Inhibition of Afferent Transmission in the Feeding Circuitry of Aplysia: Persistence Can Be as Important as Size

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We are studying afferent transmission from a mechanoefferent, B21, to a follower, B8. During motor programs, afferent transmission is regulated so that it does not always occur. Afferent transmission is eliminated when spike propagation in B21 fails, i.e., when spike initiation is inhibited in one output region—B21’s lateral process. Spike initiation in the lateral process is inhibited by the B52 and B4/5 cells. Individual B52- and B4/5-induced inhibitory postsynaptic potentials (IPSPs) in B21 differ. For example, the peak amplitude of a B4/5-induced IPSP is four times the amplitude of a B52 IPSP. Nevertheless, when interneurons fire in bursts at physiological (i.e., low) frequencies, afferent transmission is most effectively reduced by B52. Although individual B52-induced IPSPs are small, they have a long time constant and summate at low firing frequencies. Once IPSPs summate, they effectively block afferent transmission. In contrast, individual B4/5-induced IPSPs have a relatively short time constant and do not summate at low frequencies. B52 and B4/5 therefore differ in that once synaptic input from B52 becomes effective, afferent transmission is continuously inhibited. In contrast, periods of B4/5-induced inhibition are interspersed with relatively long intervals in which inhibition does not occur. Consequently, the probability that afferent transmission will be inhibited is low. In conclusion, it is widely recognized that afferent transmission can be regulated by synaptic input. Our experiments are, however, unusual in that they relate specific characteristics of postsynaptic potentials to functional inhibition. In particular we demonstrate the potential importance of the IPSP time constant.

INTRODUCTION

Many studies that have provided insights into how centrally and peripherally generated activity is integrated have utilized experimentally advantageous preparations such as the one in these experiments, the mollusk Aplysia (Nusbaum and Been-hakker 2002). We study the regulation of afferent transmission from a feeding sensory neuron, the radula mechanoafferent B21 (Rosen et al. 2000b), to a follower cell, the radula closer motor neuron B8. B21 is bipolar, having major medial and lateral processes (Fig. 1) (Rosen et al. 2000b). The medial process innervates the periphery, whereas the lateral process is the primary point of contact with the follower B8 (Borovikov et al. 2000). If spikes are actively generated in both the medial and lateral processes of B21, afferent transmission to B8 will occur (Fig. 1). If spike propagation fails (i.e., if spikes are not actively generated in the lateral process), afferent transmission does not occur (Fig. 1) (Evans et al. 2003b). Afferent transmission from B21 to B8 can, therefore be regulated via the control of active spike initiation in B21’s lateral process (Evans et al. 2003b).

During feeding-like motor programs, B21 receives synaptic input that determines whether spike initiation in the lateral process occurs. The probability that spike initiation will occur is increased by depolarizing input provided through electrical coupling with other elements of the feeding circuitry (Evans et al. 2003b). The probability that spike initiation will occur is decreased by hyperpolarizing inhibitory postsynaptic potentials (IPSPs) (Evans et al. 2003a). Cholinergic interneurons, the B4/5 cells, are one source of inhibitory synaptic input to B21 (Evans et al. 2003a). In this study, we demonstrate a second source of inhibitory input, the histaminergic B52 cells (Evans et al. 1999; Plummer and Kirk 1990).

We show that B52 and B4/5-induced IPSPs in B21 differ. B52-induced IPSPs are smaller and have a longer time constant. We determine how differences in IPSP amplitude and half-width impact function when interneurons fire in bursts (as they do during motor programs). Perhaps unexpectedly we demonstrate that the smaller amplitude B52 induced IPSPs are most effective at inhibiting afferent transmission at physiological firing frequencies (which are relatively low).

Our results are relevant to the growing number of other systems where data indicate that spike propagation is dynamically regulated by synaptic input (e.g., Gingl et al. 2004; Verdier et al. 2003; Wachowiak and Cohen 1999; Wall 1995; Xiong and Chen 2002). Our study makes an important contribution to this body of work in that we characterize two patterns of afferent inhibition that have very different functional consequences. These patterns of inhibition are likely to be observed in other species, making our work of relevance both to other studies of afferent transmission and to other studies of synaptic inhibition in general.

METHODS

Animals

Experiments were conducted in 200–300 g Aplysia californica (Marinus, CA) that had been maintained in 14–16°C holding tanks. Animals were anesthetized by injection of isotonic MgCl2 then dissected to create reduced preparations described below. The nomenclature used in this study follows that of Gardner (1971).

Preparations

Most experiments were conducted in preparations that consisted of the buccal ganglion and the isolated subradula tissue (SRT), i.e., the...
microelectrodes were high resistance (generally in injection. Stimulator Model S88 or S48 (Grass Instruments) or via DC current injection of brief depolarizing current pulses triggered via a Grass triggering of action potentials. Action potentials were induced via uncontrolled spiking, while B4/5 was depolarized to facilitate reliable cells were impaled with two electrodes. One was used for current citrate with 10 mM probenecid (to verify recording sites as described was filled with 3% 5(6)-carboxyfluorescein dye in 0.1 M potassium chloride. Electrodes were beveled so that their impedances were generally 100 MΩ. Using single-barrel electrodes fabricated from thin-walled capillary (Axon Instruments), and either a Macintosh G4 computer or a Sony analyzed using Axograph version 4.6 or pClamp version 9 software Digidata (Axon Instruments, Union City, CA) and were acquired and oscillo- in the lateral process afferent transmission to B8 does not occur (bottom) (Evans et al. 2003b). Buccal mass was dissected so that the SRT could be removed from the radula, which it underlies. The sensory innervation of the SRT passes through the radula nerve; consequently this nerve was left intact. All other buccal nerves were severed. Motor program experiments were conducted in the isolated nervous system with only the buccal and cerebral ganglia present. In general, experiments were conducted at ~16°C.

Electrophysiology

Standard current-clamp intracellular techniques were used to obtain up to four recordings simultaneously. Equipment used included the following: Getting Model 5A amplifiers modified for 100-nA current injection (Getting Instruments, Iowa City, IA), Tektronix AM 502 amplifiers (Tektronix, Wilsonville, OR), a Tektronix storage oscillo- scope (model 5111), and an Astro-Med Chart Recorder (model 9500, Grass Instruments, Quincy, MA). Some data were digitized [using a Digidata (Axon Instruments, Union City, CA)] and were acquired and analyzed using Axograph version 4.6 or pClamp version 9 software (Axon Instruments), and either a Macintosh G4 computer or a Sony Vaio PCG-GRT Notebook. To record from the somata of neurons, we used single-barrel electrodes fabricated from thin-walled capillary tubing and filled with 3 M potassium acetate and 30 mM potassium chloride. Electrodes were beveled so that their impedances were generally <10 MΩ. To record from the lateral process of B21, microelectrodes were high resistance (generally ~50 MΩ), and the tip was filled with 3% 5(6)-carboxyfluorescein dye in 0.1 M potassium citrate with 10 mM probenecid (to verify recording sites as described in the following text).

When constant frequency bursts were generated in interneurons, cells were impaled with two electrodes. One was used for current passing; the other electrode was used for recording. In general B52 was hyperpolarized below its resting membrane potential to prevent uncontrolled spiking, while B4/5 was depolarized to facilitate reliable triggering of action potentials. Action potentials were induced via injection of brief depolarizing current pulses triggered via a Grass Stimulator Model S88 or S48 (Grass Instruments) or via DC current injection.

Lateral process recordings

Recordings from the lateral process were obtained as described (Evans et al. 2003b). Briefly, we injected Fast Green dye into the B21 soma for ~5–10 min to inject the smallest amount of dye that would permit visualization. After an additional 15–30 min, the lateral and medial processes could be visualized. To facilitate penetration of the lateral process, we often removed some of the overlying connective tissue and small cells, using a glass micropipette. At the conclusion of experiments, we verified recording sites by injecting carboxyfluorescein dye. Dye-filled cells were viewed with a Nikon Labphot2 microscope equipped with a filter set to visualize fluorescein (B-2A; EX 450–490/DM 505/BA 520). Unless otherwise noted, B21 was centrally depolarized prior to activation of B4/5 and/or B52 just to the point where active spike initiation was observed in the lateral process. In all cases, conditions were as similar as possible when we compared effects of B4/5 and B52.

Induction of motor programs

Motor programs were induced by application of carbachol to the cerebral ganglion (Susswein et al. 1996). The protraction phase of motor programs was monitored by recording intracellularly from the I2 motor neuron, B61 (Hurwitz et al. 1996), and the retraction phase was monitored by recording intracellularly from the retraction phase interneuron B64 (Hurwitz and Susswein 1996).

Carbachol-induced motor programs have been described as being primarily ingestive-like (Susswein et al. 1996). To confirm that this was true in our case, we periodically recorded intracellularly from the radula closing motor neuron B8 (Morton and Chiel 1993a,b). If B8 fires at <3.5 Hz during protraction and >4.5 Hz during retraction (ratio of protraction to retraction activity <0.65), activity is classified as ingestive-like (Morgan et al. 2002). In all of the preparations tested (i.e., 5/11), activity was ingestive-like. The mean ratio of protraction to retraction activity was 0.16 ± 0.03 (mean ± SE). Mean B52 firing frequencies recorded during ingestive-like activity were also not significantly different from frequencies recorded during unclassified activity (mean frequency during ingestive-like activity: 3.13 ± 0.55 Hz; mean frequency during unclassified activity: 3.15 ± 0.42 Hz).

Peripheral stimulation of the SRT

The SRT was peripherally stimulated as has been described (Cropp et al. 1996). Briefly, mechanical stimuli were delivered by means of a mini-speaker (Quam) that had a wooden stick (tip diameter: 1 mm) that was perpendicularly attached to the speaker membrane. Reproducible movements of the membrane were regularly elicited by driving the speaker with a stimulator (Grass Instruments, S48 or S88).

Data analysis

PSP measurements were made using either Axograph or pClamp software (Axon Instruments). Specifically, we determined peak amplitude by measuring the maximum change in potential with respect to the preceding baseline potential (which was not necessarily resting membrane potential). We determined time course by measuring PSP half-width, and by measuring the 0–50% rise time. To quantify the effectiveness of a particular B4/5 or B52 frequency, we generated a number of trials by repeatedly activating B21 during each burst of interneuron activity. Additionally, at least five bursts of interneuron activity were delivered at each frequency. To pool data, we computed the mean percent of trials where inhibition of lateral process spike initiation was observed for each preparation, then combined data from different preparations as indicated.

Statistical tests were performed with Kaleidagraph version 3.6 (Synergy Software, Essex Junction, VT), or StatView version 5.01 (SAS Institute, Cary, NC). Unless otherwise noted, two group com-
parisons utilized a paired t-test and n’s provided indicate the number of preparations in which data were obtained. Data are reported as means ± SE.

RESULTS

B52 makes a synaptic connection with B21

There are two identical B52 neurons in each buccal hemi-ganglion (Evans et al. 1999) that both induce one-for-one IPSPs in B21 (n = 3). These PSPs are monosynaptic and chemically mediated because they persist when preparations are placed in high-divalent cation solutions (Fig. 2A; n = 4) and are increased in amplitude when the extracellular calcium concentration is increased (Fig. 2B; n = 5). There is no indication of electrical coupling between B52 and B21.

Afferent transmission from B21 to B8 is altered when spike initiation in B21’s lateral process is regulated (Evans et al. 2003b). It was, therefore of interest to determine whether the lateral process receives synaptic input from B52. In simultaneous lateral process and somatic recordings, IPSPs were apparent in both places, and were similar (Fig. 2C). The peak PSP amplitude in the lateral process was 0.790 ± 0.050 mV, in the soma it was 0.789 ± 0.06 mV (no significant difference; P = 0.97; t = 0.03; df = 4; n = 5). IPSP half-width in the lateral process was 205.3 ± 33.6, in the soma, it was 205.7 ± 32.7 (no significant difference; P = 0.9; t = 0.1; df = 4; n = 5). To confirm that B52-induced IPSPs recorded in the lateral process are at least in part produced via direct input (and not by input to the soma), we severed the soma/lateral process connection. After the lesion, B52 stimulation induced IPSPs in both the isolated lateral process and the rest of the neuron, i.e., the soma and medial process (Fig. 2D; n = 4). The B52 neurons do, therefore provide synaptic input to the lateral process of B21 so potentially could inhibit afferent transmission.

Individual B52-induced IPSPs in the lateral process of B21 are small (Fig. 2E). For example, the peak amplitude is generally about one-fourth the amplitude of IPSPs induced by the B4/5 neurons (Fig. 3A; neurons that induce relatively well characterized IPSPs in a number of buccal neurons including B21) (Evans et al. 2003a; Gardner 1977b, 1980a,b 1986; Gardner and Kandel 1977; Gardner and Stevens 1980). In our studies of afferent transmission (which involved depolarization of the lateral process by ~5 mV), the peak amplitude of IPSPs induced by stimulation of B4/5 was 3.2 ± 0.2 mV. The peak amplitude of B52-induced IPSPs was 0.8 ± 0.1 mV (Fig. 3A; P = 0.0002; t = 12.98; df = 4; n = 5).

Although B52-induced IPSPs are small, they have a relatively long time constant. For example, in the preparations in which we compared peak amplitude, half widths of B52-induced IPSPs were 169.7 ± 18.4 ms (Fig. 3B). Half widths of B4/5-induced IPSPs were 48.2 ± 2.6 ms (Fig. 3B; P = 0.003; t = −6.5; df = 4; n = 5). Rise times of B52 and B4/5-induced IPSPs also differed (30.9 ± 7.5 ms for B52-induced IPSPs and 4.9 ± 0.8 ms for B4/5-induced IPSPs; Fig. 3C; P = 0.02; t = 3.67; df = 4; n = 5).

To summarize, the B52 neurons produce IPSPs in the lateral process of B21 (a spike initiation site important for mecha-no-afferent transmission to the follower motor neuron B8). These IPSPs are relatively small but have a long time constant.

B52 firing frequencies during carbachol-evoked motor programs

Although individual B52-induced IPSPs in B21 are small, the B52 neurons could fire at high frequencies during feeding-
like motor programs. Although this is not the case during motor programs induced by stimulation of the afferent nerve n.2,3 (Nargeot et al. 2002), it has not been determined whether it is true when activity is evoked in other ways. Carbachol-induced motor programs were of particular interest because they have been used to study afferent transmission in B21 (Borovikov et al. 2000; Evans et al. 2003b) and feeding movements generated by carbachol application have been characterized (Susswein et al. 1996).

The B52 neurons fire at two points during feeding-like motor programs (Nargeot et al. 2002) (also Fig. 4). Burst 1 occurs during the radula protraction phase of the motor program. Burst 2 occurs immediately after radula retraction. During carbachol-evoked activity, a mechanism has been described that peripherally activates B21 during burst 1 activity in B52 (protraction phase of motor program) (Borovikov et al. 2000). Because we have no data that indicate that B21 is peripherally activated during burst 2, we only included burst 1 activity in our analysis of the B52 firing frequency. During carbachol-induced motor programs, instantaneous burst 1 firing frequencies ranged from ~2-4Hz (Fig. 4). The mean frequency was 3.14 ± 0.32 Hz (n = 11). B52 firing frequencies are, therefore relatively low at the time when regulation of afferent transmission would be expected to occur.

B52 inhibits B21-B8 mechanoafferent transmission in a frequency-dependent manner

To determine whether B52 is capable of inhibiting mechanoafferent transmission, we peripherally activated B21 and recorded PSPs in B8 with and without concurrent B52 stimulation (Fig. 5A, and 2). B52 activity either eliminated B21-induced PSPs in B8 or reduced PSP size (Fig. 5A2). The B52 neurons are, therefore capable of inhibiting B21 mechanoafferent transmission to B8.

The B52 neurons make connections with both B21 (Fig. 2A) and B8 (Evans et al. 1999). Consequently, effects could have been mediated presynaptically (on B21) or postsynaptically (on B8). Potential presynaptic effects were of particular interest because presynaptic elimination of afferent transmission would obviously make postsynaptic effects irrelevant (for mechanoafferent transmission). Consistent with the idea that there is a presynaptic effect, we found that B52 stimulation decreased the size of depolarizations evoked by DC injection into B8 (Fig. 5B, I and 2) but that decreases in pulse size were much smaller than decreases in PSP amplitude (the PSP amplitude was decreased by 62.5 ± 5.5%, the pulse amplitude by 9.3 ± 4.6%; Fig. 5C, P = 0.005; t = 7.6;df = 3; n = 4).

We definitively confirmed that the B52 neurons can act presynaptically to virtually eliminate afferent transmission in a second set of experiments that took advantage of previous work that has shown that spike initiation in the lateral process is necessary for B21 mechanoafferent transmission to B8 (Evans et al. 2003b). We peripherally activated B21 and recorded intracellularly from both the soma and lateral process (Fig. 6A). When spike initiation is inhibited, depolarizing potentials are recorded in the lateral process when peripheral stimuli are applied (Fig. 1). These depolarizations are attenuated, electrotonic versions of action potentials generated in more medial parts of the cell (Evans et al. 2003b). We, therefore stimulated B52 to determine whether we would record full size action potentials or attenuated depolarizations in the lateral process. Single B52-induced IPSPs in B21 were not sufficient to inhibit lateral process spike initiation (Fig. 6b1). When we triggered a burst of action potentials in B52, however, inhibition did occur (Fig. 6b2; n = 10). Thus with repeated activation, B52 can act presynaptically to inhibit lateral process spike initiation in B21. When this occurs,
mechanoafferent transmission to B8 will be virtually eliminated (Evans et al. 2003b).

To characterize frequency-dependent effects of B52 spike trains, we stimulated single B52 neurons at 2, 4, 8, and 12 Hz (Fig. 7A; n = 5). The 4- to 8-Hz frequencies were chosen because they are approximately twice observed instantaneous frequencies during motor programs, and there are two B52 neurons in each buccal hemiganglion (Evans et al. 1999). When the firing frequencies of the B52 neurons are relatively low, the two cells tend to fire out of phase (Evans et al. 1999), and both cells provide synaptic input to B21. Additionally, we tested a higher firing frequency, 12 Hz, and a lower frequency, 2 Hz. Effects of B52 were frequency dependent [1-way ANOVA, F(4,19) = 15.8; P < 0.0001], and as expected, higher firing frequencies produced greater suppression of lateral process spike initiation (Fig. 7, A and B). At 2 Hz, we observed inhibition 0.5 ± 0.5% of the time. At 4 Hz, we observed inhibition 68.5 ± 23.6% of the time. At 8 Hz, we observed inhibition 78.2 ± 13.6% of the time. Finally, at 12 Hz, we observed inhibition 89.7 ± 4.9% of the time. Individual comparisons with Bonferroni corrections showed that inhibition at 4, 8, and 12 Hz was significant (at 4 Hz, t = 4.72; P = 0.0084; at 8 Hz, t = 5.04; P = 0.003, at 12 Hz, t = 5.78; P = 0.0009). Inhibition at 4, 8, and 12 Hz was also different from inhibition observed at 2 Hz (for 2 vs. 4, t = 4.38; P = 0.009, for 2 vs. 8 t = 5.01; P = 0.003, for 2 vs. 12 t = 5.75; P = 0.0009). Other comparisons did not produce statistically significant results.

We show, therefore that the B52 neurons can act presynaptically to inhibit afferent transmission when they fire in bursts at physiologically relevant frequencies (e.g., 4 and 8 Hz). Interestingly these frequencies are relatively low.

**Effects of B4/5 are also frequency dependent but the pattern of inhibition differs**

As noted previously, the B52 neurons are not the only cells that can block lateral process spike initiation in B21 and inhibit afferent transmission to B8. The B4/5 neurons can do the same (Evans et al. 2003a). Results of a previous study suggest, however, that B4/5 are unlike B52 in that they are relatively ineffective at low frequencies (Evans et al. 2003a) [despite the fact that individual B4/5-induced IPSPs are 4 times larger than B52-induced IPSPs (Figs. 2E and 3A)]. Differences between experimental paradigms in previous work and this study, however, make it impossible to directly compare B52 and B4/5 efficacy at specific frequencies. To make these comparisons, we performed experiments as shown in Fig. 7A but stimulated B4/5 (n = 4). Higher B4/5 firing frequencies produced greater suppression of afferent transmission [as expected; F(4,19) = 19.6; P < 0.001; Fig. 8, A and B]. Individual comparisons with Bonferroni corrections showed that inhibition at 4, 8, and 12 Hz was significant (at 4 Hz, t = 3.41, P = 0.05; at 8 Hz, t = 3.6, P = 0.04; at 12 Hz, t = 8.0, P < 0.0001). Inhibition at 12 Hz was also different from inhibition observed at other fre-

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**FIG. 5.** A1: paradigm for A2. R, recording; S, stimulation. **A2**: effect of B52 stimulation on B21-induced PSPs in B8. B21 was peripherally activated at −1.5 Hz when mechanical stimuli were applied to the SRT. Subthreshold DC current was injected into the B21 soma to verify that peripheral stimuli activated B21. B52 was then stimulated via intracellular injection of DC current. Note that B52 stimulation decreased PSP amplitude. B1: paradigm for B2. **B2**: effect of B52 stimulation on depolarizations triggered by DC injection into B8. Brief pulses of depolarizing current were directly injected into B8 at −1.5 Hz via a 2nd stimulating electrode. Stimulation parameters (pulse duration and size) were adjusted so resulting depolarizations were as similar as possible to EPSPs within a given preparation. B52 activation as in A2 decreased pulse amplitude, but decreases in pulse amplitude were less than decreases in PSP amplitude. A2 and B2 are from the same preparation. C: group data showing that B52-induced decreases in PSP size were greater than B52-induced decreases in pulse size.
quencies (for 2 vs. 12, \( t = 7.2, P = 0.0001 \); for 4 vs. 12, \( t = 4.6; P = 0.006 \); for 8 vs. 12, \( t = 4.4, P = 0.009 \)). Other comparisons did not produce statistically significant results.

In comparing B52 and B4/5 data, the most striking difference is in the pattern of inhibition that is seen at 4 and 8 Hz. We most extensively analyzed the 8-Hz data because there were more inhibited responses. With B52 activation, once inhibition was observed, subsequent responses were also inhibited (as illustrated in Fig. 9A). This was true 97.2 \( \pm \) 2.8% of the time (Fig. 9B, black bar on left; \( n = 5 \)). In contrast, when B4/5 was stimulated at 8 Hz, an inhibited response was most commonly not followed by subsequent inhibition (as illustrated in Fig. 9A2). Inhibition of a subsequent response occurred 22.0 \( \pm \) 9.6% of the time (Fig. 9B, white bar on left; \( n = 4 \)). Results obtained by stimulation of B52 were significantly different from results obtained by stimulating B4/5 (unmatched pairs; \( P = 0.003; t = -7.5; df = 3 \)).

These results are not unexpected given the fact that temporal summation of B52-induced PSPs occurs at 4 and 8 Hz (Fig. 10, A and B). Consequently, it would be predicted that inhibition would occur as schematically illustrated in Fig. 11A 2 and 3. Once a response is affected, it would be expected that subsequent responses would continue to be inhibited (as long as stimulation is maintained). In contrast, temporal summation of B4/5 induced PSPs does not occur at 4 and 8 Hz (Fig. 10, A and B). The pattern of inhibition, therefore would be predicted to be as shown in Fig. 11B, 1 and 2. After every B4/5 action potential there would be a relatively brief (\(~50\) ms) period of potential inhibition (Evans et al. 2003a). Between periods of potential inhibition, however, inhibition cannot occur (unless there is additional input to B21). When B4/5 fire at low frequencies, the total time that inhibition can occur is less than the total time that inhibition cannot occur. For each trial, therefore the probability that inhibition will occur is relatively low and is not related to what happened in the previous trial. Consequently, subsequent inhibition can occur but there is a relatively low probability that it will. Thus the pattern of inhibition presumably differs because in one case (B52), temporal summation occurs at 8 Hz, whereas in the other case (B4/5) it does not.

At 12 Hz, temporal summation of both B4/5- and B52-induced PSPs occurs. As expected, therefore there is a relatively high probability that inhibition will occur as a result of activity in both types of interneurons (Fig. 9B). Specifically, inhibition occurred 87.5 \( \pm \) 9.6% of the time for B4/5, and 100% of the time for B52 (no significant difference; unmatched...
Because there are so few potential periods of inhibition (e.g., Afferent transmission will be altered is, however, very low

In general, there is no statistical difference in results obtained in trials where inhibition was not observed (2 and 3), intervals between B4/5 action potentials and peripheral spikes were longer.

In trials where inhibition was not observed (2 and 3), intervals between B4/5 action potentials and peripheral spikes were longer.

To summarize, results of B52 and B4/5 stimulation are only significantly different when there is a difference in temporal summation. This occurs at 4 and 8 Hz.

**DISCUSSION**

Previous experiments demonstrated that B21 mechanoafferent transmission to the motor neuron B8 can be inhibited by B4/5 (Evans et al. 2003a). We now demonstrate a second source of inhibitory input to B21, the B52 neurons (Plummer and Kirk 1990).

Presynaptic inhibition of axonal spike propagation

B52 and B4/5 make both pre- and postsynaptic contacts at the B21-to-B8 synapse, i.e., both B21 and B8 are contacted (Evans et al. 1999, 2003a; Gardner 1971, 1977a). Currently we have no data concerning the significance of the postsynaptic (B8) input for mechanoafferent transmission. We have, however, demonstrated that presynaptic (B21) effects are physiologically relevant. When mechanoafferent transmission occurs, spikes are actively triggered at least twice in B21, first in the medial process, and second in the lateral process (Fig. 1) (Evans et al. 2003b). Synaptic input can inhibit lateral process spike initiation, and thereby prevent B21-induced excitation of B8 (Evans et al. 2003a). When this occurs, postsynaptic effects are irrelevant (for mechanoafferent transmission). We, therefore demonstrate a presynaptic mechanism whereby afferent transmission is inhibited as a result of an axonal spike propagation failure.

Variability in axonal conduction has been described in a number of preparations and is generally regarded as an important mechanism by which information processing can occur (Debanne 2004). This has been largely demonstrated in experiments where conduction is altered by geometrical properties of axons, or by activity-dependent alterations in conductances. Our work differs in that we demonstrate that conduction can be altered by heterosynaptic input. Although this type of regulation has been less commonly described, it has been reported in pairs; P = 0.4; t = −1; df = 3). At 2 Hz, temporal summation does not occur for either B52 or B4/5. Temporal summation is, however, not the sole determinant of inhibition. The efficacy of a single spike is also important. When B4/5 fire at 2 Hz there is no temporal summation, but a single spike can be effective (Evans et al. 2003a). Some inhibition of afferent transmission is, therefore observed (Fig. 8, A and B). The probability that afferent will be altered is, however, very low because there are so few potential periods of inhibition (e.g., Fig. 11B, 1 vs. 2). In contrast, when B52, a single spike is never effective (Fig. 6B1), therefore virtually no inhibition of afferent transmission is observed at 2 Hz (Figs. 7, A and B, and 11A1). In general, there is no statistical difference in results obtained when B4/5 and B52 are stimulated at 2 Hz.
other systems, e.g., in spinal afferents (Lamotte d’Incamps et al. 1999; Wall 1995), trigeminal afferents (Verdier et al. 2003), leech mechanosensory neurons (Mar and Drapeau 1996), starlette afferents in *Tritonia* (Lee et al. 2003), lobster olfactory neurons (Wachowiak and Cohen 1999), and spider mechanoreceptors (Gingl et al. 2004). To summarize, presynaptic mechanisms that regulate synaptic transmission are often mediated by inputs that are electrically close to sites of transmitter release (e.g., Nusbaum et al. 1997). It is, therefore well established that neurotransmitter release can be altered by synaptic input. It is becoming increasingly apparent, however, that presynaptic input can also modify axonal spike propagation before spikes reach sites of synaptic release.

**FIG. 10.** Temporal summation of B4/5 and B52-induced IPSPs in B21. A: At 1 Hz, temporal summation of B52-induced IPSPs occurs, while temporal summation of B4/5-induced IPSPs does not. B: similar results are obtained at 8 Hz.

**B52 and B4/5 produce different patterns of afferent inhibition**

An important advantage of our preparation is that we are able to manipulate identified interneurons and study the regulation of afferent transmission as a result of physiologically relevant synaptic input. We are therefore able to address issues that are difficult to approach when transmitters that regulate afferent transmission are exogenously applied. In particular inhibitory input from multiple sources is commonly observed (e.g., Clarac et al. 2000; Wachowiak and Cohen 1999), yet its functional significance is poorly understood. In this study, we generate trains of action potentials in different interneurons and

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**FIG. 11.** Schematic representation of effects of spike trains in B52 (A) and B4/5 (B) on B21 mechanoafferent transmission to B8. Vertical lines represent action potentials. **Top rows** (labeled B52 in A and B4/5 in B): interneuronal firing frequencies as low (~2 Hz), intermediate (~8 Hz), or high (~12 Hz). **Second rows** (labeled B21 inhibition): the resulting period of inhibition in B21. In A1, inhibition is not shown because single B52-induced IPSPs do not inhibit afferent transmission. Inhibition is not induced without temporal summation, which does not occur at 2 Hz. In A, 1 and 2, a relatively brief (i.e., ~50 ms) period of inhibition is shown every time B4/5 is active. These brief periods of inhibition are effects of single B4/5-induced IPSPs in B21. Note, however, that at 2- and 8-Hz periods of inhibition are interspersed with periods when inhibition does not occur. In B3, the B4/5 firing frequency (12 Hz) is sufficient for summation to occur therefore inhibition becomes continuous. The **3rd row**: peripherally triggered action potentials in B21, which in all cases is arbitrarily represented as ~10 Hz, a reasonable frequency (Borovikov et al. 2000). Physiologically, this activity would represent spiking in medial parts of B21. **Third rows** also include representations of periods of inhibition in B21 (in gray). **Fourth rows** are modifications of 3rd rows that were generated by removing any triggered afferent activity that occurred during a period of inhibition. **Bottom rows**: spiking activity in B21’s lateral process. Effects of a particular pattern of interneuron activity can be determined by comparing the bottom 2 rows. Note that low-frequency activity in B25 does not alter afferent transmission (in A1 the bottom 2 rows are the same). In contrast, high-frequency B52 activity completely inhibits afferent transmission (in A3 there are no spikes in the bottom row). Low-frequency activity in B4/5 can modify afferent transmission (in B1 the bottom row has 1 less spike than the 3rd row). High-frequency activity is, however, much more effective (there are no spikes in the bottom row in B3).

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demonstrate that resulting patterns of inhibition can differ strikingly.

We characterize two patterns of inhibition. In one case, once inhibition is observed, it occurs repeatedly (Fig. 11, A2, A3, and B3). Alternatively, discrete periods of inhibition are intermixed with intervals in which inhibition does not occur (Fig. 11B, 1 and 2). Properties of the B52 IPSP bias it toward continuous inhibition. The relatively long time constant promotes temporal summation at relatively low firing frequencies. Discrete inhibition as a result of a single action potential is never observed (presumably as a result of the small amplitude of the IPSP). In contrast, properties of the B4/5 IPSP make discrete inhibition possible. IPSPs have a relatively large amplitude and short time constant. We show, therefore, that physiological consequences of interneuron activity can differ. The B52 neurons can produce continuous inhibition of afferent transmission at firing frequencies where only discrete inhibition of afferent transmission is observed when B4/5 are active.

**Inhibition of mechanoafferent transmission during motor programs**

We have primarily studied the functional significance of the regulation of mechanoafferent transmission during ingestive motor programs. These programs are essentially two phase (Cropper et al. 2004). First, the radula opens and protracts so that food can be grasped. Second, the radula closes and retracts pulling food into the buccal cavity. During ingestive activity B52 and B4/5 are not coactive. The B52 cells are protraction phase interneurons (Evans et al. 1999; Nargeot et al. 2002), whereas B4/5 are retraction phase interneurons (Church and Lloyd 1994; Jing and Weiss 2001; Rosen et al. 1991). The two patterns of afferent inhibition described in the preceding text, therefore will be manifested at different times during ingestive motor programs.

During radula protraction, B21 is peripherally activated when a radula opener muscle contracts (Borovikov et al. 2000). If this activity is transmitted to B8, ingestive motor programs will be disrupted. B8 is a radula closer motor neuron (Morton and Chiel 1993a,b). If B8 is excited during protraction, the radula will tend to close as it moves forward. This tends to push food out of the buccal cavity (instead of pulling it in). Our data suggest, however, that interneuronal (B52) regulation of afferent transmission prevents this from occurring. At physiologically relevant firing frequencies, the B52 neurons tend to produce continuous inhibition of afferent transmission to B8; the only exception to this being that there is a delay before temporal summation occurs and inhibitory effects are manifested. The delay may, however, be irrelevant because relatively little peripherally generated activity may be generated in B21 at this point. Protraction phase excitation of B21 is not triggered until tension is developed in the innervated muscle (Borovikov et al. 2000). To summarize, although protraction phase excitation of B21 occurs during ingestive motor programs, our results suggest that interneuronal (B52) regulation of afferent transmission prevents this activity from being transmitted to B8. B52-induced inhibition of afferent transmission to B8 is therefore likely to be of physiological importance because it prevents disruption of ingestive activity.

During radula retraction, B21 is presumably peripherally activated when food contacts the radula surface. If this activity is transmitted to B8, ingestive behavior will not be disrupted. In fact, it will be enhanced, i.e., the radula will close more tightly as food is pulled into the buccal cavity. It is therefore not surprising that physiologically relevant activity in B4/5 does not simply eliminate retraction phase mechanoafferent transmission to B8. Instead B4/5 produce a somewhat complex pattern of inhibition, which changes as retraction progresses.

The probability that B4/5 will inhibit afferent transmission is highest when retraction is initiated, i.e., ~60% (Evans et al. 2003a). It has been suggested that early inhibition delays radula closing with respect to radula retraction, which may in some cases improve the efficiency of food ingestion (Rosen et al. 2000a). As retraction progresses, however, there is an almost immediate exponential decrease in the instantaneous B4/5 firing frequency (Evans et al. 2003a). For most (87%) of retraction, B4/5 activity is below the point where B4/5-induced IPSPs in B21 temporally summate (i.e., <10 Hz) (Evans et al. 2003a). For most of retraction, the B4/5 neurons will therefore be firing at frequencies where relatively few discrete periods of afferent inhibition will be observed (Evans et al. 2003a). Consequently, the probability that afferent transmission will be inhibited is relatively low (~20–30%) (Evans et al. 2003a). In general, therefore physiologically relevant B4/5 activity and B52 activity are likely to differ in that B52 activity appears to virtually prevent transmission of afferent activity to B8. In contrast, B4/5 activity does not prevent afferent transmission. Instead it alters it, e.g., initially it delays it.

**Concluding remarks**

We show that IPSPs induced by interneuron activity have different properties (i.e., IPSPs induced by the histaminergic B52 neurons differ from IPSPs induced by the cholinergic B4/5 neurons). Similarly vertebrate IPSPs can differ, e.g., glycine- and GABA<sub>A</sub>-induced IPSPs generally have different time constants (e.g., Dumoulin et al. 2001; Jonas et al. 1998; O’Brien and Berger 1999; Rüssier et al. 2002). Although physiological roles of different types inhibition have not yet been extensively studied in the context of afferent transmission, they have been studied in terms of effects on spike initiation in general, e.g., in terms of effects on neuron discharge in response to current injection. Our work is relevant to this literature because our mechanism for regulating afferent transmission is one where spike initiation is inhibited.

Experiments in vertebrate preparations have most extensively studied inhibitory transmitter corelease, e.g., demonstrating that GABA and glycine corelease can optimize functional inhibition (Rüssier et al. 2002). Data indicate, however, that pure gycineric or pure GABAergic transmission can also occur (Ornung et al. 1994; Yang et al. 1997). We demonstrate that different types of synaptic input can produce different patterns of inhibition, i.e., continuous versus discrete. These types of inhibition are, in turn, likely to serve different physiological functions, i.e., elimination as opposed to modification of activity. Our work, therefore makes an important contribution to studies of the functional significance of chemical complexity in both afferent transmission and inhibitory synaptic transmission in general.

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