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

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Abstract

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 report 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). Further 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 where electrical and chemical transmission can be selectively controlled by membrane potential.

Keywords: Aplysia, mechanoafferent, invertebrate, sensorimotor transmission
Introduction

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; McMahon et al. 1989; Yang et al. 1990; Johnson et al. 1993; Johnson et al. 1994). Further 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 issue to be addressed by directly comparing the two types of transmission under physiologically relevant conditions.

In this report we study a molluscan radula mechanoafferent (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 mechanoafferent B21 have established that it is membrane potential dependent (Evans et al. 2003; Evans et al. 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 mechanoafferent 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 approximately 100 ml isotonic MgCl₂. Either the isolated buccal ganglion or the buccal ganglion with attached radula nerve and subradula tissue (SRT) (Fig. 1A) were 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 using Getting Model 5A amplifiers (Getting Instruments, Iowa City, Iowa) 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 using a Digidata (Axon Instruments, Union City, CA) and were acquired using 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 about 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 and using a custom written Spike II script to replace the spike data points via linear interpolation.
interpolation (Spike II software: Cambridge Electronic Design (CED), Cambridge, UK) (Fig. 1C1 red trace, 1C2 red trace).

**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 cover slipped and cells were visualized and photographed using a Nikon microscope and camera (Nikon Instruments, Inc., Melville, NY). In other cases non-cover slipped ganglia were imaged using a water-immersion lens and a custom-built focus stepper. Stacks were processed with Helicon Focus Pro software (Helicon Soft Ltd., 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 servo motor (S9650, Futaba, Champaign, IL). The servomotor was driven by a custom build 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 calculated for each animal. Data were then binned and pooled across preparations. We recorded either from the soma of B21, or 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 (Cropper 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 speaker membrane were
regularly elicited by driving the speaker with a stimulator at approximately 0.5-1 Hz (Grass/Astro-Med, West Warwick, RI).

Data analysis
Experiments were analyzed using either pClamp software (Axon Instruments) or Spike II (CED). Data are reported as means ± SE, and n’s refer to numbers of preparations. Statistical significance was determined using 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 occurs (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 was progressively altered (either increased or decreased) (Fig 1C1). The amplitude of the small stretch was similar to a contraction evoked by one SRT motor neuron 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 row) (at 2.5 mm/sec n=7, P = 0.5; at 5.0 mm/sec n= 7, P = 0.4; at 7.5 mm/sec n= 6, P = 0.9; and at 10 mm/sec n=4, P = 0.3). There was, however, an increase in the number of spikes evoked (Fig. 2A1, bottom row) (presumably as a result of the increase in stimulus duration (e.g., Fig. 2A2, left) (at 2.5 mm/sec n=7, P < 0.0001; at 5.0
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/sec) the mean B21 firing frequency was $12.8 \pm 0.4$ Hz. At the highest velocity (10 mm/sec) the mean firing frequency was $31.1 \pm 0.9$ 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. 2B, 2A2, 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 mechanoafferent 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; Evans et al. 2008; Evans et al. 2007). 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; Evans et al. 2008; Evans et al. 2007). 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 where 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. 1C1, red trace, 1C2, red
trace). 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 mechanoafferent cluster when stretch amplitude is large. Specifically, in 5/6
preparations, other radula mechanoafferents were progressively recruited as shown in
Fig. 3. Mechanoafferents recorded from 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. 4A1, 4A2). 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. 5A1). 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. 5A1, 5A2). Further, 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 mechanoafferents.

**Sensorimotor transmission to follower neurons**

**Chemical follower**

The fact that spikes can propagate with application of the stretch stimulus suggests that mechanoafferent 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 motor neurons (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 using stimuli that permitted spike propagation to the lateral process (i.e., the largest amplitude stretch at the two slowest velocities). This stimulus did in fact coactivate other RMs. When B21 was peripherally activated at its resting membrane potential, evoked spikes either did not induce measurable PSPs, or induced PSPs that were less than 1 mV and did not induce spiking in B8 (n=4) (Fig. 6A, left). To verify the integrity of the B21-B8 connection we reapplied stretches under conditions where 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).
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 interneuron, 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 EPSP, mediated by release of glutamate. In this case both chemical and electrical transmission can be co-regulated. For example, both types of contacts display a form of long-term potentiation that requires an increase in intracellular calcium and activation of NMDA receptors (Yang et al. 1990). Further, 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 mechanoefferent B21, makes an excitatory chemical connection with the B8 motor neurons. The electrical contact is with an interneuron, B64. In this report we ask whether differential regulation of electrical and chemical transmission can be used to selectively control transmission to two different types of follower neurons.

*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 motor neurons 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 three to four seconds (Cropper et al. 1990). In the isolated nervous system, protraction duration can be as long as 25 seconds (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 insure that feeding occurs at an appropriate rate (Fig. 7A).

Why don’t we see transmission to B8?

For B21 mechanoafferent 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 less than 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.

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 the intracellular calcium concentration are most apparent when central depolarizations are more than 10 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. 6B2). In contrast there are small coupling potentials in B64. This difference is presumably a reflection of the anatomical 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. 6B2). In contrast, coupling potentials in B64 are maximally potentiated. The B8 PSPs are presumably small due to 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 inexcitable 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.
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Figure Legends

Fig. 1. Preparation and experimental design. A: B21 (gray) is a bipolar neuron with major medial and lateral processes. It was peripherally activated when the muscle it innervates, the subradula tissue (SRT) was stretched. This initiates spikes, which propagate towards the CNS (red arrows). When B21 is peripherally activated at its resting membrane potential there is a propagation failure and spikes are not actively initiated in the lateral process (top recordings). When the somatic region is depolarized, however spike propagation to the lateral process occurs (bottom recordings). B: In separate sets of experiments we altered either the stretch velocity or amplitude. We recorded either from the soma of B21, or monitored spike propagation by recording from both the soma and lateral process (as schematically indicated in (A)). We determined the number of spikes evoked, and the mean and maximal firing frequency. C1: Stimulus parameters were progressively altered (i.e., decreased or increased). In the experiment shown, stretch velocity was fixed and stretch amplitude progressively decreased. In this and all subsequent figures the bottom trace is the command pulse used to drive the stimulator. Envelopes of depolarization were apparent in recordings of membrane potential (top trace), particularly when a script was used to ‘remove’ the spikes after recordings were made (red trace). C2: The response indicated in (C1) at a faster sweep speed with a superimposition of the original recording (black) and the trace after the spikes were removed (red).

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/sec). At all four velocities tested, stretch amplitude and firing frequency were not correlated (top row), whereas stretch amplitude and number of spikes were (bottom row). 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 (solid bar) and maximum (clear bar). Note both were higher at faster stretch velocities.

**Fig. 3.** Radula mechanoafferent recruitment with increases in stretch amplitude.

Recordings from the somata of four radula mechanoafferents, a B21 ipsilateral to the stretched muscle (iB21), an ipsilateral B22 (iB22), an ipsilateral unidentified radula mechanoafferent (iRM), and a B22 contralateral to the stretched tissue (cB22). The plots above each recording are instantaneous frequency. In this experiment the stretch velocity was kept constant and the amplitude of the stretch progressively increased. Note that B21 was activated by even the small amplitude stretches. In contrast, the other radula mechanoafferents were recruited as stretch amplitude increased.

**Fig. 4.** Radula mechanoafferents recorded from in this study. A1: Digital photograph of an unidentified radula mechanoafferent (RM) injected with carboxyfluorescein, and B21 injected with Alexa Flour 568 illustrating the anatomy of the RM and the relative position of B21. A2: Digital photograph of an unidentified RM injected with Alexa Fluor 568, and B21 injected with carboxyfluorescein illustrating the relative position of the two cells in the buccal ganglion. (A1) and (A2) are two different cells from two different preparations. B: The RMs tested did not make a chemical synaptic connection with B8 (left), unlike B21 (right). B21 was centrally depolarized by ~20 mV prior to the induction of spiking.

**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.

**Fig. 6.** Sensorimotor transmission with application of the stretch stimulus. A: Somatic recordings from the two B21 neurons, an unidentified radula mechanoafferent (RM), and B8. The plot under the B21 trace indicates the amount of current injected. Current was injected into only one of the B21’s. Initially the stretch stimulus was applied with B21 at its resting membrane potential (left). 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 three 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 (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.

**Fig. 7.** Schematic diagrams illustrating the postulated role of B21 mechanoafferent 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 motor neuron (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 radula mechanoafferent 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).
buccal hemiganglion

SRT

radula nerve

stretch

AB

B21

lateral

# spikes (counts)

frequency (mean, max)

B21 soma

Stretch

amplitude

Vm

resting potential

centrally depolarized

C1  Progressive change in amplitude with velocity fixed

Vm

Vm (no spikes)

Stretch

20 mV

10 mV

2.5 mm

10 s

200 ms

B21 soma

C2
A1
Velocity 2.5 mm/sec  Velocity 5.0 mm/sec  Velocity 7.5 mm/sec  Velocity 10 mm/sec

Frequency (Hz)

# Spikes

Stretch (mm)

A2
Amplitude increase...

4 spikes  21 spikes  11 spikes  8 spikes

Velocity increase...

B

mean frequency
maximum frequency
Progressive increase in amplitude with velocity fixed

i B22

i RM

c B22

i B21

Stretch
Depolarization increases

Resting potential 1st PSP max dep

B2
B64
B8

PSP amp (% max value)
A

**Isolated Nervous System**

- **CNS**
- **Ingestive motor program**
  - **Protraction Retraction**
  - **B66**
- **SRT**

**Intact Animals**

- **CNS**
- **Ingestive motor program**
  - **Pro -- Retraction**
- **B66**
- **SRT**

**Excitatory input to** **B64 from** **B21**

B

**weak central depolarization**

- **B21**
- **B64**

**more central depolarization**

- **B21**
- **B64**
- **B66**