Short-Term Synaptic Enhancement Modulates Ingestion Motor Programs of *Aplysia*

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Activity-dependent synaptic plasticity regulates the flow of information in neuronal networks and has important implications for the expression of behavior. We find a functional role for short-term synaptic enhancement (STE) such as facilitation, augmentation, and post-tetanic potentiation at central synapses in the sea slug *Aplysia californica*. Consummatory feeding in *Aplysia* such as rhythmic biting is controlled by command-like cerebral-buccal interneurons (CBIs) that drive rhythmic motor output in the buccal ganglia. CBI interneuron-2 (CBI-2) makes monosynaptic connections onto buccal neurons, including premotor neurons B31/32 and B34 and motor neurons B61/62. Stimulating CBI-2 at a physiological firing frequency of 10 Hz for 30 sec causes these synapses to increase their EPSP amplitude by ~200%. This STE persists for nearly 2 min, during which time there is an increased cycle frequency of rhythmic ingestion buccal motor programs (iBMPs) elicited by CBI-2. This increase does not occur if the contralateral CBI-2 is trained and the test is performed with the ipsilateral CBI-2; therefore, the effect on motor programs only occurs in CBI-2 pathways in which STE is elicited. Furthermore, we find that STE elicited at CBI-2 buccal synapses permits iBMPs to be initiated at lower firing frequencies. Thus, STE of CBI-2 synapses appears to contribute to the initiation or modulation, or both, of buccal motor programs for rhythmic ingestion in *Aplysia*.

Key words: facilitation; augmentation; post-tetanic potentiation; synaptic plasticity; synaptic modulation; buccal ganglia; Aplysia californica

Long-term synaptic plasticity is thought to underlie learning and memory as well as refinement of synaptic connections during neural development (Kandel and Schwartz, 1982; Shatz, 1990; Madison et al., 1991; Malenka and Nicoll, 1999). Homosynaptic short-term plasticity occurs when repeated firing in the presynaptic neuron causes a change in synaptic efficacy (Atwood and Wojtowicz, 1986). Homosynaptic short-term plasticity has received increased attention recently because of its important roles in the modulation of information processing by neurons (Delaney and Tank, 1994; Regehr et al., 1994; Fisher et al., 1997; Zucker, 1999; Buonomano, 2000).

Short-term synaptic enhancement (STE) is a common form of homosynaptic plasticity whereby synaptic efficacy is increased with repeated presynaptic activity. It affords neuronal networks the ability to modify information processing on a time scale of milliseconds to minutes and may be involved in short-term memory (Zucker, 1989; Regehr et al., 1994; Fisher et al., 1997). Four components of STE have been defined primarily by their decay time constants (τ); they are fast-decaying (F1) and slow-decaying (F2) facilitation ($\tau \sim$ tens to hundreds of milliseconds), augmentation (AUG, $\tau \sim$ seconds), and post-tetanic potentiation (PTP, $\tau \sim$ tens of seconds to minutes) (for review, see Fisher et al., 1997).

Although STE is common and mechanisms responsible for it have been studied extensively (Zucker, 1999), behavioral roles for short-term synaptic plasticity are less well known (Zucker, 1989; Fischer et al., 1997b). Here we report STE of synaptic connections made by cerebral-buccal interneuron-2 (CBI-2) in *Aplysia* and the potential behavioral roles of STE at CBI-2 synapses in modulation of ingestion motor programs.

CBI-2 is strongly excited after seaweed application to the inner lips and responds with high-frequency (>10 Hz) discharges that last many tens of seconds (Rosen et al., 1991). When fired repetitively with extrinsic current in preparations consisting of isolated cerebral and buccal ganglia, CBI-2 elicits fictive ingestion. CBI-2 appears to act as a command-like cell to initiate and maintain ingestion motor programs in the buccal ganglia (Rosen et al., 1991; Church and Lloyd, 1994; Hurwitz et al., 1999; Rosen et al., 2000). We find that CBI-2 synaptic connections in the buccal ganglia exhibit homosynaptic short-term enhancement, including connections with premotor neurons B31/32 and B34 and with protractor motor neurons B61/62. During this STE, CBI-2 can initiate motor programs at lower frequencies of stimulation, whereas at higher stimulus frequencies STE contributes to an increase in cycle frequency of buccal motor programs. These results suggest that STE at CBI-2 synapses contributes to the initiation and modulation of rhythmic ingestion.

Some of these results have appeared previously in abstract form (Sánchez and Kirk, 1998).

MATERIALS AND METHODS

Aplysia californica (100–250 gm) were purchased from Marinus, Inc. (Long Beach, CA) and maintained in recirculating Instant Ocean (Aquarium Systems, Mentor, OH). Animals were held at 13–16°C, whereas all experiments were performed at room temperature (22–24°C).

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The dissection procedure has been described in detail elsewhere (Plummer and Kirk, 1990). Briefly, the cerebral ganglia, pedal-pleural ganglia, and the buccal ganglia with a portion of I2 muscle attached were removed from anesthetized animals (see Fig. 1*A*), and the caudal surface of the buccal ganglia and ventral surface of the cerebral ganglia were surgically desheathed for intracellular recordings. The preparations were continuously perfused with normal ASW (NASW) (Plummer and Kirk, 1990) when testing for effects of STE on buccal motor programs. Saline containing 3× Ca^{2+}/3× Mg^{2+} (Hi Ca^{+2}/Hi Mg^{+2}) (Jordan et al., 1993) to reduce polysynaptic activity (Cohen et al., 1978) was used when testing for the directness of synaptic connections and in one set of experiments to document STE of CBI-2 connections.

Intracellular recordings were made from CBI-2, buccal premotor neurons B31/32 and B34, protractor motor neurons B61/62, multifunction neurons B4/5, and closure motor neurons B8a,b. CBI-2, B31/32, and B34 were identified by previously established morphological and/or physiological criteria (Hurwitz et al., 1999). The pedal-pleural ganglia were left attached to the cerebral ganglion because an important criterion for CBI-2 identification is indirect inhibitory input elicited by neuron C-PR. The polysynaptic inhibitory input in CBI-2, recruited by C-PR, appears to be mediated by interneurons located in the pedal-pleural ganglia (Teyke et al., 1997; Hurwitz et al., 1999). B4/5 were identified based on soma position and corresponding large spikes in BN3 (Jahan-Parwar et al., 1983). B8a,b were identified by soma position and their large axon spikes in one branch of the RN (Morton and Chiel, 1993). B61/62 were identified by soma position and input to the I2 muscle (Hurwitz et al., 1996).

To mimic physiological firing patterns of CBI-2 and to elicit STE, a train of CBI-2 spikes for 30 sec (Train) was used. Train stimulation consisted of 25 msec current pulses, each giving rise to a single action potential in CBI-2, delivered at 10 Hz [firing in CBI-2 during the Train was not altered by synaptic input to CBI-2 coincident with ingestion buccal motor programs (iBMPs)]. Therefore, suprathreshold CBI-2 activity during the Train consisted entirely of repetitive action potentials elicited at 10 Hz for 30 sec. In all cases in which CBI-2 was driven with current pulses, subthreshold depolarizing current was continuously applied to the CBI-2s to facilitate one for one firing with current pulses during the Pre-Train Tests, Trains, and Post-Train Tests. This included experiments that tested for effects on iBMPs of contralateral CBI-2 training (with simultaneous bilateral impalements of CBI-2s). The specific synaptic pathways required for CBI-2 initiation of iBMPs are not known; however, CBI-2 makes excitatory monosynaptic connections with premotor neurons B31/32 (Rosen et al., 1991) and with premotor neuron B34 as well as motor neurons B61/62 (see below). Therefore, we used B31/32, B34, and B61/62 to quantify STE magnitude and time course. The amplitudes of EPSPs were sampled before (Pre-Train Test) and immediately after (Post-Train Test) the tetanus by stimulating CBI-2 at 1 Hz until the EPSPs returned to Pre-Train amplitudes. The B31/32, B34, and B61/62 EPSP amplitudes during Pre-Train and Post-Train tests were recorded at mean resting membrane potentials of 53.8 ± 3.8, 53.8 ± 3.1, and 55.8 ± 1.6 mV, respectively. The training paradigm was repeated a minimum of two times in each preparation.

The decay time constant for STE was determined by plotting the natural logarithm of the decaying phase of "Percent Increase in EPSP Amplitudes" (see below) against time and establishing a first-order regression line through the points. The slope of this line is equal to $-1/\tau$, where τ is the decay time constant (Jordan et al., 1993). The n values given in the text represent the number of preparations from which data were collected. Data are given as means ± SE. Changes in B34 EPSP amplitudes, in B61/62 EPSP amplitudes, and in iBMP cycle frequency were analyzed using paired-samples t test. Comparisons among B31/32, B34, and B6/62 of STE parameters were performed using ANOVA with a least significant difference (LSD) *post hoc* test.

RESULTS

CBI-2 input to specific buccal premotor and motor neurons is monosynaptic

Recently, we reported that CBI-2 makes bilateral monosynaptic connections with buccal motor neurons B61/62 (Sánchez and Kirk, 1998) (Fig. 1*Bii*), and here we show that buccal premotor neuron B34 receives similar synaptic input. The EPSPs produced in B34 exhibited a constant latency after CBI-2 spikes elicited at high frequency (e.g., 10 Hz) (Fig. 1*Bi*). Conduction delay within the cerebral-buccal connective accounts for most of the mean EPSP latency, which was 27.3 ± 0.6 msec (n = 9) and 25.9 ± 0.7 msec (n = 5) for B61/62 and B34, respectively. In addition, these

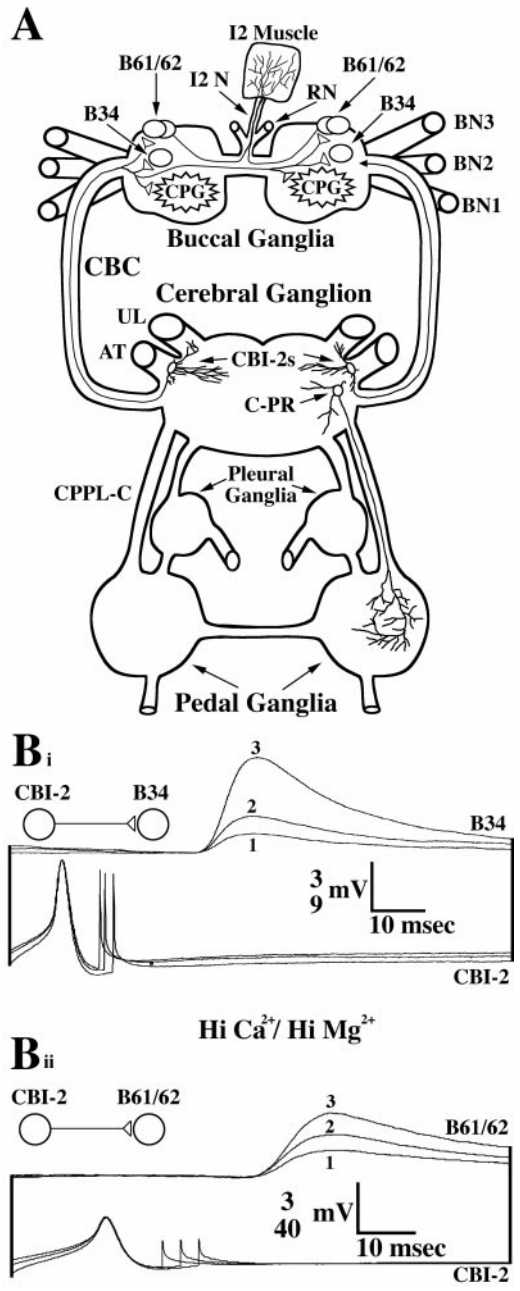

Figure 1. CBI-2 makes monosynaptic and facilitating connections with buccal premotor neuron B34 and with protractor motor neurons B61/62. *A*, Schematic of the preparation used, illustrating the neurons, axonal pathways, and synaptic connections studied. *Bi*, CBI-2 elicits facilitating EPSPs in premotor neuron B34, elicited at 10 Hz; this premotor neuron likely contributes to the CPG in the buccal ganglia (Hurwitz et al., 1997). *Bii*, CBI-2 elicits facilitating EPSPs in B61/62, during 7 Hz stimulation of CBI-2. In *Bi* and *Bii*, three traces were selected in the order indicated (*1-3*), illustrating the constant latency and facilitation of EPSPs. The preparations used in *Bi* and *Bii* were perfused with saline containing high divalent cations (Hi Ca^{+2}/Hi Mg^{+2}). *CPG*, Central pattern generator; *BN*, buccal nerve; *CBC*, cerebral-buccal connective; *CPPL-C*, cerebral-pedal-pleural connectives; *RN*, radular nerve; *UL*, upper labial nerve; *AT*, anterior tentacular nerve; *C-PR*, cerebral-pedal regulator; *CBI*, cerebral-buccal interneuron; *I2 N*, I2 branch of radular nerve.

EPSPs persisted in saline containing Hi Ca^{2+}/Hi Mg^{2+}, indicating that CBI-2 makes bilateral, monosynaptic excitatory chemical connections with B34 as well as B61/62. We also confirmed previous results that CBI-2 makes monosynaptic connections with premotor neurons B31/32 (n = 11) (Rosen et al., 1991).

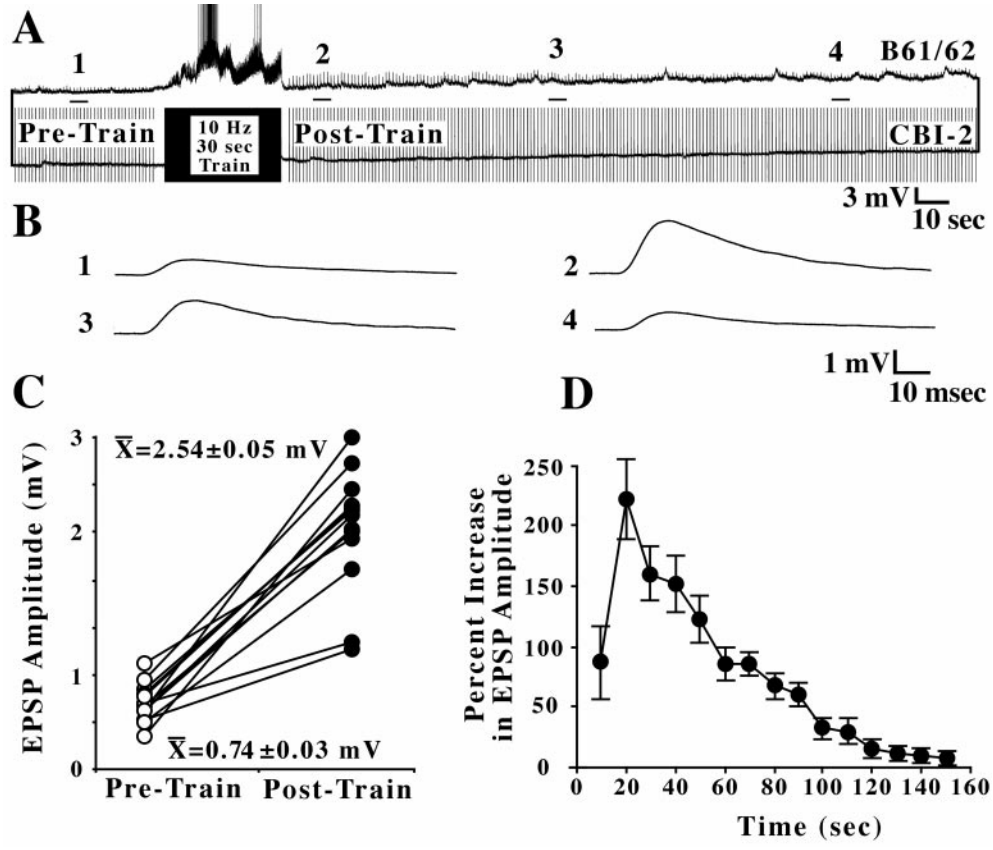

Figure 2. CBI-2 to B61/62 synapses exhibit AUG/PTP. *A*, A 10 Hz, 30 sec Train applied to CBI-2 elicits AUG/PTP. Suprathreshold current pulses were applied to CBI-2 at the indicated firing frequencies shown here and in subsequent figures. *B*, EPSPs selected from the Pre-Train and Post-Train Tests at the times indicated in *A* (*1-4*). *C*, Plot of B61/62 EPSP amplitudes before (*Pre-Train*) and during (*Post-Train*) peak AUG/PTP. Individual trials are shown from five preparations. B61/62 EPSPs recorded during AUG/PTP were significantly ($p < 0.05$) larger than Pre-Train. *D*, The peak increase in EPSP amplitude during AUG/PTP occurs at ~20 sec, and AUG/PTP lasts ~2 min. *Percent Increase in EPSP Amplitude* = [(EPSPpost-EPSPpre)/EPSPpre] × 100. EPSP amplitudes were quantified every 10 sec after the Train, and the averages (±SEM) are shown ($n = 5$). These experiments were performed in saline containing Hi Ca^{+2}/Hi Mg^{+2}.

CBI-2 connections with buccal premotor and motor neurons exhibit STE

The monosynaptic EPSPs produced in B31/32 (data not shown), B34, and B61/62 exhibited frequency facilitation (Fig. 1*B*) that appeared at frequencies >1 Hz and decayed rapidly (<1 sec); this form of STE will not be addressed further here. AUG/PTP, considered here as a single, combined form of STE (Fischer et al., 1997a), was elicited in B31/32 ($n = 3$), B34 ($n = 7$), and B61/62 ($n = 8$) when CBI-2 was fired at 10 Hz for 30 sec. The 30 sec Train mimics reported physiological responses to brief sensory stimulation (Rosen et al., 1991) and elicited STE in all preparations.

In saline containing high divalent cations, EPSPs recorded in B61/62 were increased in amplitude during and immediately after the 10 Hz train (Fig. 2). No depression of EPSPs was recorded at any time during or after the 10 Hz train applied to CBI-2 (Fig. 2*A,B,D*). In fact, the first Post-Train EPSP mean amplitude recorded in B61/62 increased by 89.8 ± 24.3% ($p < 0.005$) when compared with Pre-Train EPSP mean amplitude, and the mean EPSP amplitudes progressively increased, after the Train with peak EPSP enhancement occurring at 18.2 ± 1.7 sec (Fig. 2*D*). When quantified at the time of peak enhancement, the average percentage increase in B61/62 EPSP amplitude was 240.6 ± 71.0% ($n = 5$) (Fig. 2*C,D*). The mean decay time constant (see Materials and Methods) of this STE was 55.9 ± 4.6 sec. It is important to note that similar durations of AUG/PTP have been reported previously for other *Aplysia* synapses (Fisher et al., 1997).

Comparable results were obtained for CBI-2 synaptic input to premotor neuron B34. The 10 Hz, 30 sec Train in CBI-2 induced STE in B34 ($n = 7$), with a mean peak percentage increase in EPSP amplitude of 253.4 ± 58.9% in saline containing elevated divalent cations. The AUG/PTP peaked at 18.8 ± 0.9 sec after the Train and exhibited a mean decay time constant of 62.7 ± 6.0 sec.

We also observed similar magnitudes and time courses for AUG/PTP at synapses made by CBI-2 with buccal premotor neurons B31/32 (Susswein and Byrne, 1988; Rosen et al., 1991). The 10 Hz, 30 sec Train in CBI-2 induced STE in B31/32 ($n = 3$), with a mean peak percentage increase in EPSP amplitude of 183 ± 32% in saline containing elevated divalent cations. The AUG/PTP peaked at 18.3 ± 0.2 sec after the Train and exhibited a mean decay time constant of 71 ± 7.0 sec. When comparisons of AUG/PTP elicited in 31/32, B34, and B61/62 were made (all obtained in Hi Ca^{2+}/Hi Mg^{2+} saline), we found no significant differences in mean peak percentage increase in EPSP amplitudes ($p > 0.41$) or mean decay time constants ($p > 0.49$).

To mimic *in vivo* conditions and to determine whether the magnitude and time course of AUG/PTP were modified by saline containing high divalent cations, normal saline (NASW) was used to examine changes in synaptic efficacy in response to the Train. We used B34 and B61/62 for this set of experiments because these cells receive large-amplitude EPSPs at low frequencies of stimulation, and they comprise both premotor and motor elements, respectively, of the ingestion buccal motor circuitry. No significant differences ($p > 0.05$; $n = 4$ for B34 and $n = 3$ for B61/62) were observed in mean percentage increase in peak EPSP amplitude or mean decay time constant of AUG/PTP when values in NASW were compared with those in saline containing Hi Ca^{2+}/Hi Mg^{2+}. These results indicate that saline containing high divalent cations does not significantly alter the magnitude and time course of AUG/PTP recorded in B34 or B61/62 after CBI-2 trains.

Saline with elevated divalent cations was used in many of the experiments described above to suppress polysynaptic activity. To test whether an occult polysynaptic component contributed to the STE, we used one CBI-2 to Train the preparation and tested for heterosynaptic effects on EPSPs in B61/62 elicited by the contralateral CBI-2. After heterosynaptic stimulation (i.e., a 10 Hz, 30 sec train in the first CBI-2), the mean Post-Train EPSP amplitude in B61/62 (0.93 ± 0.06 mV) elicited by the second CBI-2 was not

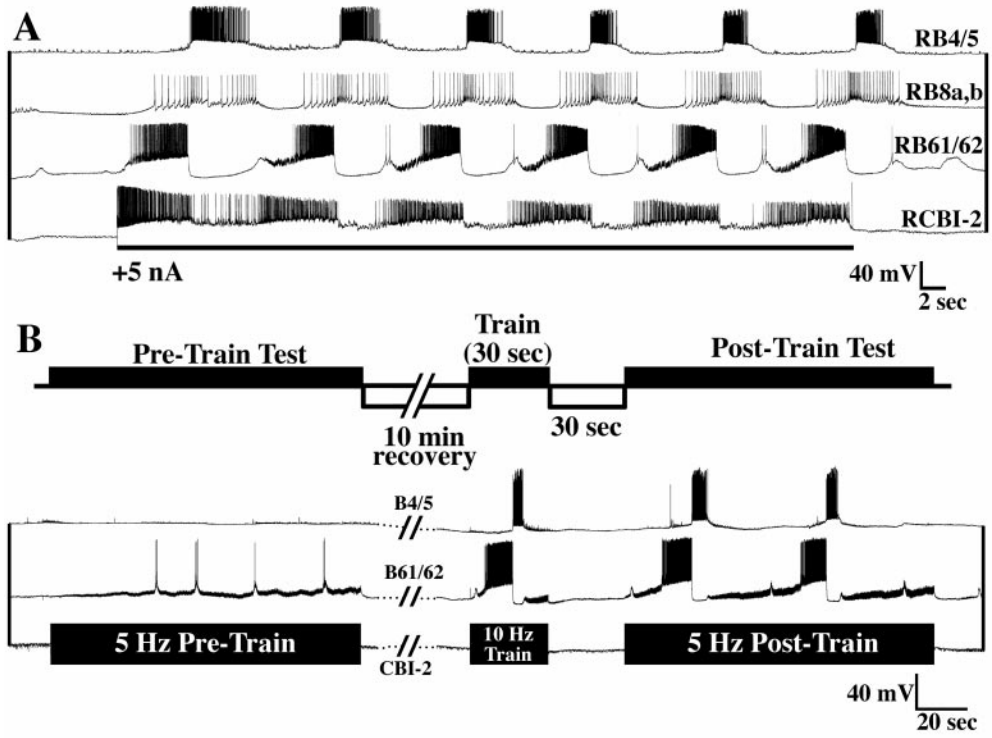

Figure 3. A, CBI-2 elicits fictive iBMPs. CBI-2 was driven with a 5 nA step of depolarizing current injected during the time indicated by the *black bar* (see Results for details). *B*, Short-term synaptic enhancement contributes to initiation of CBI-2-elicited iBMPs. The firing frequency of CBI-2 was adjusted to a level just below that required to elicit iBMP (5 Hz in this preparation; *Pre-Train Test*). The preparation was allowed to rest for 10 min and then a 10 Hz, 30 sec Train was applied to CBI-2. The *Post-Train Test* was initiated 30 sec after the conditioning *Train* using a CBI-2 firing frequency and duration identical to that of the Pre-Train Test. Note that driving CBI-2 initiates two cycles of iBMPs during the Post-Train Test.

significantly different from the mean Pre-Train EPSP amplitude (0.89 ± 0.05 mV; $p = 0.20$, $n = 2$; data not shown). We also tested CBI-2 to B61/62 synapses for changes in the magnitude of paired-pulse facilitation, which is dependent on residual presynaptic calcium (Zucker, 1989; Schulz et al., 1994; Katz and Frost, 1995; Jiang and Abrams, 1998). We observed a significant ($p < 0.01$; $n = 5$) increase in paired-pulse facilitation ratio obtained at the time of maximal AUG/PTP (mean percentage increase = $27.6 \pm 7.3\%$; using a 50 msec interval between the paired pulses). These results are consistent with a presynaptic mechanism for AUG/PTP and indicate that the increase in CBI-2 synaptic efficacy was caused primarily by homosynaptic mechanisms.

iBMPs elicited by CBI-2

Driving CBI-2 at physiological firing frequencies (Rosen et al., 1991) elicited rhythmic ingestion buccal motor programs (Fig. 3*A*), identified as iBMPs by previously established criteria (Morton and Chiel, 1993; Nargeot et al., 1997). Briefly, rhythmic ingestion occurs when the radula is closed during retraction and open during protraction. Thus, during fictive iBMPs elicited by CBI-2, iBMPs always began with a burst of action potentials in B61/62 (Hurwitz et al., 1996), indicative of the protraction phase of fictive ingestion followed by bursts of action potentials in radula retractor motor neurons (Fig. 3*A*, *RB4/5*), which overlapped with firing in radula closer motor neurons (Fig. 3*A*, *RB8a,b*). We also found that during programs elicited by CBI-2, B34 always fired during the protraction phase ($n = 12$; data not shown), whereas simultaneous recordings of *B8a,b* demonstrated that they were active during the retraction phase (Fig. 3*A*), as expected of fictive ingestion motor programs.

AUG/PTP contributes to initiation and modulation of CBI-2-elicited iBMPs

We examined whether STE of CBI-2 buccal connections influenced the central pattern generator (CPG) for ingestion. First, we tested whether STE enabled CBI-2 to initiate iBMPs at firing frequencies below that normally required to initiate rhythmic iBMPs. Figure 3*B* shows an example of one such experiment in which the CBI-2 firing frequency was adjusted to a level just below that required to reliably elicit iBMPs. CBI-2 was driven at 5 Hz for 2 min (Pre-Train Test), a frequency and duration that, in this preparation, was below that required to elicit rhythmic iBMPs. After a 10 min recovery period (all STE decays completely within this time), a Train of 10 Hz for 30 sec was applied to CBI-2. After a 30 sec rest, CBI-2 was again driven at 5 Hz for 2 min (Post-Train Test; a stimulus frequency and duration identical to that of the Pre-Train Test was always used). In all four preparations tested in this manner, CBI-2 reliably elicited rhythmic iBMPs after the Train (Fig. 3*B*). In NASW the conditioning Train occasionally elicited a single cycle of iBMP within 30 sec after the train. Therefore, to reduce the influence of residual CPG activity while maximizing the likelihood of observing a change in iBMP attributable to AUG/PTP, the Post-Train Test was initiated 30 sec after the conditioning train. In two preparations such as that shown in Figure 3*B* where accurate measurements could be made at peak AUG/PTP, the mean percentage increase in peak B61/62 EPSP amplitude after a 10 Hz, 30 sec train to CBI-2 was $231.0 \pm 19.1\%$. These results on STE induced by trains of CBI-2 action potentials suggest that AUG/PTP may act *in vivo* to enhance the ability of CBI-2 to initiate motor program generation.

We next tested whether AUG/PTP could modulate motor programs produced when CBI-2 was driven at frequencies that reliably elicited rhythmic iBMPs without previous training. In these experiments, CBI-2 was initially fired at a minimum frequency (usually 5 or 7 Hz) and duration (up to 3 min) that elicited three or four cycles of iBMP (Pre-Train Test). As described above, after 10 min of rest, a conditioning Train of CBI-2 spikes (10 Hz, 30 sec) was applied, followed by 30 sec of rest. The Post-Train Test was applied with a CBI-2 stimulus frequency and duration identical to that of the Pre-Train Test. After a conditioning train in CBI-2, iBMP cycles per minute increased by an average of $40.0 \pm 5.0\%$ ($p < 0.001$, $n = 5$) (Fig. 4*A*,*C*). In three preparations such as that shown in Figure 4*A*, the mean percentage increase in peak B61/62 EPSP amplitude after the Train was $245.0 \pm 17.5\%$.

To determine whether changes in iBMP cpm were elicited by the Pre-Train test alone, two consecutive Pre-Train Tests were

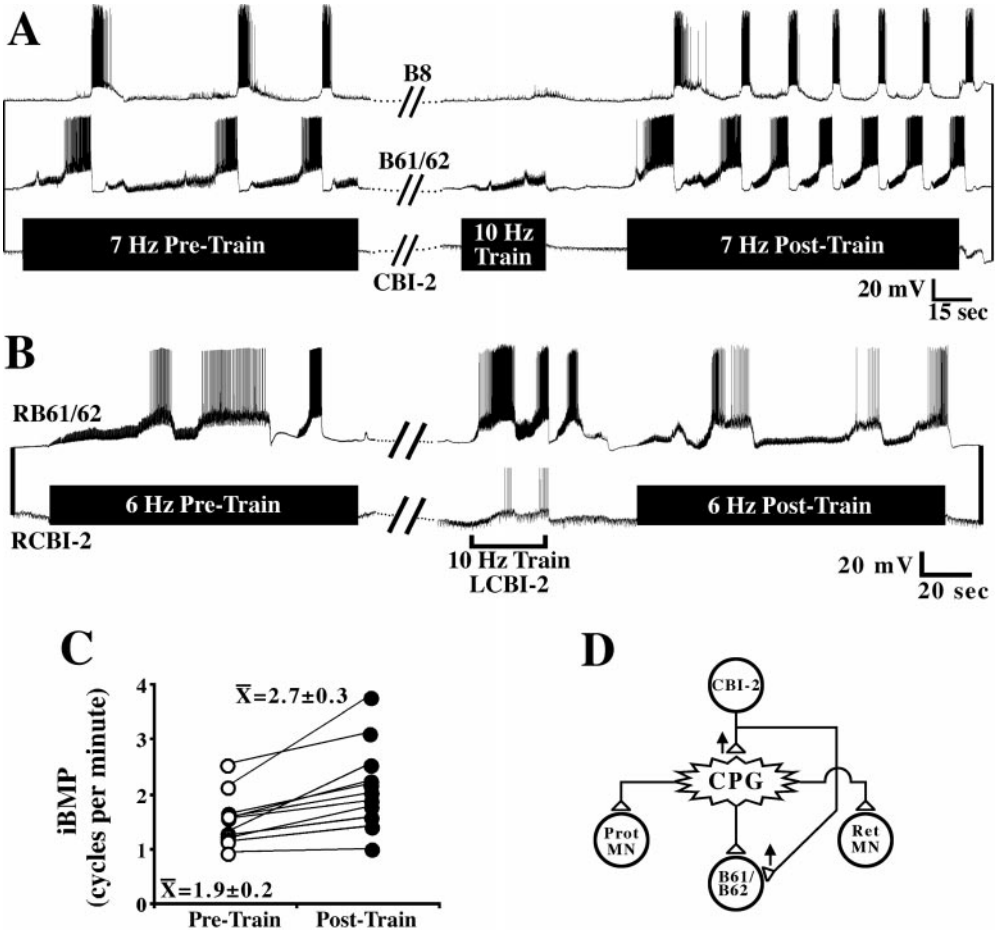

Figure 4. The cycles per minute of CBI-2-elicited iBMPs are increased during AUG/PTP *A*, Cycle frequency of ingestion buccal motor program is enhanced during AUG/PTP elicited by the Train in CBI-2. *B*, The Training effect on iBMP cycles per minute is pathway specific. Training the contralateral CBI-2 homolog (*LCBI-2*) does not produce the increase in iBMP cycles per minute. Note that when LCBI-2 was Trained and the RCBI-2 was given a continuous, subthreshold depolarization (see Materials and Methods), RCBI-2 received synaptic excitation during the Train and fired weak bursts in phase with B61/62 bursts, possibly caused by synaptic feedback from the buccal ganglia. *C*, The number of cycles per minute of iBMP increased significantly ($p < 0.001$) when elicited during peak AUG/PTP. Values for individual trials are shown for five preparations. *D*, Effects on buccal motor output reflect feedforward summation inherent in CBI-2 buccal connections and STE (*up arrows*) exhibited by these synapses. *CPG*, Central pattern generator; *Prot MN*, protractor motor neurons; *Ret MN*, retractor motor neurons.

performed without an intervening Train. Cycles per minute elicited by these consecutive test stimuli were compared, and no significant difference in iBMP cpm was observed ($p = 0.39$; $n = 3$).

The increased iBMP cycles per minute after training CBI-2 could have resulted from a nonspecific increased excitability of the buccal CPG. For instance, high-frequency stimulation of CBI-2 could have released modulatory neuropeptides that acted on premotor neurons to cause the increased burst frequency during iBMPs recruited by CBI-2. To examine this possibility, we performed the following control experiment. The effect on iBMPs elicited by CBI-2 was tested after a 10 Hz, 30 sec Train was applied to the contralateral CBI-2. The bilaterally symmetrical CBI-2s project bilaterally into the buccal ganglia (Rosen et al., 1991), and motor programs are coordinated bilaterally within the buccal ganglia (Kirk, 1989). Therefore, if the increased cycles per minute of iBMPs described above were caused by modulation of CPG excitability unrelated to STE at CBI-2 buccal synapses, a Train applied to the contralateral CBI-2 should have an equivalent effect on iBMP cycles per minute when tested with the ipsilateral CBI-2. However, no significant increase in iBMP cycles per minute was observed after training the contralateral CBI-2 (Fig. 4*B*) ($n = 3$, $p = 0.30$), whereas in these same preparations, training the ipsilateral CBI-2 resulted in a mean percentage increase in cycles per minute of 39.6 ± 2.1%. These results strongly support the hypothesis that AUG/PTP of CBI-2 synapses modulates iBMPs through presynaptic- and pathway-specific mechanisms.

DISCUSSION

We show that synaptic connections made in the buccal ganglia by command-like neuron CBI-2 exhibit short-term synaptic enhancement, including frequency facilitation and AUG/PTP. Our results support the hypothesis that AUG/PTP of CBI-2 synapses regulates iBMPs through presynaptic- and pathway-specific mechanisms. Short-term synaptic enhancement, such as facilitation and AUG/PTP, is a prevalent form of synaptic plasticity, and its roles in modulation of behavior are receiving increased attention (Fisher et al., 1997). The potential effects on rhythmic ingestion motor programs shown here are new examples of how STE may contribute to the initiation and modulation of consummatory behavior in *Aplysia*.

STE at CBI-2 synapses contributes to iBMP initiation and modulation

Each CBI-2 axon projects bilaterally into the paired buccal ganglia (Rosen et al., 1991), and the known connections to buccal neurons made by CBI-2 are found bilaterally and exhibit equivalent STE. In addition, buccal motor programs are coordinated across the midline by several synaptic pathways (Kirk, 1989; Hurwitz et al., 1997), so modulatory effects on iBMPs are likely to be induced bilaterally. Although the exact mechanisms underlying motor pattern initiation by CBI-2 are not known, STE is described here for synaptic input to premotor neurons B31/32 and B34; these neurons appear to contribute to consummatory pattern generation in the buccal ganglia (Susswein and Byrne, 1988; Hurwitz et al., 1997). We also describe STE at CBI-2 synapses with protractor motor neurons B61/62 (see also Sánchez and Kirk, 1998).

The initiation of iBMPs at low CBI-2 firing frequencies and the increased cycle frequency of iBMPs, both caused by training CBI-2, could have resulted from an increase in excitability of the buccal CPG unrelated to STE. If the effects of training CBI-2

were attributable to modulatory actions other than STE of CBI-2 buccal synapses, one would predict that training either of the bilaterally symmetrical CBI-2s would produce the same effects on iBMP cycles per minute. However, the effects of the Train on iBMPs are specific to the tetanized CBI-2. Training the contralateral CBI-2 does not cause changes in iBMPs when testing with the ipsilateral CBI-2 (Fig. 4*B*), indicating that the modulatory effects are pathway specific and likely caused by STE of the synapses of CBI-2s in the buccal ganglia.

Potential behavioral significance of CBI-2-elicited iBMPs during STE

The AUG/PTP exhibited by CBI-2 synapses decays with a time constant of ~1 min. This form of STE is recruited by training CBI-2 at a firing frequency and duration typical of the response of CBI-2s to sensory stimulation (Rosen et al., 1991) and thus is likely to be produced naturally in the intact animal. In an intact animal, AUG/PTP could modulate the number of bites per unit of time and contribute to the increased bite frequency observed during food-induced feeding arousal (Weiss et al., 1980).

In addition, STE would decrease the latency to burst onset in B61/62s and therefore decrease the latency to radula protraction after a sensory stimulus. The latency to radula protraction would be determined in part by input to B61/62 from the buccal CPG, and STE at CBI-2 synapses onto CPG neurons could indirectly lead to decreased latency of firing in B61/62. A decreased latency to radula protraction would contribute to decreased bite latency in response to a food stimulus (Rosen et al., 1989; Scott et al., 1995).

The fact that CBI-2 makes excitatory connections with premotor neurons such as B34, which may contribute to the buccal CPG (Hurwitz et al., 1997), as well as with motor neurons B61/62 represents a neural configuration known as feedforward summation (Fig. 4*D*) (Gardner and Kandel, 1972; Kandel, 1976). Premotor neuron B34 also makes facilitating monosynaptic connections with CPG neurons B31/32 (Hurwitz et al., 1997). A unique aspect of feedforward summation here is that STE of CBI-2 synaptic connections modulates CPG activity and directly influences firing in buccal motor neurons (i.e., B61/62). An analysis of firing frequency in protractor motor neurons B61/62 at the time of peak AUG/PTP reveals a significant increase in intraburst firing frequency during iBMP (our unpublished observations). The increased intraburst firing in B61/62 during STE would lead to increased contraction of muscle I2 (Hurwitz et al., 1996) and therefore to increased bite magnitude, as observed in the intact animal in which a buildup in bite magnitude occurs at the start of rhythmic biting (Weiss et al., 1980).

Although the cellular mechanisms responsible for homosynaptic STE have been studied extensively (Atwood and Wojtowicz, 1986; Zucker, 1989; Regehr et al., 1994; Fischer et al., 1997b; Fisher et al., 1997; Jiang and Abrams, 1998), few studies have documented the behavioral roles of STEs (for review, see Fisher et al., 1997). In most cases, potential behavioral roles for STE are largely implied (Trimmer and Weeks, 1991; Fisher et al., 1997), such as STE at the neuromuscular junctions of frogs, crayfish, lobster, and *Aplysia* where STE mediates enhanced muscle contraction during increased frequency of motor neuron firing (Atwood and Wojtowicz, 1986; Katz et al., 1993; Qian and Delaney, 1997; Brezina et al., 2000). An important form of STE modulates the output of the siphon-withdrawal reflex in *Aplysia*, and this STE is in turn subject to modulation, providing an example of metaplasticity (Byrne, 1997; Fischer et al., 1997a). In vertebrate cortex, short-term synaptic plasticity influences temporal-filtering properties of cortical neurons and appears to mediate contrast adaptation and enhanced sensitivity to changing cortical inputs (Dobrunz et al., 1997; Varela et al., 1997; Buonomano, 2000). In addition, short-term plasticity can contribute to functional reorganization of cortical pathways after sensory deprivation (Finnerty et al., 1999). Our results on STE in *Aplysia* CNS suggest that homosynaptic plasticity may be a fundamental mechanism used to modulate motor programs by acting at multiple levels within motor systems.

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