Multiple Contributions of an Input-Representing Neuron to the Dynamics of the Aplysia Feeding Network

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Submitted 12 December 2006; accepted in final form 12 February 2007

Proekt A, Jing J, Weiss KR. Multiple contributions of an input-representing neuron to the dynamics of the Aplysia feeding network. J Neurophysiol 97: 3046–3056, 2007. First published February 21, 2007; doi:10.1152/jn.01301.2006. In Aplysia, mutually antagonistic ingestive and egestive behaviors are produced by the same multifunctional central pattern generator (CPG) circuit. Interestingly, higher-order inputs that activate the CPG do not directly specify whether the resulting motor program is ingestive or egestive because the slow dynamics of the network intervene. One input, the commandlike cerebral–buccal interneuron 2 (CBI-2), slowly drives the motor output toward ingestion, whereas another input, the esophageal nerve (EN), drives the motor output toward egestion. When the input is switched from EN to CBI-2, the motor output does not switch immediately and remains egestive. Here, we investigated how these slow dynamics are implemented on the interneuronal level. We found that activity of two CPG interneurons, B20 and B40, tracked the input regardless of the motor output. Furthermore, we show that the slow dynamics of the network are implemented, at least in part, in the slow dynamics of the interaction between the input-representing and the output-representing neurons. We conclude that 1) a population of CPG interneurons, recruited during a particular motor program, simultaneously encodes both the input that is used to elicit the motor program and the output elicited by this input; and 2) activity of the input-representing neurons may serve to bias the future motor programs.

INTRODUCTION

It is well known that many neuronal networks are multifunctional, i.e., involved in the generation of multiple related behaviors such as different locomotor gaits. One well-characterized observation is that different inputs that activate the same network can elicit different behaviors. For instance, different proprioceptive neurons can elicit different versions of the gastric mill rhythm in the stomatogastric ganglion of the crab (Beenakker and Nusbaum 2004). In other experimental systems, the type of stimulus does not completely specify the type of response. The response may depend on the context in which the behavior is elicited. For instance, when the cricket is in the air, stimulation of a command neuron elicits flying, whereas activation of the same neuron elicits running while the cricket is on the ground (Nolen and Hoy 1984). Context-dependent differences in behavioral responses elicited by specific command neurons were also previously observed in the medicinal leech (Esch et al. 2002). In addition to external contextual cues, application of hormones and neuropeptide modulators can influence behavioral responses elicited by the same stimulus. This was shown in the crustacean stomatogastric nervous system (Harris-Warrick and Marder 1991) and in the stridulation responses in insects (Heinrich et al. 2001). Similar mechanisms were also shown to operate in control of the feeding network in Aplysia (Jing and Weiss 2002; Jing et al. 2003; Morgan et al. 2002; Wu et al. 2003). In addition to these mechanisms, another mode of control over feeding motor programs was recently described. Proekt et al. (2004) demonstrated that previous history of activity of the feeding network also influences the subsequent motor outputs.

The feeding network in Aplysia can be activated to produce both ingestive and egestive motor programs by two input pathways: the commandlike cerebral–buccal interneuron 2 (CBI-2) and the esophageal nerve (EN) (Chiel et al. 1988; Rosen et al. 1991). However, these inputs do not unequivocally specify the nature of the motor program. CBI-2 and EN drive the motor output toward a particular steady state: ingestive in the case of CBI-2 and egestive in the case of EN. Furthermore, when the input is switched to CBI-2 after repeated stimulation of EN, the motor output does not switch immediately but remains egestive (Proekt et al. 2004).

Here, we have investigated how these slow dynamics are implemented on the interneuronal level. We focused on neurons B20 and B40 because these neurons are preferentially active in, and contribute to, egestive and ingestive responses, respectively (Jing and Weiss 2001, 2002). We found that B20 continued to fire at high frequency and B40 continued to fire at low frequency in egestive motor programs when the input was switched from EN to CBI-2. Thus firing frequency of B20 and B40 expressed the motor output, regardless of the input. Activity of another interneuron, B65, however, expressed the input regardless of the output; B65 was strongly active in EN-elicited egestive programs, but did not fire at all in egestive programs elicited immediately afterward by CBI-2. We also investigated the nature of the interaction between input- and output-representing neurons. We found that B65 produced changes in the excitability and synaptic connections of B20 and B40 and that these changes outlasted B65 firing. B65-elicited changes in B20 and B40 were similar to those that followed EN-elicited programs. These persistent changes in B20 and B40 may contribute to the persistence of the egestive responses after the input is switched from EN to CBI-2.

Taken together, our results suggest that the population of interneurons within the behavior-generating network separately encodes the higher-order input used to activate it, as well

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as the behavioral response elicited by that input. Furthermore, the slow dynamics of the feeding network may at least in part be reflected in the slow modulatory actions that input-representing neurons exert on output-representing neurons and their synapses.

**METHODS**

**Animals and recording techniques**

Experiments were performed on *Aplysia californica* obtained from either Marinus (Long Beach, CA) or the University of Miami *Aplysia* Resource Facility (Miami, FL). *Aplysia* were maintained in circulating artificial sea water (ASW) made from Instant Ocean (Aquarium Systems, Mentor, OH), at 14–15°C. Animals weighing 150–250 g were anesthetized by injection of isotonic MgCl₂ (337 mM). Buccal and cerebral ganglia were dissected out of the animal and desheathed in a dissection chamber coated with Sylgard. The ganglia were then transferred to a recording chamber, lined with Sylgard, containing about 1.5 ml of ASW (in mM: 460 NaCl, 10 KCl, 55 MgCl₂, 11 CaCl₂, and 10 HEPES buffer; pH 7.6) (Trudeau and Castellucci 1992). Buccal and cerebral ganglia were maintained at 14–17°C and continuously perfused with ASW at the rate of about 0.3 ml/min. Polysynaptic activity was reduced using a high concentration of divalent cations (HiDi) solution (in mM: 420 NaCl, 10 KCl, 121 MgCl₂, 13.8 CaCl₂, and 10 HEPES buffer; pH 7.6) (Trudeau and Castellucci 1992).

Standard intracellular and extracellular recording techniques were used. Intracellular electrodes were filled with an electrolyte containing 2 M K acetate and 100 mM KCl and beveled to the resistance of 6–10 MΩ. Intracellular recordings were performed using either the Axoclamp 2B amplifier (Molecular Devices, Foster City, CA) or Getting 5A amplifiers (Getting Instruments, Iowa City, IA). All neurons were identified as described previously (Jing and Weiss 2001, 2002; Kabotyanski et al. 1998). Extracellular recordings were performed by applying suction electrodes constructed from polyethylene tubing on buccal nerves. Signals were then amplified by an AC amplifier model 1700 (A-M Systems, Carlsborg, WA).

All recordings were acquired using a Digidata 1322A (Molecular Devices) data acquisition system at the sampling rate of 5 kHz and recorded on a PC computer (Dell, Houston, TX). Digitized recordings were then plotted using Sigma Plot 5. Statistics were performed using either SigmaPlot 5 (SPSS) or Excel (Microsoft) software.

**Statistical analysis**

Unless otherwise indicated, one-way ANOVA was used to establish statistical significance. ANOVA was performed using Prism software package (GraphPad Software, San Diego, CA). The ANOVA was followed by a post hoc two-tail *t*-test with Bonferroni correction. The data sets directly compared in the post hoc analysis are indicated by brackets in the corresponding figures (*P < 0.05, **P < 0.01, ***P < 0.001).

**Characterization of feeding motor programs in Aplysia**

During the consummatory phase of feeding, *Aplysia* produces stereotyped behaviors that were earlier described as either ingestive or egestive (Künzle 1974a,b). In both behaviors *Aplysia* first protract and then retract their radula, a food-grasping organ. During ingestive behaviors, the radula closes in the retraction phase to pull food in, whereas during egestive behaviors, the radula closes in the protraction phase to push materials out. Ingestive and egestive behaviors can be distinguished using extracellular recordings obtained in vivo by differences in coordination between the activity of neuron B8 and the activity of neurons that produce radula retraction (Morton and Chiel 1993a,b). The central pattern generator (CPG) that produces these feeding behaviors is located in the buccal ganglion. In vitro this feeding CPG is capable of producing coordinated patterns of activity referred to as feeding motor programs. Although not identical, these in vitro elicited motor programs share essential characteristics with patterns of activity recorded in vivo. During ingestive motor programs, the radula closer motoneuron B8 is predominantly active in the retraction phase, whereas during egestive motor programs, B8 is predominantly active in the protraction phase (Jing and Weiss 2001, 2002; Morgan et al. 2002; Proekt et al. 2004). Based on previous findings, we defined the protraction phase as activity recorded in the I2 nerve (Hurwitz et al. 1996; Jing and Weiss 2001, 2002; Morgan et al. 2002; Nargeot et al. 1999a,b). The retraction phase was defined as high frequency of activity recorded in the buccal nerve 2 (B.n.2) after the end of activity recorded in the I2 nerve (Morton and Chiel 1993a,b; Nargeot et al. 1999a,b).

The differences in the coordination between the radula protraction, retraction, and closure activity in ingestive and egestive motor programs are clearly seen when the firing frequency of B8 during the protraction phase is plotted against the firing frequency of B8 in the retraction phase. Morgan et al. (2002) demonstrated that, in this plane, ingestive and egestive motor programs form two clusters (Fig. 1). Approximate cluster boundaries are shown as gray ovals; representative recordings of ingestive and egestive motor programs are shown in their corresponding clusters.

Motor programs were elicited by stimulating either one of two input pathways: the CBI-2 neuron (Rosen et al. 1991), which is located in the cerebral ganglion, or the EN (Chiel et al. 1988; Morton et al. 1991) that connects the esophagus and the gut with the buccal ganglion. The CPG for feeding-related responses is located in the buccal ganglia and, except for CBI-2, all of the neurons studied herein are located in the buccal ganglion. Both CBI-2 and EN are thought to be important in feeding behavior and are capable of eliciting motor programs (Due et al. 2004; Evans et al. 2003; Horn et al. 2004; Jing and Weiss 2005, 2002, 2005; Morgan et al. 2002; Proekt et al. 2004; Rosen et al. 1991) and functional ingestive and egestive responses (Chiel et al. 1988; Church and Lloyd 1994; Jing and Weiss 2005; Zhurov et al. 2005) in semi-intact preparations. CBI-2 is known to be activated by touching food (e.g., seaweed) to the lips of the animal (Jing and Weiss 2005; Morgan et al. 2002; Rosen et al. 1991), whereas the EN conveys sensory information concerning the stretch of the esophagus (Kuslan sky et al. 1987; Morgan et al. 2002) (although it may also convey other signals). CBI-2 was stimulated to fire action potentials at 9 Hz for the duration of the protraction phase. Short (20– to 30-ms duration), square current pulses, each of which triggered a single action potential, were used. No DC predepolarization was used during CBI-2 stimulation. Because DC prestimulation is required to recruit CBI-3 activity (Morgan et al. 2002), very little if any CBI-3 firing was recruited during CBI-2–elicited motor programs. EN was stimulated at about 2 Hz using 3-ms current pulses delivered by an extracellular electrode. All stimulations were performed using a Grass S48 Stimulator (Grass Instruments, Quincy, MA).

**RESULTS**

Normally the feeding CPG is quiescent, but can be activated to produce ingestive and egestive motor programs by stimulation of one of the input pathways. Figure 1 shows examples of ingestive and egestive motor programs elicited by stimulation of CBI-2. Both the ingestive and the egestive motor program in Fig. 1 were elicited in the same preparation using the same frequency of CBI-2 stimulation. CBI-2 is able to elicit both types of feeding motor programs because the type of motor program elicited by CBI-2 is determined by the previous history of activity of the feeding CPG (Proekt et al. 2004). In the following experiments, using the experimental paradigm developed by Proekt et al. (2004), we characterized how this
history dependency is reflected in the activity of several CPG interneurons.

We first focused on interneurons B20 and B40 because activity of these neurons is critical for the expression of egestive and ingestive motor programs, respectively (Jing and Weiss 2001, 2002). We repeatedly stimulated CBI-2 throughout the protraction phase with an interprogram interval of 30 s. Nine motor programs were elicited in this fashion. As shown previously (Proekt et al. 2004), after nine consecutive CBI-2 stimulations at 30-s intervals the system settles into a steady state defined by consistent ingestive responses elicited by CBI-2 stimulation. A representative example of one such ingestive response is shown in Fig. 2A; CBI-2 control. The EN was then stimulated for 5 min; this stimulation routinely elicited nearly 10 motor programs. In these egestive motor programs, B8 fired predominantly in the protraction phase and B20 fired at higher frequency than in ingestive CBI-2–elicited motor programs (Fig. 2A, box). We then switched the stimulation back to CBI-2. Because of the slow dynamics, the network disregarded the switch in the stimulus and continued to generate egestive motor programs (Fig. 2A, CBI-2, 0 min). The firing frequency of B20 also did not switch immediately;
B20 continued to fire at higher frequency than it did in CBI-2–elicited ingestive programs. On repeated CBI-2 stimulation, the motor programs once again became ingestive (Fig. 2A). This was accompanied by a decrease in the firing frequency of B20 back to control levels.

We then characterized the activity of B40 in the same experimental paradigm (Fig. 2B). In CBI-2–elicited ingestive motor programs, B40 fired at relatively high frequency (Fig. 2B, control), whereas in EN-elicited egestive motor programs, B40 did not fire at all (Fig. 2B, box). After EN stimulation, B40 firing frequency remained decreased in egestive motor programs elicited by CBI-2 (Fig. 2B, CBI-2, 0 min). On repeated stimulation of CBI-2, as the motor programs became ingestive again, the firing frequency of B40 returned to its control values (Fig. 2B, CBI-2, 5 min).

Grouped data for the two preceding experiments (Fig. 3A) demonstrate that changes in B20 (filled circle) and B40 (empty circle) firing frequencies paralleled changes in the ingestive versus egestive characteristics of the motor output, as measured by B8 firing frequency in the protraction (filled square) and the retraction (empty square) phases (Fig. 3B). Specifically, the increase in the B20 firing frequency paralleled the increase in the B8 firing frequency in the protraction phase, whereas the decrease in the B40 firing frequency paralleled the decrease in the B8 firing frequency in the retraction phase. In Fig. 3C, we replotted the same data as in Fig. 3B to demonstrate that these changes in the firing frequency of B8 amount to qualitative changes in the nature of the motor programs. The history-dependent changes in B8 firing shown in Fig. 3, B and C were similar to those shown in Proekt et al. (2004). Taken together, the data shown in Fig. 3 indicate that the firing frequency of B20 and B40 tracked the slow dynamics of the motor output of the feeding network.

Activity of another interneuron in the circuit, neuron B65 (Due et al. 2004; Jing and Weiss 2005; Kabotyanski et al. 1998), showed a dramatically different pattern of firing in the same experimental paradigm. In ingestive motor programs elicited by CBI-2 before EN stimulation, B65 fired very weakly in the protraction phase (Fig. 4A1), whereas in EN-elicited egestive motor programs B65 fired robustly during the protraction phase (Fig. 4A2). When the stimulus was switched back to CBI-2, although the network continued to generate egestive motor programs, B65 firing was immediately shut down (Fig. 4A3). Grouped data for this experiment (Fig. 4B) show that B65 fired at the same frequency in motor programs elicited by CBI-2, irrespective of whether they were ingestive or egestive. In contrast, B65 fired at very different frequencies in similar egestive motor programs elicited by EN and CBI-2. Thus in contrast to B20 and B40, the firing frequency of B65 tracked the input that activates the CPG, regardless of the motor output elicited by this input.

A switch in the input from EN to CBI-2 does not elicit an immediate switch in the motor output because the slow dynamics of the network intervene. We hypothesized that on the neuronal level, the slow dynamics of the network may be expressed in the persistent changes in the properties of the neuronal network.
output-representing neurons B20 and B40 and their synaptic connections. To address the possible mechanisms that underlie the persistent increase in the firing frequency of B20 after repeated EN stimulation, we measured the excitability of B20 before and after EN stimulation. The excitability was measured by injecting 1.5-nA, 3-s current pulses into the soma of B20 every 30 s. A representative recording (Fig. 5A) and grouped data (Fig. 5B) show that the excitability of B20 was increased after EN stimulation. Consistent with the time course of the persistent increase in the firing frequency of B20 after EN stimulation, the increase in the excitability recovered over the course of 2 min (Fig. 5). Activity of B20 is critical for the expression of egestive motor programs (Jing and Weiss 2001) and one of the defining features of egestive motor programs is that B8 fires predominantly during the protraction phase (Morgan et al. 2002). B20 is known to contribute to this egestive firing pattern of B8 by exciting it during the protraction phase by a direct synaptic connection (Diaz-Rios and Miller 2005; Jing and Weiss 2001; Proekt et al. 2004). Figure 5A demonstrates that, as previously shown by Proekt et al. (2004), EN stimulation also leads to the persistent potentiation of the B20-to-B8 synapse. The increase in the excitability of B20 and the potentiation of the B20-to-B8 synapse may act synergistically to increase the excitatory drive to B8 during the protraction phase and thus may contribute to the persistence of egestive motor programs after the switch in the stimulus.

We then characterized the effect of EN stimulation on the excitability and synaptic connections of B40 (Fig. 6A, representative recordings; Fig. 6B, grouped data). The EN-stimulation–elicited decrease in B40 excitability recovered over the course of 3 min (Fig. 6). B40 firing is known to promote ingestive motor programs (Jing and Weiss 2002). In ingestive motor programs B8 fires predominantly in the retraction phase (Morgan et al. 2002). B40 promotes this ingestive firing pattern of B8 by producing a complex postsynaptic potential (PSP) in B8 that consists of the fast inhibitory postsynaptic potential (PSP) in B8.
FIG. 6. Stimulation of EN leads to a persistent decrease in B40 excitability and suppression of the B40-to-B8 synapse. A: representative recordings of B40 firing before and after EN stimulation: 3-s, 5-nA current pulses were injected into B40 before (control) and 0, 30, 60, 90, 150, and 180 s after EN stimulation. EN was stimulated as in Fig. 2. V_m for B40 at these time points was −65, −66, −66, −66, and −66 mV, respectively. B: normalized grouped data. Number of action potentials elicited by the current injection into B40 after EN stimulation was normalized by the number of action potentials elicited before EN stimulation in the same preparation. Average (circles) and SEs (n = 5) are shown. Dashed line indicates the control level of excitability. Statistical significance of the decrease in excitability of B40 was tested using one-way ANOVA (F = 21.83; P < 0.0001). C: representative recordings of B40 and B8. B40 was stimulated with brief current pulses to fire action potentials at 10 Hz for 0.5 s. B8 was hyperpolarized to the reversal potential of the inhibitory postsynaptic potential (IPSP) to prevent B8 firing. Thus only the excitatory postsynaptic potential (EPSP) is seen in the recordings. B40 was stimulated before EN stimulation (control), and again stimulated at 30 s, 60 s, 90 s, and 180 s after EN stimulation. Recordings elicited 60 s and 180 s after EN stimulation are shown. D: grouped data. EPSP amplitude was normalized by the amplitude of EPSP elicited before EN stimulation in each preparation. Average and SEs (n = 3) are shown. Statistical significance of the decrease in EPSP amplitude was tested using one-way ANOVA (F = 6.49; P < 0.05).

(IPSP) and a slow excitatory postsynaptic potential (EPSP) that outlasts B40 firing (Jing and Weiss 2002; Jing et al. 2003). Whereas the fast IPSP suppresses B8 firing in protraction, the slow EPSP promotes B8 firing in the retraction phase (Jing and Weiss 2002; Jing et al. 2003). In the experiment shown in Fig. 6C, we stimulated B40 to fire a fixed number of action potentials and characterized the resulting EPSP before and after EN stimulation. Because the slow EPSP tends to elicit action potentials in B8, B8 was hyperpolarized to about −80 mV (Jing et al. 2003) during this experiment; thus only the excitatory component of the B40-to-B8 PSP is seen in these recordings. After EN stimulation, the slow EPSP was suppressed. This suppression recovered over the course of 3 min (Fig. 6, C and D). Thus repeated stimulation of EN elicits persistent potentiation of neuronal mechanisms that promote egestive motor programs and coordinated persistent suppression of mechanisms that promote ingestive motor programs.

On the phenomenological level, the slow dynamics of the network intervene between the input and output of the feeding CPG. We hypothesized that on the mechanistic level, these slow dynamics could be implemented in the properties of the connections between input-representing and output-representing neurons. To determine whether firing of input-representing neuron B65 could contribute to the slow dynamics, we first characterized the effect of B65 stimulation on the excitability of B40. In this experiment, B65 was stimulated to fire action potentials at 10 Hz. This firing frequency is similar to the firing frequency of B65 in EN-elicited egestive programs (Fig. 4) (Due et al. 2004). We injected current pulses into B40 as in Fig. 6A. After several control current injections, during the 30-s interval between these current injections, we stimulated B65 to fire action potentials at 10 Hz for 20 s. Three such bursts of action potentials were elicited in B65. B65 stimulation resulted in a decrease in the excitability of B40 (Fig. 7A, representative recordings; Fig. 7B, grouped data). This decrease in the excitability of B40 recovered over the course of 90 s and appeared to be elicited by B65 by a monosynaptic pathway because the stimulation of B65 in solution containing HiDi also resulted in a decrease in the excitability of B40 (n = 3; data not shown).

In a separate group of experiments, we characterized the effect of B65 stimulation on the B40-to-B8 synapse. B40 was stimulated with brief current pulses to fire action potentials for 2 s at 10 Hz. A single burst of B40 firing was elicited every 30 s. This experiment was performed in HiDi to decrease neuronal excitability. Because of the decrease in the excitability we did not need to hyperpolarize B8. Thus both the fast IPSP and slow EPSP are clearly seen in these recordings (Fig. 7C). B65 was stimulated to fire action potentials at 10 Hz for 10 s. B65 stimulation was started 10 s after the end of B40 stimulation and ended 10 s before the next B40 stimulation. After a single burst of B65 firing, the amplitude of the B40-to-B8 EPSP was decreased (Fig. 7C). Because B65 stimulation suppresses the B40-to-B8 EPSP in HiDi, this effect likely represents a direct heterosynaptic modulation of the synapse by B65; grouped data for this experiment are shown in Fig. 7D.

We then characterized the effect of B65 stimulation on the excitability of B20 in a similar paradigm and found that this resulted in the progressive increase in B20 excitability (Fig. 8A, representative recordings; Fig. 8B, grouped data). This increase in the excitability dissipated within 30 s after the end of B65 stimulation.
FIG. 7. Stimulation of B65 elicits a persistent decrease in B40 excitability and suppression of the B40-to-B8 synapse: 3-s, 5-nA current pulses were injected into B40 every 30 s (control). B65 was then stimulated with 25-ms current pulses to fire action potentials at 10 Hz for 20 s. Stimulation of B65 was started 5 s after the end of the current pulse delivered to B40 and ended 5 s before the next B40 stimulation. Three such bursts of action potentials were elicited in B65. A: representative recordings. B40 stimulations after the 1st, 2nd, and 3rd burst of action potentials elicited in B65 are labeled 1, 2, and 3, respectively; B40 stimulations 30, 60, and 90 s after the last B65 burst are also shown. V_{rest} for B40 at these time points was −55, −54, −54, −50, −50, −51 mV, respectively. B: normalized grouped data. Number of action potentials elicited in B40 by current injection after B65 stimulation was normalized by the number of action potentials elicited before B65 stimulation in each preparation. Average and SEs (n = 4) are shown. Statistical significance of the decrease in the B40 excitability was tested using one-way ANOVA (F = 12.88; P < 0.01). C: representative recordings of B8 and B40 firing in high concentration of divalent cations (100 mM). B40 was stimulated to fire action potentials at 10 Hz for 2 s. A single such burst of B40 action potentials was elicited every 30 s. Once reproducible EPSPs were established, B65 was stimulated in the 30-s interval between B40 stimulation. B65 was stimulated to fire action potentials at 10 Hz for 10 s. B65 stimulation began 10 s after the end of B40 stimulation and ended 10 s before the subsequent B40 stimulation. Representative recordings of the PSP elicited before (control), and at 10, 40, and 130 s after B65 stimulation are shown. V_{rest} for B40 at these time points was −54, −55, −55, and −55 mV, respectively. Dashed line shows the peak of the baseline EPSP for comparison purposes. D: normalized grouped data. Amplitude of the B40-to-B8 EPSP was normalized by the amplitude of the EPSP elicited by B40 stimulation before B65 stimulation in each preparation. Average and SE (n = 4) are shown. Statistical significance of the decrease in the PSP was tested using one-way ANOVA (F = 22.01; P < 0.0005).

**DISCUSSION**

In this work, we have studied state dependency in the feeding circuitry of Aplysia. Repeated EN stimulation resulted in persistent changes in the level of activity, excitability, and strength of synaptic connections of multiple CPG elements. Once established, these changes persist. Thus when the stimulus is switched, the network output does not immediately switch. Indeed, after repeated EN stimulation, when the input is switched to CBI-2, the network completely disregards the switch and continues to generate egestive motor programs. To account for these observations, we propose a conceptual scheme as illustrated in Fig. 9A. The inputs drive the network toward a particular steady state; in the case of CBI-2, ingestive, and in the case of EN, egestive. The slow dynamics of the network state prevent the system from instantaneously switching the motor output when the input is switched.

Figure 9B shows a simplified schematic of the relevant feeding CPG neurons and their synaptic connections and summarizes our findings on network reconfiguration that parallels the slow CPG dynamics. As the conceptual scheme in Fig. 9A illustrates, the inputs do not directly specify the outputs—the slow dynamics of the network state intervene. Consistent with this framework, we discovered two distinct elements within the feeding circuitry: neurons that express the input regardless of the output (i.e., B65) and neurons that express the output regardless of the input (i.e., B20 and B40) (Fig. 9B). Previously Proekt et al. (2004) suggested that state dependency may be mediated by the plasticity of the synaptic connections of the output-representing neuron B20. Here, we have extended these observations to show that plasticity associated with state dependency also involves other neurons and synaptic connections. Our results further suggest that these coordinated changes in multiple loci within the feeding network may, in part, be orchestrated by the input-representing neuron B65.

Previous work identified many neurons within the feeding circuitry of Aplysia (reviewed in Cropper et al. 2004). Firing of some neurons is important for the initiation and maintenance of the protraction and the retraction phases, irrespective of whether the motor program is ingestive or egestive (Hurwitz and Susswein 1996; Hurwitz et al. 1996, 1997; Jing and Weiss 2001). These neurons were not directly investigated here and are shown schematically as mutually inhibitory P and R neurons (Fig. 9B). Yet, other neurons shape the egestive versus ingestive nature of the motor programs. Several lines of evidence point to the importance of B20 and B40 in shaping these ingestive versus egestive characteristics: 1) B20 is more active in egestive motor programs, whereas B40 is more active in ingestive motor programs; 2) stimulation of B20 leads to the generation of more egestive motor programs, whereas stimulation of B40 results in more ingestive motor programs; and 3) suppression of B20 disrupts the generation of egestive motor programs, whereas suppression of B40 disrupts ingestive motor programs (Jing and Weiss 2001, 2002). Although P neurons directly excite B20 and B40 by electrical and chemical syn-
apses (Fig. 9B) (Jing and Weiss 2001, 2002), they are not thought to contribute to specifying the ingestive versus egestive nature of the motor programs because they fire similarly during ingestive and egestive programs (Jing and Weiss 2001, 2002).

We find that firing of both B20 and B40 was persistently altered after repeated EN stimulation (compare Fig. 9, B2 and B3). In EN-elicted egestive programs, B20 fired at high frequency (Fig. 2A), whereas B40 fired at low frequency (Fig. 2B). After the input was switched from EN to CBI-2, B20

FIG. 8. Stimulation of B65 elicits persistent increase in B20 excitability. Same paradigm as described for Fig. 7A. A: representative recordings of B20 firing after B65 stimulation. B20 stimulation after the 1st, 2nd, and 3rd burst of action potentials elicited in B65 are labeled 1, 2, and 3, respectively. Current injection into B20 30 s after the end of the last stimulation of B65 is also shown. V_{rest} for B20 in Control, 1, 2, 3, and 30 s. Recordings were −81, −81, −79, −79, and −79 mV, respectively. B: normalized grouped data. Number of action potentials elicited in B20 by current injection after B65 stimulation was normalized by the number of action potentials elicited before B65 stimulation in each preparation. Average and SEs (n = 5) are shown. Statistical significance of the increase in the excitability was tested using one-way ANOVA (F = 5.81; P < 0.01).

FIG. 9. Slow dynamics intervene between input and output representing neurons. A: inputs that activate the network (CBI-2 and EN) do not directly specify the motor output—the slow dynamics of the network intervene. Network shuttles between 2 steady-state configurations—eggestive and ingestive (shown by red and blue circles, respectively). CBI-2 drives the network toward ingestive configuration, whereas EN drives the network toward egestive configuration. Because the dynamics of the transition from egestive to ingestive configuration is slow, the motor output does not switch immediately when the input is switched from EN to CBI-2. B: neuron P represents mutually excitatory protraction neurons B63, B34, B61/62, and B31/32. Neuron R represents retraction neuron B64. Thick outline of a neuron denotes high firing frequency. Thin outline of a neuron denotes low firing frequency. Gray outline of a neuron signifies virtually no activity. Triangles denote excitatory connections. Circles denote inhibitory connections. Synaptic connections shown by dashed lines denote modulatory synaptic connections. Thick dashed line through the middle of the diagram denotes the transition between the protraction and the retraction phases. Neurons are shown in the left or right part of the diagram depending on whether they are active in the protraction or the retraction phase. Neurons are shown by 10.220.32.246 on September 27, 2016 http://jn.physiology.org/ Downloaded from
firing frequency remained high, whereas B40 firing frequency remained low. Consistent with the persistent changes in the firing frequency of B20 and B40, we found that the excitability of these neurons was persistently altered after repeated EN stimulation—B20 excitability was increased (Fig. 5), whereas B40 excitability was decreased (Fig. 6). Thus the persistence of eggestive responses after the switch in the stimulus is accompanied by both the potentiation of processes that promote eggestion and the concurrent suppression of processes that promote ingestion.

This conclusion is further supported by experiments with the synapses through which B20 and B40 act to shape the ingestive versus eggestive characteristics of the motor output. We previously demonstrated that the B20-to-B8 synapse is facilitated after repeated EN stimulation and that this synaptic facilitation is accompanied by an increase in the functional efficacy of the synapse (Proekt et al. 2004) (Fig. 9, B2 and B3). This synapse serves to excite B8 during the protraction phase. Because high frequency of B8 firing in the protraction phase is a defining characteristic of eggestive responses (Morgan et al. 2002), the potentiation of the B20-to-B8 synapse serves to promote egestion.

Here we have shown that the excitatory component of the B40-to-B8 synapse was suppressed after EN stimulation. B40 fires during the protraction phase of ingestive programs, but promotes B8 firing in the subsequent retraction phase (Fig. 9B1). B40 accomplishes this by eliciting a complex synaptic response in B8 that consists of the fast inhibitory component and the slow excitatory component. Although the fast inhibition serves to suppress B8 firing during the protraction phase, the slow excitation outlasts B40 firing and promotes B8 firing in the retraction phase (Jing and Weiss 2002; Jing et al. 2003). The high firing frequency of B8 in the retraction phase defines ingestive responses (Morgan et al. 2002). Thus suppression of the excitatory component of the B40-to-B8 synapse serves to suppress ingestive responses after repeated EN stimulation.

These experiments illustrate that the reconfiguration of the feeding network by repeated EN stimulation involves simultaneous coordinated changes in several neurons and synapses. Similar conclusions have been reached in the analysis of network reconfiguration in the leech CPG that produces crawling and swimming motor programs. By simultaneously observing the firing of a population of neurons, Briggman et al. (2005) were able to effectively predict which motor pattern would be produced, earlier than by observing any individual neuron. Here, we demonstrate that these coherent changes in firing of a population of neurons are paralleled by changes in their excitability and synaptic connections.

Our experiments further suggest that firing of neuron B65 can produce these coordinated changes in neuronal excitability and synaptic connections. Stimulation of B65 resulted in the persistent increase in the excitability of B20 (Fig. 9B2). Potentiation of the B20-to-B8 synapse is known to be activity dependent (Proekt et al. 2004); thus the increase in the excitability and the consequent increase in the firing frequency of B20 may also indirectly lead to the potentiation of the B20-to-B8 synapse (Fig. 9B2). Additionally, B65 firing leads to the persistent suppression of the B40 excitability and the direct suppression of the B40-to-B8 synapse.

B65 synapses onto many CPG neurons and receives input from them (Due et al. 2004; Jing and Weiss 2005; Kabotyanski et al. 1998). Stimulation of B65 can elicit motor programs (Kabotyanski et al. 1998). Furthermore, suppression of B65 firing produces a modest effect on the protraction duration and B8 firing frequency in EN-elicited eggestive motor programs (Due et al. 2004). Thus B65 is a component of the CPG. Here we found that B65 did not fire during ingestive programs. Furthermore, although B65 was strongly active in EN-elicited eggestive programs, it did not fire in eggestive programs elicited by CBI-2. Thus B65 firing is not essential for eggestive programs—the only type of motor output that recruits its activity. The lack of firing of B65 in CBI-2-elicited motor programs may be explained by direct inhibition of B65 by CBI-2 (Jing and Weiss 2005).

Thus although B65 is a bona fide component of the CPG, its firing does not have strong significance for the immediate motor output. Instead, activity of B65 most closely followed the input. It remains to be determined, however, whether additional neurons are also selectively activated in EN-elicited programs. This is an interesting question because the EN is the only nerve that connects the feeding network with the gut and the esophagus. As such, EN is likely to carry information about the presence of chemo-stimuli in the gut (Susswein et al. 1984) and about the degree of stretch of the gut and the esophagus (Kuslansky et al. 1987; Morgan et al. 2002). It is possible that these different sensory modalities recruit activity of distinct populations of CPG elements. In addition, EN is known to contain neuropeptides and two of these neuropeptides have been shown to convert ingestive CBI-2–elicited motor programs to eggestive ones (Jing et al. 2007; Wu et al. 2006). If these neuropeptides are released during EN stimulation, they could exert persistent action on CPG elements. Through this mechanism, neuropeptides released by EN stimulation could provide a parallel means of input representation in the feeding CPG.

Previous work that examined how the same CPG produces different behaviors focused on identifying command neurons that activate the CPG (e.g., Beenakker and Nusbaum 2004 Croll et al. 1985; Morgan et al. 2002; Rosen et al. 1991). Previously it was argued that control over which behavior is produced is achieved by a population code (Georgopoulos et al. 1986; Kristan and Shaw 1997; Lewis 1999; Lewis and Kristan 1998), meaning that a population of higher-order neurons activated by the stimulus determines the nature of the response. A similar scheme was proposed for Aplysia feeding (Jing and Weiss 2005; Morgan et al. 2002). Nonetheless, in the feeding system, the decisions as to which behavior to generate is controlled not just by the population of higher-order neurons, but also by the state of the feeding network itself. By focusing on this state dependency, we discovered two fundamentally distinct populations of neurons—input- and output-representing interneurons. This distinction is well established in networks involved in decision making. For instance, when a monkey is trained to indicate the direction of movement of dots on a screen by a saccade, the activity of neurons in the extrastriate visual cortex reflects the net movement of dots, whereas the activity of neurons in the frontal eye fields reflects the direction of the saccade (Cohen and Newsome 2004). Interestingly, electrical stimulation of the frontal eye fields immediately before the voluntary saccade elicits a saccade that deviates from the control stimulation in the same direction as the subsequent voluntary action (Gold and Shadlen 2000). This
suggests that the behavior-generating neurons are reconfigured before the onset of action and this reconfiguration dictates the nature of the subsequent behavioral response.

There is considerable debate concerning the role of individual brain areas in decision making. For instance, it was argued that neurons in the lateral intraparietal area (LIP) encode the perception of motion (Assad and Maunsell 1995; Williams et al. 2003), visual attention (Bisley and Goldberg 2003), decisional processes (Shadlen and Newsome 2001), and forming intentions (i.e., plans for future behavior) (Andersen and Buneo 2002). Here, we used the simplicity of the invertebrate preparation to show that firing of B65 reflects the instantaneous stimulus, although its actions may shape the future behavior of the feeding network. Thus firing of B65 may provide a link between the instantaneous stimulus and future behaviors.

Virtually all animals, from simple invertebrates to humans, live in complex environments. Animals’ survival depends on their ability to respond differentially and efficiently to the different stimuli to which they are exposed. However, making an animal respond immediately to a stimulus may leave it at the mercy of transient stimuli and prevent it from achieving a specific goal. This may have led to evolution of strategies that allow animals to balance the need to respond to sensory cues with the need to pursue their internal goals. The slow dynamics of switching between different behavioral responses could serve to suppress responses to transient stimuli and emphasize responses to persistent and repeated stimuli. Because environmental stimuli are not distributed randomly, but are clustered or clumped together, the slow dynamics exhibited by the feeding CPG could embody an adaptive behavioral strategy.

ACKNOWLEDGMENTS

We thank Drs. Elizabeth Cropper, Alex Kentsis, Ellen Yang, and Michael Due for helpful and critical discussion.

GRANTS

This work was funded by National Institute of Mental Health Grant MH-036730.

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