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ORIGINAL PAPER

Positive feedback loops sustain repeating bursts in neuronal circuits

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Abstract Voluntary movements in animals are often episodic, with abrupt onset and termination. Elevated neuronal excitation is required to drive the neuronal circuits underlying such movements; however, the mechanisms that sustain this increased excitation are largely unknown. In the medicinal leech, an identified cascade of excitation has been traced from mechanosensory neurons to the swim oscillator circuit. Although this cascade explains the initiation of excitatory drive (and hence swim initiation), it cannot account for the prolonged excitation (10–100 s) that underlies swim episodes. We present results of physiological and theoretical investigations into the mechanisms that maintain swimming activity in the leech. Although *intra*segmental mechanisms can prolong stimulus-evoked excitation for more than one second, maintained excitation and sustained swimming activity requires chains of several ganglia. Experimental and modeling studies suggest that mutually excitatory *inter*segmental interactions can drive bouts of swimming activity in leeches. Our model neuronal circuits, which incorporated mutually excitatory neurons whose activity was limited by impulse adaptation, also replicated the following major experimental findings: (1) swimming can be initiated and terminated by a single neuron, (2) swim duration decreases with experimental reduction in nerve cord length, and (3) swim duration decreases as the interval between swim episodes is reduced.

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Abbreviations

ADO	Adenosine;
BPE	Bursts per episode;
DP	Dorsal posterior (nerve);
CNS	Central nervous system;
CPG	Central pattern generator;
IN	Interneuron;
MN	Motoneurons;
RCI	Recurrent cyclic inhibition;
RE	Reciprocally excitatory

1 Introduction

Our aim is to identify mechanisms that sustain an episode of animal locomotion following a brief sensory stimulus, a topic that has intrigued neurobiologists for many decades. An early success in this area was the identification of crayfish command neurons, whose stimulation activates and sustains the rhythmic movements of swimmerets [1]. Nevertheless, the neuronal interactions that maintain crayfish command cell activity, despite much progress on the oscillator system [2], remain unknown. In fact, much has been learned from the seminal experiments of Wilson on locust flight [3] and more recent research on fictive swimming in many other species [4–12], and the investigation of rhythmic oscillations in the spinal cords of neonatal rodents [13]. Nevertheless, little has been learned about the neuronal mechanisms that maintain excitatory activity in any preparation [6, 14, 15]. Electronic circuits are easily constructed to achieve sustained excitation, but what are the neuronal properties and neuronal circuits that drive episodic behaviors?

Experimental data suggest that both cellular properties, such as plateau potentials, and circuit properties, such as positive feedback, might generate sustained excitation [16, 17]. Swimming activity in the isolated CNS (fictive swimming) in *Xenopus* [18–24], for example, might be maintained when swimming activity releases ATP, which de-activates a K^+ current leading to increased neuronal excitation. This positive feedback excitation persists until the ATP is converted to adenosine (ADO). ADO de-activates a Ca^{2+} current, countermanding and terminating the prolonged excitation. There is a delay in this latter step because the ectonucleotidase that converts ADP to ADO is blocked by feed-forward inhibition from ATP. Experimental and modeling studies demonstrate that the elements required for this mechanism are indeed present in the CNS of *Xenopus* embryos, although the ATP-releasing neurons are not identified. Maintenance of excitation in lampreys is likely to be driven in large part from plateau potentials that are induced when stimulation of the skin evokes Ca^{2+} influx that in turn, activates a cation channel in the reticulospinal (RS) command system [25, 26]. Thus cellular processes in RS cells aid in converting brief stimulation into protracted depolarization, and thereby engender sustained excitation in the spinal cord swim circuitry for the duration of the swim episode. In addition, there is positive feedback among neurons in the brain stem and upper spinal cord in *Xenopus* [27, 28]. These studies suggested that a combination of circuit and cellular properties might be sufficient to generate the sustained excitation that causes bouts of locomotion, but neither

positive feedback nor cellular properties are definitively linked to swim maintenance in these animals. Moreover, the proposed mechanisms are not associated with individually identified neurons.

In the medicinal leech, an identified cascade of excitation that initiates fictive swimming has been traced from mechanosensory neurons to the swim oscillator circuit [29–33]. Tactile stimulation of the body wall or depolarization of individual mechanosensory neurons activates swim-control neurons in the subesophageal ganglion, which include trigger neurons (Tr1 and Tr2) [30], the swim exciter neuron SE1 [34], and the R3b1 neuron [35]. Excitatory synapses connect Tr1 to swim-gating neurons cells 204/205 located in the posterior midbody ganglia (M9–M16) [36, 37]. Depolarization of a single cell 204 by intracellular current injection is sufficient to drive fictive swimming; such episodes usually end with, but may continue beyond, the evoking cell 204 stimulus [36, 38]. Cells 204 provide excitatory drive to a set of segmentally repeated swim oscillator interneurons (INs; cells 28, 115 and 208) via excitatory synapses [39]. Similarly, cell SE1 also directly excites swim-gating cells, oscillator cells, and swim-related MNs [34]. Because the swim-gating neurons (and SE1 cells) project to most, if not all, segmental ganglia, they provide massive excitation to activate the swim circuitry.

Although the cascade of identified synaptic connections accounts for the initiation of swimming activity, mechanisms that maintain swimming are less clear. The synaptic interactions within the swim-initiating cascade have decay time constants of about 20 ms, and cannot account for the sustained depolarization observed in the swim-gating neurons, Retzius (Rz) cells and swim oscillator neurons. What mechanisms give rise to this sustained output? We propose that a set of intersegmental interneurons with extensive, mutually excitatory intersegmental interactions could provide the sustained drive to maintain swimming activity. Experimental data presented here show that (1) brief stimulation initiates prolonged bursting, (2) maintained bursting activity requires multiple ganglia, (3) mutually excitatory synaptic interactions exhibit long latencies and (4) there are intersegmental positive feedback circuits. To determine whether these mechanisms could give rise to fictive swimming, we constructed a model comprising realistic three-compartment neurons that includes the neuronal interactions in the swim-initiating cascade together with a set of mutually excitatory intersegmental neurons. The impulse frequency of the excitatory neurons is limited by impulse adaptation. We show that (1) this model generates prolonged excitation that drives neuronal bursting, (2) that swim duration depends on cellular properties (impulse adaptation), (3) repeated stimulation initiates and then may terminate excitation, (4) swim duration increases with the length of the ganglion chain and (5) swim duration depends on the interval between swim episodes.

2 Materials and methods

2.1 Physiological experiments

Experiments were carried out on adult medicinal leeches, *Hirudo verbana*, obtained from Leeches USA (Westbury, NY) and Niagara Leeches (Cheyenne, WY). Leeches were maintained in aquaria in a light-and-temperature-controlled room. Prior to dissection, leeches were anesthetized with cold (4°C) saline with composition 115 mM NaCl, 4 mM KCl, 1.8 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM Hepes buffer, pH 7.4 [40]. Preparations were superfused with saline during the experiments, which for some experiments contained

50 μM serotonin. Preparations were pinned in glass-bottomed dishes to visualize neurons for intracellular recording. Ganglia were desheathed for experiments requiring intracellular recording. General techniques for dissection and recording are described in previous publications [40, 41].

2.1.1 Leech anatomy and nomenclature

The leech CNS comprises a head ganglion (H), a chain of 21 midbody ganglia—M1 through M21—and a tail ganglion (T). Two lateral intersegmental connectives convey the axons of (1) the oscillator interneurons (INs) and (2) cephalic neurons that control swim expression. A median tract (Faivre's nerve) conveys the axons of swim-gating neurons, cells 204 and cell 205.

2.1.2 Preparations and electrophysiology

Experiments were carried out on the isolated ventral nerve cord, with the length of the cord ranging from a single ganglion to midbody ganglion 2 through the tail ganglion (M2-T preparation). We monitored impulse activity at several sites along the nerve cord in the mixed motor–sensory dorsal posterior (DP) nerves with extracellular suction electrodes (Fig. 1a). These electrodes were also used for electrical stimulation to initiate swim episodes. Fictive swimming activity in isolated nerve cord preparations is indicated by repeating bursts of large spikes in DP nerve records. These bursts are the impulses of an identified motoneuron (MN), cell DE-3, which elicits rhythmic muscle contraction [42].

Intracellular recordings of membrane potentials from individually identifiable neurons were obtained via sharp electrodes (resistance about 40 MΩ) using an Axoclamp-2A amplifier (Axon Instruments, Sunnyvale, CA) in bridge mode. Electrical signals were amplified, digitized and stored using a Powerlab instrument and Chart software (AD Instruments, Colorado Springs, CO). Records were archived on CD-ROM discs. To initiate swimming, we usually stimulated a caudal DP nerve with a brief (about 1 s) train of 1–5 V, 5 ms pulses at 25 Hz, or by injected depolarizing current into a neuronal soma.

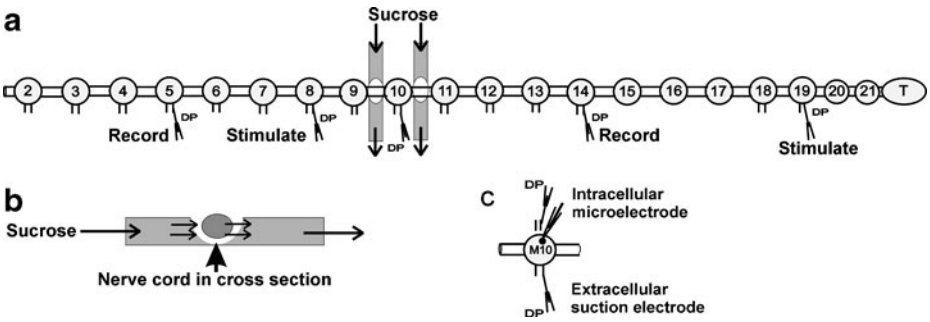

Fig. 1 Preparations and sucrose knife. **a** Nerve cord preparations comprised the entire nerve cord from midbody ganglion 2 through the tail ganglion (*T*), or reduced chains of ganglia. Extracellular recordings are from several DP nerves drawn into suction electrodes. These were used for electrical stimulation that evoked swimming and to record impulses from motor neurons. **b** Detail of the sucrose knife configuration in which isotonic sucrose flows (*horizontal arrows*) across the intersegmental nerve cord (*vertical arrow*) to quickly and reversibly block axon conduction; the nerve cord is seen in cross section. **c** Isolated ganglion M10, with intersegmental connectives severed. Intracellular recording is with sharp glass microelectrodes. *Numbered circles* represent individual segmental ganglia

2.1.3 Sucrose knife technique

The methods described here are commonly employed in our lab. We have, however, refined one previously described technique, the sucrose gap here labeled the "sucrose knife," to reversibly block impulse traffic more quickly in the ventral nerve cord [43]. Briefly, we made a notch in a plastic tube (1 mm i.d.) placed at right angles to the ventral nerve cord. Either normal saline or isotonic sucrose with fast green dye (to detect undesirable turbulence) was passed through the tube to generate a laminar stream locally across the nerve cord at one or two selected sites (Fig. 1a, b). Axon impulses in the nerve cord are quickly (within about 3 s) and reversibly blocked when the isotonic sucrose flows across the nerve cord. For some experiments, we used two sucrose knives to functionally isolate a single ganglion; in others, we physically isolated ganglia by cutting the intersegmental connectives on either side (Fig. 1c).

2.1.4 Procedure for determining the effects of chain length on swim duration

We determined the effect of preparation length on the duration of swimming episodes in a series of experiments on the isolated nerve cord. First, we established the duration of swimming in a full-length M2-T control preparation ($n = 4$ repetitions separated by 60 s). We then severed the connectives between M2 and M3 to shorten the preparation to M3-T. The duration of swim episodes were then measured with another four swim-initiating DP nerve stimuli. These procedures were then repeated until the preparation was reduced to M7-T in four leeches. In an additional three preparations, we first determined the control episode duration in the M2-T cord, and then the swim duration in partial nerve cords, sequentially reduced from M8-T to M12-T. Finally, we performed some experiments in which a series of intermediate lengths of the nerve cord were examined for swim duration. The metric for swim length in these experiments was the number of bursts per swim episode (BPE). Because of the inter-preparation variability in swim duration, we normalized all values to that of the control, M2-T, condition. Preparations shorter than M12-T normally do not exhibit fictive swimming [44]. We analyzed our physiological data with Chart software. Cycle period was computed with our custom rhythm analysis system [44]. Results are reported as means and standard error.

For examining changes in chain length during ongoing swim episodes, we used standard M2-T preparations with a sucrose knife placed between M17–M18. DP nerve recordings were taken on either side of the knife to ensure its effectiveness, and swimming was initiated by stimulation of a nerve on the rostral side of the knife. Swimming was initiated with the knife either "off" (caudal ganglia connected) or "on" (caudal ganglia disconnected). Then the knife was either left continuously "off" or "on" for the duration of the swim (the "static" condition), or the knife was switched "on" (connected, then disconnected) or "off" (disconnected, then connected), respectively, after the 4th–6th DP nerve burst of swim episodes (the "dynamic" condition). Swim duration in all four conditions was scored by counting the BPE.

2.2 Modeling methods

2.2.1 Model neurons

We previously published a series of successively more realistic and more complete simulations of the leech swim oscillator [45–48].The model presented here includes elements not

included in those simulations: three-compartment neurons with Hodgkin–Huxley conductance structures, the set of identified intersegmental swim-gating neurons, a trigger neuron and a set of intersegmental excitatory interneurons connected via reciprocal excitation. Simulations were carried out with *NeuroDynamix II* software (www.neurodynamix.net; [49]), which allows construction of conductance-based circuits with up to 100 neurons, each with up to 100 synaptic interactions. Individual neurons have invertebrate-like structures comprising three compartments: soma, neurite and axon, which are linked by electronic conductances (Fig. 2a). Individual compartments are constructed from a capacitor and one or more batteries and conductances. The site of all synaptic input is the neurite. Resting conductance is constant, whereas variable conductances are controlled either by compartment membrane potential, impulses or the membrane potentials of presynaptic neurons. Excitatory and inhibitory synaptic inputs are modeled by variable conductances associated with synaptic reversal potentials that are, respectively, above or below impulse threshold. Variable potassium and sodium conductances in the axon compartment generate impulses with a simplified conductance scheme that does not incorporate the full set of Hodgkin–Huxley equations, but does generate sodium–potassium conductance-based impulses with realistic overshoot, undershoot and duration.

2.2.2 Model overview

Sustained swimming oscillations in the isolated nerve cord of the leech are generated within chains of ventral nerve cord ganglia. The currently identified segmental neuronal circuit comprises more than 13 pairs of inhibitory intersegmental interneurons and one excitatory interneuron. In the leech nerve cord, oscillatory interneurons project to rostral or caudal ganglia with a span of about five segments [14, 50, 51]. We followed our previous practice

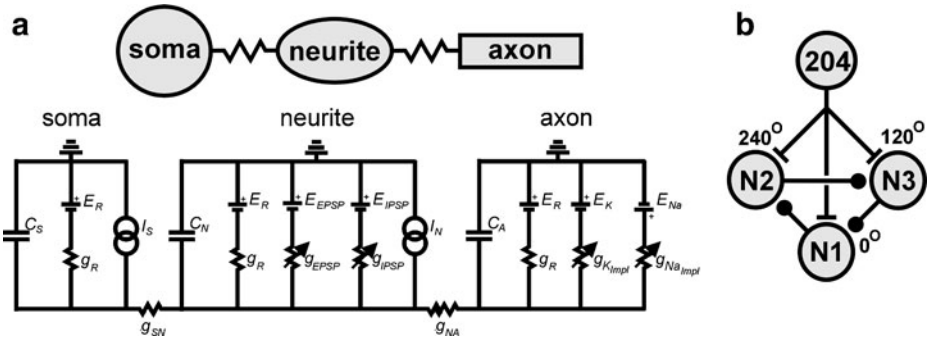

Fig. 2 Model schematics. **a** Individual neurons are simulated by three compartments, soma, neurite and axon, which are linked by passive conductances (g_{SN} and g_{NA}). Compartments comprise capacitors (C), batteries (E_R, E_{EPSP}, E_{IPSP}, E_K, and E_{Na}), and many conductances (g). The resting conductance g_R is constant; the soma and neurite include current sources (I_S and I_N). Variable conductances in the neurite compartment (g_{EPSP} and g_{IPSP}) simulate excitatory and inhibitory synaptic conductances. Variable potassium and sodium conductances (g_{KImpl} and g_{NaImpl}) in the axon compartment generate action potentials when the membrane potential is above threshold. **b** Segmental oscillators. A three-neuron ring (N1–N3; three-compartment units are denoted by circles) incorporates recurrent cyclic inhibition (relative phases are indicated) to generate the swim oscillations. Interactions within each ring are inhibitory, non-spike-mediated synapses between neurite compartments. The complete oscillator comprises 17 such units. Swim-gating neurons (204; also 205) provide the excitatory input to activate the system. Neuron terminals with filled circles are inhibitory; the 'T' ending denotes an excitatory synapse

of simulating the intrasegmental circuit as a three-neuron ring that generates oscillations through the mechanism of recurrent cyclic inhibition (RCI; Fig. 2b) [48]; these segmental circuits represent not only the rhythm-generating circuit but also the output of the excitatory MN, DE-3, used as a measure of fictive swimming in physiological preparations. The physiologically identified intersegmental connection scheme is embodied in our model (Fig. 3) and results in appropriate rostro-caudal phase delays in the MN bursts of successive segments.

The complete model circuit (Fig. 3) comprises the following. (1) One trigger neuron, combining the properties of Tr1 and Tr2 [30, 52]. (2) A total of 17 reciprocally excitatory (RE) interneurons (one per segment). These RE neurons are suggested by our physiological experiments but are currently unidentified. (3) Eight swim-gating neurons, one cell 205 and seven cells 204 located, respectively, in M9 and M10–M16 [37] and (4) 51 interneurons grouped to form 17 segmental three-neuron RCI loops. The sequence of swim initiation and swim maintenance arises from the cascade of excitation mediated by excitatory synapses. First, in the model, the trigger neuron, cell Tr, makes strong, excitatory connections to all 17 RE neurons and to all swim-gating neurons. Hence, brief excitation of Tr can drive all RE and swim-gating neurons above impulse threshold; note that the excitation among the cells postsynaptic to Tr outlasts Tr activity due to the reciprocal excitation. The 17 RE interneurons excite each other over a span of three segments, making moderately strong reciprocally excitatory synapses with a total of six other RE cells (in midcord), three in either direction (there is no self-excitation). All RE neurons also have weak excitatory connections to each swim-gating neuron (see Table 1 for parameter values).

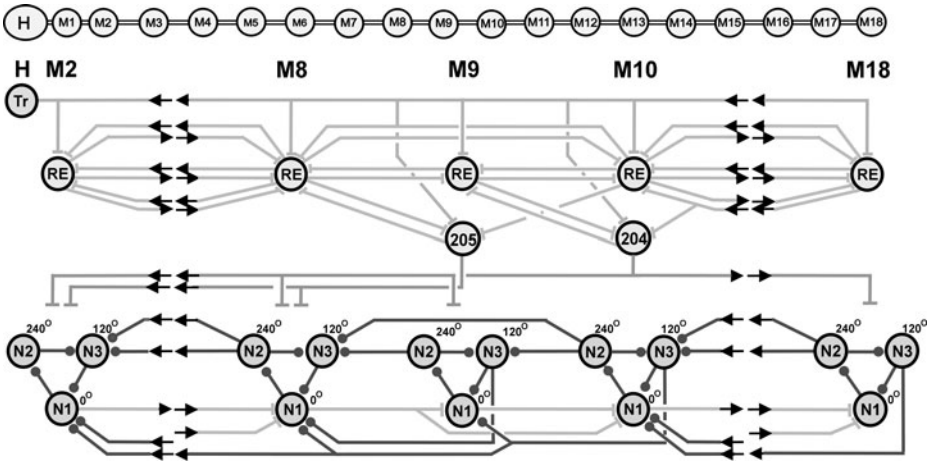

Fig. 3 Model intersegmental interactions. The complete swim-generating circuit comprises a trigger neuron (Tr; the Tr soma is located in the head ganglion, H), reciprocally excitatory interneurons (RE, one per segment), eight swim-gating neurons (205 in M9; 204 in M10–M16), and 17 segmental RCI circuits (M2–M18). Tr provides excitatory input to all RE neurons, whereas RE neurons are interconnected with others up to three segments away in either direction. Cell 205 excites segmental oscillators in M2–M8. Cells 204 drive RCI neurons in all segments except their own. Oscillator interneurons interact over a span of five segments in the direction indicated. Unlike the inhibitory intrasegmental interactions, intersegmental synapses are spike-mediated and incorporate impulse conduction times of 15 ms per intersegmental span. Neuron terminals with filled circles are inhibitory; those with 'T' ending are excitatory

Table 1 Model parameters

Parameter name	Value
Resting potential	−40.0 mV
Threshold	
Impulse	−39.5 mV
Synaptic	−40 mV
Impulse adaptation	
Magnitude	0.021 mV
Time constant	2.10 s
Impulse travel time/segment	15 ms
Stimulus parameters	
Amplitude	2 nA
Duration	0.2 s

Swim-gating neuron 205 has very weak excitatory synapses with model RE neurons in segments M2 through M8 and helps maintain excitation through reciprocal excitation. Likewise, cells 204 have very weak excitatory synapses with RE neurons in each segment except their own, and weak excitatory synapses with all RCI neurons, except in those in their own segment (Table 1).

2.2.3 Model parameters

Although the set of interneurons that generate the swim oscillations in the nerve cord have axons that generate impulses, intrasegmental synaptic interactions among swim-related neurons are largely chemotonic; that is, they are not greatly influenced by impulses, which are generated in the axon, not in the soma or neurite [53]. In the model circuit, these synapses are simulated by neurite-to-neurite interactions in which postsynaptic potentials are set by the synaptic strength parameter (relative strength) and by the difference between the membrane potential and synaptic threshold in presynaptic neurons. These intrasegmental synaptic circuits generate swim oscillations with three phases of activity (0°, 120° and 240°) and with cycle period near 0.7 s [14, 50]. Leech ganglia exhibit a U-shaped intrinsic period gradient [44]. This gradient is embodied in the model by setting the intrasegmental synaptic time constants to 120 ms in M2, deceasing stepwise (2 ms/segment) along the nerve cord to 100 ms in M12 and then increasing stepwise (7 ms/segment) to 142 ms in M18 [48] (Table 2).

Fictive swimming in leech preparations is episodic. Whether it is evoked by sensory stimulation, activation of a trigger neuron or arises spontaneously, swimming activity ceases abruptly after a few or many cycles. The number of bursts in an episode typically averages around 15, but can be under ten or well over 30. We ensured that activity in the positive feedback circuits is self-limiting by endowing RE neurons with impulse adaptation, defined as a reduction in impulse frequency in the face of constant excitatory input. We implemented the impulse adaptation property in the model as follows: after each impulse we incremented an "adaptation" parameter by some fixed amount denoted as the "adaptation amplitude." Impulse threshold was then set to decay with a time constant set by another parameter (Table 2). With the incorporation of impulse adaptation, run-away excitation, which might be expected from positive feedback loops, does not occur.

The resting membrane potential of all simulated neurons was set below impulse threshold; hence, all model neurons are silent unless driven by excitatory input (Table 2). A small random signal (maximum value ±0.1 mV) was added to the neurite potential during each simulation cycle to simulate random fluctuations in neuron resting potential and to avoid

Table 2 Model synaptic parameters

Presynaptic cell (location)	Postsynaptic cell (location)	Relative strength	Time constant (ms)
Tr (H)	RE (M2–M18)	500	20
Tr (H)	205/204 (M9–M16)	500	20
RE (M2–M18)	RE ($\pm\{1$–$3\}$[a])	10	20
RE (M2–M18)	205/204	2	20
205/204 (M9–M16)	RE (M2–M18)	1	20
205/204 (M9–M16)	RCI (M2–M18)	5	30
RCI (M2–M18)	RCI (M2–M18)	−1,000	120…100…142
RCI (M2–M18)	RCI ($+\{1$–$5\}$[b])	10	30
RCI (M2–M18)	RCI ($-\{1$–$5\}$[b])	−10	30

[a]Connection span is to other RE neurons within three-segment distance

[b]Connection span is five segments, specific connection and sign indicated in Fig. 3.

metastable states arising from numerical computation. Travel time for all model impulses passing between simulated ganglia was set to 15 ms per segment. This value is derived from published data on the intersegmental travel times in leech oscillator interneurons of medium-sized (1–3 g) animals [39, 50, 51].

3 Experimental results

3.1 Physiology

3.1.1 Brief stimulation initiates prolonged bursting

Although we understand some of the mechanisms that initiate behavior, the processes that maintain the neuronal activity are less well understood. What is clear is that duration of the output is not determined by the stimulus intensity or duration. In preparations of the leech ventral nerve cord (M2-T; Fig. 4a), extracellular stimulation of peripheral nerves can elicit neuronal bursting with a cycle period of about 0.7 s [42]. This rhythmic neuronal activity, fictive swimming, resembles that observed in nearly intact animals [54]. Usually, a train of electrical pulses is applied to a DP nerve to generate fictive swimming; however, a single 5 ms stimulus to a DP nerve can elicit the behavior, leading to responses that outlast the stimulus by many seconds (Fig. 4b). Previously, it has been shown that gating neurons 204/205 can trigger swimming when the preparation is bathed in serotonin [55]. We confirmed these results ($n = 9$ preparations; see Fig. 6), and also demonstrate that brief excitation of excitatory oscillator neuron 208 ($n = 4$ preparations; Fig. 4c) and oscillator interneuron cell 33 ($n = 1$ preparation; Fig. 4d) trigger swimming in serotonin saline. The disparity between the duration of the initiating stimulus and the ensuing behavioral response leaves little doubt that the source of the sustaining excitation, clearly evident in swim-gating neuron 205 (top trace, Fig. 4c), is internal to the nerve cord and is not provided by the external sensory stimulus. This is consistent with earlier findings that the intensity of trigger neuron excitation does not correlate with swim duration [30], and our results showing that swim duration is also independent of the voltage amplitude of a swim-initiating DP nerve shock ($n = 5$, linear regression $p = 0.82$, not illustrated).

The central swim oscillator in the leech nerve cord is driven by a set of segmentally repeated swim-gating neurons [37, 56, 57]. Although their activity is critical for driving the swim oscillator neurons, depolarization of these cells by intracellular current injection is not self-sustaining; that is, there is no evidence for plateau potentials. This suggests that circuit properties underlie gating cell depolarization; we explore here mechanisms that might generate this swim-sustaining excitation.

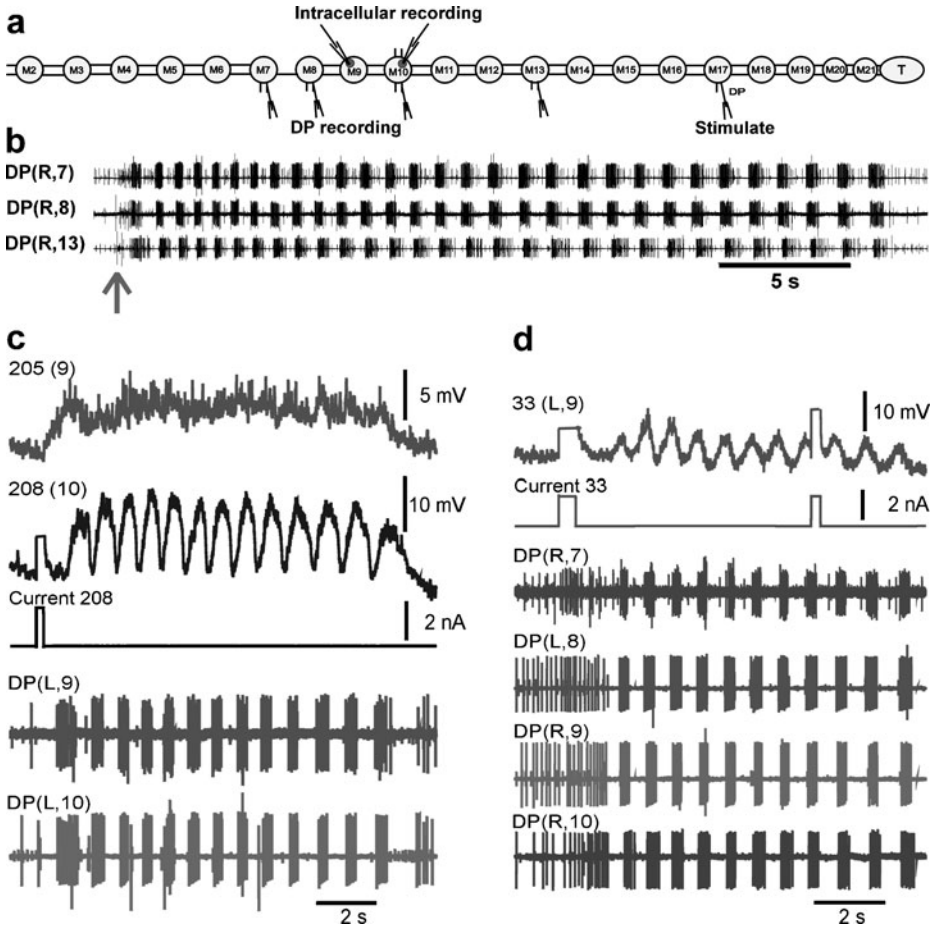

Fig. 4 Brief excitation initiates a prolonged episode of impulse bursting. **a** Schematic of leech nerve cord preparation for physiological experiments. Intracellular recordings from neuronal somata were obtained with microelectrodes. Extracellular records are from DP nerves. **b** Swim episode comprising 30 bursts, evoked by a single 5 ms electrical stimulus pulse (*arrow*) applied to a DP nerve. Repeating, clustered spikes are those of excitatory MNs recorded by suction electrodes. Labels at left indicate laterality (R-right) and ganglion identity (M7, M8, and M13). **c** Swim episode evoked by brief depolarization of cell 208. Upper and second traces are intracellular soma recordings from swim-gating cell 205 and interneuron cell 208, respectively. Both of these cells are depolarized throughout the swim episode. **d** Swim episode evoked by brief depolarization of a CPG interneuron, cell 33. Nerve cords in c and d were bathed in saline containing 50 μM serotonin. Because these recordings were made at high amplifier gain, traces saturate during current injection. The second current pulse injected into cell 33 is extraneous

3.1.2 Maintained bursting activity requires multiple ganglia

Previous experiments on the leeches showed that single isolated or nearly isolated ganglia comprise sufficient components of the central swim oscillator to generate at least rudimentary swim oscillations [44, 56, 58]. These oscillations in isolated leech ganglia are weak, erratic, and not sustained. Perhaps not surprisingly, when we compared isolated ganglia with more extended preparations, we found that the swim maintenance system

reflects these differences. The first question we addressed was whether individual, isolated ganglia can generate sustained excitation in response to external inputs in two types of preparations: individually isolated ganglia and single ganglia that were physically connected with the ventral nerve cord, but functionally isolated through the use of dual sucrose knives. The sucrose knife can reversibly disconnect and then reconnect ganglia from the rest of the nerve cord without physical trauma.

We were unable to evoke sustained activity in swim-gating neurons in physically isolated ganglia (Fig. 5a1), even when these were exposed to 50 μM serotonin. When such ganglia were stimulated, either by DP nerve shock or by P cell stimulation ($n = 5$), cell 204 was

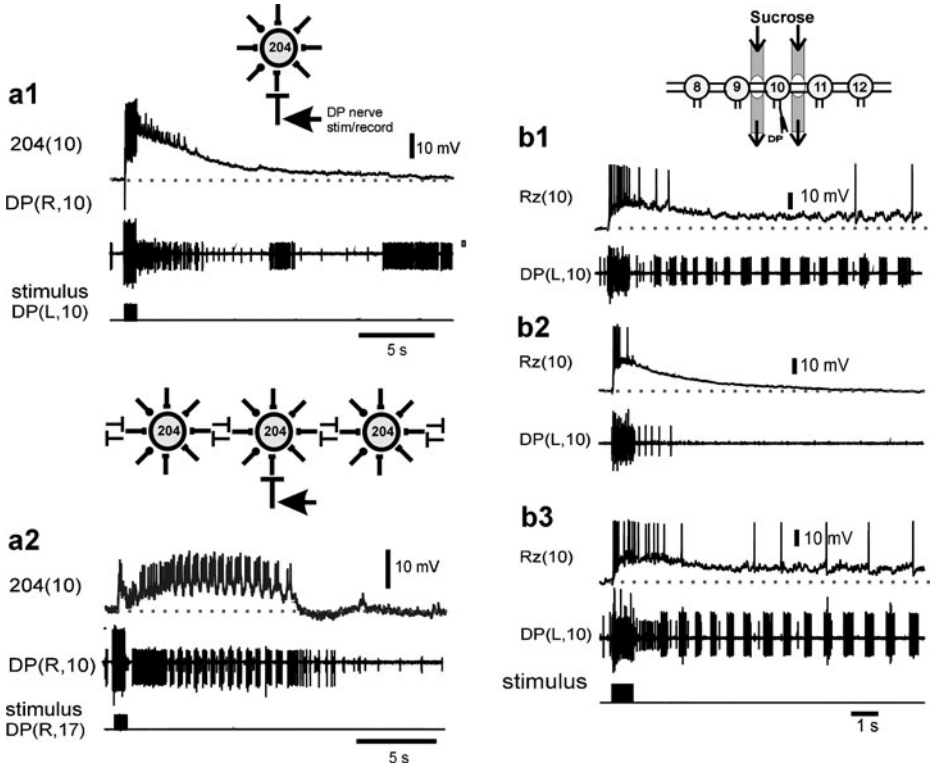

Fig. 5 Swim initiation requires a chain of ganglia. **a** Cell 204 responses to DP nerve stimulation. **a1** Transient cell 204 depolarization in the isolated ganglion. DP stimulation (*bottom trace*) causes a large, but transient, depolarization in cell 204 (*top trace*). Swimming is not evoked (*middle trace*). The inset indicates that cell 204 has multiple inputs even in the isolated ganglion. **a2** M2-T chain, swimming is initiated by DP nerve stimulation (*bottom trace*). Cell 204 responds to DP stimulation with a prolonged depolarization (intracellular recording, *top trace*) during the evoked swim episode (*middle trace*). The inset illustrates that cell 204 receives intersegmental inputs that are not available in the isolated ganglia. **b** Responses of Retzius (Rz) cells to DP nerve stimulation. **b1** Brief DP nerve stimulation (*bottom-most trace*) evokes a swim episode; a low-level sustained depolarization of the Rz cell is seen (*top trace*). **b2** Double sucrose block activated, functionally isolating ganglion M10. The Rz cell response is transient depolarization. **b3** With the removal of the sucrose block, swimming is again initiated and the depolarization of the Rz cell is maintained. *Dashed lines* indicate the membrane potential prior to stimulation. Preparations bathed in normal saline. Data in *a* are representative of eleven experiments. Data in **b** are similar to those observed in seven Rz cells in four preparations. Stimulus trace and time calibration apply to all parts of **b**

depolarized, but fictive swimming did not occur and the depolarization of cell 204 was not sustained. Rather, the depolarization decayed with a half-time of about 2 s. In the control extended nerve cord (M2-T) stimulation of a DP nerve induced sustained excitation in cell 204 and evoked a swim episode ($n = 5$; Fig. 5a2). The local neuronal interactions (inset, Fig. 5a1) that activate cell 204 following DP nerve stimulation are not identified. The intersegmental interactions (inset, Fig. 5a2) that give rise to sustained excitation are also unknown.

We carried out analogous experiments in M2-T preparations, but now implemented two sucrose knives simultaneously to block impulse traffic in the M9–M10 and the M10–M11 intersegmental connectives (Fig. 5b inset). When normal saline flowed across these connectives, impulse traffic was unimpaired. However with a latency of less than 3 s, initiation of the flow of isotonic sucrose blocked impulse traffic. In these experiments, we stimulated one of the M10 DP nerves to activate the swim circuit and recorded the ensuing swimming activity with three suction electrodes, one placed rostral and one caudal to the sucrose knives, as well as one on a DP(10) nerve. Because recording from swim-gating neurons when using dual sucrose knives is difficult, we monitored intracellular activity in the large Retzius cells (Rz), which are easily penetrated and whose depolarization response to DP nerve stimulation is similar to that of cell 204 (n = 7 Rz neurons). We found that, in the control M2-T condition (Fig. 5b1), stimulation of the DP nerve depolarized the Rz cell throughout the swim episode. In contrast, the acutely isolated M10 ganglion (Fig. 5b2) did not exhibit fictive swimming, and intracellular depolarization in the Rz cell was not sustained, but rather decayed with a time constant of about 2 s (Fig. 5b2). Reconnection of M10 by saline flow in the sucrose knives restored the control response (Fig. 5b3). Similar depolarization profiles were observed in one successful cell 204 penetration (not shown). Results from these experiments indicate that intrasegmental interactions cannot sustain excitation.

Interactions that might account for the differences in the activities of isolated and extended nerve cords are shown in the insets of Fig. 5a. In isolated ganglia (upper inset), input from DP nerve stimulation transiently depolarizes segmental cells. In full-length preparations, interganglionic excitatory interactions may prolong this activity because of positive feedback connections (lower inset). We next present experimental data that suggest the existence of such intersegmental positive feedback circuits.

3.1.3 Excitatory synaptic effects exhibit long latencies

Swim-gating neurons can trigger swimming in nerve cord preparations exposed to bath-applied serotonin [55]. These neurons are themselves depolarized during swim episodes ([36]; Fig. 5a2), suggesting the existence of excitatory loops. In Fig. 6a, we show that a brief depolarization of cell 205 in ganglion M9 first gave rise to a small depolarization in cell 204 located one segment posterior. These cells do not have direct synaptic connections [57] and the large latency of 0.3 s implies that the effect is polysynaptic. With a considerably greater latency (1.2 s), cell 204 undergoes an abrupt, very large depolarization that is concomitant with the large depolarization of cell 205 itself. Clearly, cell 205 has activated some cells that that in turn excite 204 and 205. The excitation is subthreshold for swimming in this example; nevertheless, it does not subside completely for several seconds. Whatever the source of these long-latency inputs, they do not require the activation of the swim oscillator.

Another striking example of such delayed excitation is illustrated in Fig. 6b, where brief depolarization of cell 204 in M10 evokes a strong depolarization in cell 208 in the same

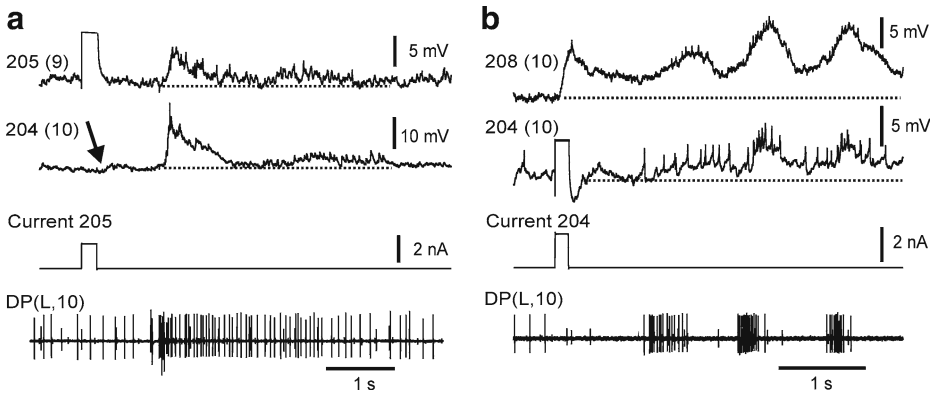

Fig. 6 Depolarizations in excitatory swim neurons arise with long latency. **a** Depolarization of swim-gating neuron cell 205 (*top trace*) evokes a small depolarization in cell 204 with a delay of 0.3 s (*arrow*, *second trace*) followed by a larger depolarization in itself and in cell 204, with a delay of 1.2 s. DP nerve excitation (*bottom trace*) also begins with long latency. The stimulus intensity was below threshold for swim initiation. **b** Depolarization of cell 204 (*second trace*) evokes excitation in cell 208 (*top trace*) with a delay of 0.044 ms. Swim bursting (*bottom trace*) has long latency. Nerve cords were bathed in saline containing 50 μM serotonin. To increase the amplitude of evoked responses, cell 204 was hyperpolarized by −0.3 nA (**a**) and cell 208 by −0.2 nA (**b**). *Dashed lines* indicate the membrane potential prior to stimulation. Because these recordings were made at high amplifier gain, traces saturate during current injection. Data shown are representative of ten experiments

ganglion. The delay is 44 ms, incompatible with a direct connection, which was already ruled out by earlier experiments [39].

It also seems unlikely that the long latency is the sum of multiple synaptic delays that might occur in a cascade of interactions. In the leech, synaptic delays are about 5 ms [53]; hence, the 44-ms latency caused by serial synapses would require a chain of at least eight neurons. The long latency of the excitatory responses might arise from another source, impulse travel times. Travel times for impulses conducted between segments for leech oscillator interneurons in medium-sized (1–3 g) animals are about 15 ms [39, 50, 51], although the precise conduction velocity of axons depends on many factors, including temperature and axon diameter. For a chain of 21 ganglia, the impulse travel time from end to end is about 300 ms. The very substantial latencies between swim-initiating stimulation of swim-gating neurons and the abrupt onset of sustained depolarization in these neurons suggest that positive feedback between neurons located at least one, but perhaps many segments apart could be important in swim initiation and maintenance. Multiple searches for direct, intersegmental excitatory synaptic loops have so far failed. We posit the existence of such intersegmental excitatory interactions as the source of the sustained drive that maintains swimming activity. This conjecture is incorporated into the model by a set of intersegmental excitatory neurons, one per segment, with broad, reciprocal interactions.

3.2 Comparison of modeling results with physiological data

3.2.1 Brief input engenders prolonged excitation that drives bursting behavior

Bouts of fictive swimming in H-M18 (and H-T) leech preparations can be evoked by brief activation of trigger neurons, whose somata are located in the rostral (brain) ganglia and

whose axons extend to the caudal nerve cord via the intersegmental connectives [30]. Provided that the intensity and duration of trigger neuron activation is suprathreshold, the duration of evoked swim episodes is independent of stimulus parameters. In our simulation of the swim circuits the multiple trigger neurons identified by physiological experiments are represented by a single neuron. This model trigger neuron, Tr, evokes swimming activity in the simulated circuits much as in nerve cord preparations. For example, brief (0.2 s) Tr stimulation evokes a simulated swim episode of seven or eight BPE (Fig. 7). Traces shown in Fig. 7 are those of the axon compartment of the indicated model cells, which simulate the recorded MN spikes in DP nerves (RCI traces show activity of one neuron per segment). In the model, the swimming activity is initiated because Tr impulses (top trace) depolarize all RE neurons (second to sixth traces show a subset) and the swim-gating

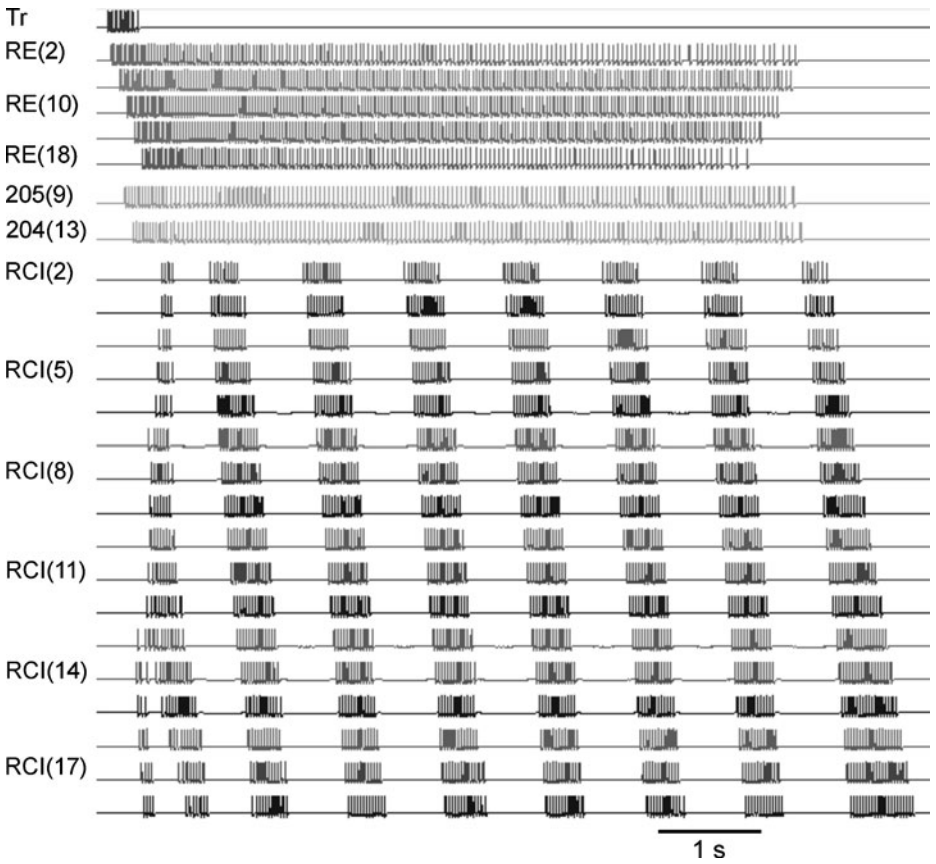

Fig. 7 Model swim episode. Brief Tr stimulation (*top trace*) evokes prolonged RE cell activation (next five traces; output of every fourth RE neuron is shown) and subsequently swim-gating neuron activity (cell 205 and a representative cell 204). The cells 205 and 204 make excitatory synaptic connections with segmental RCI neurons to generate a swim episode with eight bursts (RCI 2–18). Termination of the RE activity and consequently the activity in swim-gating neurons occurs autonomously because of impulse adaptation in RE neurons. Loss of excitatory drive terminates bursting in RCI circuits. All traces are those of axon compartments and hence simulate extracellular recordings

neurons (two of the eight are shown). The swim-gating neurons provide some positive feedback to the RE circuit and depolarize the RCI neurons, which go into the oscillatory state when depolarized beyond synaptic threshold. Segmental RCI output is phase delayed along the simulated chain of ganglia from M2 through M18 because of their intersegmental interactions (Fig. 3). The RE neurons continue to fire impulses following the cessation of Tr activity because of their own positive feedback interactions and their positive feedback from swim-gating neurons. The duration of the swim episode and the swim cycle period depend on the properties of the RE, not on the output of the Tr neuron.

In mid-chain, the initial bursts in RCI circuits resemble those of fully-activated swim episodes, but the initial bursts in the rostral and caudal chain are brief and would not be viewed as fully developed bursts in a physiological preparation. For consistency, we always determined BPE in a swim episode from the RCI(11) trace (Fig. 7). Using the first burst of RCI(11) as a marker for the initiation of swimming, the latency between Tr stimulation and swim onset is 0.33 s. This large latency arises from intersegmental impulse travel times, which are set at 15 ms/segment; synaptic delay in these simulations is less than 1 ms. The swim episode terminates when RE activity drops out (sequentially from rostral to caudal in this episode) and consequently the activity in swim-gating neurons ceases because of the loss of excitatory drive from RE neurons (model swim-gating neurons, as in the leech, do not interact with each other).

3.2.2 Swim duration depends on impulse adaptation parameters

The swim-maintaining excitatory drive in these simulations arises from the 17 RE neurons. These neurons are quiescent until stimulated; then impulses in any one RE neuron can activate the entire set through the reciprocally excitatory synaptic interactions that generate positive feedback. The effectiveness of the feedback is a function of parameters that set synaptic amplitude and time constant (constant, except as noted, throughout these experiments) and of the impulse frequency of the cells. The latter is a function of membrane potential and of impulse threshold. Depolarization of RE cell membrane potential above rest is entirely due to synaptic input—a circuit characteristic, whereas the firing rate also depends on impulse threshold and impulse adaptation—cellular properties. Impulse adaptation, the time and voltage-dependent reduction of nerve impulse frequency in the face of a constant stimulus, is observed in many leech neurons. Quantitative studies of impulse adaptation have been conducted only on MNs [59]; however, other records [37, 57] demonstrate that excitatory interneurons also exhibit impulse adaptation. The RE cells explicitly embody this property, which in these simulations is controlled by impulse adaptation time constant and magnitude parameters. The relationship between these parameters, the duration of swim-gating cell activity and swim duration are roughly hyperbolic (Fig. 8). Within the range of permissible values, decreasing values for both time constant and magnitude generate increasingly greater output values; that is, longer-lasting excitation and more protracted swim episodes. As the values of adaptation parameters are increased, RCI activity is eventually reduced to brief bursts before failing entirely.

3.2.3 Mechanisms that initiate and terminate excitation by a single input

We selected model parameters for neurons in these simulations to mimic the physiology of identified leech neurons. Similarly, synaptic interactions were modeled on physiological

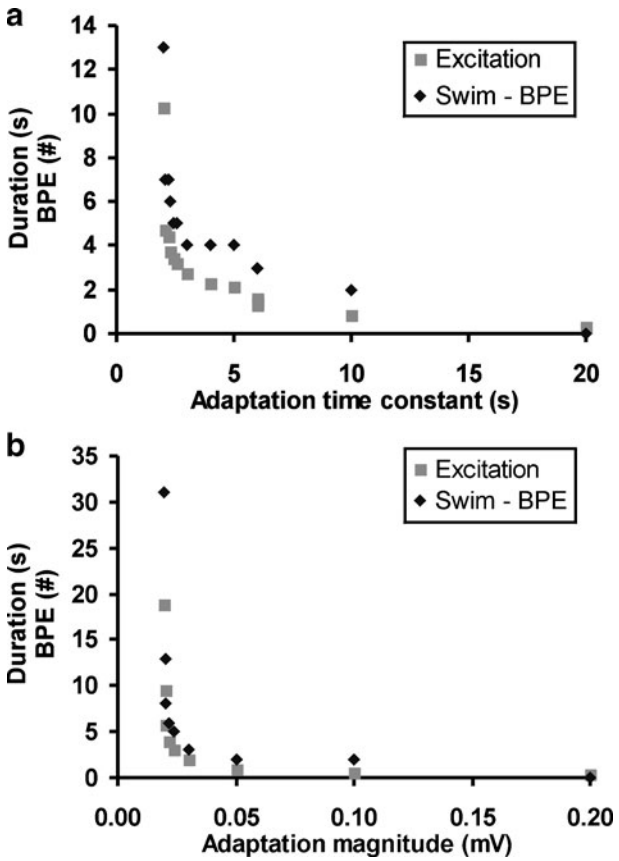

Fig. 8 Duration of swim activity is controlled by impulse adaptation time constant and magnitude. **a** Duration of model swim episodes versus impulse adaptation time constant. The ordinate gives the duration of excitation in simulated cell 204(13) (*squares*) and swimming activity (*diamonds*) of swim episodes evoked by Tr. Impulse adaptation magnitude is constant (0.021 mV), while the time constant is varied from 1.95 to 20 s. Positive feedback, and hence also swimming activity, does not terminate if the adaptation time constant is less than 1.95 s. Excitation of RE neurons is too brief to evoke bursting when the time constant is 20 s or greater. **b** Activity duration versus adaptation magnitude. Impulse adaptation time constant is held constant (2.1 s) while the magnitude of adaptation is varied from 0.019 to 0.2 mV. When the amplitude parameter is set to 0.019 mV or less, activity does not terminate; for values 0.2 mV or greater positive bursting does not occur. *Squares* indicate the duration of the impulse train in swim-gating cells [values are those for 204 (13)]. The metric for swim duration is bursts per episode (BPE; *diamonds*)

data. The parameters that defined impulse adaptation properties were chosen such that input from a short burst of trigger neuron impulses would generate a swim episode of moderate length, seven or eight bursts. Once selected, this set of parameters was not changed for the additional simulations described below.

Swimming locomotion in leeches is initiated, and can also be terminated, by sensory triggers [38, 60]. During normal leech behavior, such sensory-induced swim termination occurs when the leech encounters some physical barrier. A neuron downstream of sensory neurons, the trigger neuron Tr2, shares this fascinating property, whereby excitation of

this neuron can both initiate swimming *and* terminate an ongoing swim episode [52]. Termination does not occur if the second Tr2 depolarization is timed to occur near the beginning of a swim episode.

The "toggle" property of trigger neuron Tr2 is captured by our model. We varied the interval between two identical current pulses (0.2 s in duration) applied to activate the Tr neuron. We found that when the interval between pulses was 1–2 s, the duration of the evoked episode was enhanced from a control value of 7 to 8 BPE. For larger intervals, the swim episode terminated prematurely and the swim duration was reduced (Fig. 9). The maximum reduction was from 8 to 5 BPE when the inter-pulse interval was 2.5 s. As the spacing between pulses was increased, the swim length returned and even exceeded control values.

Termination of swimming by the swim-initiating Tr input seems paradoxical. One might expect that a second excitatory pulse from Tr would increase swim duration, rather than truncate swims. An advantage of neuronal modeling is that mechanisms that might underlie such puzzling and non-intuitive results can be investigated in detail.

The crucial factor that leads to swim termination is the nature of impulse adaptation. Were it not for impulse adaptation RE swim activity in the model would persist indefinitely; it would not be episodic. Rather, once initiated swimming would be sustained by the excitation generated through positive feedback. Impulse adaptation alters the properties of positive feedback in an interesting manner. The initial stimulus from cell Tr drives all RE neurons above impulse threshold (Fig. 10). The RE impulse frequencies are initially high; activity then drops to a lower level sustained by positive feedback. The impulse frequency in RE neurons does not remain constant; rather, it decays because of accumulating impulse adaptation (Fig. 10). Activation of Tr in mid-swim depolarizes RE cells and increases their impulse frequency. Summing with ongoing impulse adaptation, the high impulse frequency induces increased impulse adaptation. Hence, RE neuron impulse frequency drops below the threshold level required to maintain positive feedback with a gain of 1.0, leading, abruptly, to the cessation of RE activity and to early swim termination (Fig. 10).

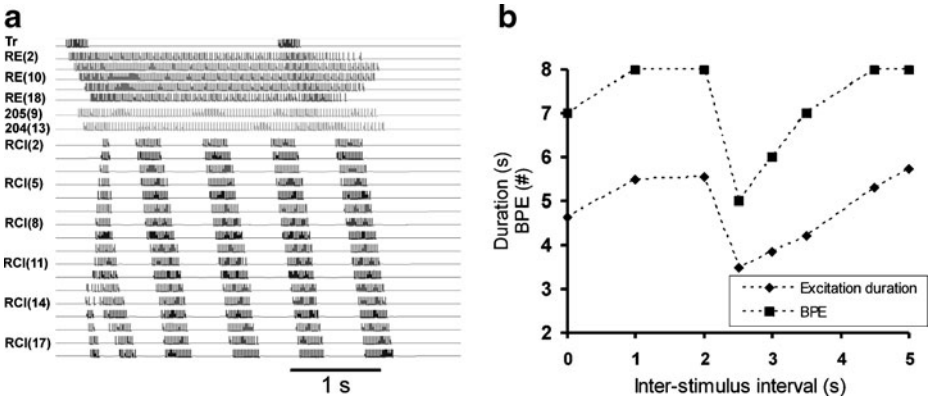

Fig. 9 Swim initiation and termination by Tr. **a** Swim termination occurred when the inter-stimulus interval was of intermediate length (2.5 s). **b** Swim duration versus inter-stimulus interval. The spacing of Tr activation (onset to onset) was varied from 0 (no second pulse) to 5 s. At both short and long, but not intermediate, intervals, the duration of swim-gating neuron excitation (*diamonds*) and burst per swim episode (BPE; *squares*) are greater than control. At intermediate intervals they are shortened

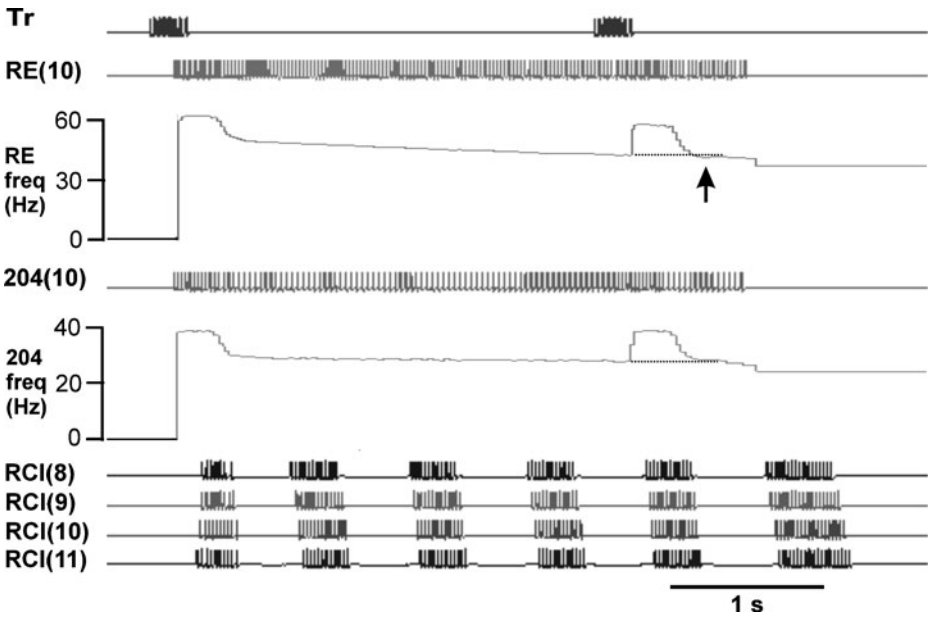

Fig. 10 Details of model swim termination by Tr. Following Tr activation (*first trace*), the RE cell (*second trace*) and cell 204 (*fourth trace*) begin firing at high frequency (RE freq, *third trace* and 204 freq, *fifth trace*). Impulse frequencies in RE cells gradually decrease because of impulse adaptation. The second Tr activation again generates a high-frequency burst, causing rapid impulse adaptation and a reduction in RE firing rates that cannot sustain positive feedback. The result is an abrupt cessation of activity in individual RE cells and a cessation of activity in cell 204. Frequency is the reciprocal of inter-impulse interval, with the initial frequency set to zero Hz. *Dashed lines* denote impulse frequencies just prior to the frequency increase. The *arrow* in the RE frequency trace indicates the adaptation-induced dip in impulse frequency (*third trace*)

3.2.4 Swim duration is correlated with chain length

We compared model output to physiological leech data in four additional simulations. First, we compared the duration of swimming activity as a function of chain length in the model with similar experiments performed on the isolated nerve cord of the leech. For one set of physiological experiments, we measured swim duration in control M2-T preparations and again after removal of individual ganglia from the rostral end. The shortest chain examined extended from M12 through T; shorter *posterior* chains do not support fictive swimming [44]. The metric for swim duration in these experiments was the number of BPE normalized by the swim duration in the control M2-T preparations. Swim episodes were evoked by DP nerve stimulation. We found that the number of BPE decreased roughly linearly as the length was reduced, although the sequential decreases in BPE were non-uniform (Fig. 11a). These, and additional experiments with similar results in which posterior, rather than rostral, ganglia were removed from the nerve cord (not shown), demonstrate that mechanisms that sustain swimming are widely distributed, without any individual ganglion having control of swim duration.

We performed analogous experiments on the swim model. In these, we determined swim duration (again BPE) in the M2–M18 control chain and then sequentially reduced chain length. Our reduction procedure was simply to hyperpolarize all model neurons in segments

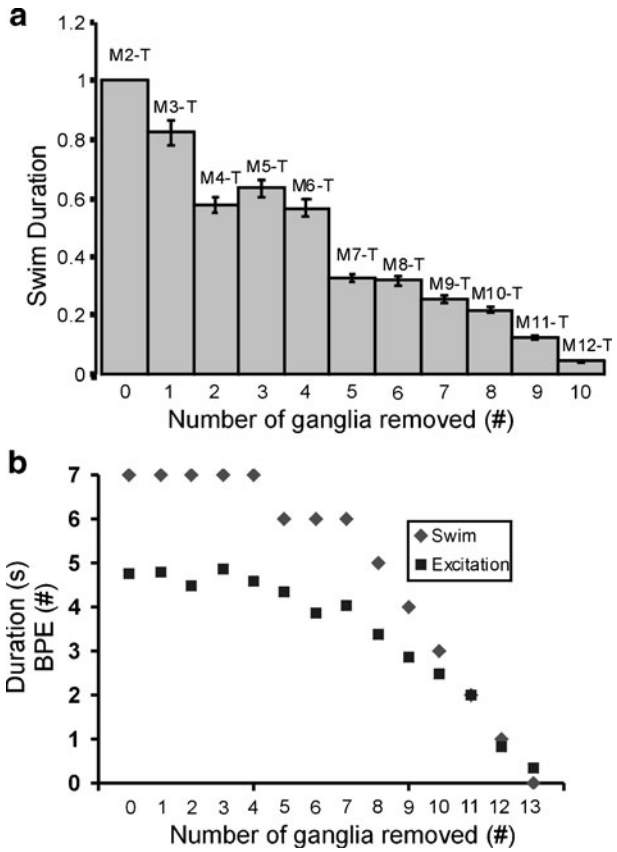

Fig. 11 Duration of swim episodes depends on chain length. **a** Experiments on isolated leech nerve cords. Leech nerve cords were successively shortened from an initial extent of M2-T (in some experiments, M8-T) by successively removing the most rostral ganglion (*abscissa*). Swim episodes were evoked by DP nerve stimulation for each of the preparation lengths, M2-T through M12-T. Swim duration (BPE) is normalized to swim duration in the M2-T preparation prior to removal of ganglia. Shortening the nerve cord leads to briefer swim episodes. Data (means and ±SE) from seven nerve cords exposed to normal saline (not all reductions were achieved with each preparation). **b** Model experiments. The model oscillator chain was successively shortened, from 17 to four segments. The *abscissa* indicates number of ganglia removed from the anterior end of a M2-M18 chain. Data shown are the duration of swim-gating cell excitation (*squares*) and swim duration in BPE (*diamonds*). Swim activity remains at control length until more than four ganglia are removed. Swimming does not occur when 12 or more ganglia are removed

to be removed. This approach simultaneously eliminated all oscillator neurons, RE neurons and gating neurons (if present), one segment at a time; swim initiation was via stimulation of the Tr neuron. Unlike the physiological data, removal of the first four anterior segments had little effect on the duration of excitatory activity in swim-gating neurons or on swim duration (Fig. 11b). Further shortening of the simulated nerve cord, however, led to a nearly linear decrease in the duration of gating cell activation and swim duration. With a swim episode defined as a minimum of two RCI bursts within 2 s, no swimming occurred in model chains with less than six segments; a single burst with increasingly briefer duration occurred

in chains comprising five or four segments. These experiments show that maintenance of RE positive feedback requires a minimum of six segments. Considering that the model structure and model parameters were not selected to generate these results, the congruence between the physiological and model data is remarkable. It appears that swim maintenance in the leech is a mass-action phenomenon that can be accounted for by intersegmental mutually excitatory loops.

3.2.5 Re-initiated impulse duration depends on swim interval

Swim episodes in isolated leech nerve cords can occur spontaneously, or be evoked, among other means, by DP nerve stimulation. The duration of these episodes varies from several

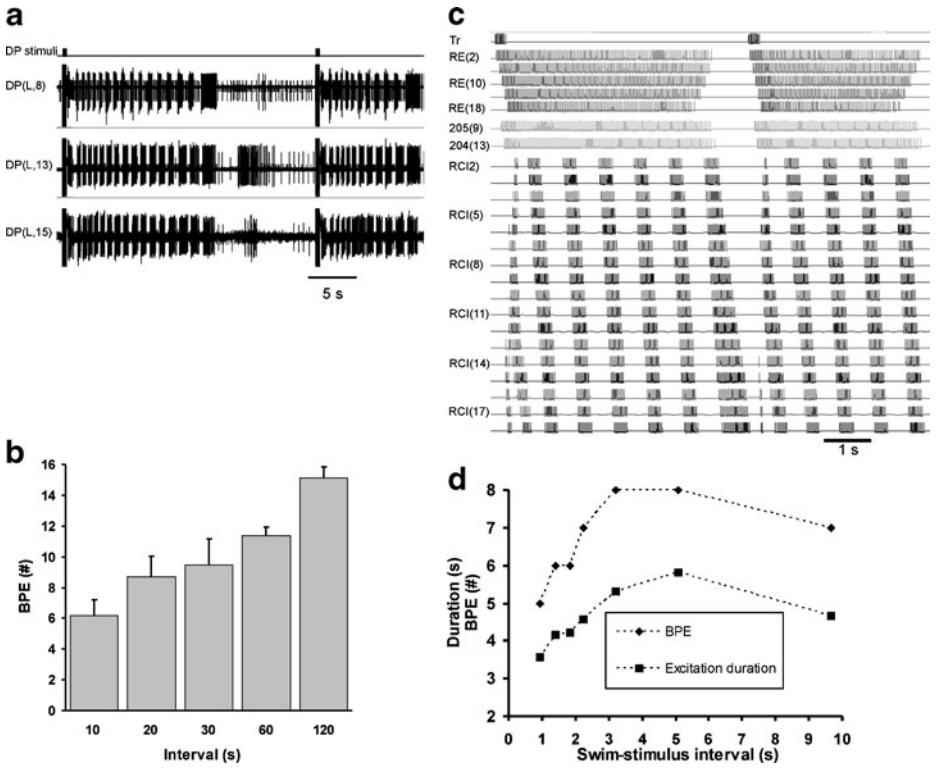

Fig. 12 Swim duration depends on inter-swim interval. **a** Swim initiation and re-initiation in a leech M2-T preparation in response to DP stimulation. The initial episode following a 60 s inter-swim interval comprised 16 bursts; the second, initiated with a delay of 10 s, comprised only 12 bursts. **b** Swim duration (BPE) versus the interval between swim cessation and the re-initiating stimuli in the leech nerve cord. $n = 5$ preparations. **c** Swim re-initiation in the model in response to Tr activation. Swim episodes with short inter-swim intervals exhibit a reduction in swim duration similar to that seen in the leech. **d** Swim duration versus swim-stimulus interval in model data. Control durations were 7 BPE. At short intervals, swim excitation duration (cell 204 activation) and BPE are relatively small

bursts to prolonged bouts with 30 or more cycles. Termination of swimming activity can be expected to leave residual effects that are not directly observable. To detect such effects, we performed physiological experiments on M2-T ventral nerve cords. Our aim was to determine whether residual effects alter the duration of swim episodes that follow swimming. Our procedure was to initiate swim episodes, with variable delay, after the termination of a previous episode ($n = 5$ preparations; Fig. 12a). Initial swim episodes either occurred spontaneously or were evoked by DP nerve stimulation; the subsequent test episode was evoked by DP nerve stimulation. The interval between swim episodes varied between 10 and 120 s. We found that swim duration was relatively shorter for short inter-swim intervals—an average of 6 BPE at the shortest interval tested (10 s) compared to an average of 15 BPE at the longest interval (120 s; Fig. 12b). The results suggest that mechanisms underlying swim maintenance in the leech nerve cord do not allow the maximum swim duration even 60 s after swim termination.

We carried out similar experiments on the M2–M18 model chain. Here, we first initiated a swim episode by activating the Tr neuron and then, with variable delay after the end of this episode, initiated a second one (Fig. 12c). The results of the model experiments were similar to those of physiological experiments. At short delays between swim episodes, the duration of the excitation and the BPE of the second episode was less than that of the initial one (Fig. 12d). At greater delays (4–6 s), however, both of these variables were increased. A notable difference between physiological experiments and modeling results is that the time scale is different. Residual effects on swim duration were observable at delays of up to 60 s in nerve cord preparations, but not in the model studies, where swimming was back to control after a delay of 10s. Moreover, an enhancement of swim duration above control levels at intermediate delays (4–6 s in model studies) was not observed in the leech nerve cord.

3.2.6 Dynamics of swim modulation

As demonstrated above, swim maintenance, in terms of swim duration and cell 204 excitation, is a function of the length of the leech nerve cord, in both physiological preparations and in the model. We asked "Is maintenance a dynamic function of chain length, with length a critical determinant of the number of BPE, or is length only important at swim initiation?" We answered this question in leech nerve cord preparations by dynamically altering chain length during ongoing swim episodes through the use of the sucrose knife ($n = 4$; inset, Fig. 13). We obtained data under two static conditions, the complete M2-T chain (Fig. 13a1) and a functionally shortened preparation, via the sucrose knife, M2–M17 (Fig. 13a4). As expected from previous experiments (Fig. 11), when swimming was evoked by DP nerve stimulation these preparations generated long and reduced swim episodes, respectively. In the first dynamic condition we initiated swimming in functional M2-M17 preparations with the sucrose knife activated. With swimming ongoing, we turned the sucrose knife off after the sixth burst (Fig. 13a2, D/C) to rapidly attain the functional full-length preparation (M2-T). The result was a swim episode whose duration was indistinguishable from those observed in full-length chains. In the converse experiment, we initiated swimming in the full-length cord (M2-T) and then functionally reduced it to M2–M17 with the sucrose knife turned on following the sixth burst (Fig. 13a3; C/D). Swim duration now was close to that of the static short chain. Results combined from four preparations showed that altering the chain length during swimming significantly changed swim duration (Fig. 13b). These

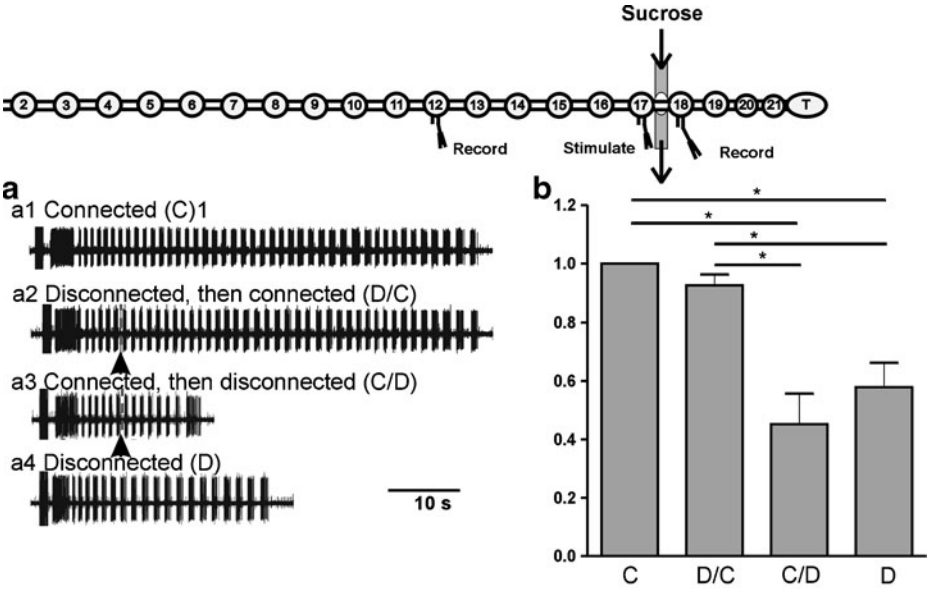

Fig. 13 Dynamics of swim modulation by caudal ganglia in leech preparations. **a** Series of swim initiation sequences in which a single sucrose knife was placed between M17 and M18 (*inset*). DP records shown are from M12, anterior to the knife. Recordings at M18 (not shown) ensured that that the sucrose knife was effective. **a1** Sucrose knife not activated (connected) and DP stimulation (*arrow*) evoked a long swim episode (43 bursts). **a2** Knife initially on, then turned off after the sixth burst (disconnected, then connected; *arrow head*), resulting in a long swim (43 bursts). **a3** Knife activated after sixth burst (connected, then disconnected, *arrow head*), resulting in an abbreviated swim (13 bursts). **a4** DP nerve stimulated while sucrose knife was continuously active (disconnected), resulting in short swim (19 bursts). **b** Summary graph of normalized swim duration for the four cases shown in **a**. Error bars are S.E.; * indicates significant differences; $p < 0.05$; $n = 4$ preparations

results indicate that the presence of caudal ganglia is necessary throughout a swim episode to achieve long swim episodes.

We performed analogous experiments on the model (Fig. 14) with swimming initiated by Tr activation. First, we performed the static experiments by determining swim duration in a full-length model chain (M2–M18) and in a shortened chain (M11–M18). In the latter, all neurons associated with M2–M10 were removed by hyperpolarization. Swim durations under these static conditions were 7 and 3 BPE, respectively (Fig. 14a,d). We then initiated swimming in the reduced M11–M18 model and then, following the second RCI cycle, restored the full-length model by removing the hyperpolarization (Fig. 14b). As a consequence of this dynamic lengthening of the model, swim duration increased to 8 BPE. Conversely, we initiated swimming in the full-length (M2–M18) chain and then removed M2–M10 by hyperpolarization after the second cycle to dynamically generate a shortened preparation (M11–M18; Fig. 14c). The result was, as in physiological experiments, a reduction in swim duration. Thus, the model mimics the activity patterns observed in leech preparations, indicating that the length of the nerve cord, in both leech preparation and model, determines swim duration, not the length of the system at swim initiation. Thus mutual excitation between caudal ganglion neurons and the more anterior nerve cord is required to maintain the excitation that drives swimming in the leech and in the model.

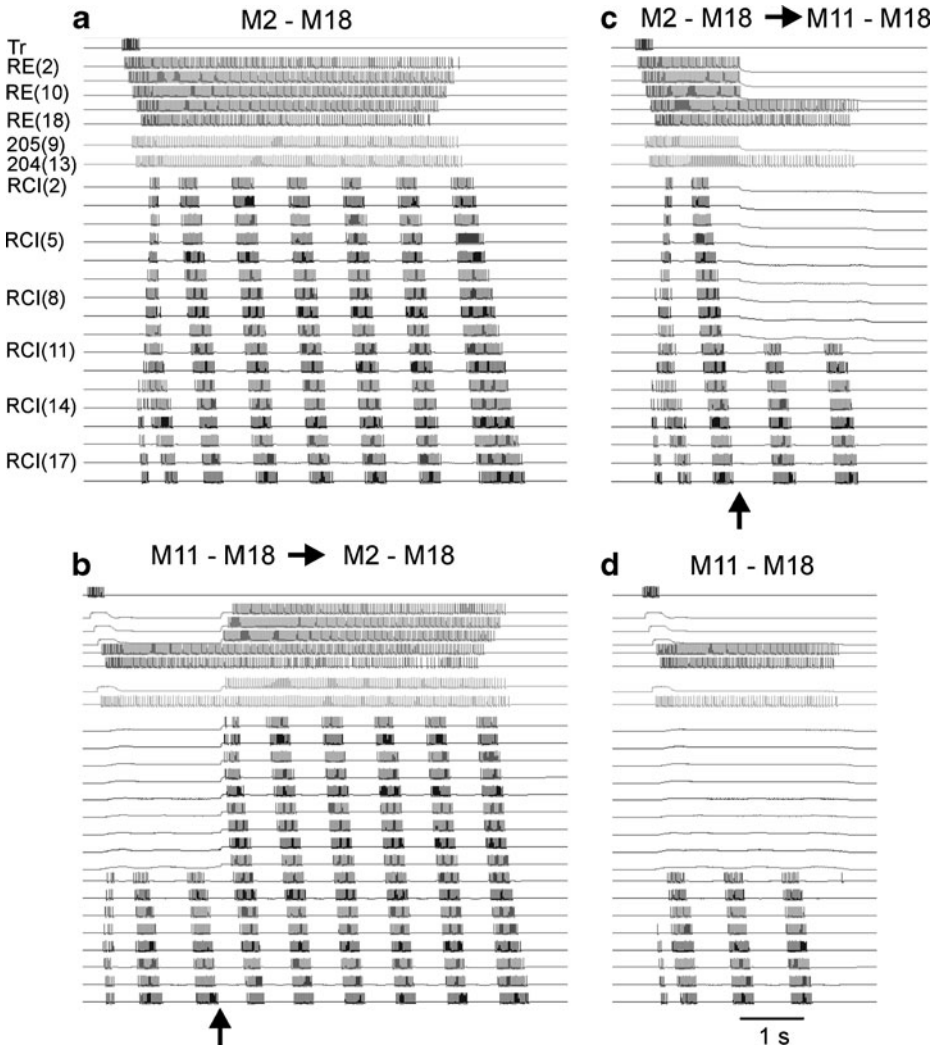

Fig. 14 Dynamics of swim modulation by caudal ganglia in model experiments. **a** Control swim evoked by Tr stimulation in an M2–M18 ganglion chain (7 BPE). **b** Tr activation in a reduced preparation (M11–M18) which was altered to the control condition after two bursts (*arrow*), resulting in an episode with eight bursts. **c** Swim initiation with M2–M18 chain, reduced to an M11–M18 chain after two bursts (*arrow*). **d** Swim initiation in an M11–M18 chain. The relative lengths of swim episodes closely parallel those observed in the leech preparation

4 Discussion

4.1 Summary

We carried out a series of physiological and modeling experiments to explore mechanisms that underlie the sustained excitation that drives swimming activity in nerve cords of the

medicinal leech. Earlier experiments had shown that a set of swim-gating neurons, seven cells 204 and a single cell 205, are the immediate source of excitation to the oscillatory circuit that generates the rhythm [37, 56]. Other physiological experiments showed that swimming can be initiated and terminated by an individual trigger neuron [29, 52]. Experiments on leech nerve cords described here showed that (1) the swim-gating neurons and some cells of the oscillator circuit have long latency, and have mutually excitatory interactions with unidentified intersegmental interneurons, (2) swim duration is a nearly linear function of the length of experimental nerve cords, (3) the duration of swim episodes evoked following any swim episode is a function of the interval between swim termination and initiation of the subsequent episode and (4) that swim duration in the nerve cord is determined not by the length of the cord at initiation, but by continuous interactions among segments. We constructed a computer model that embodies many of the known interactions of the leech swim oscillator circuits. In addition, the model included a set of intersegmental positive feedback loops. Without adjustment of parameters, this model replicated many of the results obtained in the physiological experiments.

4.2 Positive feedback and delayed swim onset

A puzzle about leech swimming is the long latency that often occurs between stimulation and swim onset. Swimming usually begins abruptly, but the latency can exceed 1 s (Figs. 4, 5, and 6). The large latencies, and the lack of sustained excitation in isolated ganglia, imply that the sustaining, excitatory interactions are intersegmental. The implication is that there are complex intersegmental processes intervening between the initiation of excitation and the activation of the swim-maintaining system. Our model studies provide a partial explanation for the curious delay as follows. First, the excitatory interactions among the RE cells are intersegmental (one to three segments in the model) with conduction times of 15 ms. Impulses therefore require up to 45 ms to reach their target cells and up to another 45 ms to return to their source. Second, the interactions between RE neurons are relatively weak with a modest decay time constant (20 ms). Therefore, considerable temporal and spatial summation is required to generate high impulse frequencies. Consequently, positive feedback with a gain of at least one requires coactivation of a large fraction of the RE neurons. In our model, very brief trigger neuron activation can bring about this result, but the process may require up to 300 ms. Had we increased the projection distance of the RE neurons with a concomitant reduction in synaptic strength, one could expect the latency to be even greater. Based on these considerations, we suggest that an intersegmental network of widely projecting excitatory interneurons underlies long-latency swim initiation in the leech nerve cord.

4.3 Swim maintenance via positive feedback

Although our model replicates physiological data qualitatively well, there are quantitative differences. Model swim durations were briefer than those of most nerve cord preparations—7 or 8 BPE in the model compared with the 15 or more BPE in the nerve cord. No doubt manipulation of model parameters can generate longer episodes, but we have not explored the effects of such parameter changes on swim termination and other physiological experiments. The model impulse adaptation time constant, 2.1 s, is

considerably larger than the adaptation time constant (88 ms) in MNs determined from physiological experiments [59]. It remains to be determined whether the time constant for impulse adaptation in swim-gating neurons is large enough to generate protracted swims. The model predicts the existence of mutually excitatory interneurons widely distributed along the nerve cord, perhaps in every segment. Gating cells 204 and 205 are likely participants in mutual excitation as is the oscillatory interneuron cell 208. Each of these cells can trigger swim episodes; hence, they have access to the swim-maintaining circuits. Each also is continuously excited during fictive swimming; hence, each one is driven by the excitatory circuit. One cell 208 soma occurs in every midbody segment and many (perhaps all) receive excitatory synaptic input from the gating cells 204 and 205 [57] and from the cephalic swim excitor neuron cell SE1 [34]. Nevertheless, cell 208 does not directly feed excitation back onto these neurons; similarly, the gating neurons do not interact directly with each other.

Our physiological data strongly suggests that intersegmental circuit properties play a large role in sustaining swim excitation and our model data is consistent with this view. The view that mutually excitatory circuits sustain locomotory behavior is gaining traction. They are thought to contribute to swimming in the *Xenopus* tadpole [27, 61] and are viewed as one source of excitation for burst generation in lamprey swimming [62–64]. We posit that there is at least one additional mutually excitatory interneuron in many, perhaps all, segmental ganglia that excite the swim-gating cells, and this is perhaps cell 208. Searching for such neurons seems warranted given the results presented here. We predict that these neurons would be strongly depolarized during swim episodes and would individually be capable of triggering swim episodes, particularly in the presence of bath-applied serotonin.

4.4 Comparison to the lamprey hemicord

Interestingly, results from the lamprey hemicord are remarkably similar to ones found here. Hemicords are generated by a longitudinal cut down the lamprey spinal cord, removing crossed inhibitory inputs. Following electrical stimulation to the hemicord, an episode of bursting is generated by swim CPGs, but at a much higher frequency due to the removal of the crossed inhibitory projections [63]. Like the results in the leech nerve cord presented here, swim episode duration in the lamprey hemicord is reduced when the length of the cord is reduced and when the interval between swim episodes is reduced [64]. Further, like the leech, the duration of the swim episode is independent of the intensity of the initiating stimulus. In these reduced lamprey preparations, the bursting is thought to be sustained by positive feedback among the excitatory interneurons (EIN; [65]). EINs are shown to excite other EINs, and in one case a mutually excitatory pair was observed [62]. Although it is unclear what role these processes observed in the hemicord play in the intact swim system, it is intriguing that such similar results were observed in the two species.

4.5 Limits of our model

Our model was constructed specifically to determine whether reciprocal excitation, limited by impulse adaptation, can account for our observations regarding initiation, maintenance and termination of fictive swimming in the leech nerve cord. Our model is realistic in that

the unit neurons have multiple compartments that simulate soma, neurite and axon dynamics and which mimic the physiology of identified cells. The circuit interactions approach those identified in the leech, but do not include some potentially important elements, such as the SE1 [34] and Rb31 [35] neurons in the subesophageal ganglion, cells 208 [57] in midcord ganglia, and the recently discovered caudal neuron E21 [66].Cell 208 is broadly excitatory; its properties are combined in our model with those of cell 204 and 205. Trigger neurons are combined into one generic Tr neuron. We have not explored how more detailed modeling of these neurons would alter the output of the simulations. A different parameter set for the model RE neurons would lead to alternative, perhaps even more realistic, results. Nevertheless, there is good qualitative and sometimes quantitative agreement between independent experiments on the leech nerve cord and the neuronal model without parameter tuning. The similarity between experimental results and model output suggest that the time is right for more experimentation rather than for a more detailed model.

4.6 Generality of the results

Although the data are not shown, our model generates similar output when the limiting factor for reciprocal excitation is fatigue in synaptic connections between RE neurons. It seems likely that many neuronal configurations that include the two essential elements of our model, positive feedback and self-limitation, could generate many of the features of our simulation. Specific structures incorporated into our model—positive feedback loops, impulse adaptation, and intersegmental circuits—are already indentified in the leech nerve cord. Reciprocal excitation between IN 208 and a swim-initiating neuron, cell 21, one segment away, was identified in another leech species [67], although not in *Hirudo* (W.O. Friesen, unpublished observations). Such properties are known also in the *Xenopus* swim system [27, 28]. The challenge is to identify positive feedback loops in other preparations. Given such loops, our modeling study points to the properties to be expected of such circuits.

5 Conclusion

Activity that is driven by positive feedback is characterized by an abrupt transition from quiescence to full, maintained activity and abrupt termination when inhibitory processes reduce positive feedback below a gain of 1.0. Our modeling experiments show that positive feedback is a feasible means of generating continuous excitation when activated by a brief trigger. Because model experiments replicate results obtained in a series of experiments not used to construct the model, the model achieves a certain validity. Essential features of our model are positive feedback interactions combined with a cellular, negative property in the form of impulse adaptation. The model is readily generalized by incorporating synaptic fatigue in the positive feedback circuit. In fact, any circuit incorporating positive feedback with negative, self-limiting processes could generate triggered bouts of excitation. Although the excitation in leech swim-gating neurons is thought to arise from circuit properties, these processes can also be found at the level of the individual neuron, for example in plateau potentials mediated by calcium-activated inward currents (I_{CAN}). An additional cellular process that can limit positive feedback loops is the calcium-activated potassium current (I_{KCa}). The activity-sustaining neuronal circuits in animals are likely to incorporate several of these mechanisms.

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