Effect of presynaptic membrane potential on electrical vs. chemical synaptic transmission

Colin G. Evans, Bjoern Ch. Ludwar, Timothy Kang, and Elizabeth C. Cropper

Fishberg Department of Neuroscience and Friedman Brain Institute, Mt. Sinai School of Medicine and Phase Five Communications Inc., New York, New York

Submitted 12 April 2011; accepted in final form 14 May 2011

Evans CG, Ludwar BC, Kang T, Cropper EC. Effect of presynaptic membrane potential on electrical vs. chemical synaptic transmission. J Neurophysiol 106: 680–689, 2011. First published May 18, 2011; doi:10.1152/jn.00340.2011.—The growing realization that electrical coupling is present in the mammalian brain has sparked renewed interest in determining its functional significance and contrasting it with chemical transmission. One question of interest is whether the two types of transmission can be selectively regulated, e.g., if a cell makes both types of connections can electrical transmission occur in the absence of chemical transmission? We explore this issue in an experimentally advantageous preparation. B21, the neuron we study, is an Aplysia sensory neuron involved in feeding that makes electrical and chemical connections with other identified cells. Previously we demonstrated that chemical synaptic transmission is membrane potential dependent. It occurs when B21 is centrally depolarized prior to and during peripheral activation, but does not occur if B21 is peripherally activated at its resting membrane potential. In this article we study effects of membrane potential on electrical transmission. We demonstrate that maximal potentiation occurs in different voltage ranges for the two types of transmission, with potentiation of electrical transmission occurring at more hyperpolarized potentials (i.e., requiring less central depolarization). Furthermore, we describe a physiologically relevant type of stimulus that induces both spiking and an envelope of depolarization in the somatic region of B21. This depolarization does not induce functional chemical synaptic transmission but is comparable to the depolarization needed to maximally potentiate electrical transmission. In this study we therefore characterize a situation in which electrical and chemical transmission can be selectively controlled by membrane potential.

Aplysia; mechanosensory; invertebrate; sensorimotor transmission

The growing realization that electrical coupling is present in the mammalian brain has sparked renewed interest in determining its functional significance, e.g., contrasting it with chemical synaptic transmission. Features of electrical coupling that have been emphasized are its potential for bidirectional communication and its potential for synchronizing the activity of coupled neurons (e.g., Bennett and Zukin 2004; Connors and Long 2004). Additionally, an intriguing suggestion is that electrical synapses may be more reliable than chemical synapses (Connors and Long 2004). This is suggested by the fact that chemical synaptic transmission is stochastic and release probability can be low. In contrast, it might be expected that coupling potentials would be induced in follower neurons whenever there is a presynaptic action potential.

Other data suggest, however, that the situation may not always be so simple. In some systems electrical transmission, like chemical synaptic transmission, can be modulated (e.g., Cachope et al. 2007; Johnson et al. 1993, 1994; McMahon et al. 1989; Yang et al. 1990). Furthermore, both types of transmission can be altered by the same type of event, e.g., increases in intracellular calcium (Yang et al. 1990) or effects of a modulator (Cachope et al. 2007). This suggests that it cannot be assumed, a priori, that electrical transmission will be more reliable. Instead, it is a question to be addressed by directly comparing the two types of transmission under physiologically relevant conditions.

In this article we study a molluscan radula mechanosensory (B21) involved in a rhythmic motor behavior (feeding) that makes both electrical and chemical connections (Rosen et al. 2000b). Considerations of ingestive feeding suggest advantages of differential control of the two types of transmission (Cropper et al. 2004), namely, chemical transmission would be expected to be regulated in a phase-dependent manner. In contrast, transmission to at least one class of electrically coupled followers would be expected to be phase independent. Thus ingestive behavior would be promoted if there is transmission to electrically coupled followers without transmission to neurons receiving chemical input. In this study we sought to determine whether this occurs.

Previous experiments that have studied the regulation of chemical synaptic transmission from the mechanosensory B21 have established that it is membrane potential dependent (Evans et al. 2003, 2007; Rosen et al. 2000a). Postsynaptic responses are not recorded in follower neurons if B21 is peripherally activated at its resting membrane potential. Postsynaptic responses are, however, observed if current is injected into the somatic region of B21 so that it is centrally depolarized prior to and during peripheral activation. Under physiological conditions B21 is centrally depolarized via input from pattern-generating interneurons during one of the two phases of feeding motor programs (radula retraction) (Evans et al. 2003; Ludwar et al. 2009; Rosen et al. 2000a). This input is ~15 mV peak amplitude (Ludwar et al. 2009). Central depolarizations of this magnitude are sufficient to gate both chemical and electrical transmission (Borovikov et al. 2000; Ludwar et al. 2009).

Experiments in this study were concerned with the protraction phase of motor programs. During protraction B21 does not receive depolarizing heterosynaptic input (Evans et al. 2003; Ludwar et al. 2009; Rosen et al. 2000a). It is, however, peripherally activated when the muscle it innervates, the subradula tissue (SRT), contracts (Borovikov et al. 2000). We simulate this stimulus and demonstrate that it coactivates B21 and other neurons of the radula mechanosensory cluster. Consequently, spiking and an envelope of depolarization are both recorded centrally from B21. The peak amplitude of the central depolarization is considerably
less than the change in membrane potential observed during the retraction phase of a motor program. It is sufficient for the induction of coupling potentials in follower neurons but is not sufficient to induce functional chemical synaptic transmission. We therefore suggest that transmission during protraction is preferentially to electrically coupled followers.

MATERIALS AND METHODS

Preparation

Experiments were conducted on *Aplysia californica* (200–250 g) obtained from Marinus Scientific (Garden Grove, CA) maintained in tanks at 14–16°C for several days. Animals were anesthetized by injection of ~100 ml of isotonic MgCl₂. Either the isolated buccal ganglion or the buccal ganglion with attached radula nerve and SRT (Fig. 1A) was removed from the animal and pinned in a Sylgard (Dow Corning, Midland, MI)-lined dish. Experiments were conducted at ~16°C in artificial seawater that had the following composition (in mM): 460 NaCl, 10 KCl, 11 CaCl₂, 55 MgCl₂, 10 HEPES, pH = 7.6.

Electrophysiology

Up to four simultaneous intracellular recordings were amplified and displayed with Getting Model 5A amplifiers (Getting Instruments, Iowa City, IA) modified for 100-nA current injection, an AxoClamp 2B amplifier (Molecular Devices, Sunnyvale, CA) in bridge mode, Tektronix AM 502 amplifiers, and a four-channel Tektronix storage oscilloscope (model 5111). Data were digitized with a Digidata (Axon Instruments, Union City, CA) and were acquired with Axoscope software (Axon Instruments).

To record from the somata of neurons we used single-barrel electrodes fabricated from thin-walled glass capillary tubing filled with 3 M KAc and 30 mM KCl. Electrodes were beveled so that their impedances were ~5–10 MΩ. To record from the lateral process of B21, microelectrodes had a higher resistance (generally ~50 MΩ) and contained 3% 5(6)-carboxyfluorescein dye in 0.1 M potassium citrate (to verify recording sites). In some experiments we injected Fast Green dye into the soma of B21 to facilitate impalement of the lateral process.

In experiments in which we measured the peak amplitude of the envelope of depolarization that develops in the soma of B21, we determined the typical duration of a spike, using a custom-written Spike II script to replace the spike data points via linear interpolation.
Imaging

To visualize radula mechanoafferents, they were iontophoretically injected with either 3% 5(6)-carboxyfluorescein (Sigma, St. Louis, MO) or Alexa Fluor 568 (Invitrogen, Carlsbad, CA). In some cases ganglia were coverslipped and cells were visualized and photographed with a Nikon microscope and camera (Nikon Instruments, Melville, NY). In other cases noncoverslipped ganglia were imaged with a water-immersion lens and a custom-built focus stepper. Stacks were processed with Helicon Focus Pro software (Helicon Soft, Kharkov, Ukraine) to obtain in-focus projections.

Peripheral Stimulation

In some experiments B21 was peripherally activated by attaching the SRT to a pushrod that was linearly moved by a servomotor (S9650, Futaba, Champaign, IL). The servomotor was driven by a custom-built controller (based on a PIC16F690 MCU) that enabled us to reproducibly stretch the SRT with defined velocity and amplitude (Fig. 1B). In separate sets of experiments we progressively altered either the stretch velocity or amplitude (e.g., Fig. 1C1). In some experiments velocity or amplitude was progressively increased; in other experiments velocity or amplitude was decreased. Each stimulus was applied at least twice, and mean values were calculated for each animal. Data were then binned and pooled across preparations. We recorded either from the soma of B21 or from both the soma and lateral process. We counted the number of spikes evoked by the stretch and determined the mean and maximal firing frequency (Fig. 1B). Additionally, in a number of experiments we plotted instantaneous firing frequency.

In other experiments the SRT was peripherally stimulated as has been described previously (Cropper et al. 1996). Briefly, mechanical stimuli were delivered by means of a minispeaker (Quam) that had a wooden stick (tip diameter 1 mm) that was perpendicularly attached to the speaker membrane. Reproducible movements of the speaker membrane were regularly elicited by driving the speaker with a stimulator at ~0.5–1 Hz (Grass/Astro-Med, West Warwick, RI).

Data Analysis

Experiments were analyzed with either pCLAMP software (Axon Instruments) or Spike II (CED). Data are reported as means ± SE, and n refers to numbers of preparations. Statistical significance was determined with a repeated-measures one-way ANOVA and was defined as P < 0.05.

RESULTS

In this report we study sensorimotor transmission as it occurs during the protraction phase of feeding motor programs. During protraction B21 is peripherally activated when stretching and contraction of the SRT occur (Borovikov et al. 2000). To simulate this stimulus we utilized a device that stretches the SRT (Fig. 1A).

Responses to the Stretch Stimulus

To characterize the B21 response to the stimulus, initially the stretch velocity was fixed and the amplitude of the stretch

![Fig. 2. Somatic recordings of the B21 response to the stretch stimulus. A1: group data from experiments in which stretch velocity was fixed and stretch amplitude was progressively altered (e.g., increased from 2.5 to 5.0 mm). Four different stretch velocities were tested (2.5, 5.0, 7.5, and 10 mm/s). At all four velocities tested, stretch amplitude and firing frequency were not correlated (top), whereas stretch amplitude and number of spikes were (bottom). A2: sample recordings and plots of instantaneous frequency showing that an increase in amplitude increases the number of spikes evoked but does not significantly alter firing frequency (left). Firing frequency is, however, increased by an increase in stretch velocity (right). B: group data from experiments in which stretch amplitude was fixed and velocity progressively altered. Firing frequencies plotted are both mean and maximum. Note that both were higher at faster stretch velocities.](image-url)
was progressively altered (either increased or decreased) (Fig. 1C1). The amplitude of the small stretch was similar to a contraction evoked by one SRT motoneuron in a reduced preparation (Borovikov et al. 2000). The largest stretch was similar to contractions observed during a motor program (Borovikov et al. 2000).

Experiments were performed at four stretch velocities, which spanned the range of SRT contraction rates that have been reported. We found that there was no correlation between stretch amplitude and B21 firing frequency at any of the stretch velocities tested (Fig. 2A1, top) (at 2.5 mm/s n = 7, P = 0.5; at 5.0 mm/s n = 7, P = 0.4; at 7.5 mm/s n = 6, P = 0.9; and at 10 mm/s n = 4, P = 0.3). There was, however, an increase in the number of spikes evoked (Fig. 2A1, bottom), presumably as a result of the increase in stimulus duration (e.g., Fig. 2A2, left) (at 2.5 mm/s n = 7, P < 0.0001; at 5.0 mm/s n = 7, P < 0.0001; at 7.5 mm/s n = 6, P < 0.0001; and at 10 mm/s n = 4, P < 0.05).

Although the B21 firing rate was not correlated with stretch amplitude, data suggested a correlation with stretch rate (Fig. 2A1, top). For example, at the lowest velocity (2.5 mm/s) the mean B21 firing frequency was 12.8 ± 0.4 Hz. At the highest velocity (10 mm/s) the mean firing frequency was 31.1 ± 0.9 Hz. To confirm this correlation we performed experiments in which the stretch amplitude was kept constant and the stretch velocity was progressively altered. As expected, firing frequency increased with increasing stretch velocity (Fig. 2, B and A2, right). This was true for both the mean and the maximal firing frequency (Fig. 2B) (for mean frequency n = 9, P < 0.0001; for maximal frequency n = 9, P < 0.0001).

**Spike Propagation in B21**

Previous experiments that studied the regulation of mechanoefferent transmission to chemical follower neurons demonstrated that in part transmission fails at resting membrane potential as a result of a spike propagation failure within B21 (Evans et al. 2003, 2007, 2008). B21 is a bipolar neuron with major medial and lateral processes (Fig. 1A). The lateral process is the primary point of contact with neurons receiving chemical synaptic input (the B8 neurons) (Borovikov et al. 2000). When B21 is at its resting membrane potential and is peripherally activated, spikes fail to actively propagate to the lateral process (Evans et al. 2003, 2007, 2008). In contrast, if B21 is centrally depolarized and then peripherally activated spike propagation to the lateral process occurs. An essential “first step” in inducing B21 sensorimotor transmission is, therefore, a modification of spike propagation.

We have characterized one physiologically relevant situation in which B21 is centrally depolarized and spikes propagate to the lateral process. Central depolarization and spike propagation occur during the retraction phase of feeding motor programs (Evans et al. 2003). We now show that central depolarization is also observed with application of the stretch stimulus. In the latter case, the central depolarization is in the form of an envelope of depolarization that is recorded in the soma of B21. To more clearly visualize and measure the amplitude of this depolarization, we utilized a script that removed the spikes from the B21 recording (after recordings were made) (Fig. 1, C1 and C2, red traces). With large-amplitude stretches, the peak depolarization was 6.6 ± 0.5 mV; with smaller stretches...
it was 3.0 ± 0.8 mV (n = 4). This difference in amplitude is presumably at least in part due to the fact that B21 tends to be coactivated with other cells of the radula mechanoreceptor cluster when stretch amplitude is large. Specifically, in 5/6 preparations, other radula mechanoreceptors were progressively recruited, as shown in Fig. 3. Mechanoreceptors recorded in this study include the identified neuron B22 (Rosen et al. 2000b) and cells that respond to light touch of the SRT that are in relatively close proximity to B21 (Fig. 4, A1 and A2). These cells are weakly electrically coupled to B21 (Miller et al. 1994). Photographs of these neurons are included to illustrate previously unreported features of their anatomy (e.g., they have processes that extend laterally in buccal ganglion toward B8). Interestingly, however, cells tested did not make a chemical synaptic connection with B8 (Fig. 4B) (n = 9).

Central depolarizations of a few millivolts can permit spike propagation to the lateral process when DC current injection is used to alter the B21 membrane potential. To determine whether the central depolarizations that develop when the SRT is stretched can also modify spike propagation, we performed experiments in which we simultaneously recorded from the soma and lateral process (n = 3). The SRT was stretched at a fixed velocity and the amplitude of the stretch progressively altered (Fig. 5A). At all four stretch velocities tested, spike propagation could occur (Fig. 5B). Whether or not it did was determined by stimulus properties. Spikes did not propagate when stretches were small but did propagate when stretches were larger (Fig. 5, A1 and A2). Furthermore, at a given amplitude more spikes propagated when the stretch velocity was slower.

When single responses where spike propagation did occur are viewed at a fast sweep speed it becomes apparent that not all spikes triggered peripherally propagate to the lateral process (Fig. 5A2). This was true at all velocities and amplitudes tested (Fig. 5C). Most commonly, the first spikes did not propagate (Fig. 5A2). Thus ~96% (178/186) of the spikes that failed to propagate were either the first spike evoked or a spike that followed a propagation failure. This is not surprising given the fact that it takes time for the envelope of depolarization to develop (Fig. 1C2). Subsequent to the initial failure, spikes generally propagated one for one (Fig. 5A2).

To summarize, we demonstrate that whether or not spike propagation occurs depends on stimulus properties. With large-amplitude stretches the envelope of depolarization that develops centrally in B21 is larger and spikes propagate. In part the difference in central depolarization is likely to be due to the pattern of afferent activation, i.e., the progressive recruitment of the other radula mechanoreceptors.

Sensorimotor Transmission to Follower Neurons

Chemical follower. The fact that spikes can propagate with application of the stretch stimulus suggests that mechanoreceptor information could be transmitted to follower neurons. During ingestive behavior, we would not, however, expect transmission to neurons that receive chemical synaptic input, the B8 radula closer motoneurons (Cropper et al. 2004). The stretch stimulus mimics a protraction phase event. When animals feed, the radula closes during retraction (not protraction) (Kupfermann and Carew 1974; Morton and Chiel 1993a).

To study transmission to B8 we recorded from it and stretched the SRT with stimuli that permitted spike propagation to the lateral process (i.e., the largest-amplitude stretch at the 2 slowest velocities). This stimulus did in fact coactivate other
Fig. 5. Soma and lateral process recordings of the B21 response to the stretch stimulus. A1: sample recordings from an experiment in which stretch velocity was fixed and stretch amplitude progressively decreased. The plots above the soma and lateral process recordings are instantaneous frequency. A2: responses 1–3 from A1 shown at a faster sweep speed. Note that spike propagation occurs when the stretch amplitude is sufficiently large (e.g., it occurs during responses 1 and 2 but not response 3). B: group data from experiments in which stretch velocity was fixed and stretch amplitude was progressively altered. Note that in general spikes propagated with larger stretches when stretch velocity was slow. C: group data from experiments in which spike propagation to the lateral process was monitored. Data from each animal are plotted with a unique symbol. Note that propagation failures occurred at all velocities and amplitudes tested.
radula mechanosensory B21 became apparent (Fig. 6, left). To verify the integrity of the B21-B8 connection we reapplied stretches under conditions in which B21 was centrally depolarized via current injection prior to and during the peripheral activation. This increases the total central depolarization. Under these conditions postsynaptic responses in B8 became apparent (Fig. 6A, right).

Electrically coupled follower. In a final set of experiments we sought to compare afferent transmission to B8 to transmission to a second type of “follower” neuron, i.e., an electrically coupled internuncial neuron, B64 (Rosen et al. 2000b). This connection is bidirectional, i.e., a spike in B21 will induce a coupling potential in B64 and vice versa. However, it has been postulated that transmission in the B21-B64 direction has an important role during the protraction phase of feeding (as is described in more detail below). We induced single spikes in B21 and recorded PSPs in B8 and coupling potentials in B64 under two conditions, i.e., when B21 was depolarized to the point where very small PSPs are first apparent in B8 (which is approximately the membrane potential where spikes begin to propagate) (Ludwar et al. 2009) and when B21 was sufficiently depolarized to maximally potentiate synaptic transmission to B8 (Fig. 6B). Interestingly, we found that coupling to B64 was maximally potentiated when B21 was depolarized to a level just sufficient for allowing propagation to the lateral process. The additional depolarizations that maximally potentiated PSPs in B8 did not produce further increases in coupling potential amplitude. Thus maximal potentiation of the two types of transmission occurred in different voltage ranges, with the effect on coupling potentials occurring at more hyperpolarized potentials.

DISCUSSION

An intriguing suggestion is that electrical synapses may be more “reliable” than chemical synapses (Connors and Long 2004). This is suggested by the well-known fact that chemical synaptic transmission is a stochastic process and release probability at a number of synapses is very low. In contrast, electrical coupling might be expected to occur whenever there is a presynaptic action potential. Potentially problematic for this idea is a growing body of work that indicates that electrical transmission, like chemical synaptic transmission, can be modulated (Cachope et al. 2007; McMahon et al. 1989; Yang et al. 1990). This suggests that it cannot be assumed, a priori, that electrical transmission will be more reliable. Instead, it is a question to be addressed experimentally by directly comparing the two types of transmission under physiologically relevant conditions.

This type of comparison has been made in the goldfish (Cachope et al. 2007; Yang et al. 1990). Here individual eighth nerve afferents make both chemical and electrical connections with an identified reticulospinal neuron, the Mauthner cell. Eighth nerve stimulation produces a biphasic effect consisting of a fast electrotonic response followed by a chemical excitatory PSP (EPSP), mediated by release of glutamate. In this case both chemical and electrical transmission can be coregulated. For example, both types of contacts display a form of long-term potentiation that requires an increase in intracellular calcium and activation of N-methyl-D-aspartate (NMDA) receptors (Yang et al. 1990). Furthermore, the two types of transmission can be simultaneously potentiated by endogenous modulators (Cachope et al. 2007). In this case coregulation of the two types of transmission clearly makes functional sense. Afferents contact a single postsynaptic neuron that mediates an important escape behavior. The parallel potentiation of electrical and chemical transmission increases the potential for the sensitization of this response. As studies of the regulation of electrical transmission in the mammalian brain progress, similar types of coregulation are likely to be identified (e.g., Cruikshank et al. 2005).

Our work differs in that electrical and chemical contacts are made with two different types of follower neurons. Thus the sensory neuron we study, the radula mechanosensory B21, makes an excitatory chemical connection with the B8 mo-

Fig. 6. Sensorimotor transmission with application of the stretch stimulus. A: somatic recordings from the 2 B21 neurons, an unidentified RM, and B8. The plot under the B21 trace indicates the amount of current injected. Current was injected into only 1 B21. Initially the stretch stimulus was applied with B21 at its resting membrane potential (left). Postsynaptic potentials (PSPs) in B8 were very small, and B8 did not spike. To test the integrity of the connection, the stimulus was reapplied and DC current injected into B21 (middle and right). Note that PSPs became apparent in B8. B1: sample data from an experiment in which we simultaneously recorded from an electrically coupled neuron (B64) and a neuron receiving chemical synaptic input (B8). B21 was peripherally activated at 3 different membrane potentials, i.e., resting membrane potential (left), with central depolarization to the point where PSPs first became apparent in B8 (which is approximately where spike propagation is first observed) (middle), and with central depolarization that maximally potentiated synaptic transmission to B8 (max dep; right). B2: group data for the experiment shown in B1. Note that potentiation of electrical transmission occurred at a more hyperpolarized potential than chemical transmission. With maximal depolarization coupling potential amplitude actually decreased.

J Neurophysiol • VOL 106 • AUGUST 2011 • www.jn.org
Behavioral and electrophysiological studies have demonstrated the critical role of mechanical signals in the control of feeding in mollusks. The feeding apparatus, including the radula and the buccal cavity, is moved forward and backward in a coordinated manner, driven by two major muscle groups: the salivary radula transport (SRT) and the buccal mass (BM). The SRT muscle group is responsible for the retraction phase, while the BM drives the protraction phase. The SRT is activated during the protraction phase of motor programs, and its movement results in the ejection of food from the buccal cavity. The BM is activated during the retraction phase, causing the radula to close and the food to be ingested.

**Functional Considerations of Transmission to B8 vs. B64**

To peripherally activate B21, we mimicked an event that occurs during the protraction phase of ingestive motor programs (SRT stretch). During protraction, differential transmission to B8 and B64 has been predicted (Cropper et al. 2004). With respect to B8, it would be expected that transmission would not occur. The B8s are motoneurons that close the radula (Morton and Chiel 1993b). If the radula closes as it moves forward, it pushes food out of the buccal cavity (egestion occurs). During protraction, increased excitatory drive from B21 to B8 is therefore not expected since it will tend to promote egestive rather than ingestive behavior. In contrast, transmission to B64 is expected to occur (Cropper et al. 2004). B64 is an interneuron that plays an important role in determining temporal characteristics of motor programs (Hurwitz et al. 1996; Wu et al. 2007). It begins to spike early in retraction and inhibits the protraction circuitry. When it is prematurely activated, phase transitions occur earlier. A cell that provides excitatory input to B64 (like B21) can therefore modify temporal characteristics of motor programs by phase advancing retraction and thereby shortening protraction duration. This type of a role for afferent feedback in initiating phase transitions has been described in a number of other preparations (e.g., Pearson 1995; Rossignol et al. 2006).

Consistent with the idea that phase transitions are influenced by sensory feedback, it has been noted that there are marked differences in the cycle period of feeding in intact animals and the duration of the protraction phase of motor programs generated in the isolated nervous system. When intact animals feed, ingestive responses can be triggered every 3–4 s (Cropper et al. 1990). In the isolated nervous system, protraction duration can be as long as 25 s (e.g., Jing and Weiss 2005). These data suggest a behaviorally relevant role for afferent input in general. With respect to B21, it has been demonstrated that spiking triggered during the protraction phase of motor programs significantly reduces protraction duration (Borovikov et al. 2000). Thus data suggest that B21-B64 input during protraction will be beneficial. It will tend to couple the initiation of retraction to the current state of the periphery, which is likely to ensure that feeding occurs at an appropriate rate (Fig. 7A).

**Why Don't We See Transmission to B8?**

For B21 mechanoefferent transmission to B8 to occur, spikes must propagate to the lateral process of B21 (Evans et al. 2003). In this study we demonstrate that spikes can propagate when the SRT is stretched (e.g., propagation occurs with large-amplitude stretches). Nevertheless, PSPs in B8 are <1 mV and B8 does not spike. Functional chemical synaptic transmission is not induced. Results of this study taken together with earlier work suggest that this is a result of the fact that central depolarizations that are generated in B21 when the SRT is stretched are relatively small (i.e., are 6 mV or less). Thus a number of synapses (including B21-B8) display a type of plasticity that is manifested as a graded, potentiating effect of holding potential on chemical synaptic transmission (Alle and Geiger 2006; Ludwar et al. 2009; Nicholls and Wallace 1978; Shimahara and Tauc 1975; Shu et al. 2006). What is particularly striking about B21-B8, however, is the range of variation in PSP amplitude. With relatively little central depolarization PSPs are not simply reduced in amplitude, they are virtually nonexistent (Ludwar et al. 2009). A consequence of this arrangement is that there is a voltage range in which spikes propagate in B21 but where chemical synaptic transmission is not induced.

![Schematic diagrams illustrating the postulated role of B21 mechanoefferent transmission to B64 (A) and effects of central depolarization on electrical vs. chemical transmission (B).](image-url)

**Fig. 7.** Schematic diagrams illustrating the postulated role of B21 mechanoefferent transmission to B64 (A) and effects of central depolarization on electrical vs. chemical transmission (B). A: in the isolated nervous system there is no afferent feedback from the periphery, and the duration of the protraction phase of the motor program is relatively long (left). In contrast, in intact animals the SRT motoneuron (B66) is activated during protraction. Consequently, the SRT contracts during protraction. This will provide increased excitatory drive to B64 (via B21). We suggest that this phase advances retraction and shortens protraction duration (right). B: with weak central depolarizations (such as those induced by RM coactivation), spike propagation in B21 can be modified and coupling potentials induced in the electrically coupled B64. In contrast, chemical synaptic transmission to B8 does not occur (left). With larger central depolarizations (such as those induced during the retraction phase of feeding motor programs) spikes propagate and postsynaptic responses are generated in both B64 and B8 (right).
Experiments designed to characterize mechanisms underlying potentiating effects of membrane potential on B21-B8 chemical synaptic transmission are currently ongoing. In part, however, it has become apparent that they are mediated by modulation of a “background” calcium current [as has been described in other systems (e.g., Ivanov and Calabrese 2003)]. These currents can be activated by subthreshold depolarizations. Their induction produces a widespread increase in the intracellular calcium concentration, which potentiates subsequent spike-mediated synaptic transmission. In B21, increases in intracellular calcium concentration are most apparent when central depolarizations are $>10 \text{mV}$ (Ludwar et al. 2009). This is greater than the largest central depolarization observed with the stretch stimulus. To summarize, in this report we demonstrate that the central depolarization induced by stretch of the SRT is insufficient to activate the processes necessary to sufficiently upmodulate chemical synaptic transmission so that measurable PSPs are induced in B8.

**Transmission Between B21 and the B64 Neurons**

The B21-B64 connection differs from the B8 connection in that the B64 connection is electrical. Interestingly, differences in transmission to the two types of followers are observed, namely, at membrane potentials where PSPs are virtually nonexistent in B8 coupling potentials are recorded in B64. Explanations for this difference in transmission presumably depend on the membrane potential in question. At B21’s resting potential there are no PSPs in B8 (Fig. 6B). In contrast, there are small coupling potentials in B64. This difference is presumably a reflection of the anatomic specifics of the system. Thus there is a significant “medial” component to the B21-B64 contact (Borovikov et al. 2000). In contrast, the B21-B8 contact is primarily between B21’s lateral process and B8 (Borovikov et al. 2000). It is spike propagation to the lateral process that is modified by central depolarization (Evans et al. 2003). Consequently, spike propagation to the lateral process is necessary for transmission to B8 but is not necessary for transmission to B64.

When B21 is centrally depolarized to the membrane potential where spike propagation is first observed, very small PSPs become apparent in B8 (Fig. 6B). In contrast, coupling potentials in B64 are maximally potentiated. The B8 PSPs are presumably small because of the absence of the processes that upregulate chemical synaptic transmission (e.g., background calcium currents). It is likely that the potentiation of the coupling potentials in B64 is a consequence of the change in spike propagation. When spikes propagate (i.e., are triggered in the lateral process), the laterally initiated spike is reflected and impacts medial regions of the cell (Evans et al. 2003). Medial spikes are broader and have an increased amplitude. This reflection is particularly pronounced in parts of B21 that contact B64, i.e., in the inexitable soma and proximal medial process.

To summarize, we suggest that the difference in transmission to the electrically coupled B64 and the chemical follower B8 results from the fact that transmission to the electrically coupled B64 is determined by spike propagation. When spikes propagate, coupling potentials are maximally potentiated. In contrast, transmission to B8 is not simply determined by spike propagation. Instead, further depolarization is required to induce and maximally potentiate chemical synaptic transmission (Fig. 7B). The mechanism we characterize could operate in any system where there is a low probability for synaptic release. It is likely to be particularly important in situations such as this one where chemical and electrical connections are with different sets of follower neurons and there are behavioral advantages for selective transmission of information.

**ACKNOWLEDGMENTS**

We thank K. R. Weiss for valuable comments on an earlier version of this manuscript.

**GRANTS**

National Institute of Mental Health Grant MH-51393 supported this work. Some of the *Aplysia* used in this study were provided by the National Resource for *Aplysia* of the University of Miami under National Center for Research Resources Grant RR-10294.

**DISCLOSURES**

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

**REFERENCES**


Hurwitz I, Susswein AJ. B64, a newly identified central pattern generator element producing a phase switch from protraction to retraction in buccal programs of *Aplysia californica*. *J Neurophysiol* 75: 1327–1344, 1996.


*J Neurophysiol* • VOL 106 • AUGUST 2011 • www.jn.org


Morton DW, Chiel HJ. 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, 1993a.


