Benjamin Shanahan, Luigim Vargas, Tariq Cannonier, Nischal Acharya 20 April 2016

Lab 3: Aplysia

Examination of the Intracellular Electrophysiological Properties of *Aplysia caeli*

Abstract

Here we describe the neuronal firing properties of the newly discovered *Aplysia caeli*, which were recently found by National Aeronautics and Space Administration (NASA) rovers on Mars. We compare the electrophysiology of *Aplysia caeli* neurons to that of similar terrestrial organisms by intracellularly recording neuronal activity from individual cells found in abdominal ganglion. We characterized the neurons' firing properties under depolarizing and hyperpolarizing currents and varied stimulation frequencies. We observed excitatory postsynaptic potentials, action potentials, and signs of neuronal adaptation similar in duration and size to neurons from terrestrial alphysia. The conserved neuronal properties between *Aplysia caeli* and Earth aplysia suggest a common ancestor between the two species.

Introduction

Intracellular recordings allow for measuring precise changes in the membrane potential of a single cell. This recording method provides a unique opportunity to obtain data about the internal voltage changes and the integration processes within neurons. Due to their availability and easy dissection, the anatomy of terrestrial aplysia is well-documented and their neurons are a staple for intracellular studies in modern neuroscience.

We compared intracellular recordings of *Aplysia caeli* with those of Earth aplysia. Neurons in the abdominal ganglion of these organisms communicate via bioelectrical signals, typically in the form of action potentials (APs). APs are initiated at the axon hillock of a neuron when voltage-gated ion channels undergo a conformational change due to membrane depolarization, typically between -40mV and -60mV for earth organisms. The opening (activation) of these channels allow an influx of positively-charged sodium ions which increase the membrane potential from its negative resting value to around 30 mV or 40 mV.

We characterized the shape of APs, the duration and amplitude of excitatory postsynaptic potentials (EPSPs), and the spontaneous firing rate of a single neuron. We stimulated the neuron with hyperpolarizing and depolarizing currents, observing their respective changes in membrane potential. We analyzed the spontaneous firing rate of the cell, the adaptability of the neuron, and alterations to APs in the neuron. By understanding the electrophysiological properties of the *Aplysia caeli* we can better describe the mechanisms utilized by these organisms for neuronal communication.

Methods

We obtained the aptly named *Aplysia caeli* from NASA after their recent mission returned with invertebrate organisms found by rovers on the surface of Mars. We were tasked, along with numerous other research teams, to compare this species of Aplysia-like organism to those found on Earth. Our objective was to characterize the electrophysiological activity of neurons found within the abdominal ganglia and compare the neuronal properties to similar species on Earth.

Dissection

The anatomy of the *Aplysia caeli* is very similar to aplysia found on Earth (Figure 1). In order to record from neurons, we had to access the ventral side of the aplysia and dissect the abdominal ganglion. To access the ventral side of the organism, we began by inserting pins into the head and tail and pinned the aplysia with its dorsal side against the dissection mat, exposing the foot. We made a long incision spanning the length of the foot, which gave us access to all of the internal organs and several ganglion. Throughout the dissection process we did not use anesthesia, which is in accordance with previous Earth aplysia preparations and is the accepted methodology for invertebrate organisms. Aplysia lack a significant number of synapses between nociceptors and relevant somatosensory areas, suggesting that they react to nociception but do not experience the sensation we know as pain (Harvey-Clark, 2011).

We chose to excise the abdominal ganglia and record intracellularly from neurons located therein (Stein, 2016). The abdominal ganglion is easily identifiable by its bright orange color and is located near the anterior gizzard and spermatheca (Figure 1). The neurons within are large, easy to access, and are easily visualized beneath the connective tissue sheath of the ganglion with a standard dissecting microscope. Following excision, the ganglion was preserved in a dish filled with seawater, the fluid typically found within all aplysia. The dish also had gel on the bottom that allowed for us to pin down the connective tissue. In order to produce tension across the ganglion's connective sheath, we inserted pins through the connective tissue into the gel at steep angles and moved them radially outward. Once there was sufficient tension in the protective

sheath, we cut along the length of ganglion with a #26 scalpel. This caused some neurons to spill out and created an access point for intracellular recording.

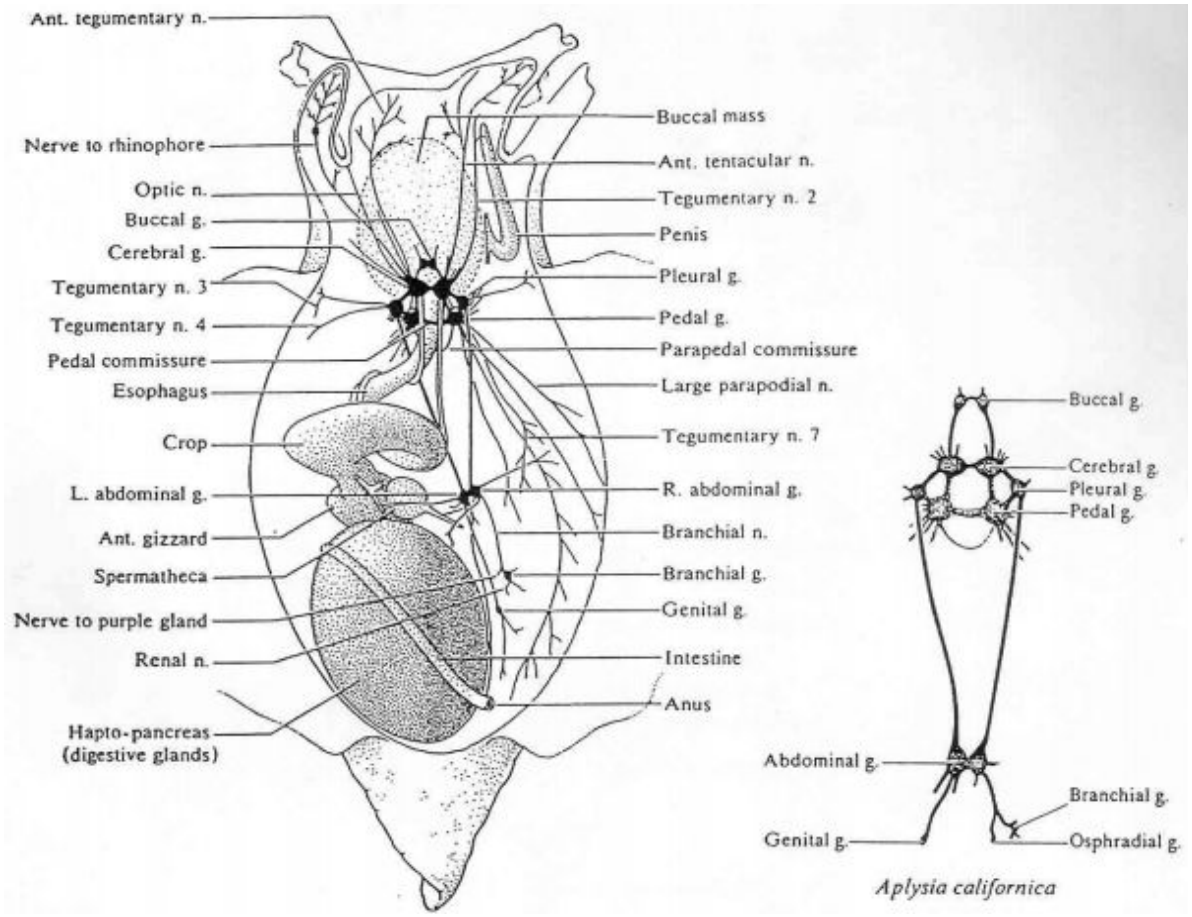

Figure 1. Internal Anatomy of the *Aplysia caeli*.

Cell Recording

It was fairly easy to locate cells in the ganglion under the dissecting microscope. In order to record from these cells intracellularly, we made fine tipped glass pipettes using a pipette puller. We turned the pipette into an electrode by filling it with KCl, a conducting fluid, and connecting a silver wire coated in AgCl to the amplifier. We measured the electrode's resistance by injecting a 1 nA current into the bath and observing changes in potential. We used a single

electrode with a resistance of 2 MΩ for all of our recordings. In order to isolate the electrode resistance from the membrane's resistance, we used the stimulus-cancellation function of the amplifier (Stein, 2016). Small junction potentials in the bath were eliminated by using the position function of the amplifier. We observed changes in cell potential using ADInstruments Data Acquisition Hardware. Recordings were taken at 40,000 samples per second. A micromanipulator allowed us to move the electrode in small, precise movements to impale the cellular membrane. Upon membrane penetration, there was a characteristic drop in resting potential and the impaled cell fired off a series of APs. If the cell did not die, we proceeded by applying a slight hyperpolarizing current to help the membrane reseal. Afterwards, we tested the neuron's response to current injections of varying amplitudes and changes in spike frequency after prolonged stimuli.

Results

General Cell Observations

Despite only being able to record from a limited number of cells from the *Aplysia caeli* due to resource and time limits, we observed a number of shared trends among the cells within the organism. All of the recorded cells appeared to fire spontaneously with resting membrane potentials of around -20 mV to -30 mV. The spontaneous firing rate of cell 2 was 0.47 spikes / second. The spike amplitude for cells 2 and 3 was 53.42 mV and 69.44 mV, respectively. The spike duration was measured from the time when the spike initially deflected upwards until the point where its trace returned to the same reading in its falling phase. The average durations of

these spikes in cells 2 and 3 were 3.07 ms and 2.02 ms and they had membrane resistances of 12.09 MΩ and 5.84 MΩ, respectively.

Cell Injury Response and Death

We found that *Aplysia caeli* cells exhibited injury responses to damage. After an electrode was inserted and the impalation was sub-optimal (sufficient tissue damage to prevent the membrane from sealing), we observed what appeared to be a cell injury response indicative of a dying cell. Early in the voltage trace, we see a high AP amplitude. This amplitude, as well as the spiking rate, rapidly decreases in just a matter of seconds (Figure 2). Over time the cell's membrane potential diminished to zero and spiking stopped.

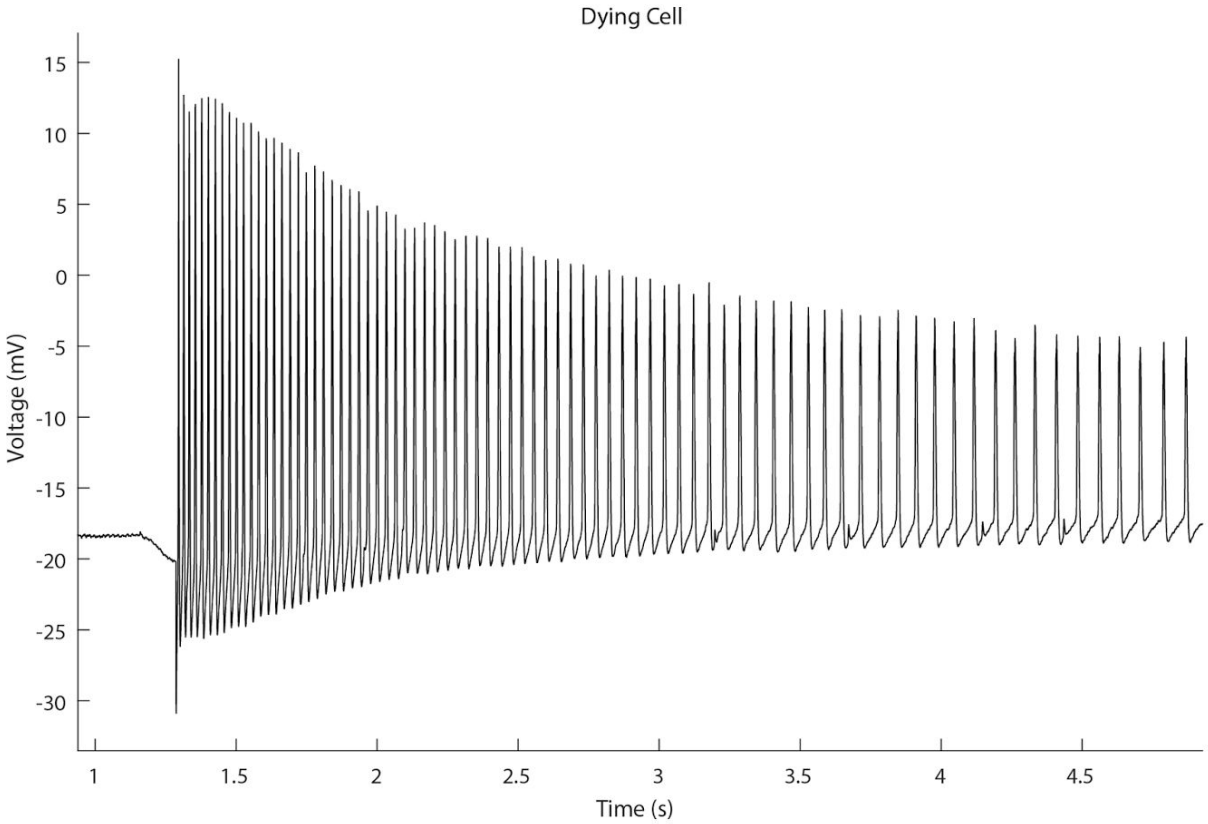

Figure 2. Suspected injury response of *Aplysia caeli* cell. All spiking gradually stopped as time continued. Note the simultaneous decrease in spike amplitude and frequency.

Spontaneous Excitatory Post-Synaptic Potentials

Both of the recorded cells showed evidence of EPSPs, which are so-called for their excitatory summative effect towards AP threshold (Bear *et al.*, 2007). Since the amplitudes and durations of EPSPs in each cell differ, we show here a single representative EPSP from one of our several recorded cells (Figure 3). It provides a good visual representation of the *Aplysia caeli* EPSP size and duration, but it is important to note that each EPSP can vary significantly.

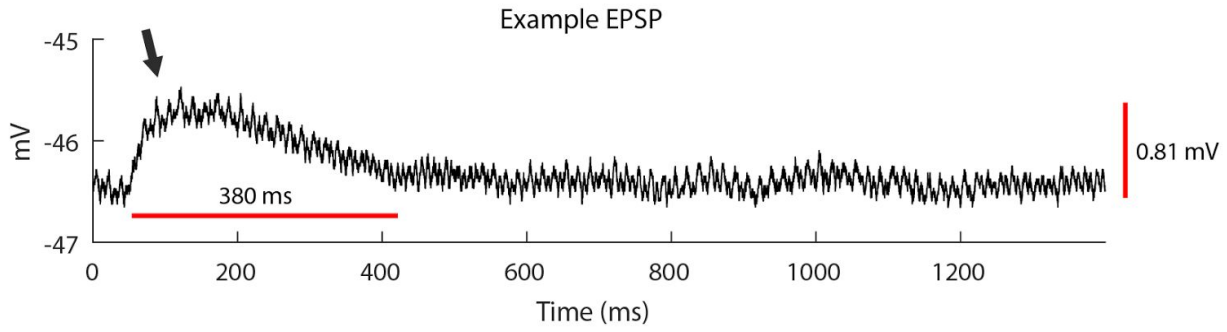

Figure 3. Representative EPSP from recorded *Aplysia caeli* voltage trace. This EPSP trace gives the general shape of EPSPs found in these space aplysia neurons. The EPSP in this trace is indicated by the black arrow: its duration is ~380 ms and it has an amplitude of ~0.81 mV.

Effect of Increasing Stimulus Amplitude on Spiking

By gradually increasing the amplitude of our injected stimulus in a stepwise fashion, we were able to elicit an AP and at the same time discern the stimulus amplitude threshold above which elicits a spike. As we increased the stimulus amplitude, we saw the resting membrane

potential of the cell depolarize more until we elicited the first spike with an input stimulus current of 5.4 nA (Figure 4).

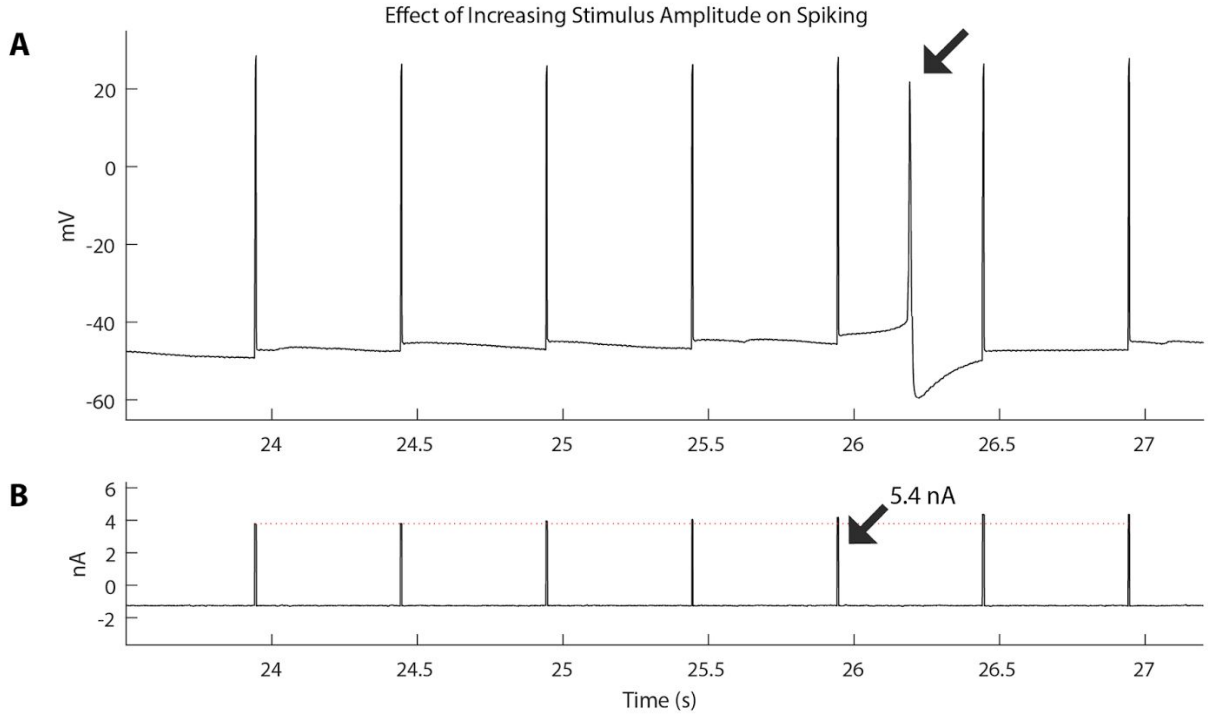

Figure 4. The effect of increasing stimulus amplitude on spiking in *Aplysia caeli* cell. **A**. Extracellular recording of voltage potential in aplysia cell. Note that the only AP in this voltage trace is identified by the black arrow. All of the other "spikes" are actually just input stimulus current artifact. **B**. The current trace of input stimuli injected into the aplysia cell. Note that the input stimuli are gradually increasing in size (the dotted red line has been included to facilitate this observation). The black arrow identifies the amplitude (5.4 nA) of the first stimuli to elicit an AP in the cell.

Adaptation with Depolarization

A cell is said to "adapt" if the firing rate progressively decreases when the cell is stimulated (Stein, 2016). We observed a decrease in firing rate in our recording cell when we removed the hyperpolarizing current injected into the cell body, allowing the cell to return to its

initial resting membrane potential. When we allowed the cell to return to its original membrane potential, we found that the cell appeared to adopt a slightly faster spontaneous firing rate, calculated to be 0.60 spikes / second. The cell, when "de-hyperpolarized," (Figure 5B) fired very rapidly for the first several seconds afterwards, but quickly returned to a steady, slower firing rate, close to what we observed in initial recordings (Figure 5A).

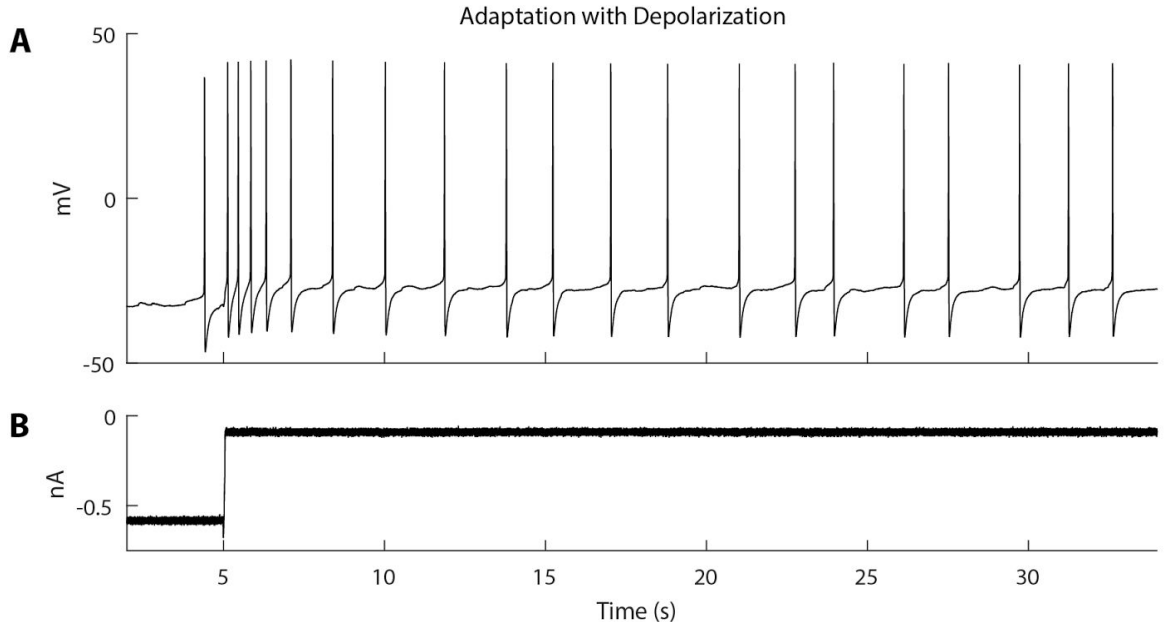

Figure 5. Adaptation of cell firing rate with depolarization. **A**. Voltage trace of cell. Note the rapid spiking at around 5 seconds and the rapid decrease in rate that leads to a steady firing rate. **B**. Trace of input current into cell. Hyperpolarizing current is removed at 5 s, causing rapid spiking which quickly slows down to a steady rate (A).

Discussion

[see individual discussion sections]

References

Bear, M. F., Connors, B. W., & Paradiso, M. A. (Eds.). (2007). Neuroscience: Exploring the Brain (Vol. 3) (117-8, 124). Lippincott Williams & Wilkins.

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