LONG-TERM SENSITIZATION TRAINING ALTERS THE BIOPHYSICAL PROPERTIES OF A DECISION-MAKING NEURON IN THE FEEDING NEURAL CIRCUIT OF *APLYSIA CALIFORNICA*

By

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ABSTRACT

The marine mollusk *Aplysia californica* is an exceptional model system to study the cellular mechanisms underlying learning-induced behavioral modifications. One particular well-studied learning paradigm in *Aplysia* is long-term sensitization (LTS), which has mainly been examined as an enhancement of defensive reflexes, such as the tail-siphon withdrawal reflex (TSWR). It was recently established that LTS is accompanied by a suppression of biting behavior, 24 h after LTS training. This LTS training-induced suppression of biting behavior is associated, at the cellular level, with a decrease in excitability of neuron B51, a key decision-making neuron that is pivotal for the elicitation of biting behavior. The decrease in excitability of B51 is expressed as an increase in the threshold to elicit regenerative bursts of action potentials (i.e., plateau potentials) and is not accompanied by changes in resting properties (i.e., resting membrane potential and input resistance), suggesting the modulation of voltage-dependent ion channels. Therefore, the goal of this study was to identify changes in voltage-dependent Na^+ and/or K^+ channels, produced by LTS training, by using pharmacological blockers of Na^+, K^+, and Ca^{2+} channels.

There were two groups in this research project: LTS trained and untrained (control). Biting behavior and the TSWR were measured before and 24 h after treatment in trained and untrained animals. After measuring biting behavior and the TSWR 24 h after training, the buccal ganglion, which houses neuron B51, was excised and prepared for intracellular recordings. Using the standard two-electrode current clamp technique,

the resting membrane potential, input resistance and burst threshold of B51 were analyzed in LTS trained and untrained animals. In order to isolate the contribution of voltage-dependent Na^+ and K^+ channels to B51 properties, the following combinations of channel blockers were used: 1) Tetraethylammonium (blocks delayed-rectifier K^+ channels), 4-Aminopyridine (blocks transient K^+ channels) and Cobalt (blocks Ca^{2+} and Ca^{2+}-dependent K^+ channels) to isolate voltage-dependent Na^+ channels, 2) Tetrodotoxin (blocks Na^+ channels) and Cobalt to isolate voltage-dependent K^+ channels. After obtaining B51 properties, the population of animals was divided into two groups: B51 with isolated voltage-dependent Na^+channels and B51 with isolated voltage-dependent K^+ channels. In each of these groups, the voltage responses to injected current were dominated by voltage-dependent Na^+ and K^+ channels, respectively. For both groups, the resting membrane potential, input resistance, burst threshold were again analyzed after the channel blockers were applied. For the Na^+ channels isolated group, the threshold to elicit the first action potential (i.e., firing threshold) was also measured. For the K^+ channels isolated group, neuron B51 did not elicit actions potentials but instead elicited K^+-dependent depolarizations. Therefore, the following parameters were measured: the area, the current-voltage responses, and the resistance to depolarizing current injections.

The data collected from this experiment show that 24 h after LTS training, *Aplysia* exhibit a suppression of biting behavior and a decrease in excitability of B51, which confirmed previous findings. The analysis of the effects of LTS training on B51 voltage-dependent channels revealed that the Na^+-dependent firing threshold was higher in B51 from trained animals as compared to untrained animals. The K^+-dependent properties of B51 were not significantly different between LTS trained and untrained

animals.

The experiment conducted shows that the effects of LTS training can biophysically manifest at the cellular level, in a decision-making neuron (i.e., neuron B51). In particular, the data from this experiment show that the increase in the Na^+ -dependent firing threshold in LTS trained B51 contributes, at least in part, to the decrease in B51 excitability observed following LTS training. This finding indicates that the LTS training-induced decrease in B51 excitability is Na^+ dependent, thus suggesting that training selectively modified the biophysical properties of voltage-dependent Na^+ channels in B51. Given that B51 is a decision-making neuron, which exhibits an all-or-nothing regenerative burst of action potentials, the change in Na^+ channels may represent one of the mechanisms underlying the decreased number of bites elicited 24 h after LTS training.

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ACKNOWLEDGMENTS

In Dr. Mozzachiodi's research laboratory, the marine mollusk *Aplysia californica* is studied to understand the fundamental mechanisms underlying learning and memory. However, studying learning and memory in *Aplysia*, at the behavioral and cellular level, was not simple. More specifically, I am referring to the main focus of my graduate research: studying neuron B51. Although neuron B51 provides the unique opportunity study the cellular properties contributing to changes in behavior; the catch was managing to finding the neuron and impaling it consistently with two electrodes. Despite all of the stress from my research, they were small prices to pay to gain a great deal of knowledge, patience, and independent working skills that will help me in my future endeavors. Also, I believe that my graduate career was an enjoyable experience because of the great people I worked with in the laboratory. Without them, it would have been a much more stressful experience.

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