. cell 208 to cells 115 (Fig. 6b) and 28) and between oscillator neurons and a dorsal excitor motor neuron (e.g. cell 208 and cells 115 to DE-3) are not blocked by the same non-NMDA receptor antagonists that block glutamatergic connections on the input side (Thorogood et al., 1996 and pers. obser.).

4.3. Distributed processing of the decision to swim

As is the case with leech swimming, the control of rhythmic motor patterns has been described primarily in terms of neurons and pathways that activate oscillatory networks (Pearson, 1993). Little attention was paid to the fact that stimulation of these neurons or pathways, even in reduced preparations, does not guarantee a fixed motor output; rather the motor response is highly variable. For example, stimulation of cell Tr1 can lead to swimming on one trial, but might not on the next trial (Brodfuehrer and Friesen, 1986c Fig. 4c). The variability observed in cell Tr1's swim-initiating ability is inconsistent with the control of swimming being dictated simply by the activation of swim-gating and oscillator interneurons. It is also doubtful that neuromodulators or prior experience are critical factors in regulating whether cell Tr1 triggers swimming since variability in swim responsiveness occurs immediately after isolating the nerve cord from the leech, when inter-stimulus intervals are short (less than 20 s) and fluctuates within a given preparation over the time course of an experiment. Furthermore, application of serotonin, the most potent neuromodulator of leech swimming, affects swimming only after bathing nerve cords for 10–20 min (Willard, 1981; Brodfuehrer and Friesen, 1986b; Hashemzadeh-Gargari and Friesen, 1989). Thus, it is likely that the short-term variations in cell Tr1's swim-initiating ability are associated with changes in the 'internal state' of the leech nervous system and are not induced by the action of known neuromodulators of leech swimming.

Attempts to correlate the activity of individual neurons with the likelihood that a given stimulus will initiate swimming led to the hypothesis that the control of swimming involves two parallel systems originating in the head ganglion that have opposite effects on the segmental swim-generating network; a swim-activating system that excites the segmental swim-generating network and a swim-inactivating system that inhibits or suppresses it (Brodfuehrer and Burns, 1995). In order for a given stimulus to initiate swimming the swim-activating system must be 'turned on' and the inactivating system 'turned off'. Evidence supporting this dual control mechanism is based primarily on the activity patterns observed in cells 204 and cell SIN1, an identified interneuron in the subesophageal ganglion (Brodfuehrer and Burns, 1995).

In quiescent preparations (i.e. when swimming is not occurring) cells SIN1 are normally tonically active. When swimming occurs, spiking activity in cells SIN1 ceases and their membrane potentials hyperpolarize approximately 0.5–1.5 s prior to the onset of the first swim cycle (Fig. 7a). In addition, depolarization of cell SIN1 during swimming generally terminates the swim episode. Although suppression of cell SIN1's activity is necessary for swimming, it is not sufficient to initiate swimming since hyperpolarization of cells SIN1 alone does not initiate swimming. A concurrent requirement is the activation of the segmental swim-generating network. Simultaneous intracellular recordings from cells SIN1 and 204 show that before swimming starts spiking activity ceases in cell SIN1 and increases in cell 204 (Fig. 7b).

To date, only a few putative members of the swim-activating and inactivating systems have been identified (Brodfuehrer et al., 1995a). A potential candidate for

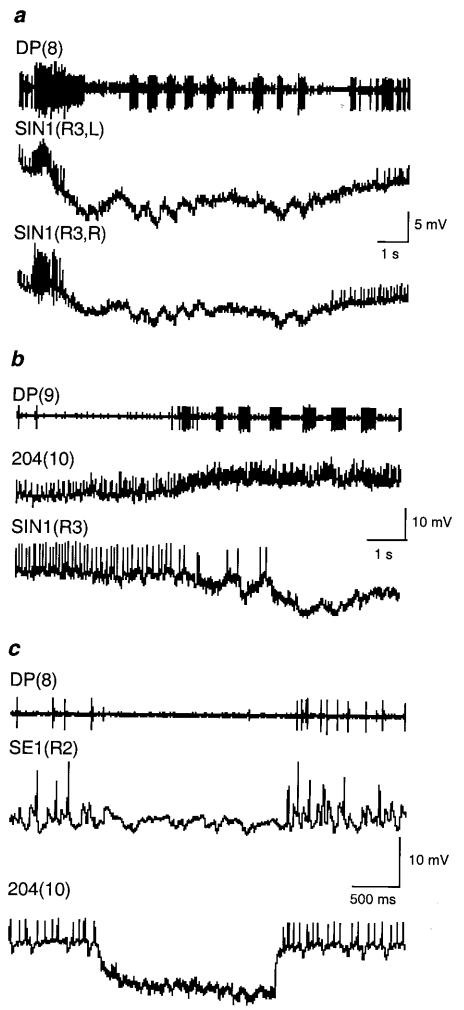

Fig. 7. Distributed control of swimming. (a) Hyperpolarization of cells SIN1 precedes the initiation of swimming. During swimming cells SIN1 are rhythmically inhibited, while after swimming the membrane potential of cells SIN1 depolarizes and spiking activity resumes (from Brodfuehrer and Burns, Neurobiol. Learning Memory 63:192-199 © 1995, Academic Press, Inc., reprinted with permission). (b) Approximately 1 s before swimming begins, spiking activity ceases in cell SIN1 and increases in cell 204 (from Brodfuehrer and Burns, ibid, reprinted with permission.). (c) Elimination of cell SE1 spiking activity results in suppression of all EPSPs with cell 204 and of large motor unit activity in the DP nerve (from Brodfuehrer et al., J. Neurophysiol. 73:983-992 © 1995, The American Physiological Society, reprinted with permission).

the swim-activating system is cells SE1, a pair of interneurons in the subesophageal ganglion (Brodfuehrer et al., 1995b). Cells SE1 are generally spontaneously active, and receive feedback from the oscillator network during swimming. Their inclusion in the swim-activating system is based on the following observations. (1) Cell 204 receives direct excitatory input from cell SE1. (2) The level of excitation in cell 204 is positively correlated with the firing frequency of cell SE1. In fact, spiking activity in cell SE1 regulates the spontaneous level of activity in cell 204 to such an extent that when the swim motor program is not active, elimination of spiking activity in cell SE1 abolishes almost all EPSPs in cell 204 (Fig. 7c). (3) The level of excitation in both cells 115 and DE-3 is positively correlated with the activity of cell SE1. (4) Cell SE1 directly excites cells 28, 208, and DE-5. Even though cells SE1 have a profound effect on a number of elements in the swim-generating network, extracellular recording of neuronal activity descending in the lateral connectives shows that cell SE1 is just one of the many neurons in the head ganglion activated prior to swimming (Brodfuehrer and Burns, 1995).

The neurons comprising the swim-inactivating system are also presently unknown. Cell SIN1 is probably only a minor component of the swim-inactivating system since its activity level is controlled by other as yet unidentified neurons, some of which are undoubtedly associated with leech behaviors that are incompatible with swimming such as whole-body shortening. Members of the swim-activating and -inactivating systems may also be part of a dynamic network that defines the 'internal state' of the leech nervous system, which is itself variable and modifiable, and influences the behavioral responsiveness of a leech to constant, repetitive stimuli (Grobstein, 1994).

Two observations document the existence of intrinsic variability within the leech nervous system, and that it affects the ability of a given input to elicit swimming. First, the motor output of isolated nerve cords intact from the head ganglion to the tail ganglion (H-T preparation) varies continuously in the absence of variations in input, but occasionally produces swimming without external input (Fig. 8a). Second, in an H-T preparation identical peripheral (dorsal posterior, DP) nerve stimulation sometimes, but not always, triggers swimming (Fig. 8b). There is no threshold stimulus voltage that consistently elicits swimming (Fig. 8c). In contrast, in a preparation consisting of a chain of ganglia from segmental ganglion 3 to the tail ganglion there is a clear threshold stimulus voltage where DP nerve stimulation reliably elicits swimming (Fig. 8c). Thus a property 'intrinsic' to the nervous system affects the behavioral responsiveness of leech preparations and is modifiable by changing

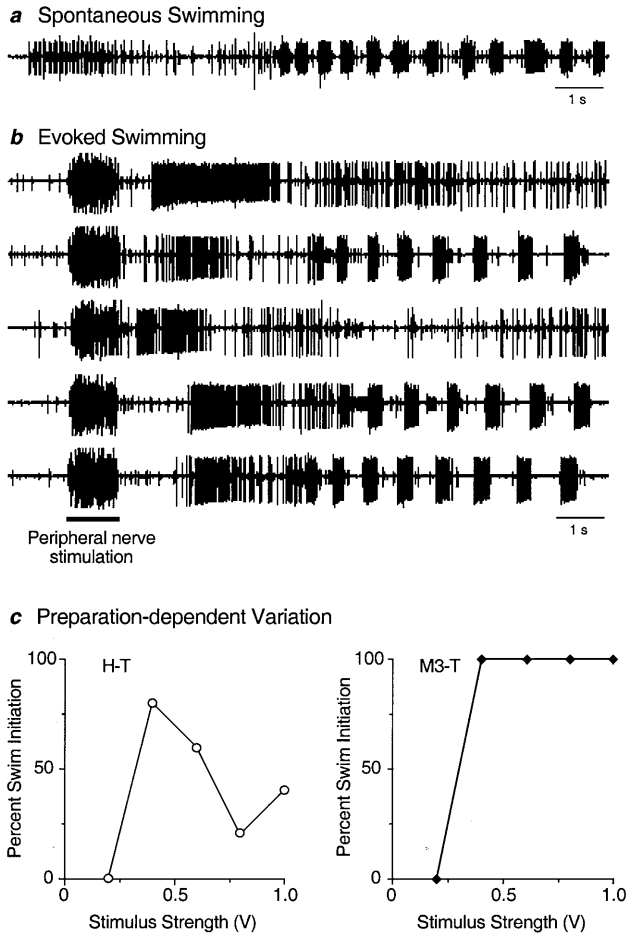

Fig. 8. Intrinsic variability. (a) In the absence of variations in the input, a period of swimming activity spontaneously occurs in an isolated nerve cord. (b) Identical trains of electrical stimuli (bar) delivered to a DP nerve produces variable motor output as recorded from a different DP nerve. On three trials DP nerve stimulation resulted in swimming, but with variable latencies; while no swimming occurred after two stimulation trials although changes in multi-unit activity were triggered. (c) Stimulus/response graphs for the isolated nervous system with (left) and without (right) the head ganglion. In a M3-T preparation (nervous system intact from segmental ganglion three to the tail ganglion) there is a clear threshold voltage (abscissa) at which the percent swim initiation (ordinate) to DP nerve stimulation is 100%. No such threshold voltage is observed an H-T preparation (nervous system intact from the head ganglion to the tail ganglion). Both sets of observations were made on the same isolated nervous system. The experiment was first performed on an H-T preparation, and then repeated on a M3-T preparation after the head ganglion and first two segmental ganglia were surgically removed (modified from Grobstein, Encyclopedia of Human Behaviour 4:447–454 © 1995 Academic Press, Inc., reprinted with permission.)

the number of ganglia comprising the preparation; specifically by eliminating the influence of the head ganglion on the rest of the nervous system. This observation suggests that the ability of cell Tr1 stimulation to trigger swimming may depend upon the internal state of the nervous system.

5. Multifunctional interneurons

As has been shown in a number of simple systems where the total number of neurons is small, identified interneurons can function in more than one behavior (Wu et al., 1994; Morton and Chiel, 1994). Identified interneurons are not necessarily dedicated to only one motor pattern, but can be multifunctional and contribute to the production of several motor patterns. With respect to leech swimming, five interneurons (cells Tr1, SE1, 204, 208 and 115) in the swim-generating network participate, to varying degrees, in one or more of the following leech behaviors, local bending, whole-body shortening, and crawling (Shaw and Kristan, 1997; Lockery and Kristan, 1990; Wittenberg and Kristan, 1992; Baader, 1997).

Cell 115 is particularly interesting in that it participates in three qualitatively different behaviors, swimming, local bending and whole-body shortening. Cell 115 was originally classified as a member of the swim oscillator network because its membrane potential oscillates in phase with the swimming rhythm, it phase shifts the swimming rhythm when depolarized and it makes direct synaptic connections with other oscillatory neurons. Cell 115 receives direct excitatory input from oscillator neuron cell 208 and forms reciprocal inhibitory connections with three other oscillator neurons, cells 28, 102 and 1 (Nusbaum et al., 1987; Friesen, 1989). With respect to local bending, Lockery and Kristan (1990) found that cell 115 is one of the premotor local bending interneurons. Pressure-sensitive mechanosensory neurons excite cell 115, and subsequently, cell 115 excites dorsal longitudinal motor neurons and inhibits ventral longitudinal motor neurons. Hyperpolarizing cell 115, which effectively removes it from the local bending circuit, reduces the amplitude of the local bending response. Similarly, cell 115 is active during whole-body shortening and hyperpolarizing cell 115 substantially decreases the magnitude of the excitation in posterior dorsal longitudinal motor neurons (Wittenberg and Kristan, 1992). Thus we conclude that cell 115 is a multifunctional interneuron since in all the three behaviors experimental manipulation of cell 115's activity functionally alters the output pattern of each behavior.

Behavioral choice experiments have also shown that cells Tr1 and SE1, which were hypothesized to be at the highest level of control in the swim-generating network and hence dedicated to the swimming, may in fact be multifunctional. Shaw and Kristan (1997) observed that cells Tr1 and SE1 are excited to the same extent by electrical stimulation of the body wall independent of whether whole-body shortening or swimming occurs (Fig. 9a and b). Surprisingly, behavior dedicated responses were associated with lower levels of controls, in the activity patterns of cells 204 and 208. Stimuli that led to swimming excited cells 204 and 208, while stimuli that led to whole-body shortening elicited fast, strong inhibition in cells 204 and 208 (Fig. 9a). Although these results imply that cells 204 and 208 are 'dedicated' to the expression of swimming and mutually exclusive with whole-body shortening, results from Baader (1997) question this notion. Baader (1997) demonstrated that during the elongation phase of crawling the firing frequency of cell 204 increases (Fig. 9c), while during the contraction phase it decreases, as does the firing frequency of cell 208. Thus it is appears that cells 204 and 208 are not dedicated to swimming, but also participate in crawling.

The multifunctional nature of many interneurons in the leech swim-generating network suggests that 'dedicated' neurons are rare even in simple systems. Classifying neurons as dedicated to a particular behavior may be a consequence of our limited understanding of the neuronal basis of all behaviors in an animal. Truly

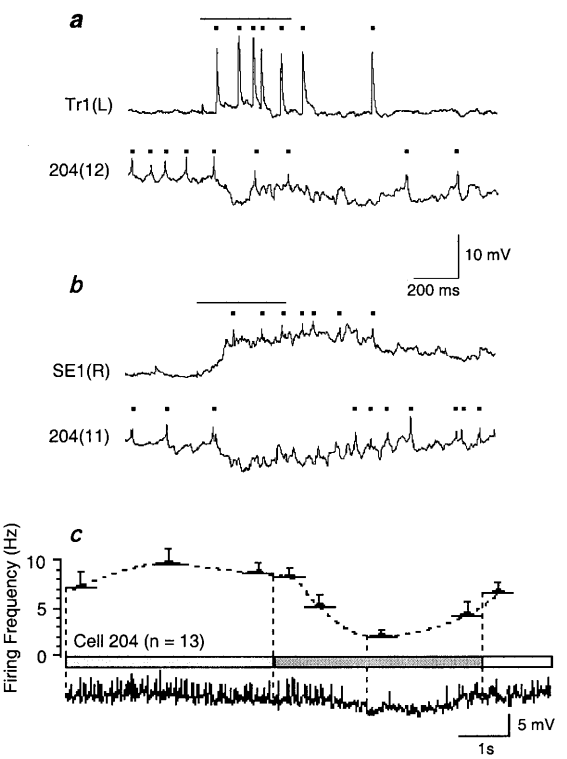

Fig. 9. Multifunctional neurons. (a and b) Response of swim circuit neurons during shortening in semi-intact preparations. Body wall stimulation that elicits shortening excites cells Tr1 and SE1, but inhibits cell 204. Bar indicates timing of electrical stimulation of the body wall and dots indicate spikes in the intracellular recordings (adapted from Shaw and Kristan, 1997 J. Neurosci. 17:786–795 ©, Society for Neuroscience, reprinted with permission). (c) Rhythmic modulation of the firing frequency of cell 204 during the elongation (light gray) and contraction (dark gray) phases of crawling. A representative example of changes in the firing frequency of cell 204 during crawling is shown below the summary graph (from Baader, J. Exp. Biol. 200:1369–1381 © 1997 The Company of Biologists Limited, reprinted with permission).

dedicated neurons may only occur in networks controlling some visceral functions such as heart interneurons in the leech heartbeat network. With respect to the concept of identified neurons, it's apparent that the multifunctional nature of neurons compels investigators to reference the 'identity' of a neuron to a specific behavior.

6. Conclusions

Since the experiments of Nicholls and Baylor, the initial characterization of identified neurons has provided significant insight into the circuitry transforming mechanosensory input into the motor output of swimming. From physiological characterization of only a small percentage of cells within the leech CNS, we have gained important information about how the decision to swim is processed and how the rhythmic motor pattern is generated. While many of the synaptic connections in the swim-generating circuit have been identified, the elucidation of the biophysical and biochemical mechanisms underlying these connections has only recently begun. The observation that constant input can result in variable motor output suggests that, in addition to describing a cell's identity in terms of structure and function, factors such as behavioral context and the 'internal state' of the nervous system must also be considered. As circuits controlling other behaviors become known, one can examine the interactions between these networks to understand issues of behavioral choice at the level of identified neurons. The leech CNS has expanded our understanding of how the nervous system produces behavior and continues to serve as an excellent model in this endeavor.

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