Reference: *Biol. Bull.* **210:** 215–229. (June 2006)

A contribution to *The Biological Bulletin Virtual Symposium on Marine Invertebrate Models of Learning and Memory.*

Target-Specific Regulation of Synaptic Efficacy in the Feeding Central Pattern Generator of *Aplysia:* Potential Substrates for Behavioral Plasticity?

MANUEL DÍAZ-RÍOS AND MARK W. MILLER*

Institute of Neurobiology and Department of Anatomy, University of Puerto Rico, 201 Blvd. del Valle, San Juan, Puerto Rico 00901

Abstract. The contributions to this symposium are unified by their focus on the role of synaptic plasticity in sensorimotor learning. Synaptic plasticities are also known to operate within the central pattern generator (CPG) circuits that produce repetitive motor programs, where their relation to adaptive behavior is less well understood. This study examined divergent synaptic plasticity in the signaling of an influential interneuron, B20, located within the CPG that controls consummatory feeding-related behaviors in *Aplysia.* Previously, B20 was shown to contain markers for catecholamines and GABA (Díaz-Ríos *et al.,* 2002), and its rapid synaptic signaling to two follower motor neurons, B16 and B8, was found to be mediated by dopamine (Díaz-Ríos and Miller, 2005). In this investigation, two incremental forms of increased synaptic efficacy, facilitation and summation, were both greater in the signaling from B20 to B8 than in the signaling from B20 to B16. Manipulation of the membrane potentials of the two postsynaptic motor neurons did not affect facilitation of excitatory postsynaptic potentials (EPSPs) to either follower cell. Striking levels of summation in B8, however, were eliminated at hyperpolarized membrane potentials and could be attributed to distinctive membrane properties of this postsynaptic cell. GABA and the $GABA_B$ agonist baclofen increased facilitation and summation of EPSPs from B20 to B8, but not to B16. The enhanced facilitation was not affected when the membrane potential of B8 was pre-set to hyperpolarized levels, but GABAergic effects on summation were eliminated by this manipulation. These observations demonstrate a target-specific amplification of synaptic efficacy that can contribute to channeling the flow of divergent information from an intrinsic interneuron within the buccal CPG. They further suggest that GABA, acting as a cotransmitter in B20, could induce coordinated and target-specific pre- and postsynaptic modulation of these signals. Finally, we speculate that target-specific plasticity and its modulation could be efficient, specific, and flexible substrates for learning-related modifications of CPG function.

Received 7 November 2005; accepted 23 January 2006.

* To whom correspondence should be addressed. E-mail: mmiller@neurobio.upr.clu.edu

Introduction

The contributions to this symposium underscore the central position of activity-dependent synaptic plasticity in our present understanding of sensorimotor learning. One attribute of such plasticities that renders them suitable for producing adaptive behavior is their own capacity to be modified (Krasne, 1978; Byrne and Kandel, 1996; Abraham and Bear, 1996). Recent investigations have broadened our appreciation for the occurrence of diverse forms of synaptic plasticity within the central pattern generator (CPG) circuits that produce repetitive behaviors (Marder, 1998; Parker and Grillner, 1999, 2000; Nadim and Manor, 2000; Sakurai and Katz, 2003; Yuste *et al.,* 2005). The contribution of such activity-dependent modifications of synaptic strength to adaptive CPG-driven motor activity, however, remains incompletely understood.

The consummatory behaviors of *Aplysia* are generated by a multifunctional or polymorphic CPG that supports several forms of non-associative and associative behavioral plasticity (Susswein and Schwarz, 1983; Kupfermann *et al.,* 1989; Colwill *et al.,* 1997; Lechner *et al.,* 2000a). Cellular analyses of *in vitro* analogs have begun to reveal potential correlates of experience-dependent plasticity at multiple levels of the feeding network (Nargeot *et al.,* 1997, 1999a, b;

Lechner *et al.,* 2000b; Brembs *et al.,* 2002, 2004; Mozzachiodi *et al.,* 2003; Proekt *et al.,* 2004). These correlates often correspond to loci where activity-dependent synaptic plasticity is known to occur. For example, command-like interganglionic projection neurons in the cerebral ganglion activate CPG circuits in the buccal ganglion *via* excitatory postsynaptic potentials (EPSPs) that exhibit various forms of short-term enhancement (Sánchez and Kirk, 2000, 2002; Hurwitz *et al.,* 2003). Signaling from the interneurons within the buccal CPG to the motor neurons that execute consummatory feeding behaviors also displays several forms of plasticity (Teyke *et al.,* 1993; Kabotyanski *et al.,* 1998; Jing and Weiss, 2002). Finally, patterned firing by the motor neurons themselves produce facilitating or depressing excitatory junction potentials in the buccal muscles (Cohen *et al.,* 1978; Cropper *et al.,* 1990; Jordan *et al.,* 1993). The possible involvement of these synaptic plasticities with feeding-related behavioral plasticities is largely unexplored.

Regulation of synaptic efficacy by neuromodulatory messengers also plays a pivotal role in learning and memory in several invertebrate model systems (Kandel and Schwartz, 1982; Hawkins *et al.,* 1993; Menzel, 2001; Balaban, 2002). Neuromodulatory control has been intensively studied in CPG circuits, where broadly acting messengers are capable of regulating the biophysical properties of constituent neurons and their synaptic connections in a coherent fashion (Kupfermann, 1979; Harris-Warrick and Marder, 1991; Katz, 1999). When modulators originate from neurons that are not *sensu stricto* participants in the CPG, they are considered extrinsic (Kupfermann *et al.,* 1979; Morgan *et al.,* 2000). When they derive from neurons that are themselves elements of the CPG (Katz and Frost, 1996), or from motor neurons (Cropper *et al.,* 1987), they are designated intrinsic. In both configurations, modulators are frequently released from neurons in which they occur as cotransmitters (Kupfermann, 1991; Weiss *et al.,* 1992; Nusbaum *et al.,* 2001).

Recently, colocalization of markers for GABA and catecholamines was demonstrated in B20 (Díaz-Ríos *et al.,* 2002), an intrinsic buccal CPG interneuron that can initiate and specify the consummatory feeding motor programs of *Aplysia* (Teyke *et al.,* 1993; Jing and Weiss, 2001; Proekt *et al.,* 2004). Pharmacological evidence supported the role of dopamine as the mediator of fast synaptic signaling from B20 to B16 and B8, two motor neurons that cause closure of the food-grasping radula (Fig. 1A; see Díaz-Ríos and Miller, 2005). GABA, acting *via* $GABA_B$-like receptors, exerted differential modulatory actions on synaptic transmission from B20 to B16 and B8 (Díaz-Ríos and Miller, 2005). In that study, the effects of GABA were tested on EPSPs that were evoked by single B20 impulses. As B20 ordinarily fires in a burst mode during feeding motor programs (Teyke *et al.,* 1993; Jing and Weiss, 2001; Proekt *et al.,* 2004), this investigation explored some plastic properties of its divergent synaptic signaling and their modification by GABA. Some of these observations have appeared in abstract form (Díaz-Ríos and Miller, 2002).

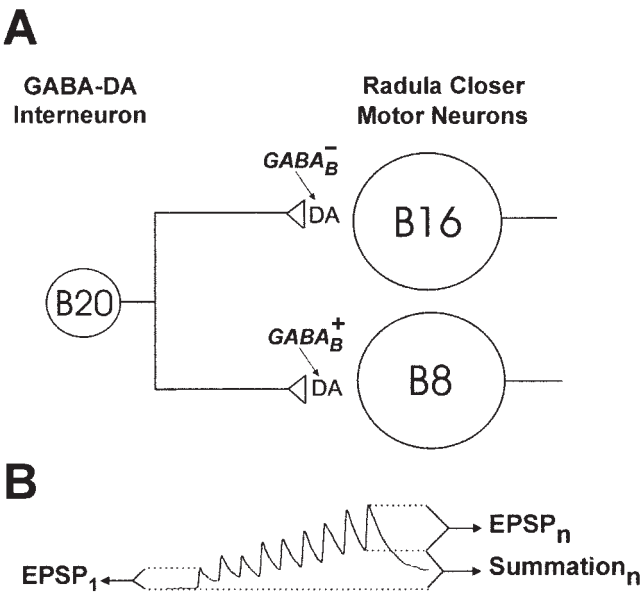

Figure 1. Divergent signaling from interneuron B20 to motor neurons B16 and B8. (A) Schematic of the synaptic signals examined in this investigation (adapted from Díaz-Ríos and Miller, 2005; used with permission of the American Physiological Society). The cell body of interneuron B20 is located in the central region of each buccal hemiganglion. It produces direct impulse-mediated EPSPs in two motor neurons (B16 and B8) that contribute to closure of the radula (food-grasping structure). In both targets, this rapid signaling is produced by dopamine (DA; Díaz-Ríos and Miller, 2005). EPSPs produced by firing single B20 impulses are modified by GABA as indicated (*italics*). (B) Calculation of summation and facilitation. Summation of $EPSP_n$ was measured as the difference between the membrane potential at the onset of $EPSP_n$ and the resting membrane potential prior to $EPSP_1$. Facilitation of $EPSP_n$ was calculated as the amplitude of $EPSP_n$, measured from its summation level to its peak, divided by the amplitude of $EPSP_1$ (EPSP values corrected for changes in driving force; see Methods).

Materials and Methods

Subjects

Experiments were conducted on specimens of *Aplysia californica* (150–250 g) that were purchased from the *Aplysia* Resource Facility and Experimental Hatchery (University of Miami, Miami, FL) or from Marinus Inc. (Long Beach, CA). Animals were maintained in a refrigerated aquarium (14–16 °C) and fed dried seaweed twice per week. Experimental protocols followed established guidelines of the Institutional Animal Care and Use Committee of the University of Puerto Rico Medical Sciences Campus.

Electrophysiology and pharmacology

Neurons were identified in preparations consisting of the paired buccal and cerebral ganglia. Intracellular microelec-

trodes filled with 2 *M* KCl (10–20 MΩ) were used to record from B20 and its direct synaptic follower motor neurons B16 and B8 (Teyke *et al.,* 1993). Current was injected into the postsynaptic cells by a second intracellular electrode (5–10 MΩ). B20 was stimulated *via* passage of current through the recording electrode across the bridge circuit of the amplifier (NeuroProbe, A-M Systems). All experiments were conducted in artificial seawater (ASW) containing high concentrations of divalent cations (Liao and Walters, 2002) to attenuate polysynaptic activity.

Solutions of drugs at the concentration to be applied were prepared in high-divalent ASW immediately before the start of each experiment. GABA and (±)-baclofen were obtained from Sigma Chemical Co. (St. Louis, MO). All experiments were performed with application of agonists at a concentration of 1 m*M,* which produced consistent and reversible effects in previous studies (see Díaz-Ríos and Miller, 2005). Drugs were delivered at a rate of 0.5 ml/min by a gravity-fed perfusion system (ALA Scientific Instruments, model VM4). Responses were recorded 2, 5, and 10 min after switching the perfusion source.

Measurements and statistics

A measurement of summation (Sum_n; unit: mV) for a particular EPSP in a train ($EPSP_n$) was obtained by subtracting the postsynaptic membrane potential prior to initiation of the train (V_{rest}) from the membrane potential immediately prior to the onset of $EPSP_n$ (Fig. 1B). An operational measure of facilitation of $EPSP_n$ (F_n, a unitless value) was defined as $(EPSP_n/EPSP_1) - 1$, so that a value of 0 signified the absence of facilitation (Fig. 1B). EPSP amplitudes were corrected for summation by subtracting Sum_n. They were also corrected for changes in driving force (Martin, 1955), using an estimated value of +5 mV as the reversal potential of B20-evoked fast EPSPs (Díaz-Ríos and Miller, 2005).

In view of the presence of long-lasting forms of plasticity and GABAergic actions in this system (Díaz-Ríos and Miller 2002, 2005), statistical tests (Student's paired *t* test; two-tailed) were performed by comparing measurements obtained prior to drug application to measurements obtained at the peak of the response. A value of $P < 0.05$ was established as the criterion for significance.

Results

Divergent synaptic signaling from B20 to B16 and B8

In a previous study, we found the rapid synaptic signaling from B20 to B16 and B8 to be mediated by dopamine (See Fig. 1A and Díaz-Ríos and Miller, 2005). During that investigation, repetitive firing of B20 revealed two forms of incremental increases in the efficacy of its excitatory signaling—facilitation and summation (Fig. 1B; see also Teyke *et al.,* 1993; Jing and Weiss, 2001). Simultaneous recording from B16 and B8 allowed us to directly compare facilitation and summation during their responses to B20 impulse trains (Fig. 2A). Facilitation values (see definition in Materials and Methods) measured at the 10th EPSP in a train in B8 (1.8 ± 0.4; mean ± SEM) were greater than those observed in B16 (1.2 ± 0.2; $t = 4.63$; $P < 0.05$; $n = 4$; Fig. 2B). Summation values (see definition in Materials and Methods) measured at the tenth EPSP in a train in B8 (6.1 ± 0.5 mV) were also greater than those observed in B16 (4.3 ± 0.3 mV; $t = 7.59$, $P < 0.05$; $n = 4$; Fig. 2C).

The activity-dependent short-term plasticity of EPSPs originating from B20 was further examined in experiments in which the postsynaptic membrane potentials of B16 and B8 were set to hyperpolarized levels. Synaptic facilitation, which is generally thought to reflect presynaptic conditions that produce increases in neurotransmitter release (del Castillo and Katz, 1954; Fisher *et al.,* 1997; Zucker and Regher, 2002), was not affected by this manipulation (Fig. 2A–C). Summation, on the other hand, was differentially affected in the two synaptic followers. Whereas the measures of summation increased in B16 when that cell was pre-set to hyperpolarized levels (Fig. 3A, D), summation was severely attenuated in B8 when it was hyperpolarized (Fig. 3B, D).

The differential effects of membrane potential on synap-

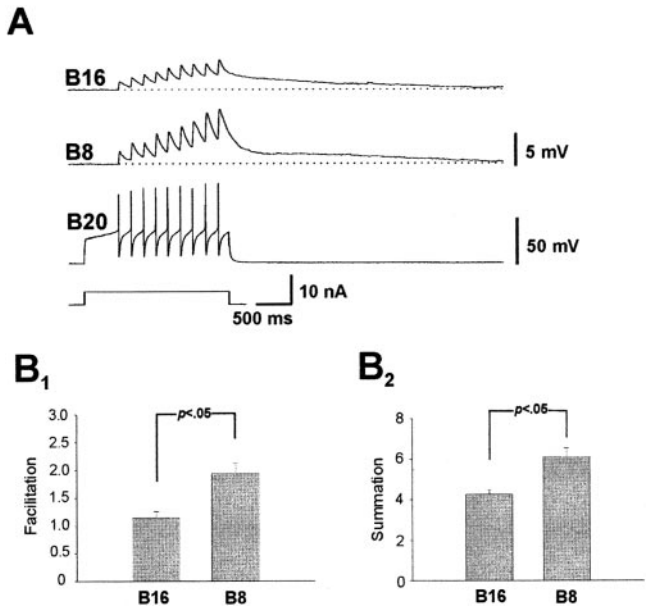

Figure 2. Divergent synaptic plasticity from interneuron B20 to two follower motor neurons. (A) Simultaneous intracellular recording from B20 (bottom record) and its two follower motor neurons, B16 (top record) and B8 (middle record). A 3-s pulse of depolarizing current (shown below recordings) was passed into B20, causing it to fire at a rate of about 10 Hz. The EPSPs recorded in both B16 and B8 exhibited summation and facilitation. (B_1) Grouped data showing that facilitation was greater in the signaling from B20 to B8 than to B16. (B_2) Grouped data showing that summation was greater in the signaling from B20 to B8 than to B16.

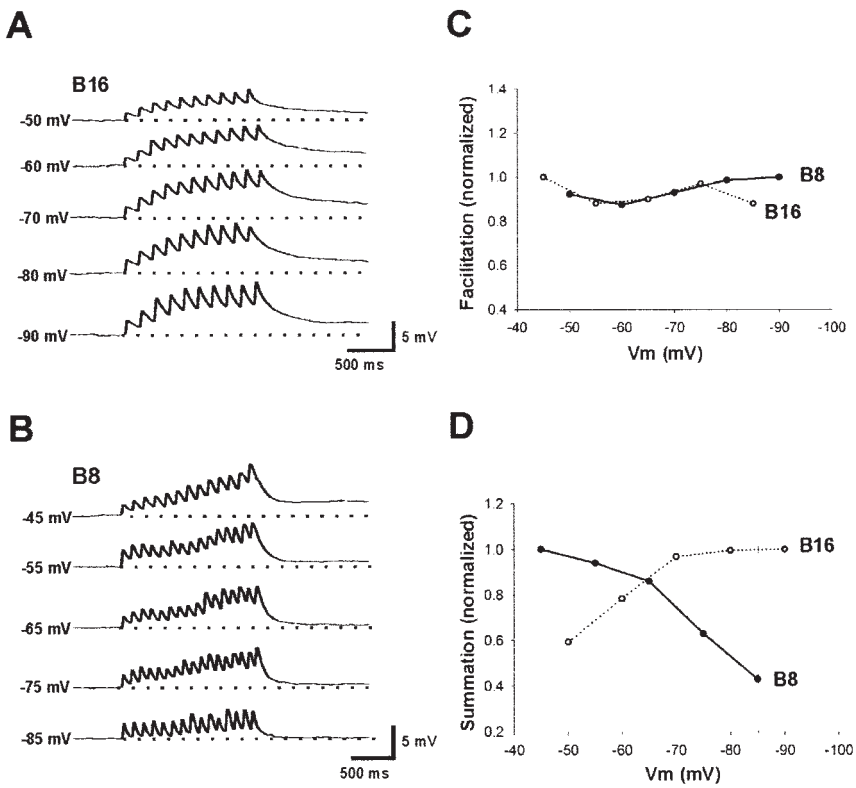

Figure 3. Effect of postsynaptic membrane potential on the plastic properties of signaling from interneuron B20 to motor neurons B16 and B8. (A) Current was injected into B16 to pre-set its membrane potential to hyperpolarized levels (shown at left of each record) prior to producing a train of impulses in B20. As the postsynaptic membrane potential was held at progressively more hyperpolarized levels, the degree to which evoked EPSPs exhibited summation was progressively increased, while the level of facilitation ($EPSP_{10}/EPSP_1$) did not vary. (B) Current was injected into B8 to pre-set its membrane potential to hyperpolarized levels (shown at left of each record) prior to producing a train of impulses in B20. As the postsynaptic membrane potential was held at more hyperpolarized levels, the degree to which evoked EPSPs exhibited summation was progressively decreased, while the level of facilitation ($EPSP_{10}/EPSP_1$) did not vary. (C) Graphed dependence of facilitation on membrane potential of B16 and B8. Facilitation values were normalized to maximal value in each neuron. (D) Graphed dependence of summation on membrane potential of B16 and B8. Summation values were normalized to maximal value in each neuron.

tic summation in B16 and B8 prompted us to compare the current-voltage relations in these two motor neurons. Voltage responses to current pulses (2 s) injected into B16 rapidly reached a stable value (Fig. 4A). A plot of these voltage displacements as a function of injected current values revealed a predominantly linear relation with limited rectification in the depolarizing quadrant (Fig. $4A_2$). Responses to current pulses in B8 were more complex (see also Klein *et al.,* 2000; Díaz-Ríos and Miller, 2005). Pulses to levels more hyperpolarized than about −70 mV exhibited a depolarizing sag (Fig. $4B_1$, arrow). Upon release from these large pulses, the membrane potential of B8 displayed a transient depolarization (Fig. $4B_1$, arrowhead) prior to reestablishing its resting level. Although not further explored here, such responses have been shown to reflect the presence of hyperpolarization-activated inward currents (I_h) in other invertebrate motor systems (Angstadt and Calabrese, 1989; Golowasch and Marder, 1992). Depolarizing current pulses also revealed time-dependent voltage responses in B8. When stepped to levels more depolarized than −35 mV, the membrane potential response exhibited a gradual depolarizing drift (Fig. $4B_1$).

In view of the time-dependent properties of the voltage deflections in B8, two values were measured for each current pulse. When initial values of voltage responses (designated "early" in Fig. $4B_2$, closed symbols) were plotted, a linear relation comparable to that observed in B16 was obtained. However, voltage values measured immediately prior to the termination of current pulses (designated "late" in Fig. $4B_2$, open symbols) revealed departures from ohmic behavior in both the hyperpolarizing and depolarizing quadrants. These deviations reflect, respectively, the sag and the drift described above.

Longer current pulses (5 s) were delivered to B8 to

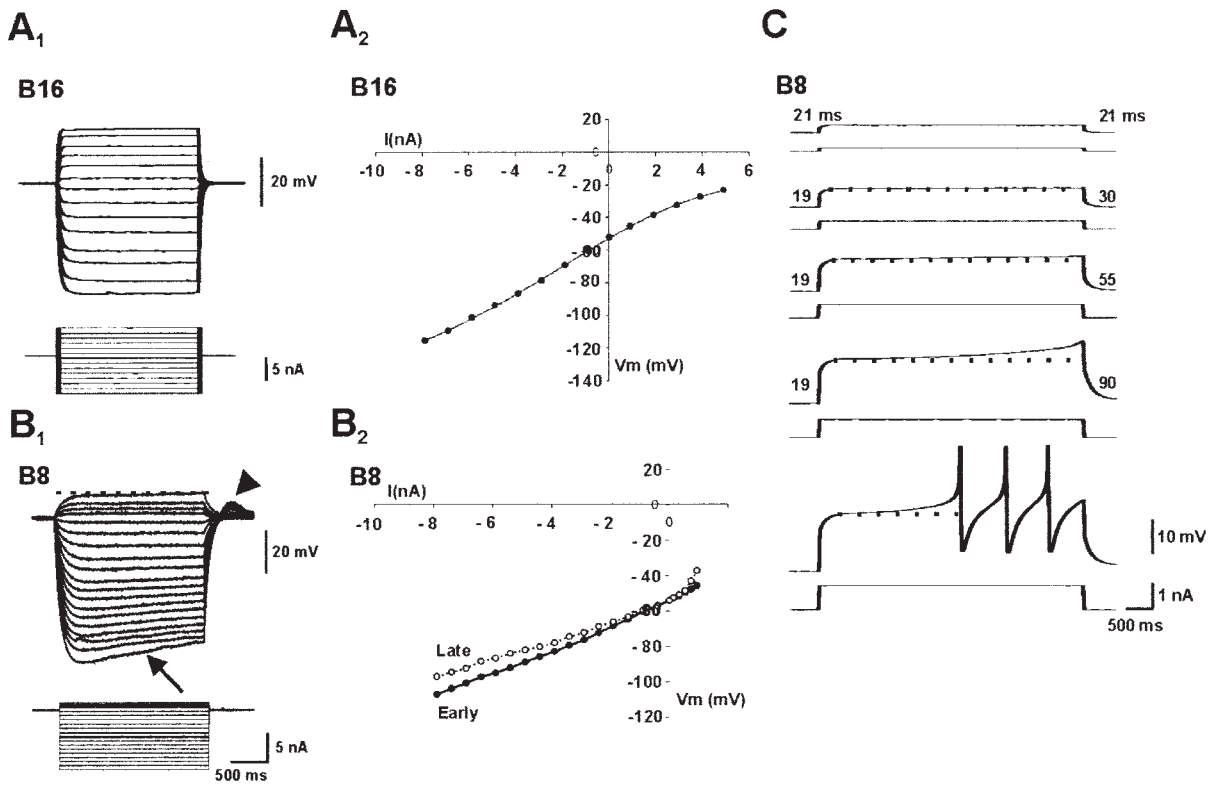

Figure 4. Current-voltage relations of B16 and B8. (A_1) Current pulses (2 s, lower records) were passed into B16 and voltage deflections (upper records) were measured with an independent electrode. (A_2) Plot of current-voltage relation from an experiment such as A_1. The deviation from linearity in the depolarizing quadrant is likely due to delayed rectification in this cell. (B_1) Voltage deflections (upper records) to current pulses (lower records) passed into B8 exhibited time-dependent characteristics. Large hyperpolarizing pulses revealed a depolarizing sag (arrow) and a transient depolarization (arrowhead) following release from the pulse. Depolarizing pulses also exhibited a slowly developing depolarization. (B_2) Plot of current-voltage relation from an experiment such as B_1. Voltage measurements were determined 150 ms after the onset of each pulse (Early) and immediately prior to its termination (Late). While peak values reflected an ohmic current-voltage relation, the late values deviated from linearity in both the hyperpolarizing and depolarizing quadrants. (C) Longer current pulses (5 s) were delivered to B8 to further examine the slowly developing response to depolarization. The time constant of the onset of voltage responses (indicated at the left of each record) remained in the range of 20 ± 1 ms as the amplitude of the current pulse was increased. The time constant of decay (indicated at the right of each record), however, increased from 21 to 90 ms as the amplitude of the current pulse was augmented. Injection of larger current pulses produced gradual depolarizations that could exceed threshold (lowest records).

further examine the slowly developing response to depolarization. With increasing pulse amplitudes, the time constant of the onset of voltage responses was unchanged (indicated at the left of each voltage trace of Fig. 4C). The time constant of decay, however, was progressively increased (indicated at the right of each record), suggesting that the depolarizing drift in B8 is associated with an increase in the membrane resistance. The gradual depolarizations produced by injection of larger current pulses could exceed threshold and elicit repetitive impulses (lowest records).

The possible influence of the membrane properties of B16 and B8 on EPSPs was examined using brief depolarizing pulses as a crude simulation of synaptic currents (Fig. 5). The amplitude and duration of a current pulse were adjusted to produce a depolarizing response that resembled a B20-evoked EPSP in B16. At the resting membrane potential of B16 (−48 mV), this pulse produced a depolarization of 2 mV with a half-time of decay of 80 ms (Fig. $5A_1$, black recording). When the membrane was hyperpolarized to a level 40 mV more hyperpolarized than rest, the amplitude of the response was increased, but its rate of decay was unchanged (Fig. $5A_1$, gray recording). In contrast, a similar manipulation of the membrane potential in B8 produced a marked increase in the decay kinetics of its response to a pulse of 4 nA. Whereas responses at rest (−46 mV) exhibited a decay half time of 85 ms (Fig. $5B_1$, black recording), those elicited with the *V*m set 40 mV more hyperpolarized than rest decayed with a half time of 35 ms (Fig. $5B_1$, gray recording). The amplitude of the voltage response in B8 was relatively unaffected by hyperpolarization (see Discussion).

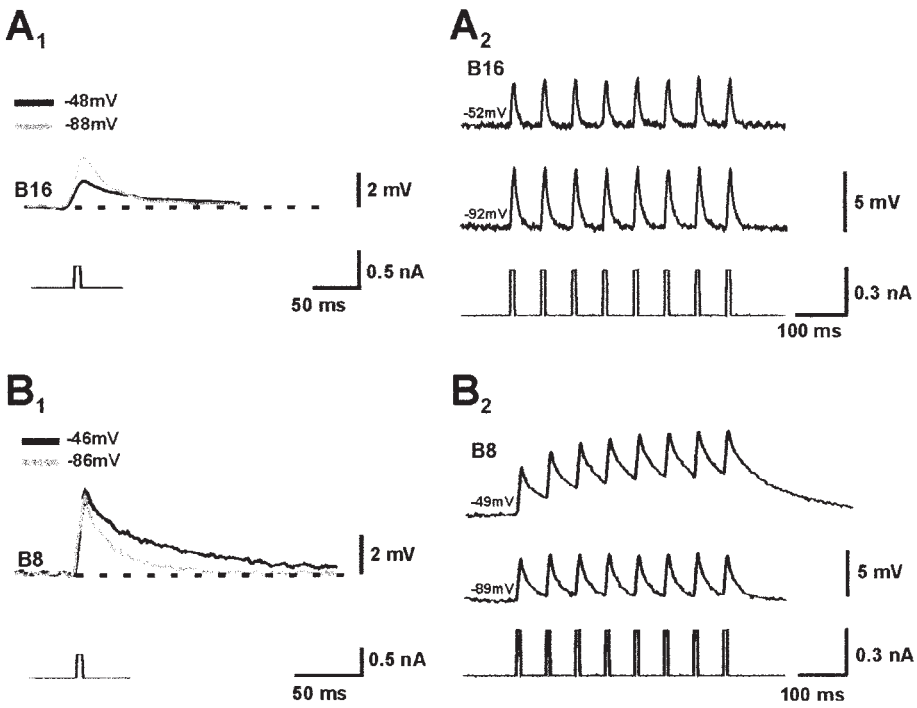

Figure 5. Differential membrane properties of B16 and B8 revealed by brief depolarizing pulses. Current pulses were injected into B16 and B8 *via* current-passing electrodes. The brief duration of these pulses (5 ms) in comparison to the membrane time constant (see Fig. 4) produced incomplete charging of the membrane. (A_1) Injection of a pulse (bottom record) into B16. The time course of the resulting potential deflection at rest (dark recording) was similar to that observed when the membrane potential of B16 was pre-set at a level 40 mV more hyperpolarized than rest (gray recording). (A_2) When a train of current pulses (5 ms, 0.3 nA) was injected into B16 (lowest record), the membrane potential returned to baseline between each response, whether the B16 was at its resting potential (upper record) or pre-set at the more hyperpolarized level (middle record). (B_1) A 5-ms pulse (bottom record) was injected into B8. The rate of decay of the resulting potential change at rest (dark recording) was substantially slower than that observed when the membrane potential was pre-set at a level 40 mV more hyperpolarized than rest (gray recording). (B_2) When a train of current pulses (same parameters as A_2) was injected into B8 (lowest record), the membrane potential did not return to pre-pulse levels between each response when B8 was at its resting potential (upper record). When B8 was pre-set at a level 40 mV more hyperpolarized than rest, each deflection returned to baseline prior to the onset of the subsequent response (middle record).

The differential effects of membrane potential on responses to current pulses suggested that the specific membrane properties of B16 and B8 could contribute to the observed differences in the summation of their EPSPs originating from B20. This possibility was examined by injecting trains of pulses to simulate the EPSP trains that occur during B20 bursts. Whereas the membrane potential of B16 decayed to rest (−52 mV) between each pulse (Fig. $5A_2$, upper trace), those in B8 exhibited summation when the membrane was initially at its resting level (−49 mV; Fig. $5B_2$, upper trace). When the membrane potential of B8 was pre-set to −89 mV, each pulse returned to this level prior to the onset of the subsequent response (Fig. $5B_2$, lower trace). These findings are consistent with the observed dependence of synaptic summation on the membrane potential of B8 (Fig. 2B, D).

Together, these results indicate that the divergent signaling from B20 to two motor neurons, B16 and B8, exhibits distinctions in two forms of use-dependent increases in efficacy, facilitation, and summation. Although the differences in facilitation appear to reflect target-specific properties of plasticity at B20 terminals, the difference in summation can be attributed to dissimilar membrane properties in the two postsynaptic cells. The facilitation and summation both bias the excitatory signaling from B20 toward B8 *versus* its signaling to B16.

GABAergic regulation of divergent synaptic enhancement from B20 to B16 and B8

GABA-like immunoreactivity is present in B20 (Díaz-Ríos *et al.,* 2002), and GABA has been shown to modify synaptic signaling of this neuron *via* $GABA_B$-like receptors (Díaz-Ríos and Miller, 2005). It was therefore of interest to examine the effects of GABA on facilitation and summation of B20-evoked EPSPs in B16 and B8. Application of ex-

ogenous GABA (up to 1 m*M*) did not affect facilitation or summation of the excitatory signaling of B20 to B16 (Fig. $6A_{1,2}$). Facilitation of $EPSP_{10}$ in the presence of GABA (0.48 ± 0.14; mean ± SEM) did not differ from control values (0.37 ± 0.16; $t = 1.22$; $P > 0.05$; $n = 4$; Fig. $6B_1$). Likewise, the level of summation of $EPSP_{10}$ in GABA (2.1 ± 0.2 mV) did not differ from control values (2.4 ± 0.3 mV; $t = 0.83$; $P > 0.05$; $n = 4$; Fig. $6B_2$).

In contrast, application of GABA had potent effects on both facilitation and summation of rapid signaling from B20 to B8 (Fig. $7A_{1,2}$). Facilitation of $EPSP_{10}$ in the presence of GABA (3.7 ± .8; mean ± SEM) was significantly greater than control values (2.6 ± 0.3; $t = 1.4$; $P < 0.05$; $n = 5$; Fig. $7B_1$). Likewise, the level of summation of $EPSP_{10}$ in GABA (4.2 ± 0.5 mV) was significantly greater than control values (2.9 ± 0.2 mV; $t = 2.69$; $P < 0.05$; $n = 5$; Fig. $7B_2$). Each of the actions observed with GABA was also produced by the $GABA_B$ agonist baclofen (Fig. $8A_{1,2}$). Facilitation of $EPSP_{10}$ in the presence of baclofen (1.6 ± 0.2) was significantly greater than control values (0.6 ± 0.1; $t = 4.43$; $P < 0.05$; $n = 5$; Fig. $8B_1$). The level of summation of $EPSP_{10}$ in baclofen (7.9 ± 0.4 mV) was also significantly greater than control values (3.3 ± 0.1 mV; $t = 7.60$; $P < 0.05$; $n = 5$; Fig. $8B_2$).

When B8 was at its resting potential, it was not possible to examine the effects of GABA on synaptic facilitation or summation individually. But adjusting the membrane potential of B8 to levels 40 mV more hyperpolarized than rest markedly diminished summation of the B20-to-B8 EPSPs (see Fig. 3), enabling a more direct examination of GABAergic effects on facilitation of this signal (Fig. 9). GABA (1 m*M*) produced a significant increase ($t = 6.89$; $P < 0.05$) in the facilitation value of $EPSP_{10}$ which was reversed upon wash with normal ASW (Fig. 9A, C_1). Baclofen (1 m*M*) produced a comparable increase ($t = 5.95$; $P < .05$) in the facilitation of $EPSP_{10}$ in B8 when it was pre-set to hyperpolarized membrane potentials (Fig. 9B, C_2). The increases in summation that were elicited by GABA and baclofen when B8 was at its resting potential (Fig. 7 and Fig. 8) were not observed at hyperpolarized levels.

Finally, the effects of baclofen on summation were examined using small current pulses (250 pA, 20 ms) injected into B8 to simulate synaptic currents without firing the presynaptic neuron B20 (Fig. 10). It was previously shown that trains of such pulses can exhibit "summation" when applied within the physiological firing frequency of B20 (Fig. 5). Application of baclofen (1 m*M*) decreased the rate of decay of voltage deflections such that successive pulses within a train were incrementally prolonged and their summed depolarization of B8 was increased (Fig. 10B–D). As the effect of baclofen developed, the cumulative depo-

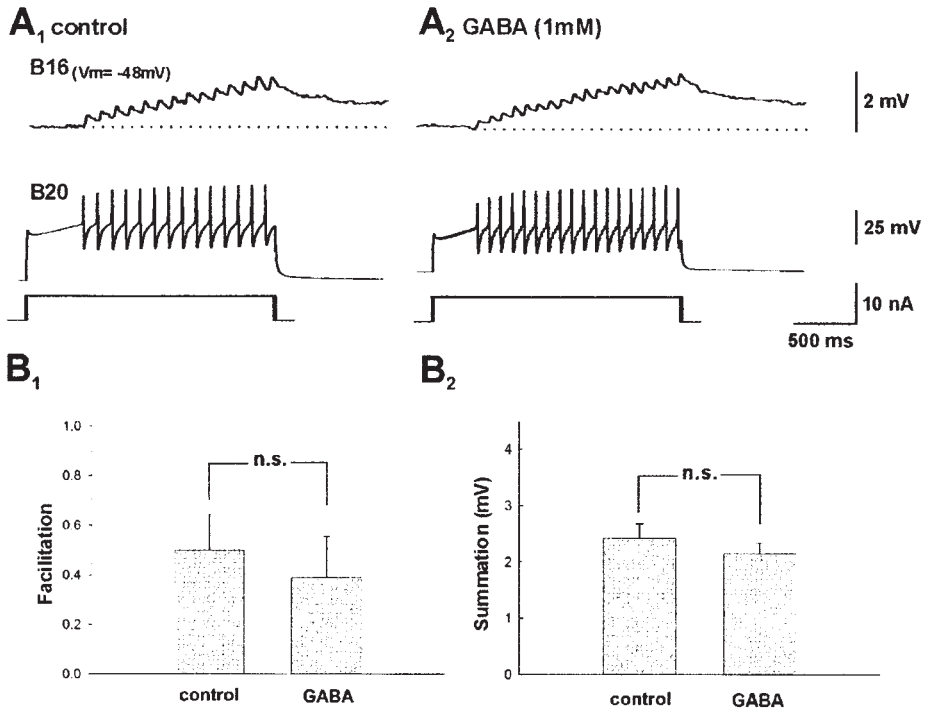

Figure 6. GABA does not modify the plastic properties of signaling from B20 to B16. (A) Application of GABA (1 m*M*) did not produce detectable effects on facilitation or summation of signaling from B20 to B16. (B_1) Grouped data did not reveal significant effects of GABA on facilitation ($n = 3$). (B_2) Grouped data did not reveal a significant effect of GABA on the value of $summation_{10}$ of the rapid excitatory signaling from B20 to B16 ($n = 3$).

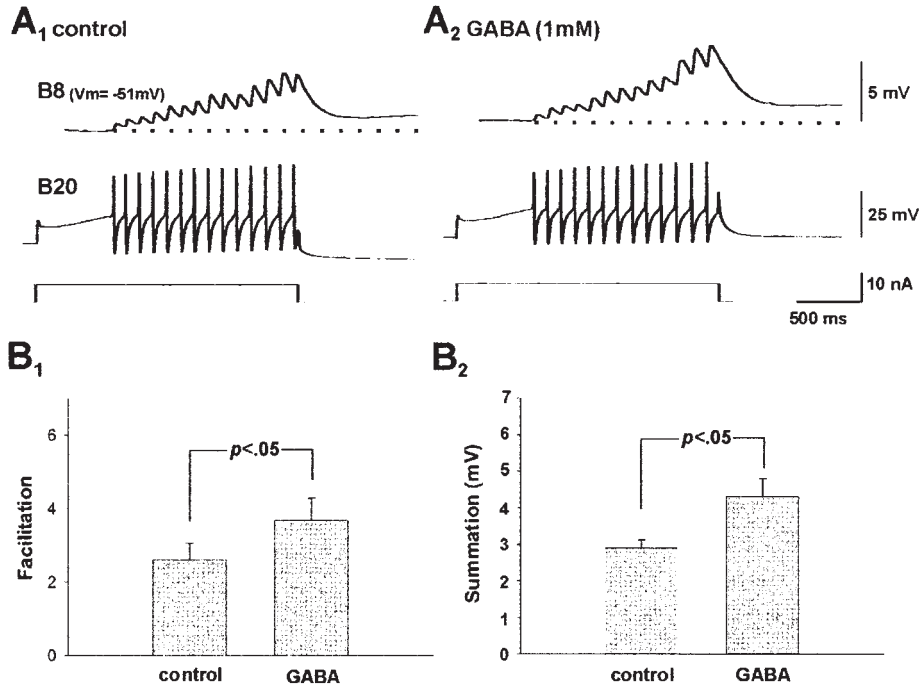

Figure 7. GABA enhances facilitation and summation of the rapid EPSP from B20 to B8. (A_1) Control response of B8 (upper trace) to a train of impulses (middle trace) evoked by a depolarizing current pulse in B20 (lower trace). Prior to the train, the membrane potential of B8 was at rest (−51 mV). EPSPs exhibited facilitation and summation. (A_2) In the presence of GABA (1 m*M*), the resting potential of B8 was depolarized by approximately 1 mV. The EPSPs produced by a train of impulses in B20 exhibited levels of facilitation and summation that were greater than those observed under control conditions. (B_1) Collective data ($n = 5$) showed that facilitation of $EPSP_{10}$ was significantly increased by GABA. (B_2) Collective data ($n = 5$) also showed that summation of $EPSP_{10}$ was significantly increased by GABA.

larization of pulse trains produced firing of B8 at progressively earlier phases (Fig. 10C, D).

Together, these results demonstrate that GABA, acting *via* $GABA_B$-like receptors, enhances facilitation and summation from B20 to B8 but not to B16. The increase in B20-to-B8 facilitation appears to reflect presynaptic properties that are independent of the postsynaptic membrane potential. The increase in summation can be attributed to postsynaptic actions in B8 that produce decreased rates of decay in successive EPSPs.

Discussion

In this study, signaling from the influential buccal interneuron B20 was progressively biased toward one of its followers during trains of action potentials. Our observations indicate that this bias resulted from presynaptic and postsynaptic mechanisms acting in a concerted fashion (Fig. 11A). Both contributions to this bias were amplified by modulatory effects of GABA, which increased facilitation and summation in a target-specific fashion (11B). These findings predict that, excluding additional factors, patterned firing of B20 will be more effective in producing excitation of B8 than of B16. This was in fact observed with simultaneous recording of the two motor neurons when trains of impulses were produced in B20 (Fig. 11C).

Previous investigations localized markers for catecholamines and GABA to B20 (Díaz-Ríos *et al.,* 2002) and showed that its fast EPSPs to both B16 and B8 were mediated by dopamine (Díaz-Ríos and Miller, 2005). While it is intriguing to consider that GABA could be acting as a cotransmitter at these synapses, its release from B20 remains to be demonstrated, and its source is therefore not specified in our present schema (Fig. 11B). However, whether the modulation of signaling from B20 is homosynaptic or heterosynaptic in origin, its presence in an interneuron that is embedded within the buccal CPG would classify it as an intrinsic form of modulation (Katz *et al.,* 1994; Katz and Frost, 1996; see Introduction). As such, its magnitude can be expected to both reflect and influence the degree to which the overall system is activated.

A type of modulation with formal similarities to that observed in this study has been demonstrated in the circuit mediating the siphon withdrawal reflex of *Aplysia* (Fischer *et al.,* 1997). In that system, serotonin acting in a heterosynaptic fashion attenuated the ability of an identified inhibitory neuron (L30) to display short-term synaptic enhancement. This action

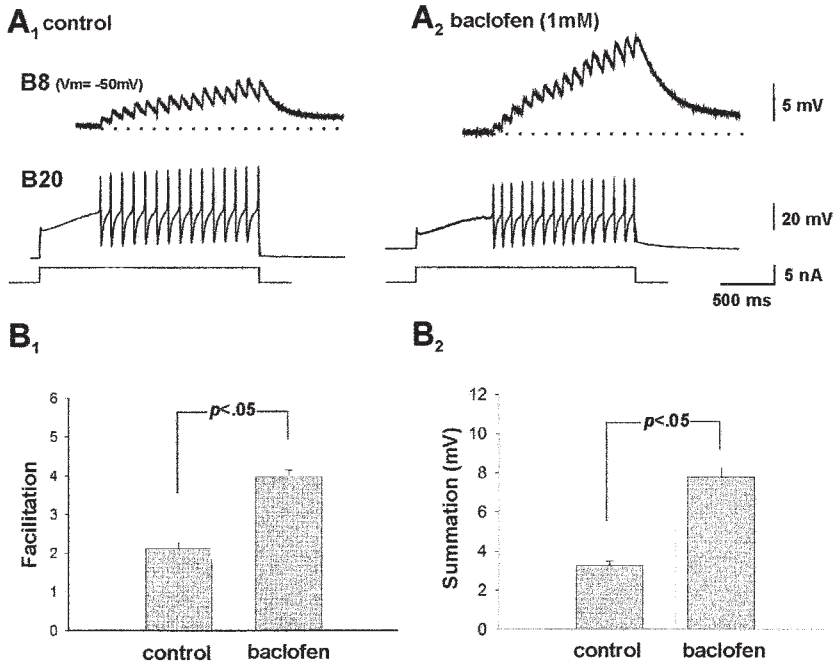

Figure 8. Baclofen enhances facilitation and summation of the rapid EPSP from B20 to B8. (A_1) Control response of B8 (upper trace) to a train of impulses (middle trace) evoked by a depolarizing current pulse in B20 (lower trace). Prior to the train, the membrane potential of B8 was at rest (−50 mV). EPSPs exhibited facilitation and summation. (A_2) In the presence of baclofen (1 m*M*) the EPSPs produced by a train of impulses in B20 exhibited levels of facilitation and summation that were greater than those observed under control conditions. (B_1) Pooled data ($n = 5$) showed that facilitation of $EPSP_{10}$ was significantly increased by baclofen. (B_2) Grouped data ($n = 5$) showed that summation of $EPSP_{10}$ was significantly increased by baclofen.

was designated "modulatory metaplasticity" to distinguish it from previously described homosynaptic forms of metaplasticity in which a neuron's firing history influenced its ability to express plasticity (Abraham and Bear, 1996). If GABA is released when B20 fires, and if that synaptically released GABA is capable of influencing subsequent rapid dopaminergic signaling, then both of these terms would be applicable to B20-to-B8 signaling during impulse trains. In the context of the existing nomenclature, such regulation would be designated "homosynaptic modulatory metaplasticity" (Fig. 11B).

Divergent synaptic plasticity

Branch, or target-specific, synaptic plasticity, such as that observed in the signaling from B20 to its two follower motor neurons, appears to be used extensively to achieve divergent channeling of information flow in motor systems. In the neuromuscular systems of crustaceans, where a small number of neurons control multiple muscles, terminals of an individual motor neuron can exhibit wide ranges of facilitation or depression (Atwood, 1967; Bittner, 1968). In an extreme case, the terminals of a single stomatogastric motor neuron in the lobster were shown to exhibit facilitation in one muscle and depression in another muscle (Katz *et al.,* 1993).

Within the *Aplysia* buccal ganglion, diverse manifestations of differential synaptic plasticity can contribute to CPG function. Gardner and Kandel (1977) identified a diphasic synaptic potential (EPSP followed by an IPSP) originating from the cholinergic interneuron B4/5. Repeated firing of B4/5 preferentially reduced the magnitude of the hyperpolarizing component of this PSP, eventually resulting in a monophasic depolarization. In that instance, differential rates of synaptic depression were attributed to properties of distinct postsynaptic acetylcholine receptors that exhibited different rates of desensitization (Gardner and Kandel, 1977). Another form of differential synaptic plasticity was recently shown to occur in the signaling of buccal interneuron B65, where EPSPs exhibit depression in one follower neuron (B4/5) and facilitation in a second (B8; Kabotyanski *et al.,* 1998). Interestingly, B65 shares the GABA-DA neurotransmitter phenotype with B20, the subject of this investigation (Díaz-Ríos *et al.,* 2002; Due *et al.,* 2004).

Complementary pre- and postsynaptic modulation of plasticity

Previously, it was found that GABA, acting *via* $GABA_B$-like receptors, produced a small depolarization and increase in the input resistance and excitability of B8 (Díaz-Ríos and Miller, 2005). GABA and baclofen were

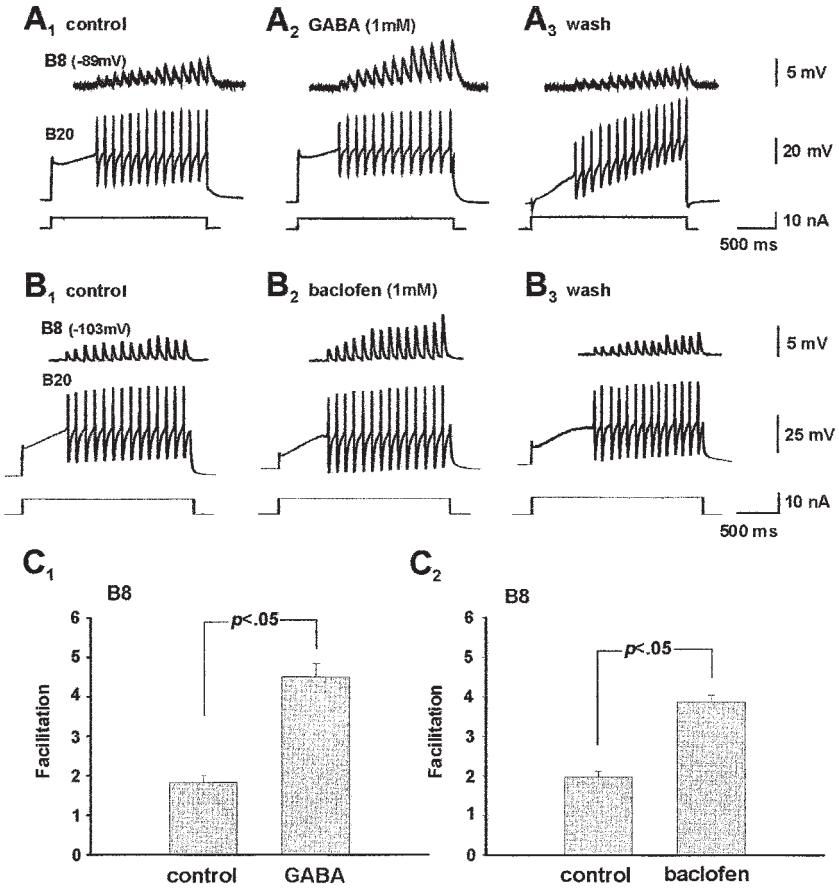

Figure 9. GABA and baclofen enhance facilitation of the B20-to-B8 EPSP. (A_1) B8 was hyperpolarized *via* current injection to −89 mV prior to eliciting a train of impulses in B20. (A_2) Facilitation of the B20-to-B8 EPSP was increased in the presence of GABA (1 m*M*). (A_3) The GABA-induced increase in facilitation was reversed by washing with normal ASW. (B_1) B8 was hyperpolarized via current injection to −103 mV prior to eliciting a train of impulses in B20. (B_2) Facilitation of the B20-to-B8 EPSP was increased in the presence of baclofen (1 m*M*). (B_3) The baclofen-induced increase in facilitation was reversed by washing with normal ASW. (C_1) Grouped data ($n = 5$) show that facilitation of $EPSP_{10}$ was significantly enhanced by GABA when B8 was hyperpolarized 40 mV more hyperpolarized than rest prior to eliciting a B20 impulse train. (C_2) Grouped data ($n = 5$) show that facilitation of $EPSP_{10}$ was significantly enhanced by baclofen when B8 was 40 mV more hyperpolarized than rest prior to eliciting a B20 impulse train.

also found to increase the amplitude of EPSPs evoked in B8 by single impulses in B20. The present investigation demonstrated that GABA can also enhance signaling from B20 to B8 by increasing facilitation and summation. Additional mechanisms, including postsynaptic interactions between the neurotransmitters present in B20, may also be operating to modify these signals. Recently, it was shown that GABA and baclofen increase inward currents produced by dopamine in B8 (Svensson *et al.,* 2004). If GABA is in fact co-released with dopamine from B20, then such synergistic influences could contribute to the enhancement of the B20-to-B8 EPSP that is observed during impulse trains.

Presynaptic modulation. $GABA_B$ receptors have been shown to regulate transmitter release in a range of systems and species (see review by Bettler *et al.,* 2004), including several invertebrates (Miwa *et al.,* 1990; Parnas *et al.,* 1999; Gutovitz *et al.,* 2001). Prior to the recognition of GABA receptor subtypes, Phillipe and coworkers (1981) found that baclofen ($3–5 \times 10^{-5}$ *M*) altered synaptic plasticities in the *Aplysia* abdominal ganglion. In that study, the effects of baclofen were thought to be indirect and were attributed to its ability to liberate biogenic amines (Philippe *et al.,* 1981). In the system studied in the present investigation, the concentrations of GABA and baclofen required to produce detectable effects were considerably higher than are normally found to activate $GABA_B$ receptors. However, if GABA originates from the terminals of B20 itself, it is likely to be capable of achieving high concentrations at or near the sites of dopamine release.

We propose that the GABA-induced increases in facil-

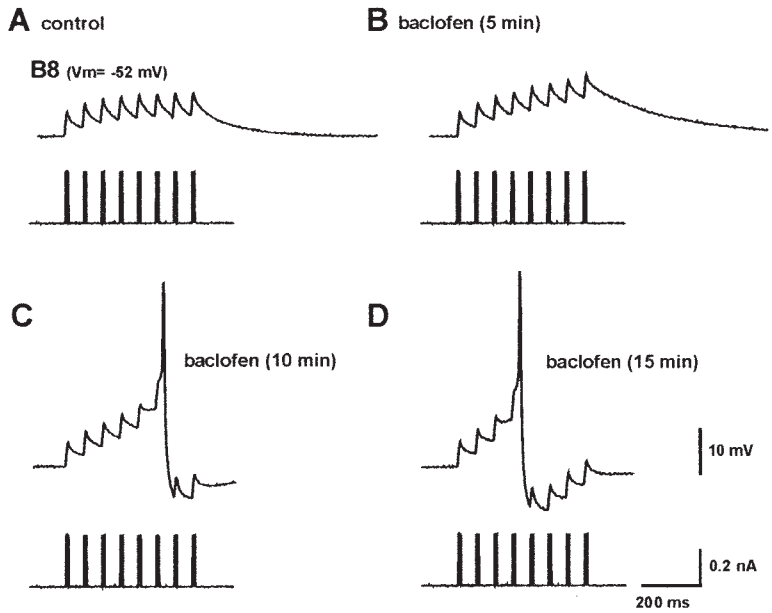

Figure 10. Baclofen increases the decay time of voltage deflections produced by current pulses in B8. (A) A train of current pulses (5 ms, 0.25 nA) was injected into B8 to mimic the EPSPs that are produced by a burst of B20 impulses. (B–D) In the presence of baclofen (1 m*M*), the decay times of the voltage deflections became successively prolonged. Pulse trains that failed to reach the threshold of B8 under control conditions became capable of producing action potentials at progressively earlier phases of their duration.

itation reflect presynaptic actions that modify the history-dependence of neurotransmitter release from B20. Similar presynaptic modulatory actions have been demonstrated with aminergic neuromodulators in *Aplysia* central synapses (Tremblay *et al.,* 1976; Woodson *et al.,* 1976; Newlin *et al.,* 1980). Their possible behavioral significance is best understood in the circuits that mediate defensive reflexes, where presynaptic modulation is implemented by serotonin acting in a heterosynaptic fashion (Carew and Kandel, 1974; Kandel and Schwartz, 1982; Byrne and Kandel, 1996).

Postsynaptic modulation. Our findings indicate that GABA, acting *via* $GABA_B$-like receptors on B8, enhances summation of the B20-to-B8 EPSP. This modulatory action did not occur when the membrane potential of B8 was set to hyperpolarized levels. GABA did, however, enhance the "summation" of voltage deflections produced by current pulses injected into B8 at its resting *V*m. Its effects appear, therefore, to reflect an action on membrane properties of B8 that are operative near its resting membrane potential. We propose that the progressive advance of the B8 *V*m into a region of increased resistance during a train of EPSPs contributes to the enhanced summation of successive EPSPs and that the modulatory effects of GABA reflect an action that augments this intrinsic property.

Several buccal interneurons and sensory neurons converge upon B8 and B16, and their synaptic actions on these motor neurons have been related to their ability to specify motor programs (Kabotyanski *et al.,* 1998; Nargeot *et al.,* 1999b; Jing and Weiss, 2001; Morgan *et al.,* 2002; Jing *et al.,* 2003; Proekt *et al.,* 2004). A previous investigation demonstrated a nonlinearity in the membrane properties of B8 that could modify EPSPs originating from the identified mechanosensory neuron B21 (Klein *et al.,* 2000). In that study, the amplitude and time constant of decay of the B21-to-B8 EPSP were both decreased when the membrane potential of B8 was set to hyperpolarized levels. The nonlinear properties of B8 were likened to the "anomalous rectification" that occurs in the serotonergic metacerebral cell (MCC) of *Aplysia* (Kandel and Tauc, 1966). In the MCC, anomalous rectification strongly influenced the ability of repetitive EPSPs to summate (Kandel and Tauc, 1966). In agreement with observations made in the MCC, we found that the effect of the B8 membrane potential on the rate of decay of the B20-to-B8 EPSP was substantially greater than its effect on the magnitude of this EPSP.

Divergent modulation of plasticity—potential contributions to behavioral plasticity

The phase of radula closure with respect to its protraction and retraction is the operational parameter that distinguishes ingestive from egestive feeding behaviors (Kupfermann, 1974; Morton and Chiel, 1993a, b; Cropper *et al.,* 2004). In experiments with both *in vivo* and *in vitro* preparations,

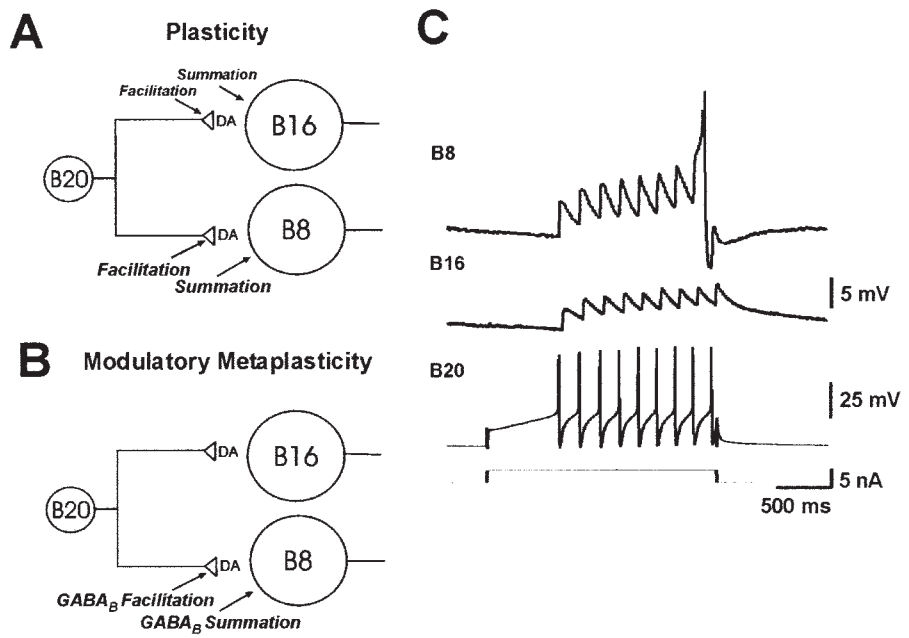

Figure 11. Divergent short-term synaptic plasticity and modulatory metaplasticity in the rapid excitatory signaling from B20 to B16 and B8. (A) Schematic of divergent excitatory signaling from B20 to the two radula closer motor neurons B16 and B8. Short-term *facilitation* and *summation* were greater in the rapid EPSPs to B8 (labeled with larger type). Differences in facilitation are attributed to target-specific properties of the B20 synapses. Differences in summation are attributed to dissimilar membrane properties of the two postsynaptic motor neurons. (B) Proposed modulatory effects of GABA on the divergent signaling of B20. Actions of GABA were not detected on either facilitation or summation of the EPSPs from B20 to B16. In B8, both of these short-term increases in the synaptic efficacy of signaling of from B20 were enhanced by GABA and baclofen. $GABA_B$ effects on facilitation are proposed to reflect actions on the B20-to-B8 presynaptic terminals. Effects on summation are proposed to reflect $GABA_B$-like actions on the membrane properties of B8. (C) When repetitive trains of B20 impulses were produced by injection of current pulses (bottom record), facilitation and summation of EPSPs contributed to firing B8 while responses in B16 remained subthreshold. Recording of the impulse in B8 was truncated by the data acquisition system.

radula closure is commonly monitored by observing the phase and pattern of B8 firing (Church and Lloyd, 1994; Nargeot *et al.,* 1997, 1999a, b; Kabotyanski *et al.,* 1998; Sweedler *et al.,* 2002). The influence of B20 on the buccal CPG is best understood in the context of egestive motor patterns produced by stimulation of cerebral-buccal command neurons (Jing and Weiss, 2001) or the esophageal nerve (Proekt *et al.,* 2004). In both instances, the direct B20-to-B8 EPSP is thought to specify egestive motor programs by promoting radula closure during protraction. The degree to which the forms of synaptic enhancement examined in this study contribute to such motor-pattern specification remains uncertain. Several observations suggest that additional more persistent forms of plasticity also modify the signaling of B20 to B8 (Teyke *et al.,* 1993; Jing and Weiss, 2001; Proekt *et al.,* 2004; our observations).

The initiation of a feeding bout in *Aplysia* is characterized by a progressive increase in the frequency and intensity of biting, a feature that is thought to reflect food-induced arousal (Kupfermannn *et al.,* 1979; Rosen *et al.,* 1989). Transitions of buccal motor programs also occur in a graded fashion and exhibit some inertia (Kabotyanski *et al.,* 1998; Brezina *et al.,* 2003a, b; Proekt *et al.,* 2004). A progressive increase in the efficacy of excitatory signaling from B20 to B8 was recently shown to accomplish the graded transition of the buccal CPG toward egestive motor patterns (Proekt *et al.,* 2004). Motor-pattern warm-up and transitions are likely to require that radula closer motor neurons be recruited in a specific sequence (Brezina *et al.,* 2003a, b; Zhurov *et al.,* 2005; Ye *et al.,* 2006). The divergent short-term synaptic plasticities from B20 to B16 and B8, and their differential modulation by GABA, could contribute to the effective and adaptive onset and transitioning of repetitive motor patterns.

Recent investigations have increased our appreciation for the scope and complexity of neural mechanisms that contribute to relatively simple forms of behavioral plasticity in *Aplysia* (Glanzman, 1995; Byrne and Kandel, 1996; Martin *et al.,* 2000). The mechanisms underlying learning and memory in the polymorphic circuits that produce repetitive behaviors are likely to exhibit comparable or even greater levels of distribution and complexity (see Benjamin *et al.,* 2000). Correlates of associative conditioning in molluscan

feeding circuits have been shown to occur in the sensory pathways that mediate food detection (Morielli *et al.,* 1986; Staras *et al.,* 1999; Lechner *et al.,* 2000a; Mozzachiodi *et al.,* 2003), in the signaling from command-like interneurons to pattern-initiating CPG elements (Kovac *et al.,* 1986; Lechner *et al.,* 2000b; Straub *et al.,* 2004), and in the biophysical properties of key buccal interneurons (Nargeot *et al.,* 1999a, b; Brembs *et al.,* 2002). The contribution of plastic synaptic signaling to the motor neurons themselves, a major determinant of non-CPG-mediated behavioral plasticity (see contributions to this symposium), has received less scrutiny. This report aims to stimulate further investigation into modifications of signaling to the final common pathway that executes such CPG-mediated motor programs when the experience of an animal causes it to adjust its actions.

Acknowledgments

Supported by the National Science Foundation: CAREER Award: IBN-9722349; National Institutes of Health DRS RCMI RR-03051, NIGMS MBRS: GM-08224, and NIMH MRISP MH48190; and funds from the University of Puerto Rico Medical Sciences Campus Deanship of Biomedical Sciences.

Literature Cited

Abraham, W. C., and M. F. Bear. 1996. Metaplasticity: the plasticity of synaptic plasticity. *Trends Neurosci.* **19:** 126–130.

Angstadt, J. D., and R. L. Calabrese. 1989. A hyperpolarization-activated inward current in heart interneurons of the medicinal leech. *J. Neurosci.* **9:** 2846–2857.

Atwood, H. L. 1967. Variation in physiological properties of crustacean motor synapses. *Nature* **215:** 57–58.

Balaban, P. M. 2002. Cellular mechanisms of behavioral plasticity in terrestrial snail. *Neurosci. Biobehav. Rev.* **26:** 597–630.

Benjamin, P. R., K. Staras, and G. Kemenes. 2000. A systems approach to the cellular analysis of associative learning in the pond snail *Lymnaea. Learn. Mem.* **7:** 124–131.

Bettler, B., K. Kaupmann, J. Mosbacher, and M. Gassmann. 2004. Molecular structure and physiological functions of GABA(B) receptors. *Physiol. Rev.* **84:** 835–867.

Bittner, G. D. 1968. Differentiation of nerve terminals in the crayfish opener and its functional significance. *J. Gen. Physiol.* **51:** 731–758.

Brembs, B., F. D. Lorenzetti, F. D. Reyes, D. A. Baxter, and J. H. Byrne. 2002. Operant reward learning in *Aplysia:* neuronal correlates and mechanisms. *Science* **296:** 1706–1709.

Brembs, B., D. A. Baxter, and J. H. Byrne. 2004. Extending *in vitro* conditioning in *Aplysia* to analyze operant and classical processes in the same preparation. *Learn. Mem.* **11:** 412–420.

Brezina, V., I. V. Orekhova, and K. R. Weiss. 2003a. Neuromuscular modulation in *Aplysia.* I. Dynamic model. *J. Neurophysiol.* **90:** 2592–2612.

Brezina, V., I. V. Orekhova, and K. R. Weiss. 2003b. Neuromuscular modulation in *Aplysia.* II. Modulation of the neuromuscular transform in behavior. *J. Neurophysiol.* **90:** 2613–2628.

Byrne, J. H., and E. R. Kandel. 1996. Presynaptic facilitation revisited: state and time dependence. *J. Neurosci.* **16:** 425–435.

Carew, T. J., and E. R. Kandel. 1974. Synaptic analysis of the interrelationships between behavioral modifications in *Aplysia.* Pp. 339–383 in *Synaptic Transmission and Neuronal Interaction,* M. V. L. Bennett, ed. Raven Press, New York.

Church, P. J., and P. E. Lloyd. 1994. Activity of multiple identified motor neurons recorded intracellularly during evoked feeding-like motor programs in *Aplysia. J. Neurophysiol.* **72:** 1794–1809.

Cohen, J. L., K. R. Weiss, and I. Kupfermann. 1978. Motor control of buccal muscles in *Aplysia. J. Neurophysiol.* **41:** 157–180.

Colwill, R., K. Goodrum, and A. Martin. 1997. Pavlovian appetitive discriminative conditioning in *Aplysia californica. Anim. Learn. Behav.* **25:** 268–276.

Cropper, E. C., P. E. Lloyd, W. Reed, R. Tenenbaum, I. Kupfermann, and K. R. Weiss. 1987. Multiple neuropeptides in cholinergic motor neurons of *Aplysia:* evidence for modulation intrinsic to the motor circuit. *Proc. Natl. Acad. Sci. USA* **84:** 3486–3490.

Cropper, E. C., I. Kupfermann, and K. R. Weiss. 1990. Differential firing patterns of the peptide-containing cholinergic neurons B15 and B16 during feeding behavior in *Aplysia. Brain Res.* **522:** 176–179.

Cropper, E. C., C. G. Evans, I. Hurwitz, J. Jing, A. Proekt, A. Romero, and S.C. Rosen. 2004. Feeding neural networks in the mollusk *Aplysia. Neurosignals* **13:** 70–86.

del Castillo, J., and B. Katz. 1954. Statistical factors involved in neuromuscular facilitation and depression. *J. Physiol. (Lond.)* **124:** 574–585.

Díaz-Ríos, M., and M. W. Miller. 2002. GABA and dopamine colocalization in interneurons of the *Aplysia* feeding circuit: presynaptic actions of GABA. Abstract 367.12. [Online: *2002 Abstract Viewer/Itinerary Planner*]. Society for Neuroscience, Washington, DC.

Díaz-Ríos, M., and M. W. Miller. 2005. Rapid dopaminergic signaling by interneurons that contain markers for catecholamines and GABA in the feeding circuitry of *Aplysia. J. Neurophysiol.* **93:** 2142–2156.

Díaz-Ríos, M., E. Suess, and M. W. Miller. 1999. Localization of GABA-like immunoreactivity in the central nervous system of *Aplysia californica. J. Comp. Neurol.* **413:** 255–270.

Díaz-Ríos, M., E. Oyola, and M. W. Miller. 2002. Colocalization of γ-aminobutyric acid-like immunoreactivity and catecholamines in the feeding network of *Aplysia californica. J. Comp. Neurol.* **445:** 29–46.

Due, M. R., J. Jing, and K. R. Weiss. 2004. Dopaminergic contributions to modulatory functions of a dual-transmitter interneuron in *Aplysia. Neurosci. Lett.* **358:** 53–57.

Fischer, T. M., T. E. J. Blazis, N. A. Priver, and T. J. Carew. 1997. Metaplasticity at identified inhibitory synapses in *Aplysia. Nature* **389:** 860–865.

Fisher, S. A., T. M. Fischer, and T. J. Carew. 1997. Multiple overlapping processes underlying short-term synaptic enhancement. *Trends Neurosci.* **20:** 170–177.

Gardner, D., and E. R. Kandel. 1977. Physiological and kinetic properties of cholinergic receptors activated by multiaction interneurons in buccal ganglia of *Aplysia. J. Neurophysiol.* **40:** 333–348.

Glanzman, D. L. 1995. The cellular basis of classical conditioning in *Aplysia californica:* It's less simple than you think. *Trends Neurosci.* **18:** 30–36.

Golowasch, J., and E. Marder. 1992. Ionic currents of the lateral pyloric neuron of the stomatogastric ganglion of the crab. *J Neurophysiol.* **67:** 318–331.

Gutovitz, S., J. T. Birmingham, J. A. Luther, D. J. Simon, and E. Marder. 2001. GABA enhances transmission at an excitatory glutamatergic synapse. *J. Neurosci.* **21:** 5935–5943.

Harris-Warrick, R. M., and E. Marder. 1991. Modulation of neural networks for behavior. *Annu. Rev. Neurosci.* **14:** 39–57.

Hawkins, R. D., E. R. Kandel, and S. A. Siegelbaum. 1993. Learning to modulate transmitter release: themes and variations in synaptic plasticity. *Annu. Rev. Neurosci.* **16:** 625–665.

Hurwitz, I., I. Kupfermann, and K. R. Weiss. 2003. Fast synaptic

connections from CBIs to pattern-generating neurons in *Aplysia:* initiation and modification of motor programs. *J. Neurophysiol.* **89:** 2120–2136.

Jing, J., and K. R. Weiss. 2001. Neural mechanisms of motor program switching in *Aplysia. J. Neurosci.* **21:** 7349–7362.

Jing, J., and K. R. Weiss. 2002. Interneuronal basis of the generation of related but distinct motor programs in *Aplysia:* implications for current neuronal models of vertebrate intralimb coordination. *J. Neurosci.* **22:** 6228–6238.

Jing, J., F. S. Vilim, J.-S. Wu, J.-H. Park, and K. R. Weiss. 2003. Concerted GABAergic actions of *Aplysia* feeding interneurons in motor program specification. *J. Neurosci.* **23:** 5283–5294.

Jordan, R., K. P. Cohen, and M. D. Kirk. 1993. Control of intrinsic buccal muscles by motoneurons B11, B15, and B16 in *Aplysia californica. J. Exp. Zool.* **265:** 496–506.

Kabotyanski, E. A., D. A. Baxter, and J. H. Byrne. 1998. Identification and characterization of catecholaminergic neuron B65 that initiates and modifies patterned activity in the buccal ganglion of *Aplysia. J. Neurophysiol.* **79:** 605–621.

Kandel, E. R., and J. H. Schwartz. 1982. Molecular biology of learning: modulation of transmitter release. *Science* **218:** 433–443.

Kandel, E. R., and L. Tauc. 1966. Anomalous rectification in the metacerebral giant cells and its consequences for synaptic transmission. *J. Physiol.* **183:** 287–304.

Katz, P. S., ed. 1999. *Beyond Neurotransmission: Neuromodulation and Its Importance for Information Processing.* Oxford University Press. New York.

Katz, P. S., and W. N. Frost. 1996. Intrinsic neuromodulation: altering neuronal circuits from within. *Trends Neurosci.* **19:** 54–61.

Katz, P. S., M. D. Kirk, and C. K. Govind. 1993. Facilitation and depression at different branches of the same motor axon: evidence for presynaptic differences in release. *J. Neurosci.* **13:** 3075–3089.

Katz, P. S., P. A. Getting, and W. N. Frost. 1994. Dynamic neuromodulation of synaptic strength intrinsic to a central pattern generator circuit. *Nature* **367:** 729–731.

Klein, A. N., K. R. Weiss, and E. C. Cropper. 2000. Glutamate is the fast excitatory neurotransmitter of small cardioactive peptide-containing *Aplysia* radula mechanoafferent neuron B21. *Neurosci. Lett.* **289:** 37–40.

Kovac, M. P., E. M. Matera, P. J. Volk, and W. J. Davis. 1986. Food avoidance learning is accompanied by synaptic attenuation in identified interneurons controlling feeding behavior in *Pleurobranchaea. J. Neurophysiol.* **56:** 891–905.

Krasne, F. B. 1978. Extrinsic control of intrinsic neuronal plasticity: an hypothesis from work on simple systems. *Brain Res.* **140:** 197–216.

Kupfermann, I. 1974. Feeding behavior in *Aplysia:* a simple system for the study of motivation. *Behav. Biol.* **10:** 1–26.

Kupfermann, I. 1979. Modulatory actions of neurotransmitters. *Annu. Rev. Neurosci.* **2:** 447–465.

Kupfermann, I. 1991. Functional studies of cotransmission. *Physiol. Rev.* **71:** 683–732.

Kupfermann, I., J. L. Cohen, D. E. Mandelbaum, M. Schonberg, A. J. Susswein, and K. R. Weiss. 1979. Functional role of serotonergic neuromodulation in *Aplysia. Fed. Proc.* **38:** 2095–2102.

Kupfermann, I., S. Rosen, T. Teyke, E. C. Cropper, M. Miller, F. Vilim, and K. R. Weiss. 1989. Neurobiology of behavioral states in *Aplysia:* non-associative forms of plasticity of feeding responses. Pp. 47–59 in *Dynamics and Plasticity in Neuronal Systems.* N. Elsner, and W. Winger, eds. Georg Thieme Verlag, New York.

Lechner, H. A., D. A. Baxter, and J. H. Byrne. 2000a. Classical conditioning of feeding in *Aplysia.* I. Behavioral analysis. *J. Neurosci.* **20:** 3369–3376.

Lechner, H. A., D. A. Baxter, and J. H. Byrne. 2000b. Classical conditioning of feeding in *Aplysia.* II. Neurophysiological correlates. *J. Neurosci.* **20:** 3377–3386.

Liao, X., and E. T. Walters. 2002. The use of elevated divalent cation solutions to isolate monosynaptic components of sensorimotor connections in *Aplysia. J. Neurosci. Meth.* **120:** 45–54.

Marder, E. 1998. From biophysics to models of network function. *Annu. Rev. Neurosci.* **21:** 25–45.

Martin, A. R. 1955. A further study of the statistical composition on the end-plate potential. *J. Physiol.* **130:** 114–122.

Martin, K. C., M. Barad, and E. R. Kandel. 2000. Local protein synthesis and its role in synapse-specific plasticity. *Curr. Opin. Neurobiol.* **10:** 587–592.

Menzel, R. 2001. Searching for the memory trace in a mini-brain, the honeybee. *Learn. Mem.* **8:** 53–62.

Miwa, A., M. Ui, and N. Kawai. 1990. G protein is coupled to presynaptic glutamate and GABA receptors in lobster neuromuscular synapse. *J. Neurophysiol.* **63:** 173–180.

Morgan, P. T., R. Perrins, P. E. Lloyd, and K. R. Weiss. 2000. Intrinsic and extrinsic modulation of a single central pattern generating circuit. *J. Neurophysiol.* **84:** 1186–1193.

Morgan, P. T., J. Jing, F. S. Vilim, and K. R. Weiss. 2002. Interneuronal and peptidergic control of motor pattern switching in *Aplysia. J. Neurophysiol.* **87:** 49–61.

Morielli, A. D., E. M. Matera, M. P. Kovac, R. G. Shrum, K. J. McCormack, and W.J. Davis. 1986. Cholinergic suppression: a postsynaptic mechanism of long-term associative learning. *Proc. Natl. Acad. Sci. USA* **83:** 4556–4560.

Morton, D. W., and H. J. Chiel. 1993a. *In vivo* buccal nerve activity that distinguishes ingestion from rejection can be used to predict behavioral transitions in *Aplysia. J. Comp. Physiol. A* **172:** 17–32.

Morton, D. W., and H. J. Chiel. 1993b. The timing of activity in motor neurons that produce radula movements distinguishes ingestion from rejection in *Aplysia. J. Comp. Physiol. A* **173:** 519–536.

Mozzachiodi, R., H. A. Lechner, D. A. Baxter, and J. H. Byrne. 2003. *In vitro* analog of classical conditioning of feeding in *Aplysia. Learn. Mem.* **10:** 478–494.

Nadim, F., and Y. Manor. 2000. The role of short-term synaptic dynamics in motor control. *Curr. Opin. Neurobiol.* **10:** 683–690.

Nargeot, R., D. A. Baxter, and J. H. Byrne. 1997. Contingent-dependent enhancement of rhythmic motor patterns: an *in vitro* analog of operant conditioning. *J. Neurosci.* **17:** 8093–8105.

Nargeot, R., D. A. Baxter, and J. H. Byrne. 1999a. *In vitro* analog of operant conditioning in *Aplysia.* I. Contingent reinforcement modifies the functional dynamics of an identified neuron. *J. Neurosci.* **19:** 2247–2260.

Nargeot, R., D. A. Baxter, and J. H. Byrne. 1999b. *In vitro* analog of operant conditioning in *Aplysia.* II. Modifications of the functional dynamics of an identified neuron contribute to motor pattern selection. *J. Neurosci.* **19:** 2261–2272.

Newlin, S. A., W. T. Schlapfer, and S. H. Barondes. 1980. Separate serotonin and dopamine receptors modulate the duration of post-tetanic potentiation at an *Aplysia* synapse without affecting other aspects of synaptic transmission. *Brain Res.* **181:**107–125.

Nusbaum, M. P., D. M. Blitz, A. M. Swensen, D. Wood, and E. Marder. 2001. The roles of co-transmission in neural network modulation. *Trends Neurosci.* **24:**146–154.

Parker, D., and S. Grillner. 1999. Activity-dependent metaplasticity of inhibitory and excitatory synaptic transmission in the lamprey spinal cord locomotor network. *J. Neurosci.* **19:**1647–1656.

Parker, D., and S. Grillner. 2000. The activity-dependent plasticity of segmental and intersegmental synaptic connections in the lamprey spinal cord. *Eur. J. Neurosci.* **12:** 2135–2146.

Parnas, I., G. Rashkovan, J. Ong, and D. I. B. Kerr. 1999. Tonic

activation of presynaptic $GABA_B$ receptors in the opener neuromuscular junction of crayfish. *J. Neurophysiol.* **81:** 1184–1191.

Philippe, E., G. Grenon, and J. P. Tremblay. 1981. Baclofen modifies via the release of monoamines the synaptic depression, frequency facilitation, and posttetanic potentiation observed at an identified cholinergic synapse of *Aplysia californica. Can. J. Physiol. Pharmacol.* **59:** 244–252.

Proekt, A., V. Brezina, and K. R. Weiss. 2004. Dynamical basis of intentions and expectations in a simple neuronal network. *Proc. Natl. Acad. Sci. USA* **101:** 9447–9452.

Rosen, S. C., K. R. Weiss, R. S. Goldstein, and I. Kupfermann. 1989. The role of a modulatory neuron in feeding and satiation in *Aplysia:* effects of lesioning of the serotonergic metacerebral cells. *J. Neurosci.* **9:** 1562–1578.

Sakurai, A., and P. S. Katz. 2003. Spike timing-dependent serotonergic neuromodulation of synaptic strength intrinsic to a central pattern generator circuit. *J. Neurosci.* **23:** 10745–10755.

Sánchez, J. A., and M. D. Kirk. 2000. Short-term enhancement modulates ingestion motor programs of *Aplysia. J. Neurosci.* **20:** RC85.

Sánchez, J. A., and M. D. Kirk. 2002. Ingestion motor programs of *Aplysia* are modulated by short-term synaptic enhancement in cerebral-buccal interneuron pathways. *Invertebr. Neurosci.* **4:** 199–212.

Staras, K., G. Kemenes, and P. R. Benjamin. 1999. Cellular traces of behavioral classical conditioning can be recorded at several specific sites in a simple nervous system. *J. Neurosci.* **19:** 347–357.

Straub, V. A., B. J. Styles, J. S. Ireland, M. O'Shea, and P. R. Benjamin. 2004. Central localization of plasticity involved in appetitive conditioning in *Lymnaea. Learn. Mem.* **11:** 787–793.

Susswein, A. J., and M. Schwarz. 1983. A learned change of response to inedible food in *Aplysia. Behav. Neural. Biol.* **39:**1–6.

Svensson, E., A. Proekt, and K. R. Weiss. 2004. Complementary effects of co-localized dopamine and GABA on synaptic transmission in the *Aplysia* feeding network. Abstract 537.4. [Online: 2004 Abstract Viewer/Itinerary Planner]. Society for Neuroscience, Washington, DC.

Sweedler, J. V., L. Li, S. S. Rubakhin, V. Alexeeva, N. C. Dembrow, O. Dowling, J. Jing, K. R. Weiss, and F. S. Vilim. 2002. Identification and characterization of the feeding circuit-activating peptides, a novel neuropeptide family of *Aplysia. J. Neurosci.* **22:** 7797–7808.

Teyke, T., S. C. Rosen, K. R. Weiss, and I. Kupfermann. 1993. Dopaminergic neuron B20 generates rhythmic neuronal activity in the feeding motor circuitry of *Aplysia. Brain Res.* **630:** 226–237.

Tremblay, J. P., P. B. Woodson, W. T. Schlapfer, and S. H. Barondes. 1976. Dopamine, serotonin and related compounds: presynaptic effects on synaptic depression, frequency facilitation, and posttetanic potentiation at a synapse in *Aplysia californica. Brain Res.* **109:** 61–81.

Weiss, K. R., V. Brezina, E. C. Cropper, S. L. Hooper, M. W. Miller, W. C. Probst, F.S. Vilim, and I. Kupfermann. 1992. Peptidergic cotransmission in *Aplysia:* functional implications for rhythmic behaviors. *Experientia* **48:** 456–463.

Woodson, P. B., J. P. Tremblay, W. T. Schlapfer, and S. H. Barondes. 1976. Inhibition modifies the presynaptic plasticities of the transmission process at the synapse in *Aplysia californica. Brain Res.* **109:** 83–95.

Ye, H., D. W. Morton, and H. J. Chiel. 2006. Neuromechanics of coordination during swallowing in *Aplysia californica. J. Neurosci.* **26:** 1470–1485.

Yuste, R., J. N. MacLean, J. Smith, and A. Lansner. 2005. The cortex as a central pattern generator. *Nature Rev. Neurosci.* **6:** 477–483.

Zhurov, Y., A. Proekt, K. R. Weiss, and V. Brezina. 2005. Changes of internal state are expressed in coherent shifts of neuromuscular activity in *Aplysia* feeding behavior. *J. Neurosci.* **25:** 1268–1280.

Zucker, R. S., and W. G. Regehr. 2002. Short-term synaptic plasticity. *Annu. Rev. Physiol.* **64:** 355–405.