

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

By

John Hernandez

July, 2012

A Thesis Submitted  
in Partial Fulfillment of  
the Requirements for the Degree of

MASTER OF SCIENCE IN BIOLOGY

THE GRADUATE BIOLOGY PROGRAM  
DEPARTMENT OF LIFE SCIENCES  
TEXAS A&M UNIVERSITY – CORPUS CHRISTI

APPROVED: \_\_\_\_\_ Date: \_\_\_\_\_

Dr. Riccardo Mozzachiodi, Chair

\_\_\_\_\_  
Dr. Kirk Cammarata, Member

\_\_\_\_\_  
Dr. Manuela Gardner, Member

\_\_\_\_\_  
Dr. Joe Fox, Chair  
Department of Life Sciences

\_\_\_\_\_  
Dr. Frank Pezold  
Dean College of Science and Engineering

## 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<sup>+</sup> and/or K<sup>+</sup> channels, produced by LTS training, by using pharmacological blockers of Na<sup>+</sup>, K<sup>+</sup>, and Ca<sup>2+</sup> 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  $\text{Na}^+$  and  $\text{K}^+$  channels to B51 properties, the following combinations of channel blockers were used: 1) Tetraethylammonium (blocks delayed-rectifier  $\text{K}^+$  channels), 4-Aminopyridine (blocks transient  $\text{K}^+$  channels) and Cobalt (blocks  $\text{Ca}^{2+}$  and  $\text{Ca}^{2+}$ -dependent  $\text{K}^+$  channels) to isolate voltage-dependent  $\text{Na}^+$  channels, 2) Tetrodotoxin (blocks  $\text{Na}^+$  channels) and Cobalt to isolate voltage-dependent  $\text{K}^+$  channels. After obtaining B51 properties, the population of animals was divided into two groups: B51 with isolated voltage-dependent  $\text{Na}^+$  channels and B51 with isolated voltage-dependent  $\text{K}^+$  channels. In each of these groups, the voltage responses to injected current were dominated by voltage-dependent  $\text{Na}^+$  and  $\text{K}^+$  channels, respectively. For both groups, the resting membrane potential, input resistance, burst threshold were again analyzed after the channel blockers were applied. For the  $\text{Na}^+$  channels isolated group, the threshold to elicit the first action potential (i.e., firing threshold) was also measured. For the  $\text{K}^+$  channels isolated group, neuron B51 did not elicit actions potentials but instead elicited  $\text{K}^+$ -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  $\text{Na}^+$ -dependent firing threshold was higher in B51 from trained animals as compared to untrained animals. The  $\text{K}^+$ -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<sup>+</sup>-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<sup>+</sup> dependent, thus suggesting that training selectively modified the biophysical properties of voltage-dependent Na<sup>+</sup> 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<sup>+</sup> channels may represent one of the mechanisms underlying the decreased number of bites elicited 24 h after LTS training.

## TABLE OF CONTENTS

ABSTRACT	ii
TABLE OF CONTENTS	v
LIST OF FIGURES	viii
ACKNOWLEDGEMENTS	x
INTRODUCTION	1
The origins of neuroscience	1
Learning and memory	2
Associative and nonassociative learning	3
Using a tractable nervous system for studying learning and memory	4
Using the tail-siphon withdrawal reflex to study sensitization in <i>Aplysia californica</i>	4
Feeding behavior of <i>Aplysia</i>	5
The neural circuitry controlling biting behavior in <i>Aplysia</i>	7
The role of B51 in the suppression of biting behavior, 24 h after LTS training	9
Voltage-dependent ion channels: The sentries regulating neuronal excitability	10
Objectives	12
MATERIALS and METHODS	12
Animals	12
Preparation of animals for behavioral testing	13

<i>Parapodectomies</i>	13
<i>Implantation of electrodes</i>	14
Behavioral testing	16
<i>Measuring the TSWR</i>	17
<i>Measuring biting behavior</i>	19
Long-term sensitization training	20
Criteria for animal use	21
Measurement of B51 membrane properties	22
Preparing channel blockers	25
<i>Making the TEA, 4-AP and Co<sup>2+</sup> solutions</i>	25
<i>Making the TTX solutions</i>	25
<i>Isolation of voltage-dependent Na<sup>+</sup> and K<sup>+</sup> channels</i>	25
<i>Isolating B51 voltage-dependent Na<sup>+</sup> channels</i>	25
<i>Isolating B51 voltage-dependent K<sup>+</sup> channels</i>	26
Internal controls	26
<i>Internal control for determining the efficacy of TEA, 4-AP and Co<sup>2+</sup> concentrations</i>	26
<i>Internal control for determining the efficacy of TTX and Co<sup>2+</sup> concentrations</i>	27
Measuring B51 properties after isolating voltage-dependent Na <sup>+</sup> and K <sup>+</sup> channels	28
<i>Measuring B51 properties after isolating voltage-dependent Na<sup>+</sup> channels</i>	28
<i>Measuring B51 properties after isolating voltage-dependent K<sup>+</sup> channels</i>	28
Experimental design	29
Statistical analysis	30

RESULTS	32
The effects of LTS training on TSWR and biting behavior	32
The effects of LTS training on B51 membrane properties	33
Pilot study: Effects of blocking voltage-dependent $K^+$ and $Ca^{2+}$ on elicited spikes in neuron B3	34
The contribution of $Na^+$ channels to B51 properties 24 h after LTS training	35
Pilot study: The effects of blocking voltage-dependent $Na^+$ and $Ca^{2+}$ channels on neuron B3 activity	39
The contribution of $K^+$ channels to B51 properties 24 h after LTS training	39
DISCUSSION	44
<i>LTS training induces a suppression of biting behavior concomitant to enhanced TSWR</i>	44
<i>LTS training decreases the excitability of B51 at 24 h</i>	45
<i>Modification to threshold of activation in voltage-dependent <math>Na^+</math> channels of B51, 24 h after LTS training</i>	46
<i>Future directions</i>	48
CONCLUSIONS	50
LITERATURE CITED	52

## LIST OF FIGURES

Figure 1. Effects of LTS training on B51 membrane properties	9
Figure 2. Checking for breaks in Teflon-coated wire using a multimeter (FLUKE 73II)	14
Figure 3. A Teflon-coated silver wire in a needle, ready for implantation	15
Figure 4. Illustration of electrode implantation	16
Figure 5. Illustration of the TSWR in <i>Aplysia</i>	17
Figure 6. Photograph illustrating electrode hook-up	17
Figure 7. Biting phases of <i>Aplysia</i>	20
Figure 8. Illustration of LTS training	21
Figure 9. Illustration of the buccal ganglion	23
Figure 10. Measuring spike duration	27
Figure 11. Measuring the area of K <sup>+</sup> -dependent B51 depolarizations	29
Figure 12. Experimental design	30
Figure 13. Effects of LTS training on TSWR and biting behavior	32
Figure 14: LTS training decreased B51 excitability	33
Figure 15: LTS training did not modify V <sub>m</sub> or R <sub>m</sub> of B51	34
Figure 16: Pilot study: Effects of isolating Na <sup>+</sup> channels on elicited B3 spike	35
Figure 17. LTS training did not modify Na <sup>+</sup> -dependent resting properties of B51	36

Figure 18. LTS training did not significantly induce a change in Na <sup>+</sup> -dependent burst threshold of B51	37
Figure 19. LTS training increased the firing threshold of B51 Na <sup>+</sup> channels	38
Figure 20. Pilot study: Effects of isolating K <sup>+</sup> channels on elicited B3 spikes	39
Figure 21. LTS training did not modify K <sup>+</sup> -dependent resting properties of B51	40
Figure 22. LTS training did not modify the amplitude of B51 depolarizations	41
Figure 23. LTS training did not significantly affect the area of B51 depolarizations	42
Figure 24. LTS training did not significantly affect B51 input resistance to depolarizing current injections	43

## 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 to study the cellular properties contributing to changes in behavior; the catch was managing to find 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.

First off, I would like to thank Dr. Riccardo Mozzachiodi and Dr. Marcy Wainwright for all of their support, patience, understanding and advice. I am extremely lucky to have worked with such great people who put up with all of my shenanigans. I also would like to thank Kathy Dickinson, Max Odem and all of the undergraduates in

our research lab for all of their help with the many hours of training (i.e., Liz Hager, Tammy Flores, Harris Weisz, and Valerie Miranda), preparatory work, and overall outstanding diligence. Finally, I would like to thank Dr. Kirk Cammarata and Dr. Manuela Gardner, my graduate committee, for their guidance and edits for my thesis proposal and my thesis. I would also like to thank the NSF grant IOS-1120304 (awarded to Riccardo Mozzachiodi), the TRDF grant 140130-10090 (awarded to Riccardo Mozzachiodi and Marcy Wainwright) for supporting my research endeavors and the Graduate Office for their funding through the Graduate Summer Research Development Program for the summer of 2011. And finally, I would like to thank my family and my fiancé for always being there for me when I needed them, and for their understanding when I had to work so many hours away from them.