BRIEF REPORT

A mechanoafferent neuron with an inexcitable somatic region: Consequences for the regulation of spike propagation and afferent transmission

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RUNNING HEAD: Somatic regulation of afferent transmission

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ABSTRACT

In the *Aplysia* mechanoafferent B21, afferent transmission is in part regulated via the control of active spike propagation. When B21 is peripherally activated at its resting membrane potential, spikes fail to propagate to an output process, and afferent transmission does not occur. In this report we show that the propagation failure is in part a result of the fact that the somatic region of B21 is relatively inexcitable. We isolate this region and demonstrate that net currents evoked by depolarizing pulses are outward. Furthermore, we show that all or none spikes are not triggered when current is injected. Previous reports have, however shown that spiking is triggered when current is somatically injected and cells are intact. We demonstrate that spikes evoked under these circumstances do not originate in the soma. Instead they originate in an adjacent part of the neuron that is excitable (the medial process). In summary we show that the mechanoafferent B21 consists of excitable input and output processes separated by a relatively inexcitable somatic region. A potential advantage of this arrangement is that somatic depolarization can be used to modify spike propagation from the input to the output processes without altering the encoding of peripherally generated activity.

Key Words: *Aplysia*, invertebrate, sensori-motor integration, spike propagation, afferent regulation
INTRODUCTION

Commonly, peripherally and centrally generated activity is integrated so that motor output accommodates changes in the peripheral environment. Mechanisms that underlie these integrative processes are only partially understood. In this report we study the regulation of afferent transmission in an experimentally advantageous *Aplysia* neuron, a mechanoafferent designated as B21 (Rosen et al. 2000b). B21 is of interest since afferent transmission is controlled via the regulation of spike propagation. Thus, when B21 is peripherally activated at its resting membrane potential, peripherally triggered activity is not transmitted to follower neurons. Spikes fail to propagate to output processes. When B21 is centrally depolarized, the failure is relieved, and afferent transmission occurs (Evans et al. 2003b). Under physiological conditions B21 is rhythmically depolarized during motor programs (Evans et al. 2003a; Evans et al. 2003b; Rosen et al. 2000a; Shetreat-Klein and Cropper 2004). Spike propagation is, therefore, dynamically regulated so that afferent transmission occurs in a phase-dependent manner.

In this study we sought to determine why spikes fail to propagate when B21 is peripherally activated at its resting membrane potential. We show that propagation fails as impulses are transmitted from B21’s peripheral (input) process to the somatic region of the cell. The failure is in part a consequence of the fact that the soma is relatively inexcitable. At resting membrane potential the inexcitable soma promotes functional compartmentalization. When the propagation failure in B21 is relieved via central depolarization, conduction occurs. Spikes are actively generated in the peripheral (input) process and although transmission through the somatic region is primarily electrotonic, spikes are reinitiated when impulses reach the output process.

In summary, we characterize a mechanism in which spike propagation is determined by the regulation of membrane potential in a relatively inexcitable part of a cell (the soma). This distinguishes the regulation of spike propagation in B21 from other characterized cells where synaptic input is axonal and can initiate activity (Bucher et al. 2003; Goaillard et al. 2004; Meyrand et al. 1992). Although central depolarizations in B21 effectively alter information transfer from the input to the output process, they generally do not directly trigger spiking during
feeding motor programs (Evans et al. 2003b). Consequently, information transmission can be regulated without disrupting peripheral encoding of afferent information.

MATERIALS AND METHODS

Experiments were conducted in 200-300 gm *Aplysia californica* (Marinus, CA) maintained in 14-16° C holding tanks. Animals were anesthetized by injection of isotonic MgCl2. Experiments were conducted at approximately 16° C in artificial seawater (in mM: 460 NaCl, 10 KCl, 11 CaCl2, 55 MgCl2, and 10 HEPES, pH 7.6).

Equipment used in current clamp experiments included; Getting Model 5A amplifiers modified for 100 nA current injection (Getting Instruments, Iowa City, IA), Tektronix AM 502 amplifiers (Tektronix, Wilsonville, OR), and a Tektronix storage oscilloscope (model 5111). Data were digitized (using a Digidata (Axon Instruments, Union City, CA)) and were acquired and analyzed using pClamp version 9 software (Axon Instruments), and a Sony Vaio PCG-GRT Notebook.

Equipment used in single electrode voltage clamp (SEVC) experiments included an NPI SEC-05LX amplifier (NPI Electronics GmbH, Tamm, Germany), a Model 410 filter amplifier (Brownlee Precision, San Jose, CA), and a CED Power 1401 AD/DA converter (Cambridge Electronic Design, Cambridge, England). Scripts, written in Spike II (version 5.15; Cambridge Electronic Design, Cambridge, UK), were used to control the amplifier, acquire the data, and subtract leak current. Voltage steps had durations of 200 ms and were given every 10 s. Between steps, the membrane potential was held at -70 mV.

To record from the somata of neurons we used single barrel electrodes filled with 3 M potassium acetate and 30 mM potassium chloride. Electrodes were beveled so that their impedances were generally below 10 MΩ. In voltage clamp experiments electrodes were coated with Sylgard (Dow Corning, Midland, MI). To record from processes, microelectrodes were generally about 50 MΩ and the tip was filled with 3% 5(6)-carboxyfluorescein dye in 0.1 M potassium citrate. Carboxyfluorescein was injected to verify recording sites. During physiological experiments
processes were visualized for impalement by injecting Fast Green dye into the B21 soma (Evans et al. 2003b).

In lesion experiments, processes were mechanically severed using a glass micropipette. In current clamp experiments, we measured the input resistance of the intact cell, and the input resistance of the cell after the medial lesion. Neurons were only tested for excitability if an increase in input resistance was observed. On average cells originally had an input resistance of $3.2 \pm 0.3 \, \text{M}\Omega$. After lesions the input resistance was $6.2 \pm 1.2 \, \text{M}\Omega$ (n=7). Voltage clamp experiments were only conducted on neurons with an input resistance above the mean observed in current clamp experiments.

TTX (Sigma-Aldrich, St. Louis, MO) was focally applied to the medial process at a concentration of $\sim 10^{-4} \, \text{M}$. In some experiments (n=2) the TTX was pressure applied via a micropipette with a large tip diameter. Fast Green was included in the TTX solution to monitor the injection. In other experiments (n=5) the TTX solution was added to a subchamber that pharmacologically separated the ASW perfusing the medial process from the ASW perfusing the rest of B21. Again Fast Green was used to test for subchamber leaks. Controls did not show any affect of Fast Green on excitability.

To evoke afferent activity, the subradula tissue (SRT) was peripherally stimulated using a mini-speaker controlled by a stimulator (Grass Instruments) (Cropper et al. 1996).

Statistical tests were performed with Kaleidagraph (Synergy Software, Essex Junction, VT). Unless otherwise noted, two group comparisons utilized a paired t-test and n’s provided indicate the number of preparations in which data were obtained. Data are reported as means $\pm$ SEMs.

RESULTS

B21 has major medial and lateral processes (Fig. 1A1). The medial process acts as an input region, innervating the periphery (Rosen et al. 2000b). The lateral process acts as an output region, being the primary point of contact with motor neurons (the B8 cells) (Borovikov et al.
2000; Gardner 1971). Peripherally triggered spikes are therefore transmitted from the medial process of B21 through the soma to the lateral process (Fig. 1A2). When B21 is peripherally activated at its resting potential, spikes fail to propagate to the lateral process (Fig. 1A1) (Evans et al. 2003b). To determine why the propagation failure occurs, we sought to determine whether there are regional differences in B21 excitability.

When the lateral process of B21 is mechanically isolated, it reseals and is excitable (Evans et al. 2003b). We found that the same is true for the isolated medial process (Fig. 2A1, right, 2A2) (n=3; average spike amplitude 48.7 ± 1.9 mV). The somatic region is however different. We mechanically isolated this region of B21 progressively, removing the medial process first and the lateral process second. In most (6/7) cells, medial lesions virtually eliminated spiking. In one preparation small depolarizations were still apparent (Fig. 2B2) that resembled impulses previously observed when spikes were triggered in the lateral process (Evans et al. 2003b). As expected, these depolarizations were eliminated when the lateral process was severed (Fig. 2B3). These data suggest that the somatic region of B21 (including the proximal medial process) is relatively inexcitable. Consistent with this idea are data we obtained in single electrode voltage clamp (SEVC) experiments in lesioned neurons. Net currents evoked by depolarizing steps in the somatic region were outward in normal saline (Fig. 3A) (n=6).

A potential paradox, however, is that spike-like depolarizations are recorded when current is injected into somata of intact B21 neurons (Fig. 2B1) (Rosen et al. 2000a; Rosen et al. 2000b; Shetreat-Klein and Cropper 2004). We hypothesized that these impulses were triggered in the medial process and electrotonically conducted to the soma (Fig. 2B1). (Previous characterizations of the lateral process length constant preclude the possibility that spikes were laterally initiated (Evans et al. 2003b).) To determine whether impulses were medially initiated, we simultaneously recorded from both the soma and the medial process and triggered spiking by injecting current into the soma. Consistent with the idea that spikes were medially triggered, impulses recorded in the medial process were observed prior to impulses recorded from the soma (Fig. 3B1). (The time difference between peak depolarizations was 1.4 ± 0.2 msec) (n=6).) Impulses in the medial process also had greater peak amplitudes (46.1 ± 2.1 mV vs. 38.1 ± 3.1 mV; t-test, p<0.05; t= 2.58; df=5) (n=6). In a second type of experiment spikes were triggered
via somatic current injection, and TTX was selectively applied to the medial process (Fig. 3B2, top). TTX application blocked spike initiation (Fig. 3B2, bottom) (n=7).

Our results suggest that when B21 is peripherally activated at its resting potential, spikes actively propagate in the medial process but that propagation fails in the relatively inexcitable somatic region (Fig. 1A2). Although the lateral process is capable of active spike generation (Evans et al. 2003b) it does not occur, presumably due to the fact that currents generated by medial spike initiation are insufficient to trigger lateral action potentials. When B21 is centrally depolarized prior to peripheral activation, active spiking again occurs through the branch point in the medial process (the T-junction region) and transmission in the somatic region is electrotonic. Events in the lateral process, however, are changed. Currents generated by medial spike initiation are sufficient to trigger active spike initiation.

DISCUSSION

In previous work we demonstrated that spikes fail to actively propagate to the lateral process (an output region) when B21 is peripherally activated at its resting membrane potential (Evans et al. 2003b). In this study we demonstrate that this propagation failure is in part a result of the somatic region of B21 being inexcitable. Thus the conduction failure is not simply a result of the impedance mismatch between the medial process and soma. Cells with excitable somata have been described in *Aplysia*, as have neurons with inexcitable somata (e.g., (Alving 1968; Hurwitz et al. 1994; Tauc 1962a, b)). It is therefore currently not clear whether an inexcitable soma is the rule or exception in this mollusc. In general it is often difficult to localize sites of spike initiation in *Aplysia*, since length constants in neurons can be surprising long. Consequently, somatic current injection often evokes relatively large amplitude spike-like impulses but without further analysis it cannot be determined whether these impulses are remotely triggered.

Our current data do not implicate a specific mechanism as the underlying cause of the somatic inexcitability in B21. It is likely however that the soma is characterized by a low density of voltage gated sodium channels, as has been demonstrated in other sensory neurons (e.g., (Safronov et al. 2000). Channels may also have an unfavorable distribution. Thus, clustering of
voltage gated sodium channels has been demonstrated in axons of *Aplysia* and is likely to decrease the number of channels required for spike initiation important for propagation (Johnston et al. 1996). In the B21 soma, channel distribution may be uniform. It is important to point out, however, that our voltage clamp data indicate that the somatic region of B21 does not simply function as a passive load, i.e., outward currents in the soma are activated within presumably physiological ranges. This phenomenon has been described, and implicated as being an important determinant of neuronal excitability (Graubard and Hartline 1991).

The regional biophysical differences in B21 produce a situation where excitable input and output processes are separated by a relatively inexcitable part of the cell. Although this arrangement is a result of a bipolar morphology in B21, unipolar or pseudo-unipolar neurons can be similar if they are characterized by a relatively short ‘stem’ process exiting from the soma (i.e., if electrical affects of the soma are not masked) (Luscher et al. 1994; Weiss et al. 1986). An interposed inexcitable region may be of functional significance in a sensory neuron like B21 since it permits the regulation of afferent transmission without disrupting peripheral encoding (Fig. 1B1 vs. 1B2). Thus, when B21 is peripherally activated at its resting potential, spikes are actively initiated in the input process but currents generated are insufficient to trigger spiking in the next region capable of spike generation, the output process. In contrast, when B21 is centrally depolarized prior to and during peripheral activation, spikes in the input process do trigger spiking in the output process. Somatic depolarization therefore promotes reinitiation of peripherally triggered spikes. Importantly, however, somatic depolarization does not in itself induce additional spikes. Thus, we characterize a mechanism whereby synaptic input can regulate afferent transmission without altering the encoding of peripherally generated signals.

Regulation of spike propagation in B21 is likely to be important for sensori-motor integration during feeding. When *Aplysia* feed, the food grasping organ (the radula) is protracted and retracted, and opened and closed (Cropper et al. 2004). Phase relationships between protraction/retraction and opening/closing determine the nature of the behavior. If the radula closes during protraction, food is pushed out (i.e., behavior is egestive). In contrast, if the radula closes during retraction, food is pulled in (i.e., behavior is ingestive). Studies of spike propagation in B21 have focused on afferent transmission to radula closer motor neurons during
ingestive activity. Under these conditions, excitatory input from B21 to closer motor neurons will tend to reinforce ingestive activity if it occurs during radula retraction (i.e., the radula will close more tightly so that food will be pulled into the buccal cavity). In contrast, excitatory input to closer motor neurons during protraction will be counterproductive. As expected we have found that B21 is continuously depolarized throughout the retraction phase of ingestive programs via input it receives from electrically coupled interneurons (Evans et al. 2003a; Evans et al. 2003b; Rosen et al. 2000a; Rosen et al. 2000b; Shetreat-Klein and Cropper 2004). Thus, the dynamic regulation of spike propagation to B21’s lateral process presumably insures that ingestive behavior is enhanced rather than disrupted.

In conclusion, we demonstrate that the somatic region of the Aplysia mechanoafferent B21 is relatively inexcitable. A consequence of this arrangement is that active spike propagation fails at resting potential. Physiologically this is likely to be important in that it permits functional compartmentalization of B21. Peripherally triggered activity is not transmitted to all B21 follower neurons. This conduction failure can, however, be relieved if B21 is centrally depolarized prior to peripheral activation. Under physiological conditions this can occur when the somatic region receives electrical synaptic input during feeding motor programs. Since the soma is inexcitable, central depolarizations do not initiate spiking and therefore do not alter the peripherally determined encoding pattern. Somatic depolarizations do however promote conduction in B21. The inexcitable B21 soma is therefore used to control transmission of afferent information without altering it.

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REFERENCES


FIGURE LEGENDS

Figure 1. Spike propagation in B21 at resting potential (A1) and with central depolarization (A2). B21 is illustrated with camera Lucida drawings in which regions in which active spike initiation occurs are indicated by black, and regions in which electrotonic transmission occurs are indicated by gray. Traces are intracellular recordings simultaneously obtained in a physiological experiment in which B21 was peripherally activated. At resting membrane potential (A1), spikes are actively generated in the medial process but thereafter propagation fails. Impulses are electrotonically transmitted through the somatic region to the lateral process. When B21 is centrally depolarized (A2), spike propagation in the medial process is unchanged, and impulse transmission through the somatic region is again electrotonic. Currents generated by medial spike initiation are, however, now sufficient to trigger spiking in the lateral process. (B) Physiological consequence of a mechanism whereby spike propagation is regulated by altering the membrane potential of an inexcitable region in a neuron. (B1) When B21 is centrally depolarized peripherally evoked activity is reinitiated in the lateral process and peripheral encoding is not altered. (B2) If central depolarization triggered spiking in B21 peripheral encoding could be altered.

Figure 2. Regional differences in B21 excitability. (A) The medial process is excitable. Neurons were injected with Fast Green, and the medial process severed (A2). To facilitate the subsequent medial process impalement, peripheral stimuli were continuously applied (A1, left). Note that spikes could be triggered when current was injected into the isolated medial process (A1, right). After recording sessions, electrode placement was verified via carboxyfluorescein injection (A2). (B) The somatic region of B21 is relatively inexcitable. In an attempt to trigger spiking, current was injected into intact cells (B1), and lesioned cells (B2 and B3). First the medial process was lesioned (B2); secondly the lateral process was lesioned (B3). After recording sessions, lesions were verified using carboxyfluorescein (B3). In (B1)-(B3) current was injected via one electrode (1 in B1-B3), while recordings were obtained via a second electrode (R). B1 shows current injection in intact cells. Note that spike-like impulses were observed. Data shown in Fig. 3 indicate that these impulses are electrotonic versions of spikes actively generated in the medial process (indicated by the flame in B1). B2 shows current injection after the lesion of the medial
process. Our data indicate that these depolarizations are electrotonic versions of spikes actively generated in the lateral process (indicated by the flame in B2), i.e., spikes were not triggered when the somatic region was isolated (B3).

Figure 3. (A) Net leak subtracted currents evoked by depolarizing steps in the somatic region of lesioned B21 neurons. SEVC experiment conducted in ASW at a holding potential of -70 mV. Note that net currents are outward. (B1) Spikes are actively triggered in the medial process of B21. Experiment in an intact neuron in which spikes were elicited by somatic current injection (DC). Cells were simultaneously impaled as indicated (top trace-soma recording; bottom traces-recordings from medial electrodes 1 and 2)). The dotted line marks the peak of the somatic recording. Note that the medial 2 spike was larger and preceded both the medial 1 and somatic spikes. (B2) Experiment in an intact neuron in which spikes were triggered by somatic current injection before, during, and after injection of $10^{-4}$ M TTX above the medial process (gray). Note that spikes are not evoked when TTX is applied.
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