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Lab 3 Aplysia – Discussion Section

Examination of the Intracellular Electrophysiological Properties of *Aplysia caeli*

Discussion

Our research team characterized various neuronal firing properties of *Aplysia caeli*, finding that in general these space organisms appear to have similar electrophysiological characteristics to analogous organisms found on Earth. These characteristics include but are not limited to action potentials and EPSPs (both of which are similar in size and duration) and neuronal adaptation with prolonged stimulation.

General Cell Observations

Recorded action potentials appeared consistent with prior knowledge of spiking in neurons, as the resting membrane potential (RMP) held a negative value and when depolarized past threshold would quickly rise to a positive value and then promptly recover. The RMPs for all recorded cells were considerably higher than that of those found in terrestrial aplysia, but still within reason. The spiking amplitude and duration also fell within acceptable ranges for Earth organisms, which would imply further evidence for an ancestral link between *Aplysia caeli* and terrestrial aplysia. It is possible that certain but not all properties of space aplysia neurons are different from Earth aplysia. Due to the very limited number of cells recorded, and due to those cells all spontaneously firing, we were unable to produce current-voltage plots for the neurons, as they were too active. Injecting hyperpolarizing current into the cell via the electrode was also not successful due to the cell rapidly dropping its firing threshold until it had regained a similar firing rate to the initial.

Cell Injury Response and Death

The observed rapid decrease in both spiking amplitude and rate (Figure 2) appears to fit with prior knowledge of cell biology: the elicited injury response would be expected to be directly related to the cell's inability to restore its original RMP. As a result, it cannot spike as rapidly or as powerfully (spike rate and amplitude). This knowledge of how cells operate in Earth-based organisms provides a possible mechanism for the injury response we observed in *Aplysia caeli*, increasing the likelihood of cell membranes being physiologically similar to those of Earth aplysia.

Spontaneous Excitatory Post-Synaptic Potentials

The EPSPs found in *Aplysia caeli* were consistent with those reported for Earth aplysia, implying a potential ancestral link between the two organisms. We note that there are a large number of EPSPs present in the data for all recorded cells and that they often vary in both amplitude and duration. This may provide some evidence that the cell being recorded has a number of inputs from different neurons (Covey, 2009), which is also found to be the case among terrestrial organisms.

Effect of Increasing Stimulus Amplitude on Spiking

We found that injecting increased stimulus amplitude into a cell resulted in progressively larger depolarizations, eventually reaching a threshold level and firing an AP. This suggests that the APs we observe in *Aplysia caeli* operate in a similar fashion to those of Earth aplysia (spiking threshold must be reached in order to fire). Additionally, it appears that our calculated spiking threshold (the depolarized potential at which the cell will fire if reached) is within biological levels which are generally less than 1 nA in current amplitude (Koch *et al.*, 1995).

Upon closer examination of this data, however, there is evidently much room for improvement and further exploration. For example, due to the fact that all of the cells we recorded were spontaneous and fired action potentials readily, did holding the cell membrane at a hyperpolarized potential affect its ability to spike, or its spiking threshold, for that matter? Additionally, why did the neuron only fire a single time once the spiking threshold was reached (black arrow in Figure 4A)? It is not likely that the neuron was still refractory because the next stimuli arrives much later in time (longer than the usual refractory period duration) has passed. We believe that this warrants further investigation and will pursue this in future studies.

Adaptation with Depolarization

All of the recorded cells showed behavior that can be interpreted as adaptation, defined as prolonged stimulation causing a progressive decrease in firing rate towards an asymptotic value. When we triggered adaptation in the cell, we observed that the newly adopted stabilized rate was slightly higher than the initial spontaneous rate pre-stimulation. It is plausible that this change implies that the cell changes to a new state and does not necessarily “recover” to its initial firing rate. To elicit adaptation, we initially hyperpolarized the cell and then waited for it to adapt to the more negative RMP. We then depolarized it to its initial RMP to trigger the adaptation. It is possible that this may have resulted in a different outcome than if we depolarized from the initial

RMP to elicit adaptation. Once again, we believe that future studies of *Aplysia caeli* will be able to better discern differences in these two approaches.

We were able to infer the presence of inhibitory post-synaptic potentials (IPSPs) through examination of the cells' adaptation with depolarization (Figure 6). IPSPs are the mechanism through which a cell makes itself less likely to fire an action potential (Bear *et al.*, 2007) and therefore we should expect an IPSP to fall wherever the voltage trace decreases outside of the falling phase of an action potential. We note the presence of tiny downward deflections (away from spiking threshold) in the voltage trace of the spontaneous activity of cell 2. These tiny deflections (noted by the black arrows in Figure 6) potentially indicate the presence of IPSPs as the voltage appears to move downwards, in a direction opposite to spiking threshold. This would consequently limit, or diminish, the spontaneous firing rate in a cell.

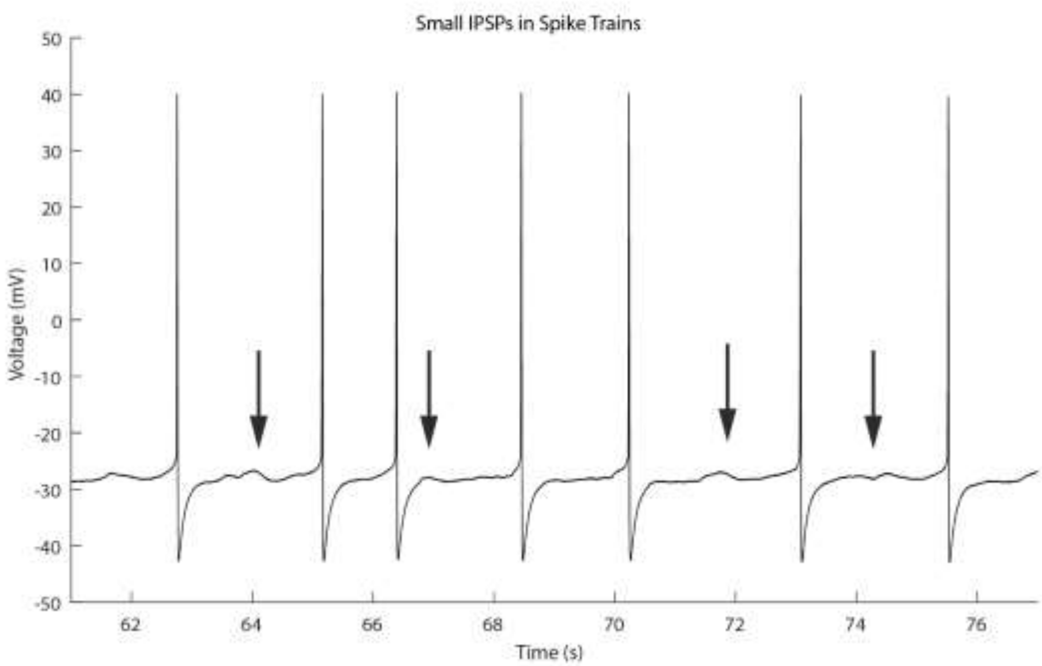

Figure 6. Potential evidence of small IPSPs in spike trains. Note the downward direction of the voltage trace indicated at each black arrow, indicating a movement away from spiking threshold, making the cell less likely to fire spontaneously.

We were unable to isolate any individual IPSPs to show as representative examples, but we believe that this would be a powerful direction to pursue for future studies. As a side note, it is likely that the IPSPs within our recorded data would be similar in size and duration to those of the EPSPs, but negative voltages instead of positive.

Sources of Error

Despite a number of potential error sources having already been mentioned, it is important to note that there is still much to learn about *Aplysia caeli*. Our study was limited to a very small sample size of cells and it would be beneficial to the scientific community for more cells from these organisms be investigated. Additionally, there were a number of space aplysia which were unhealthy upon dissection, likely due to the fact that they got sick upon entering the Earth's high pressure atmosphere. This could have in turn led to any number of unforeseen errors in our data collection (dying neurons, unhealthy cells, etc.) which can only be detected and remedied by further investigation and more data collection.

Sources

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