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The brain matters: effects of descending signals on motor control

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Mullins OJ, Friesen WO. The brain matters: effects of descending signals on motor control. *J Neurophysiol* 107: 2730–2741, 2012. First published February 29, 2012; doi:10.1152/jn.00107.2012.—The ability of nerve cords and spinal cords to exhibit fictive rhythmic locomotion in the absence of the brain is well-documented in numerous species. Although the brain is important for modulating the fictive motor output, it is broadly assumed that the functional properties of neuronal circuits identified in simplified preparations are conserved with the brain attached. We tested this assumption by examining the properties of a novel interneuron recently identified in the leech (*Hirudo verbana*) nerve cord. This neuron, cell E21, initiates and drives stereotyped fictive swimming activity in preparations of the isolated leech nerve cord deprived of the head brain. We report that, contrary to expectation, the motor output generated when cell E21 is stimulated in preparations with the brain attached is highly variable. Swim frequency and episode duration are increased in some of these preparations and decreased in others. Cell E21 controls swimming, in part, via excitatory synaptic interactions with cells 204, previously identified gating neurons that reliably initiate and strongly enhance leech swimming activity when the brain is absent. We found that in preparations with the brain present, the magnitude of the synaptic interaction from cell E21 to cell 204 is reduced by 50% and that cell 204-evoked responses also were highly variable. Intriguingly, most of this variability disappeared in semi-intact preparations. We conclude that neuronal circuit properties identified in reduced preparations might be fundamentally altered from those that occur in more physiological conditions.

descending control; leech; locomotion; neural circuits; rhythmic behavior

IT IS WELL-KNOWN THAT NERVOUS systems generate rhythmic fictive locomotion in the absence of peripheral sensory feedback or descending inputs from the brain. The circuitry necessary to create these patterns is well-identified in many species and is modulated by both proprioceptive feedback and descending projection neurons (Mulloney and Smarandache 2010; Ryczko et al. 2010; Stein 2009). Owing to the simplicity of reduced preparations, the functional assignments of many spinal or nerve cord cells have been deduced from preparations lacking the brain (Marder and Bucher 2007; Mullins et al. 2011a). To understand fully neuronal control of behavior, it is important to determine whether the circuit properties described in reduced preparations faithfully replicate those in more intact nervous systems. However, few systematic comparative studies have evaluated neuronal circuits with and without an intact brain.

The functional consequences of experimental manipulations performed on animals in one state are not necessarily replicated in other contexts (Palmer and Kristan 2011). For example, serotonin differentially alters the crayfish escape circuit depending on the social status of the animal (Yeh et al. 1996). Furthermore, individual cells can elicit different behaviors depending on the sensory environment. In the cricket, stimulating the interneuron Int-1 elicits avoidance behavior during flight, but no movement is detected if the cricket is grounded (Nolen and Hoy 1984). The leech projection neuron R3b1 initiates swimming when the animal is submersed in water but crawling if water levels are low (Esch et al. 2002). These versatile neural responses to similar inputs suggest that the behavioral consequences of stimulating high-level neurons might be influenced by the brain.

In medicinal leeches, the head brain suppresses swimming by impeding swim initiation and reducing swim duration (Brodfuehrer and Friesen 1986). In this system, control of swim expression is mediated by trigger and gating neurons (Kristan et al. 2005). A recently identified, posteriorly located neuron, cell E21, has trigger and gating functions and outputs to identified gating neurons, cells 204 (Mullins et al. 2011b). In preparations lacking the head brain, stimulation of E21 or 204 during swimming decreases cycle period and extends swim duration. It is unknown whether these functional effects persist in preparations that include the brain.

To determine whether the actions of swim-circuit neurons are altered by the head brain, we compared the responses evoked by stimulating cells E21 and 204 in leech nerve cord preparations with and without the brain. Surprisingly, we found that in isolated preparations with the brain attached, both cells had inconsistent, and sometimes opposing, effects on fictive swimming. Furthermore, the synaptic interaction between these neurons was strongly contingent on the presence of the brain. We propose that the variability of stimulus-evoked responses in intact nerve cords reflects a range of internal brain states. This view is supported by further experiments on semi-intact, brain-attached preparations in which consistent swim responses to E21 input were obtained, likely as a consequence of state-defining sensory input. We conclude that removing the brain alters the functional effects of cells E21 and 204 by removing access to swim-suppressing circuitry.

METHODS

Leech Nervous System and Terminology

The leech nervous system comprises 21 segmental (midbody) ganglia that are bordered by rostral/head (H) and caudal/tail (T) brains. Midbody ganglia are denoted by the letter "M" (midbody) and their ordinal number, with the anterior-most midbody ganglion named "M1" and the most posterior, "M21." The brain comprises the supra- (SupraEG) and subesophageal (SubEG) ganglia. Isolated and semi-intact preparations are identified by the span of their nervous system; for example, "H-T" refers to preparations that include the entire nerve cord, from head to tail brain. The nerve cord of semi-intact preparations without the head brain and ganglion M1 is denoted by "M2-T."

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For brevity, "brain" refers to the head brain; the tail brain is intact in all preparations.

Leeches

Experiments were performed on adult medicinal leeches, *Hirudo verbana*, supplied by Niagara Leeches (Cheyenne, WY) or Leeches USA (Westbury, NY). Leeches are 10–15 cm when fully extended. Leeches were maintained in aquaria in a temperature-controlled room on a 12:12-h light-dark cycle at 18–21°C. Before dissection, leeches were anesthetized with 4°C normal leech saline containing (in mmol/l) 115 NaCl, 4 KCl, 1.8 $CaCl_2$, 2 $MgCl_2$, and 10 HEPES buffer (pH 7.4) (Friesen 1981). The nervous system in all preparations was superfused with either normal leech saline or saline containing serotonin (50 μM) for the duration of the recordings.

Preparations

The experiments were carried out on preparations of the isolated nerve cord and on semi-intact leeches. All dissections were performed in a wax-bottomed dish filled with saline and surrounded by ice.

Isolated. For isolated preparations, the ventral nerve cord and some dorsal-posterior (DP) nerves were dissected free of the surrounding tissue and body wall. In some preparations, the brain and M1 were removed. The nerve cord was placed in normal leech saline in a shallow glass-bottomed dish covered with a thin layer of resin and secured by magnetic pins. The sheath was removed from the ventral side of the appropriate ganglia for intracellular recordings.

Semi-intact. In all semi-intact preparations, anterior segments of the body wall up to M11 were intact, whereas the body wall normally innervated by ganglia M11-T was removed. We used four variations of the semi-intact preparations with differing amounts of intact nerve cord and innervation: *1*) H-T, with the rostral sucker innervated; *2*) H-T, with the rostral sucker denervated; *3*) H-T, with anterior body wall (normally innervated by H-M5) denervated; and *4*) M2-T, with the head and M1 detached. Because differences in the responses to E21 stimulation among the H-T preparations were small, we combined their data under the label "H-T" semi-intact preparations.

Threads to suspend the intact portion of the leech were attached to denervated body flaps at M11. In addition, a thread was either tied to the denervated rostral sucker or two threads were attached to either side of the denervated M2 body wall. Semi-intact preparations were placed into a saline-filled dish with a deep well to accommodate the swimming movements of the anterior end of the leech and a shallow portion for intra- and extracellular recording from the posterior nerve cord (see Fig. 4*A*, *inset*). During "swim-enhancing" conditions, the anterior body wall of preparations undulated freely in the well; in other experiments, the well was filled with small pebbles. In this latter condition, the anterior body wall made contact with the substrate leading to enhanced expression of crawling behavior.

Electrophysiology

In isolated preparations, swimming activity was monitored via extracellular suction electrodes placed on several DP nerves; these contain, among others, the axon of motor neuron cell DE-3, which generates the second largest spike in DP records. The largest motor neuron spike is that of the shortener (L cell), which is usually silent in recordings from isolated nerve cord preparations. Rhythmic bursting in cell DE-3, with a cycle period between 0.5 and 2 s, is indicative of fictive swimming (Kristan and Calabrese 1976). Although recordings were taken from multiple nerves, for space reasons only one nerve recording is presented. For brevity, fictive swimming is simply called swimming in this manuscript. Suction electrodes were also used to deliver shocks (trains of 2–4 V, 5-ms pulses at 25 Hz) to DP nerves to initiate swimming. In semi-intact animals, DP nerve recordings were used in conjunction with visual observation to identify crawling and swimming.

Sharp glass microelectrodes for intracellular recording were pulled with a P-87 Flaming/Brown Micropipette Puller (Sutter Instruments, Novato, CA). They were filled with 2.7 M potassium acetate and 20 mM KCl and had resistances of 30–60 MΩ. Intracellular recording and current injection were accomplished with an Axoclamp 2A amplifier (Axon Instruments, Sunnyvale, CA) in bridge mode. Extracellular signals were amplified by preamplifiers and then, along with intracellular signals, further amplified and digitized with PowerLab and displayed with Chart software (ADInstruments, Colorado Springs, CO). Intracellular recordings were obtained from the somata of neurons identified by location, size, and electrical and functional properties.

Procedures

Isolated preparations. To evaluate the effect of cell E21 on swim maintenance, we recorded from cell E21 while monitoring fictive swimming through extracellular suction electrodes. Swim episodes were initiated by trains of pulses applied to a DP nerve, with a constant latency of 60 s between the end of one episode and the initiation of a second. (This interval was reduced to 40 s in preparations that generated a high level of spontaneous swimming to decrease the probability of spontaneous swims occurring between evoked episodes.) Each swim episode was designated either as a "control" swim (when E21 was not stimulated; impulse frequencies $<$3 Hz) or a "depolarized" swim (when E21 was injected with depolarizing current during the episode). For most experiments, the beginning of current injection was timed to occur during the middle third of an episode, as estimated from control swim lengths. In some trials, used only to analyze swim duration, current was injected before the third DP nerve burst. Current injection was terminated when swim episodes ended or their duration was at least double that of the control length (this cutoff was implemented to reduce damage to the cell from prolonged depolarizing injections). The amplitude of current was adjusted to obtain impulse frequencies $>$20 Hz and up to 55 Hz, as measured during the 1st 3 s of the current injection. [During prolonged depolarization of cell E21 ($>$5 s), a high level of firing often could not be maintained.] A linear regression comparing the change in swim duration and cycle period to cell E21 impulse frequency was insignificant ($P = 0.16$ and 0.15, respectively) so data from all impulse frequencies were grouped. Similar experiments were performed with penetrations of, and current injection into, cells 204. Serotonin saline, to enhance swim initiation, was used for H-T preparations (Willard 1981). In most preparations, swim duration and cycle period were evaluated, but in some experiments only one variable was measured. This occurred for 1 of 2 reasons. *1*) Control swims were very long with unpredictable durations. We could not depolarize cell E21 for prolonged periods without damage, so only changes in cycle periods were measured. *2*) The swim terminated shortly after cell E21 or 204 stimulation, precluding cycle period analysis.

Semi-intact preparations. The procedures outlined above were repeated with semi-intact preparations. (Serotonin saline was applied to the isolated, posterior portion of H-T, but not M2-T, preparations to replicate conditions for isolated preparations.) We used recordings from the isolated portion of the nerve cord to monitor swimming activity. However, we also made visual observations of the undulating body wall to verify that movements and DP records were coupled. To determine what behaviors cell E21 can initiate, the intact portion of semi-intact leeches were placed on a pebble substrate with the level of the fluid above the substrate adjusted during the experiment. In these preparations, the entire nerve cord was intact (H-T), but the anterior sucker was denervated. Visual observation of the leech body together with DP nerve recordings were used to identify crawling activity. DP nerve recordings during crawling reveal slow, rhythmic bursting of motor neuron DE-3 with a cycle period of 5–25 s; simultaneously, the

leech body engages in elongation and contraction cycles. These cycles are coordinated with attachment and release of the suckers in intact animals (Puhl and Mesce 2010).

Cell E21-to-cell 204 interactions. To measure the strength of the excitatory connection from cell E21 to cell 204, we obtained simultaneous intracellular recordings from the two cells. All cell 204 recordings were obtained from either M10 or M11. We injected 1- to 2-s depolarizing current of varying amplitude into cell E21 and recorded the impulse frequency in cell 204 and in cell E21. To ensure a consistent baseline activity level in cell 204, tonic current was injected into cell 204 to maintain a "resting" firing rate of 2–8 Hz. To make the comparison between H-T and M2-T preparations, recordings were first completed in the H-T preparation ($n = 5$). In four experiments, ganglia H-M1 were subsequently removed, and similar experiments were carried out in the reduced M2-T nerve cord. Because the surgery dislodged the electrode in two such preparations, these experiments were completed with a second, undamaged cell 204.

Data Analysis

Swim duration and cycle period. Swim duration was measured by enumerating cell DE-3 bursts during swim episodes. Changes in cycle period due to cell E21 or 204 stimulation were determined by normalizing the periods of the first three cycles during current injection by the average value of the two swim cycles preceding current injection. We did not analyze the cycle period that occurred during the initiation of the current injection unless the stimulation "paused" the swim during that cycle. A pause was defined as an 80% increase in cycle period from the preceding cycle. For control swims, analogous analyses were performed. Cycle period was obtained using the RAS MATLAB program (Hocker et al. 2000).

In one statistical analysis, we obtained averages from control and depolarized-swim trials from each experiment. These averaged values were compared using a paired *t*-test. For another analysis, to quantify the variability, we performed comparisons between the control and depolarized swim groups within individual experiments. In this case, swim duration and cycle period values in each experiment were compared using a Student's *t*-test, and the effect of E21 and 204 stimulation was determined for that particular preparation.

Cell E21-to-cell 204 interactions. Impulse frequencies in cells E21 and 204 were analyzed with MATLAB (The MathWorks, Natick, MA). Because each cell 204 has two independent impulse-initiating zones, intracellular records show two trains of spikes. To compute spike frequency in cell 204, we counted all spikes; simultaneous impulses (resulting in a single large spike) were both scored. Impulse frequencies in cells E21 and 204 during the 1st s of E21 stimulation were subjected to a linear regression analysis. The slopes of the lines from this analysis in the two conditions, with and without the head brain, were compared, and the r^2 values from these analyses were compared using the Fisher *z*-transform.

Statistical analyses were completed using Prism 5 (GraphPad, La Jolla, CA). This program was also used to generate all graphs. Results are reported as means with standard error.

RESULTS

Stimulation of Cell E21 in Preparations Lacking the Brain

Previously, we characterized the properties of a novel interneuron, cell E21, which lies at an intermediate position in the sensory-motor pathway, in preparations with the brain removed (Mullins et al. 2011b). Experiments on swimming have often utilized these reduced preparations because the head brain reduces the reliability of swim initiation and promotes irregular bursting (Brodfuehrer and Friesen 1986). However, here we aim to quantify behavioral responses that occur in the presence of descending inputs and to evaluate the function of cell E21 in a preparation that more closely resembles the intact animal. We first repeated experiments examining the effects of stimulating cell E21 on swim maintenance during ongoing swim episodes in preparations with the head brain and first midbody ganglion removed; such preparations extend from M2 to T (Fig. 1*A*). Swim maintenance refers to the processes that sustain swim episodes, and this system controls swim duration and cycle period (Friesen et al. 2011). As expected, continuous depolarizing current injections into cell E21 during swimming caused an increase in swim duration (Fig. 1*B*). Furthermore, this stimulation elicited an increase in burst frequency of segmental excitatory motor neurons (Fig. 1*C*), a decrease previously shown to be inversely correlated with E21 impulse frequency (Mullins et al. 2011b). We have observed enhancement of swim maintenance by cell E21 in >50 M2-T nerve

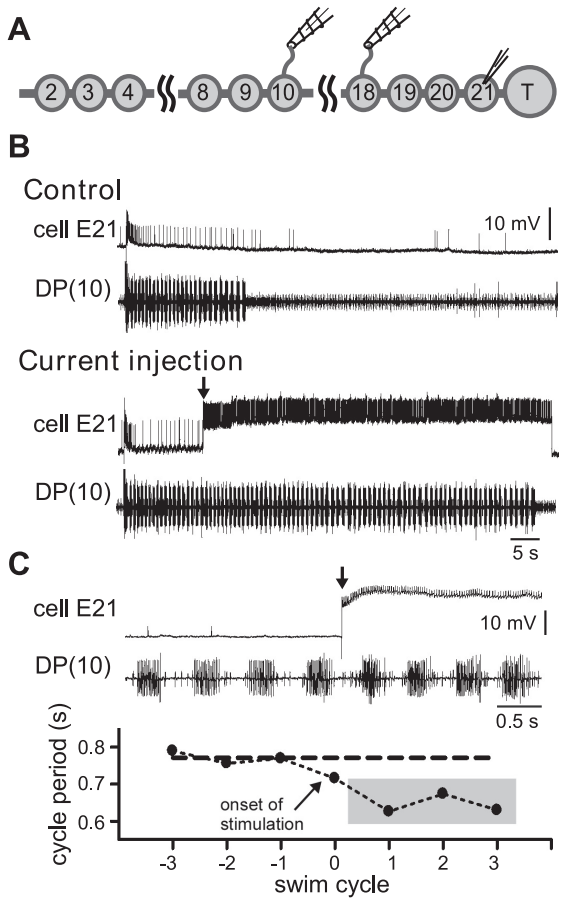

Fig. 1. Cell E21 excitation enhances swimming in midbody ganglion 2 through tail brain (M2-T) isolated preparations. *A*: schematic of M2-T isolated nerve cord. Head (H)-M1 is removed, and suction electrodes placed on dorsal-posterior (DP) nerves monitor swimming (M10) or deliver a shock for swim initiation (M18). A microelectrode is used to record from cell E21 (M21). *B*: control swim in a M2-T preparation (*top* 2 traces). Current injection into E21 (beginning at arrow, 3rd trace) during an ongoing swim extends swim duration (*bottom* trace). Bursts in DP traces here and elsewhere comprise spikes from motor neuron cell DE-3. Note that the time scale is 5 s, hence bursts are condensed in appearance. *C*: stimulation of cell E21 (*top* trace, at arrow) during an ongoing swim episode (2nd trace) decreases cycle period in a M2-T preparation. Graph shows the explicit periods of the cycles displayed above, with the "0" swim cycle indicating the cycle during which current injection was initiated. Gray shading indicates swim cycles when cell E21 was stimulated. The swim cycle at stimulus initiation is not shaded. Thick dashed line approximates cycle period before E21 stimulation.

cord preparations (quantification to follow). Therefore, in preparations of the isolated nerve cord with the head ganglia removed, depolarization of cell E21 has reliable excitatory effects on swimming.

Control of Swim Duration

The inhibitory effects of the head brain on swimming occur in the intact animal as well as the isolated nerve cord (Mullins et al. 2011a; Schlüter 1933), but it is unknown how the brain alters the locomotor response to stimulation of command-like neurons. It is of interest, therefore, to determine whether the excitatory swim response during cell E21 stimulation persists in presence of the brain. To this end, intracellular depolarizing current injections were given to cell E21 during ongoing swim episodes in preparations of the complete isolated nerve cord (H-T; Fig. 2*A*), and responses were compared with those obtained in M2-T preparations (Fig. 1*A*). Unexpectedly, cell E21 stimulation in H-T preparations often elicited responses that were the reverse of those obtained when the brain is not present. Thus, strikingly, in some H-T preparations, depolarization of cell E21 shortened or even terminated swim episodes (Fig. 2*B*), a response never observed with the brain removed. In other H-T preparations, stimulation of cell E21 prolonged the episodes (Fig. 2*C*). In still other preparations, E21 excitation had no obvious effect on swim duration (Fig. 2*D*).

We compared averaged swim durations of control swim trials, in which no current was injected, with those of depolarized-swim trials, in which E21 was depolarized by current injection during ongoing swimming, in M2-T and H-T preparations. This comparison demonstrates a clear difference between the preparation types, with a significant increase in swim duration in M2-T preparations [25.8 vs. 54.7 burst per episode (BPE), control and current injection, respectively; $P = 0.003$] but not in full-length (H-T) nerve cords (18.1 vs. 17.5 BPE, control and current injection, respectively; $P = 0.71$; Fig. 2*E*). However, the averaged H-T data did not capture the variability observed in these preparations. When the durations of control and E21 depolarized-swim trials are directly compared within each individual experiment (Fig. 2*F*), the difference between these preparations becomes clear. Namely, in 3 of 11 experiments, cell E21 stimulation significantly increased swim duration. In 2 other H-T preparations, E21 stimulation had the opposite effect, significantly decreasing swim duration. Finally, in the remaining H-T preparations ($n = 6$), current injection into cell E21 had no significant effect on swim duration (as in Fig. 2*D*). These latter results might suggest that E21 excitation did not influence swim duration in these preparations; however, an alternative interpretation is that our statistics obscure real effects because of high trial-to-trial variability in swim duration. Overall, the data indicate that in the presence of the head brain, E21 stimulation can either reverse or mimic the effects on swimming observed in brain-removed preparations, with the swim response varying widely across preparations when the brain is intact.

Control of Cycle Period

In many species, individual neurons or cell populations that control the duration of locomotor bouts also control the period of individual cycles (Arshavsky et al. 2010; Böhm and Schildberger 1992; Deliagina et al. 2000; Dembrow et al. 2003;

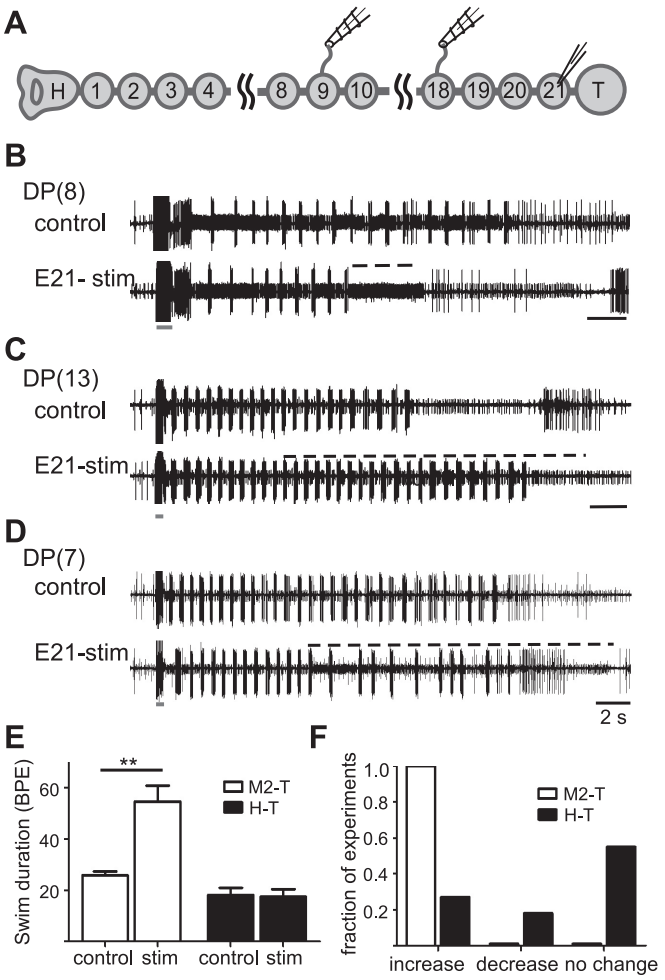

Fig. 2. Swim duration modulation by excitatory stimulation of cell E21 in H-T preparations. *A*: preparation. Extracellular nerve recordings sites vary in location. *B–D*: examples of the swim response to cell E21 stimulation (E21-stim) in H-T preparations. The *top* trace in each pair of traces is the control swim (no current injection); in the *bottom* traces, depolarizing current was injected into cell E21 (indicated by dashed lines) during fictive swimming. Swims were initiated by shock applied to a DP nerve (gray bars). These examples were taken from preparations in which cell E21 stimulation significantly decreased swim duration (*B*), increased swim duration (*C*), or had no significant effect on swim duration (*D*). *E*: averaged results from control and current-injected (stim) trials across all experiments. In M2-T, brain-removed preparations, current injection reliably increased swim duration, but effects were variable in H-T, brain-attached preparations, with no overall trend. *F*: effect on individual preparations. E21 stimulation increased swim duration in every M2-T preparation ($n = 7$). Swim duration was significantly increased ($n = 3/11$), decreased ($n = 2/11$), or unchanged ($n = 6/11$) by E21 stimulation in H-T preparations. Short bars indicate 0 values. Data in *B–D* are from 3 different leeches. BPE, bursts per swim episode. 2 s Applies to all scales. $^{**}P < 0.01$.

Hedwig 2000; Sirota et al. 2000; Weeks and Kristan 1978). Such dual actions are seen when cell E21 is depolarized in preparations lacking the head brain (Mullins et al. 2011b). We therefore performed experiments to determine whether the presence of the head brain alters the influence of cell E21 on swim cycle period in the same manner as it does on swim duration.

In some H-T preparations, stimulating E21 during an ongoing swim episode decreased cycle period (Fig. 3*A*) as it does in M2-T preparations. More commonly, however, E21 stimulation increased cycle period; the time interval between bursts

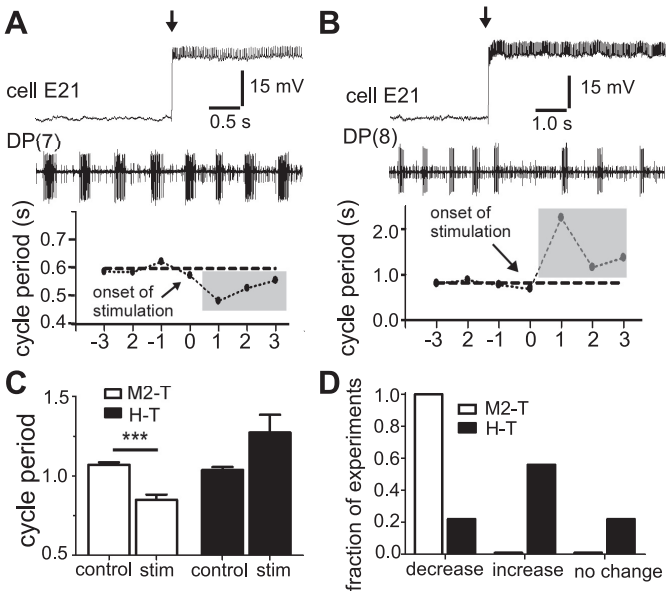

Fig. 3. Effects of cell E21 stimulation on cycle period. *A*: decreased cycle period. Current injection into cell E21 (*top* trace, at arrow) decreased cycle period (extracellular recording, *bottom* trace) in a H-T preparation. Explicit values of cycle period are shown in the graph below the traces. Gray shading indicates cycles during cell E21 stimulation; thick dashed line approximates the periods before stimulation. *B*: increased cycle period in a H-T preparation. Data are as in Fig. 2*A*. Data from *A* and *B* are from different preparations. *C*: stimulation of cell E21 during swimming (stim) decreased cycle period in M2-T preparations. The overall mean cycle periods were not significantly different in H-T preparations between the groups ($P = 0.081$). Cycle period is normalized. *D*: effect on individual preparations. E21 stimulation decreased cycle period in every M2-T preparation ($n = 7$) but only in 2/9 H-T preparations. In 5/9 H-T preparations, this stimulation increased cycle periods. In 2 preparations, there were no statistically significant effects. $***P < 0.01$.

elicited by such stimulation was often so large that this effect might be considered a pause in the swim episode (Fig. 3*B*). To quantify results from these experiments, we normalized the periods of the first three cycles during current injection by the mean period of the two cycles preceding the current injection. Thus values <1 indicate a decrease in cycle period, and values >1 denote period increases. As expected, in M2-T preparations, cell E21 excitation decreased cycle period significantly (Fig. 3*C*; 1.07 and 0.86, respectively, for control and current injection groups; paired *t*-test, $P = 0.0007$). Comparison of control and depolarized-swim trials within individual experiments show this effect was consistently observed in all M2-T preparations (Fig. 3*D*). However, in H-T preparations, the overall mean values of the control and depolarized groups from all experiments were not significantly different (Fig. 3*C*; 1.04 and 1.27, respectively, for control and current injection trials; $P = 0.081$). However, evaluation of individual H-T experiments showed that in over half ($n = 5/9$) of the experiments, cycle period was significantly increased by E21 stimulation compared with control trials. Nevertheless, a significant decrease in cycle period occurred in only two of nine of these preparations (Fig. 3*D*). Cycle period was not altered significantly by cell E21 activity in the remaining two preparations. These data show that in isolated preparations, cell E21 elicits stereotyped responses in swim maintenance parameters with the brain removed but variable and opposing responses with the brain attached. Therefore, the functional consequences of the activity in a command-like neuron can be altered by descending inputs.

Interaction Among Cell E21, the Brain, and the Environment

Animal behavior occurs within the context of a changeable, unpredictable external environment that modifies behavioral expression through a broad range of sensory modalities. The effect of these inputs on neural activity patterns depends strongly on the internal state of the animal. The experiments described above were performed on isolated nervous systems that, deprived of sensory input, lack an external basis for behavioral choice. The seemingly random variability we observed in response to depolarization of cell E21 might arise from this lack of guiding information. We have suggested previously that cell E21 may serve as one element underlying escape locomotion (Mullins et al. 2011b), in part because it fires in response to mechanical sensory input that elicits avoidance behaviors, such as shortening or swimming (Kristan et al. 1982). We hypothesized that there are specific environments in which increasing the vigor and duration of a swim episode would be the appropriate response to external threats, such as if the leech were immersed in water and lacking contact with any substratum. If activation of cell E21 does indeed underlie escape responses in the intact animal, and the variable responses in H-T isolated preparations represent alternative state-dependent escape behaviors, E21 stimulation in the described environment would be predicted to enhance swim maintenance reliably.

To determine whether this hypothesis is correct, the procedures performed on the isolated nervous system were applied to semi-intact leech preparations. In these experiments, the anterior, nearly intact portion of the leech was suspended in a saline-filled well, a swim-enhancing environment (Esch et al. 2002), whereas the posterior half of the body was removed (Fig. 4*A*). Preparations had either the head brain attached (H-T semi-intact) or removed (M2-T semi-intact). H-T semi-intact preparations had varying numbers of ganglia innervating the body wall, but as responses were similar across the preparations, their results were combined (see METHODS). The exposed posterior nervous system provided access to the soma of cell E21 for intracellular electrodes as well as to DP nerves for extracellular recordings.

We found that the effects of depolarizing cell E21 in the H-T semi-intact preparations in the swim-enhancing environment differed remarkably from those of the isolated H-T preparations. Variability in the sign of the swim response was nearly eliminated, and E21 excitation reliably extended swim duration (Fig. 4*B1*) and decreased cycle period (Fig. 4*C1*). Indeed, the effect of current injection into cell E21 in H-T semi-intact preparations was essentially that observed in isolated M2-T nerve cords. Summaries of the data for the semi-intact preparations are illustrated in Fig. 4, where the pairwise comparisons are between swim duration (Fig. 4*B2*) and cycle period (Fig. 4*C2*) in control trials (no current injection) and trials where E21 was depolarized via current injection. In all individual M2-T and H-T experiments, swim duration was significantly increased in depolarized-swim trials compared with control trials ($P < 0.05$ for all within-experiment comparisons). In 10 out of 12 H-T experiments, stimulating E21 significantly decreased cycle period compared with control trials. In 1 of the

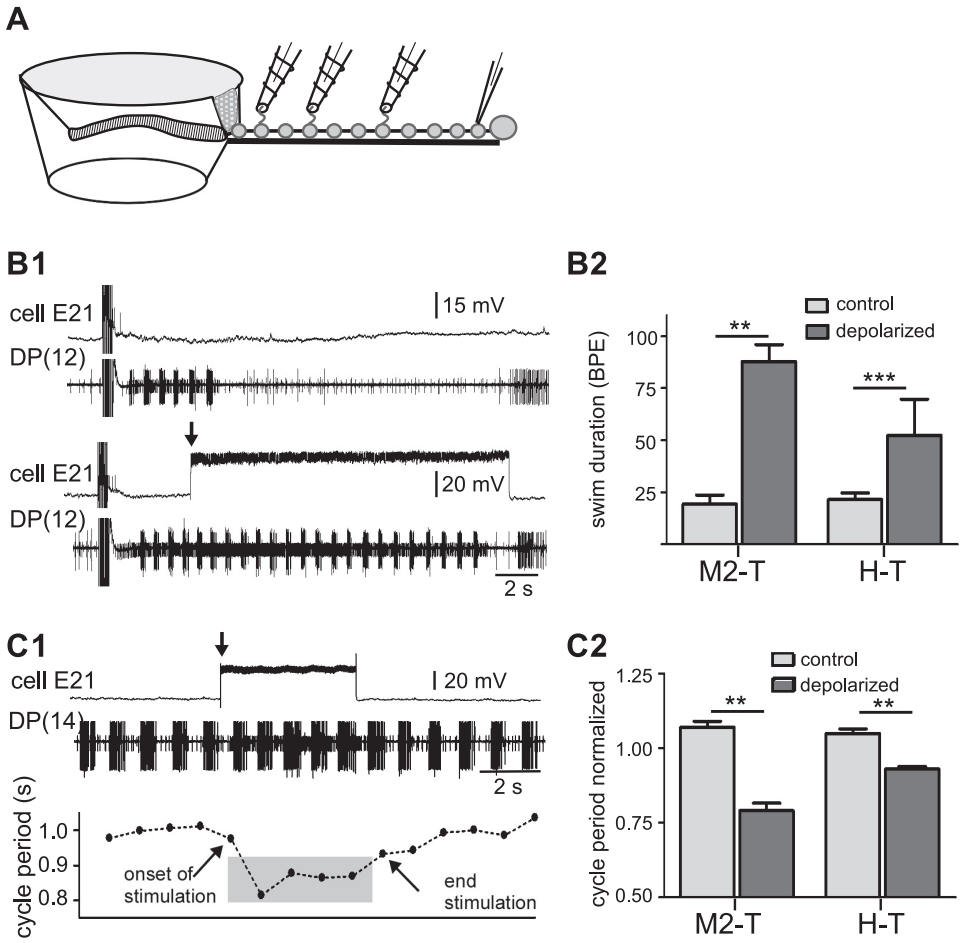

Fig. 4. Swim response variability to E21 stimulation is greatly reduced by sensory environment. *A*: semi-intact preparation. Anterior portion of the leech is partially intact and suspended in a well. The posterior end is isolated. *B1*: control of swim expression by cell E21 in an H-T semi-intact leech suspended in deep water. *Top* 2 traces show a control swim episode. When cell E21 is stimulated by current injection (3rd trace, at arrow), swim duration increases (*bottom* trace). *B2*: bar graph demonstrating the increase in swim duration from E21 stimulation (dark gray bars) in all semi-intact M2-T and H-T preparations compared with control swims (light gray bars). M2-T, $n = 3$; H-T, $n = 9$. *C1*: current injection into cell E21 (*top* trace, at arrow) during an ongoing swim episode (2nd trace) decreases the cycle period. Graph shows explicit periods of cycles in the above trace; those that occurred completely during cell E21 stimulation are shaded in gray. *C2*: bar graph demonstrating changes in cycle period from E21 stimulation, as in *B2*. Data from 1 H-T preparation in which cell E21 stimulation significantly increased cycle period are not included. M2-T, $n = 3$; H-T, $n = 11$. $^{**}P < 0.01$, $^{***}P < 0.001$. Bars are SE.

remaining 2 experiments, E21 stimulation resulted in a nonsignificant decrease in cycle period ($P = 0.057$). In the other remaining H-T experiment, E21 stimulation resulted in a significant increase in cycle period. With the exception of this outlier, these results show that the variability in the swim maintenance response to E21 stimulation observed in isolated systems is absent in semi-intact leeches suspended in a swim-enhancing environment.

To compare results from the various semi-intact and isolated H-T and M2-T preparations, a summary of the swim maintenance changes induced by the injection of depolarizing current into cell E21 are presented in Fig. 5. In each panel, pairs of data points connected by a line represent a single experiment in the given condition. For each experiment, the mean swim duration or cycle period for the E21-depolarized trials is normalized by the mean respective parameter for the control trials. Therefore, the mean control value for each experiment equals 1. As these diagrams clearly reveal, cell E21 stimulation had a consistent enhanced swim duration or cycle period (with 1 exception) in *1*) isolated M2-T, *2*) semi-intact M2-T, and *3*) semi-intact H-T preparations. However, in isolated H-T experiments, the effects on swim maintenance were variable and often swim-suppressing.

Control of Swimming by Swim-Gating Neurons

Cell E21 has direct inputs to all seven swim-gating neurons cells 204 and the homologous cell 205 (Mullins et al. 2011b). These eight swim-gating neurons are essential for the initiation and maintenance of swimming (Weeks and Kristan 1978). The cycle period of swimming is inversely correlated with the firing frequency of cells 204 (Debski and Friesen 1986), and extended depolarization of these cells via current injection is known to extend swim duration. However, those studies were performed in preparations with the head brain detached. Because cells 204 are postsynaptic to cell E21, we examined the possibility that the variable swim responses following cell E21 activation are mediated by interactions of the brain with the gating neurons.

We recorded intracellularly from a cell 204 in either segmental ganglion M10 or M11. Cells 204 fire at a high rate during swimming (Weeks and Kristan 1978) but tend to hyperpolarize during prolonged recording sessions (O. J. Mullins and W. O. Friesen, unpublished observations). Therefore, to minimize differences in trials, we injected current into cell 204 before swim initiation to set its firing level <3 Hz. Although rare, injection of depolarizing current into cell 204 in M-T preparations sometimes suppressed swim maintenance (Fig. 6*A*). A significant decrease in swim duration was observed in one out of nine H-T preparations, with a significant increase in cycle period occurring in one out of five intact isolated nerve cord preparations (Fig. 6*B*). Equally surprising, this depolarization led to a significant increase in swim duration and a decrease in cycle period in only one out of nine and one out of five preparations, respectively. In the remaining preparations, current injection into a cell 204 had no significant effect. Some of this lack of significance might be attributed to modest changes in swim

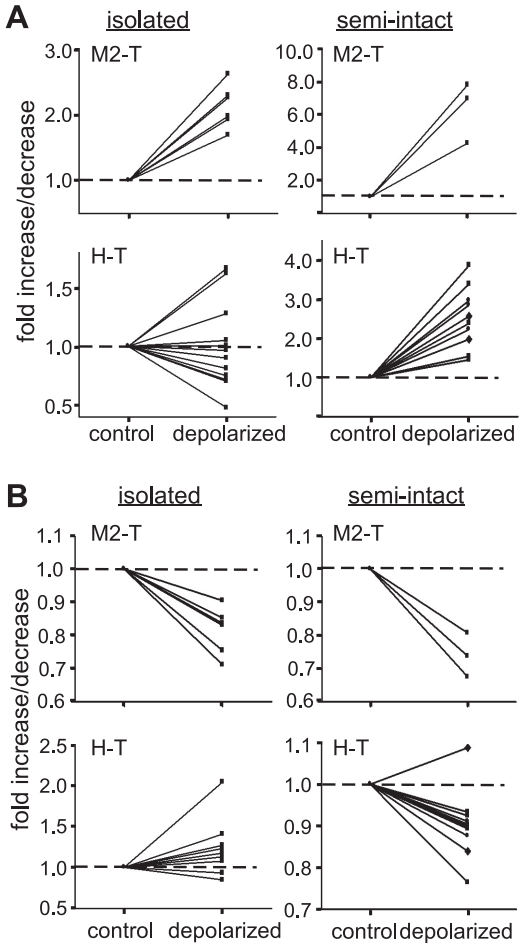

Fig. 5. Summary of E21 effects on swimming in all preparations. *A*: fold increase in swim duration in E21-depolarized trials normalized to control trials in M2-T (*top* row) and H-T (*bottom* row) and isolated (1st column) and semi-intact (2nd column) preparations. Each pair of points connected with a line represents 1 experiment, and values >1.0 represent an increase in swim duration. Unlike the other conditions, E21 excitation sometimes decreased swim duration in isolated H-T preparations. *B*: fold increase in cycle period due to E21 excitation. Data are plotted as in *A*. Values <1.0 indicate a decrease in cycle period. E21 excitation often increased cycle period in H-T isolated preparations but decreased cycle period in all other conditions, with 1 outlier in the semi-intact H-T condition.

maintenance that were not captured in statistical analysis. In all M2-T preparations (brain absent), cell 204 excitation had the expected effects: increased swim duration ($n = 9$) and decreased cycle period ($n = 3$; Fig. 6*B*).

These experiments suggest that some of the variability observed in swimming due to cell E21 stimulation is indirect and is mediated through cell 204 activity. However, subsequent experiments demonstrated that some of the effects of cell E21 must be mediated independently of cells 204. We showed recently that the two ganglia of the head brain have opposing effects on swim maintenance parameters in the leech, with the SupraEG providing the majority of inhibition to the swim system and the SubEG providing overall excitation (Mullins et al. 2012). We report here that in isolated nerve cords with the SupraEG removed but the SubEG intact (SubEG-T), the variable effects of cell E21 stimulation on swim maintenance persist. Using the same set of protocols described earlier, we found that swim duration was inhibited by cell E21 stimulation in two out of four preparations and cycle period was increased in one out of six preparations (data not shown). Conversely, excitation of cell 204 had only excitatory effects on cycle period in these preparations ($n = 3$; data not shown). (We tested the effects of cell 204 on swim duration in only 1 SubEG-T preparation, in which it significantly extended the swim episode, because swim durations tend to be long and unpredictable in duration in these preparations, and cell 204 can receive depolarizing current injections for relatively short durations without damage.) Therefore, most or all of the variable effects of cell 204 must be mediated by the SupraEG, whereas both divisions of the head brain appear to mediate the variable effects of cell E21.

Synaptic Interactions Between Cells E21 and 204

The head brain clearly has a strong impact on the ability of cell E21 to initiate behavior and influence swim maintenance. Additionally, the action of cell 204 activity is altered by the intact head brain. A further issue is whether the presence of absence of the head brain controls the cell 204 response to cell E21 excitation. To test whether this direct interaction is modified by the brain, we obtained simultaneous recordings from cells 204 and E21 in isolated H-T and M2-T preparations. Cell E21 was injected with brief depolarizing current pulses (1–2 s) of varying intensities while the impulse frequency of a cell 204 was monitored. The impulse frequency for the 1st s of stimulation in both cells was calculated. We examined 5 H-T preparations, obtaining a total of 97 individual trials, and 4 M2-T preparations with a total of 95 individual trials. In both types of preparations, cell E21 stimulation increased impulse activity in cell 204 (Fig. 7, *A* and *B*), with a linear relationship between the impulse frequencies of the 2 neurons (Fig. 7, *C* and *D*; linear regression, $P < 0.001$ for both H-T and M2-T preparations). However, the slope of regression lines for the 2 preparations differed significantly (brain-removed slope, 0.65;

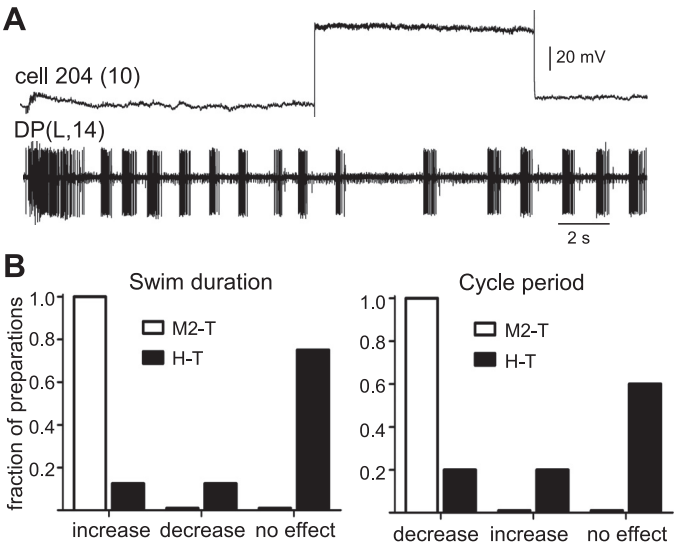

Fig. 6. Some of the variable effects of E21 on swimming in H-T preparations are mediated by cell 204. *A*: an example of cell 204 depolarization increasing cycle period. Cell 204 was hyperpolarized during the remaining portions of the swim episode. *B*: comparison of the effects of 204 depolarization on swim duration and cycle period in individual H-T and M2-T preparations. Number of preparations analyzed is as follows. H-T: swim duration, $n = 8$; cycle period, $n = 5$. M2-T: swim duration, $n = 9$; cycle period, $n = 3$. L, left.

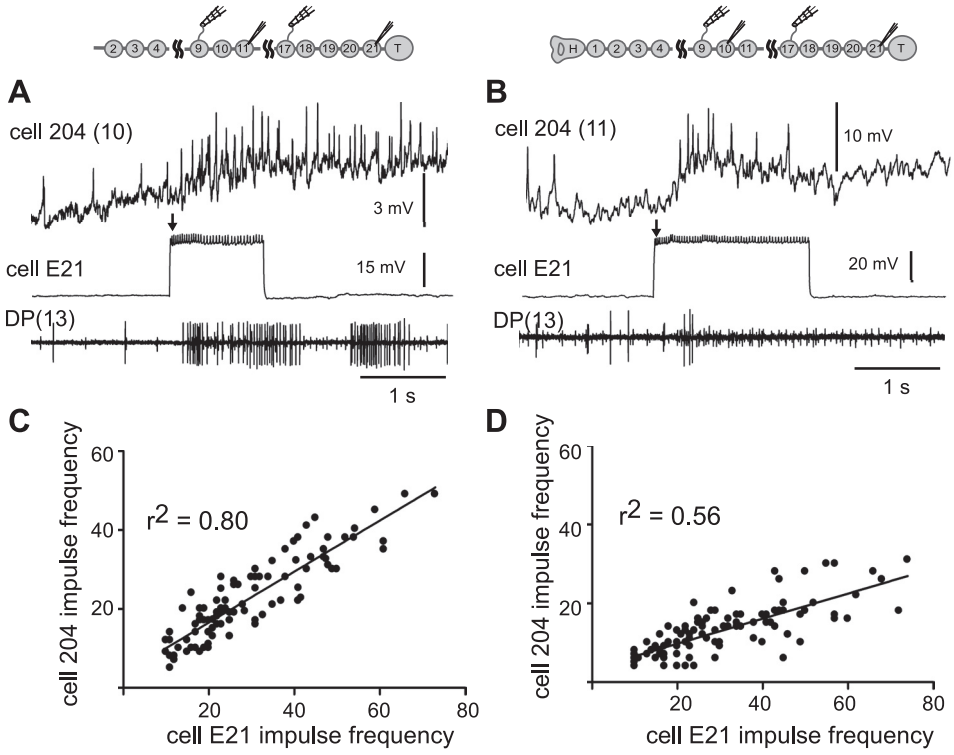

Fig. 7. The cell E21-to-204 interaction is altered by the head brain. *A*: representative response of cell 204 (*top* trace, located in M10) to stimulation of E21 (*middle* trace, at arrow) in an M2-T preparation (*inset*). This excitation initiated swimming (*bottom* trace). *B*: representative response of cell 204 (*top* trace, located in M11) to E21 stimulation (*middle* trace) in an H-T preparation (*inset*). Despite the slightly higher E21 firing frequency and longer current pulse than in *A*, the increase in impulse frequency in cell 204 is less than in the M2-T preparation. Here, excitation of cell E21 did not initiate swimming (*bottom* trace). *A* and *B* are from the same nerve cord. *C* and *D*: plot of cell 204 vs. cell E21 impulse frequency in M2-T ($n = 4$ leeches, 96 trials; *C*) and H-T ($n = 5$ leeches, 94 trials; *D*) preparations. The M2-T slope, 0.65, is significantly steeper than the H-T slope, 0.32 ($P < 0.01$), and the r^2 values are significantly different (0.80 vs. 0.56, respectively, $P = 0.038$). *Insets* at *top* refer to data in the entire column underneath each respective nerve cord.

brain-attached slope, 0.32; $P < 0.01$). Thus the strength of the excitation elicited in cell 204 by cell E21 activity is less when the brain is intact. This lowered drive might contribute to the increased threshold for swim initiation observed previously in response to DP nerve stimulation (Brodfuehrer and Friesen 1986). Our experiments did not reveal whether the brain exerts a tonic inhibitory influence on cell 204, which could elicit changes in input resistance, or whether cell 204 is inhibited by cells postsynaptic to cell E21 that are located in, or influenced by, the brain.

Interestingly, the r^2 value in the brain-off condition was large, 0.80, indicating that 80% of the variance in the cell 204 response to cell E21 stimulation can be accounted for by the impulse frequency in cell E21. However, the r^2 value, 0.56, was significantly less in brain-on preparations (2-sample correlation test, $P = 0.004$), showing that the 204 response to E21 stimulation is significantly weaker when the head brain is intact. Thus one source for the variability in the behavioral response when cell E21 is stimulated in isolated H-T preparations might be the variability of the evoked impulse frequency in swim-gating neurons.

Environment Modulates Behaviors Initiated by Cell E21

Previous research has shown that R3b1, a neuron located in the SubEG, can selectively drive swimming, crawling, or a swim-crawl hybrid behavior depending on the depth of the fluid surrounding the leech (Esch et al. 2002). Another leech neuron (Tr1) originally identified as triggering swimming can elicit crawling episodes as well (Brodfuehrer et al. 2008). These findings cast doubt on the idea that individual neurons are dedicated to the initiation of a single behavior. As E21 can elicit variable changes in ongoing behavior, we were curious about whether depolarization of cell E21 might initiate multiple behaviors as well. To determine whether cell E21 has such multifunctional properties, we placed H-T or M2-T semi-intact preparations into a dish with a well nearly filled with small pebbles (Fig. 8*A*). We then varied the saline level during the course of the experiment (Fig. 8*A*). Behavior was monitored by extracellular DP recordings and through visual observation of the intact portion of the animal.

Although swimming activity is enhanced by removal of the head brain (Brodfuehrer and Friesen 1986), its presence is required to obtain coordinated crawling (Puhl and Mesce 2010). With the brain removed in semi-intact preparations (M2-T; $n = 3$), stimulation of cell E21 under low fluid conditions often failed to evoke locomotory movements; preparations tended to remain motionless. Only with intense (>40 Hz) and prolonged (≥1 s) stimulation of cell E21 did we sometimes observe one to two swim-like cycles with the anterior end raised into the air. Rarely, with such strong stimulation, a single alternation between a partial contraction and partial elongation occurred. In striking contrast, spontaneous and cell E21-evoked swimming activity occurred in these M2-T semi-intact preparations immersed in medium and high fluid levels.

Conversely, we found that in all four H-T preparations tested, stimulating cell E21 in a low-fluid environment drove crawling behavior (Fig. 8, *B* and *C*). When cell E21 was depolarized during ongoing crawling episodes, the leech often continued crawling but with a reduced cycle period (Fig. 8*B*). Stimulation of this neuron when the preparation was quiescent sometimes initiated crawling (Fig. 8*C*), behavior that occasionally outlasted cell E21 excitation. With medium fluid levels, we observed swim-crawl hybrids, in which crawling episodes alternated with brief intervals of swimming (Fig. 8*D*). In these episodes, the leech initiated swimming in either the elongation or contraction phase of the crawl cycle. Consistent swim or crawl episodes also occurred at intermediate fluid levels. When

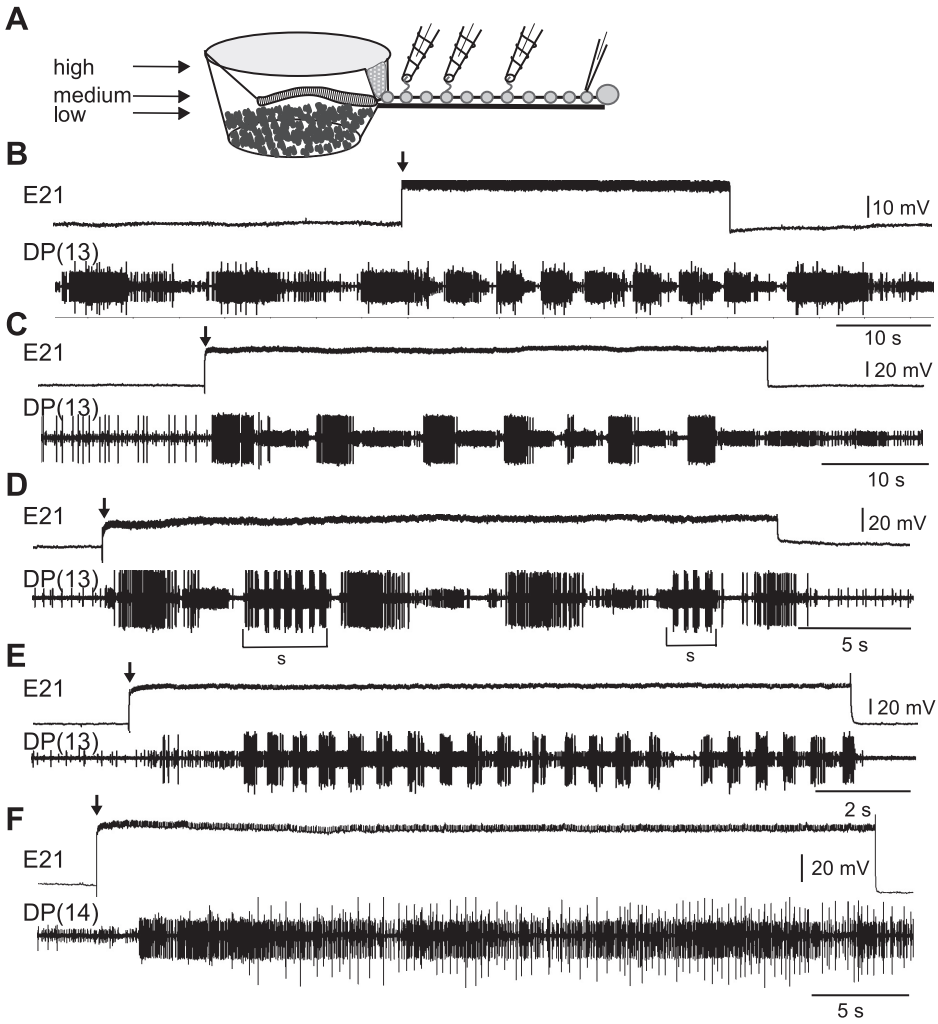

Fig. 8. Cell E21 excitation drives multiple locomotor responses dependent on the sensory environment. *A*: schematic of setup. Semi-intact H-T leeches were placed in the dish shown in Fig. 4*A*; here, the well of the dish was filled with pebbles on which the intact portion of the leech lay. Arrows approximate the "low," "medium," and "high" fluid levels. In low levels, the fluid did not rise above the pebbles. In medium levels, the fluid level approximately just covered the leech body at rest. For high levels, the fluid was ~1 cm above the leech body. *B* and *C*: cell E21 stimulation drives crawling in low fluid levels. *B*: the long DP nerve bursts before E21 excitation show spontaneous crawling activity; this stimulation reduced cycle period. *C*: stimulating cell E21 during a period of no locomotor activity in low fluid initiated and maintained a crawl episode. *D*: with a medium fluid level, a crawling episode initiated and maintained by E21 excitation was interrupted by 2 swim episodes, marked by an "s." *E*: despite the presence of a substrate, E21 excitation almost exclusively drove swimming in deep fluid. *F*: here, in low fluid, E21 excitation initiated a shortening response. The animal remained shortened for the entire 41-s stimulation and for another 90 s following current termination. Motor neuron DE-3 fires tonically; the largest spikes in this recording are those of the shortener motor neuron (L cell; Ort et al. 1974). *B* and *F* are from 1 preparation; Note difference in time scales in the traces. Arrows indicate start of depolarizing current injection.

the fluid level was high, stimulation of cell E21 induced only swimming, never crawling. A third behavioral response, shortening, was sometimes evoked in H-T preparations exposed to low fluid levels. The electrophysiological manifestation of this behavior is a continuous train of motor neuron impulses in DP nerves (Fig. 8*F*; Shaw and Kristan 1995). As illustrated, this contraction can be greatly prolonged, outlasting the stimulation. We tested whether cell E21 stimulation could elicit crawling or shortening when the body wall is removed in isolated H-T preparations. We found that in one of four such preparations, cell E21 clearly initiated crawling activity, and in a second preparation, there was weak crawl-like activity. In the remaining two experiments, cell E21 stimulation only initiated swimming (data not shown). We concluded that depolarization of cell E21 can initiate crawling in semi-intact and in isolated preparations with fully intact nerve cords.

DISCUSSION

The aim of our experiments was to elucidate the influence of the brain on motor function by examining changes in motor output caused by its removal. The experimental approach was to stimulate two identified neurons, cells E21 and 204, for which depolarization accentuates swimming when the brain is removed. We found that in preparations of the intact isolated nerve cord (i.e., including the brain but lacking sensory inputs), stimulation of these neurons resulted in remarkably variable and inconsistent responses. These variable responses were absent in brain-attached semi-intact preparations when the leech body was suspended in deep water, a swim-enhancing environment. In searching for circuit changes that may underlie the observed effects, we found that cell 204, which is postsynaptic to cell E21, had a decreased and less stereotyped response to cell E21 stimulation in the presence of the brain. Also, we found that cell E21 can drive crawling activity, in addition to swimming, in preparations that include the brain.

Brain-Induced State-Dependent Activity

Although it is obvious that the brain is necessary to modulate output generated from lower neural centers, systematic examinations of changes in motor output caused by removal of the brain are lacking. Most studies that did investigate changes in locomotion due to brain lesions examined only basic parameters, such as changes in initiation, episode duration, and cycle periods (e.g., Cohen et al. 1996; da Silva and Lange 2011; Graham 1979; Roeder 1937; Thompson 1986a,b). We showed that the brain can profoundly alter the functional actions of swim-system components previously reported as exclusively swim enhancing (Debski and Friesen 1986; Mullins et al. 2011b; Weeks and Kristan 1978). The variability in swim maintenance induced by E21 and 204 activity in isolated nerve

cords suggests that the actions of these neurons are more complex than previously imagined. Cell 204 drives swimming through direct excitatory connections to oscillator neurons (Nusbaum et al. 1987), and cell E21 directly excites all seven cell 204s and the homologous cell 205 (Mullins et al. 2011b). Thus, in preparations with the brain removed, robust swim excitation occurs in response to cell 204 or cell E21 activity (Fig. 9*A*). Swimming is controlled through swim-activating and -inactivating neurons (Brodfuehrer and Burns 1995), and we propose that cells E21 and 204 can access swim-suppressing pathways located in the brain (Fig. 9, *B* and *C*). In this scheme, these cells excite the swim circuitry and can also activate unidentified swim-suppressing neurons (SSN) in the head brain; SSN may inhibit neurons downstream from cell 204 and perhaps cell 204 itself (Fig. 9*C*). Which effect dominates is determined by the internal state of the brain. Stimulation of cells E21 or 204 during swimming can therefore depress or accentuate the behavior. Similarly, stimulation of cell E21 during quiescence can lead to activation or repression of swim circuits. Activation of the SSN could occur in conjunction with the initiation of crawling and shortening, which are incompatible with swimming and are thought to rely on suppression of swim-excitatory elements (Briggman et al. 2005; Shaw and Kristan 1997).

Sensory Influences on Neural State

The factors that underlie the state of isolated neural systems are unknown, but we hypothesize that variable swim responses in the leech represent "choices" embodied by neuronal circuits within the brain (Kristan 2008). In intact animals, the sensory environment modulates circuit activity (Blitz and Nusbaum 2011). Consistent with this schema, we obtained reliable swim initiation and enhancement of swim maintenance with the leech suspended in deep water, a swim-promoting environment. Furthermore, in environments unsuitable for swimming, E21 stimulation during periods of inactivity initiated crawling when the brain was intact, similar to results obtained for the multifunctional head brain neuron, R3b1 (Esch et al. 2002; Puhl and Mesce 2010). Interestingly, such context-dependent behavior was reduced, but not abolished, in semi-intact preparations lacking the brain. Because the brain is necessary for coordinated crawling (Puhl and Mesce 2010), crawling was not observed in semi-intact M2-T preparations. However, stimulating cell E21 in low fluid in these preparations often elicited no behavior at all. This was not due to physical inability, as intense prolonged E21 stimulation sometimes elicited one to two cycles of air swimming. Rather, these data are evidence that lower centers of the nervous system can exhibit some, albeit reduced, state-dependent behavior.

Sensory cues are not the sole arbitrator of internal circuit configuration; factors such as seasonal variability, hunger levels, reproductive state, and current activity can all influence behavioral responses (Palmer and Kristan 2011). For instance, in other leech studies, feeding suppressed the excitatory postsynaptic potential (EPSP) arising from sensory neurons in certain interneurons via presynaptic inhibition (Gaudry and Kristan 2009). In the cricket, Int-1 activity during flight elicits avoidance behavior (turning) but no such response when the cricket is grounded (Nolen and Hoy 1984). Social status can also influence circuit activity; serotonin reduced sensory-induced EPSPs in the lateral giant neuron of subordinate crayfish but enhanced it in social dominants (Yeh et al. 1996). State-dependent alterations in circuit properties are often mediated through neuromodulators, which perhaps altered the cell 204 and E21 interaction in our studies. In the leech, application of serotonin increases the incidence of swim initiation (Willard 1981); similarly, dopamine promotes crawling (Puhl and Mesce 2008). Experiments on the stomatogastric system in crustaceans illustrates the extent to which neuromodulators can reconfigure circuits; in one study, dopamine and serotonin were shown to affect nearly every synapse in the pyloric motor circuit (Johnson et al. 1995).

Roles of Cells E21 and 204 in the Intact Animal

The radically altered, even opposing responses observed in intact leech nerve cords consequent to cell E21 stimulation

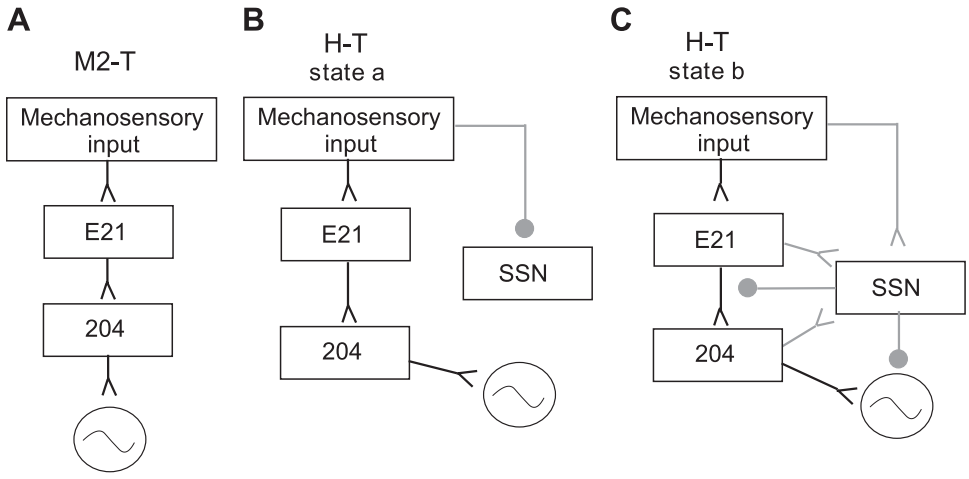

Fig. 9. Proposed mechanism underlying variability. Sensory cell input to E21 elicits activity in 204, which leads to swimming. *A*: in the absence of the brain, depolarization of E21 or 204 during swimming simply leads to an enhancement of the behavior. *B* and *C*: we propose that in the presence of the brain, cell E21 and 204 directly or indirectly stimulate cephalic neurons that suppress swimming (SSN), perhaps through inhibition of the swim oscillator interneurons, creating simultaneous competing activity in swim-activating and -inactivating systems. The expression of behavior depends on the totality of sensory inputs and the internal state of the system, which is altered by neuromodulators. *B*: in *state a*, SSNs are inhibited by sensory input and E21 and 204 stimulation activate and enhance swimming. Only dominant interactions are shown. *C*: in *state b*, SSNs are activated by sensory input and by E21 and 204. The SSNs then compete with excitation from cells 204 and inhibit swimming. SSNs may also inhibit 204 through either direct circuit connections or neuromodulatory action. Gray interactions are hypothetical.

were unexpected but are compatible with cell E21 properties in the broader context of leech behavior. The soma of cell E21 is posteriorly located, but its axon extends throughout the nerve cord; moreover, its spikes can be initiated in most or all ganglia (Mullins et al. 2011b). Therefore, cell E21 integrates sensory information from the entire body. Cell E21 is excited by mechanosensory inputs, which have variable effects on behavior in the intact animal. For example, mechanical stimulation of the swimming leech can terminate swimming or increase swimming speed (Kristan et al. 1982; O. J. Mullins and W. O. Friesen, unpublished observations). Because cell E21 activity relays the occurrence of a sensory stimulation somewhere along the body, we argue that cell E21 excitation must be interpreted in the context of and subsequent behavior must be dependent on the state of the animal as well as on the totality of sensory inputs. Coactivation of neurons is one means through which command-like neurons can activate multiple behaviors. For example, in the lobster stomatogastric nervous system, independent stimulation of the anterior gastric receptor (AGR) or the posterior stomach receptors (PSR) elicits the type I gastric motor pattern, but simultaneous stimulation elicits the type II pattern (Combes et al. 1999a,b; Barrière et al. 2008). This effect can be explained by the configuration of the synaptic connections downstream from these neurons. Perhaps a similar mechanism determines the consequences of cell E21 activation in the intact animal.

Cell 204 differs from cell E21 in that its activity is likely essential for maintaining swimming (Brodfuehrer et al. 2008; Friesen et al. 2011; Weeks and Kristan 1978). That cell 204 can also inhibit swimming was surprising but perhaps accounts for the irregular cycle periods and missed bursts sometimes observed in H-T-isolated preparations (Brodfuehrer and Friesen 1986). It can be difficult to evoke swimming in a leech, and episodes are easily terminated, by input to the anterior end (Kristan et al. 1982). The dual effects of cell 204 might serve as a negative feedback system to ensure swims are only activated and extended when appropriate and yet allow rapid termination of swimming when necessary.

Conclusion

Our initial studies using brain-removed isolated nerve cord preparations suggested that cell E21 was strictly swim-excitatory. Subsequent experiments in isolated H-T and semi-intact preparations showed that E21 has a multitude of effects on swimming and, further, can drive crawling behavior. Interestingly, in the most physiological preparation studied (semi-intact H-T), E21 effects on swim maintenance mimicked those observed in the most reduced preparation (isolated M2-T). However, the semi-intact preparations were tested in a static environment, and we predict that the variable responses seen in isolated intact nerve cords would also be observed in dynamic real-world environments.

This research demonstrates the importance of considering context when assigning functionality to neurons and neuronal circuits. Studies on leech motor systems often use reduced preparations with the brain removed (Kristan et al. 2005). Other reduced preparations often employed in motor control research include the isolated crustacean stomatogastric nervous system (Marder and Bucher 2007) and lamprey, zebrafish, and rodent isolated spinal cords or spinal cord-brain stem preparations (Grillner and Jessell 2009). Such preparations are convenient and accessible, and their simplicity is often necessary for initial identification of circuit components. However, as our study illustrates, functional roles and synaptic interactions can shift in more intact preparations. Because the final aim is to ascertain the function of neuronal circuits in the intact animal, proposed functions should eventually be tested in preparations that include the brain as well as sensory input.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

O.J.M. and W.O.F. conception and design of research; O.J.M. performed experiments; O.J.M. analyzed data; O.J.M. and W.O.F. interpreted results of experiments; O.J.M. prepared figures; O.J.M. drafted manuscript; O.J.M. and W.O.F. edited and revised manuscript; O.J.M. and W.O.F. approved final version of manuscript.

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