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Effect of Latrunculin B on Phonotaxis in Induced Selective Crickets Treated With JHIII (Acheta domesticus)

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Honors Thesis

Effect of Latrunculin B on Phonotaxis in induced selective crickets treated with JHIII (*Acheta domesticus)*

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Abstract

The purpose of this experiment is to determine the effect of the actin kinase inhibitor, Latrunculin B, on previously unselective crickets. We expected the injection of a mixture of latrunculin b andJuvenile Hormone (JHIII) into the prothoracic ganglion would cause a reduction in the affect of JHIII in older crickets, causing them to remain unselective. Based on the results we can conclude that Latrunculin B reduces the effect of JHIII, which indicates that creating new synapses is at least a partial effect of JHIII.

Introduction

Phonotaxis refers to the response of the female crickets to the male calling songs, more specifically the movement of the female cricket toward sound a stimulus. Selectivity refers to the characteristic of female crickets to respond to specific male calling song syllable periods produced by the scraper gliding over the file on the male cricket's wings (Huber and Thorson 1845). Selective crickets is a term used to denote crickets that respond to less than 4 of the 7 syllable periods tested; unselective crickets are crickets that respond to more than 4 syllable periods. Older less selective crickets, around 20-21 day old, tend to respond to more syllable periods as compared to younger more selective crickets, around 3-4 day old (Walikonis et al, 1991). In the study done by Walikonis (1991), he discovered that the selectivity of the younger crickets were a result of the high levels of Junevile hormone III naturally synthesized within the crickets; it was also determined that the older crickets categorized as less selective had low levels of JHIII in their system. In a study done by Henley, et al, (1991) they found that injecting

older crickets with JHIII produced results similar to younger crickets in term of selectivity. Later studies tested the effects of different hormones on female crickets to see if selectivity could be altered, such as: pyrilamine, and anti-histamine, which was shown to make previously unselective crickets more selective (Chung 2012). Chung's study acted as a follow up to one done by Yoon (2011), which demonstrated that histamine caused selective crickets to become more unselective. The purpose of the Yoon study was to determine which of the naturally occurring inhibitory neurotransmitters would affect to the cricket's selectivity, in which he found histamine to have an effect. These studies demonstrate that changes in the selectivity in female crickets result from changes in the strength of connections between the prothoracic L3 neurons and ON1 (Atkins et al. Manuscript in preparation)

The purpose of my research is to help determine the mechanism that JHIII uses to cause the upreguation of the ON1 neuron on the L3 neurons. Studying the effect of Latrunculin b—an actin kinase inhibitor—on the phonotactic response of female crickets to the male calling songs will shed light on the mechanism, by either disproving or approving one possible mechanism for synaptic strengthening. Two possibilities exist to explain how a neuronal synapse is strengthened. The first method requires the creation of new synapses, the second mechanism requires increasing the sensitivity of the synapse (Gluck et al, 76-77, 2008). A study done by Chand et al (2012), showed that actin polymerization is one of the most important components in filapoda growth of pyramidal cells in the hippocampus, which led to the creation of new synapses. A study done by Moscatelli et al. (2012) showed that latrunculin b acts as an actin kinase inhibitor and inhibits the polymerization of actin. Therefore, synaptic growth should be inhibited by

latruncilin b, because ideally it would stop the polymerization of more actin in the prothoracic ganglion. Thus, if successful the latrunculin b would cause a decrease in the effect of Juvenile Hormone III on older crickets, because it would stop JHIII from strengthening the connections of the ON1 neuron on the L3 neurons. The reduction in effect of JHIII in this case would indicate that the JHIII induced up-regulation in the prothracic ganglion was caused by the growth of new synapses. In conclusion, my hypothesis states that the addition of Latrunculin b at the prothoracic ganglion would negate the effect of JHIII, making unselective crickets remain unselective, because new synapses would be unable to form.

Methodology

Four to five week old male and female *Acheta domesticus* nymphs were bought from Fluker's Cricket Farm and raised in 100-L plastic containers at 22-24 °C. They were exposed to an artificial 12:12 hour light: dark cycle, with egg cartons for shelter. The crickets were given cricket food (Fluker's Cricket Farm) and water daily. Once the crickets molted, they were separated from nymphs daily. Adult males were discarded, while females were kept in 16-L plastic containers housed with the nymphs to ensure that all of the females were virgin.

Four-5 week old adults were used in this experiment. The female crickets were transferred from the colony to a climate controlled cricket arena. The arena had a sand-covered floor, with a speaker in the center that faced up, which projected sound in 360°. The arena was 152 cm in diameter, with a 10 cm high clear plastic rim to prevent the crickets from escaping. A camera hung in the ceiling above the arena, which recorded the

entire circular arena and projected the image onto a large monitor, where the movements of the crickets were manually traced with markers on transparent plastic which covered monitor. After being placed in the arena, the crickets were given a 5-min acclimation period prior to being tested. Meanwhile, I traced over the image of the arena on transparent paper on the monitor screen; in addition, I recorded relevant information such as: the temperature within the room, the date of the experiment, the age of the cricket, and the test that I was about to conduct.

. The computer played artificial digital male call songs with syllable periods of 30ms, 40ms, 50ms, 60ms, 70ms, 80ms, and 90ms. The sounds were played in a standard, non-sequential order for 5 min each with a 3-min break in between (50ms-90ms-70ms-30ms-60ms-80ms-40ms). Phonotaxis in the arena was defined if the movement of the cricket from the sides of the arena to the center speakers occurred within a 90° quadrant with no negative tracking.

After the pretest the crickets are then secured to a miniature dissection table with clay, the torso was covered and the two front legs were stabilized with smaller pieces of clay. Dissection scissors were used to expose the cricket's thoracic area. An inverted U shaped flap was opened cut above the prothoracic ganglion, to expose the ganglion. A Drummond Nanoinject II was used to inject 9.3nL of Juvenile Hormone III diluted to 10^{-5} M in acetone onto the ventral portion of the prothoracic ganglion for the control group. For the experimental group we injected 9.3nL of 10^{-5}M of a Latrunculin b and JHIII mix dissolved in acetone. After the injections the crickets' thorax closed and the hemolymph created a seal, which typically occurs within seconds.

The posttest is conducted within 30 min of the nanoinjections. The posttest was conducted the same way as the pretest. After the posttest we evaluated phonotaxis from the tracking data. We compared the pretest and the posttest using a paired t-Test. We conducted an unpaired t-Test, in order to compare the posttests of the control and the experimental groups.

Results

Twenty crickets were injected with Juvenile hormone III in order to act as the control group. The control group was treated with JHIII and the results of its effect on phonotaxis were recorded in figure 1. A paired t-test showed a significant difference between the number of syllable periods responded to in the pretest compared to the post test with a p value of 1.302772e-8 (Figure 1). This indicates that JHIII increased the selectivity of the crickets. A graphical comparison of the crickets responses to the different syllable periods showed that in the control group there was an overall reduction in the response of the crickets in the posttest as compared to the pretest. However, it showed that during the pretest the crickets were highly unselective as indicated by the relatively horozontal line (Figure 3). In addition, some selectivity was shown in the posttest syllable periods around 50ms and 60ms appeared to be preferred (Figure 3).

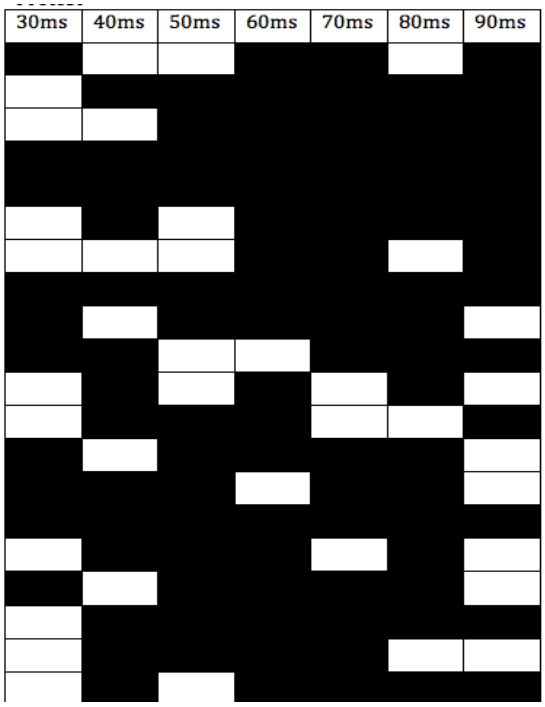
The experimental group, which was treated with a mixture of JHIII and Latrunculin B, also showed an increase in its selectivity, which is apparent in figure 2; a paired T-test on the pretest and the posttest indicated that there was significant difference in phonotaxis between its pretest and posttest with a p value of 1.99712e-09 (Table 2). In the experimental group there was a clear distinction in the activity of the pretest and

posttests of the crickets. The pretest showed definite unselective behavior thought the relatively horizontal line, and selectivity in the posttest with the peaks in the 50-70ms range (Figure 4).

A comparison of phonotaxis was then made between the control group and the experimental group, in which the pretests and posttests were compared for similarity. An unpaired T-Test, which compared the control pretest and the experimental pretest showed that there was no significant difference the two with a p value of 0.289999709. An unpaired T-test, which compared the posttest of the control group and the posttest of the experimental group showed a significant difference with a p-value of 0.01896745. The divergence from the pretest to the posttest indicated that the addition of Latrunculin B in the experimental treatment had an effect on the action of JHIII.

Figure 1: Juvenile Hormone III (Control) data, with the pre and post test comparison of the phonotactic response of the female crickets to the different syllable periods. Each row indicates a different cricket. White boxes indicate no phonotaxis was recorded, while the black boxes indicate phonotaxis occurred, the same 20 crickets conducted the pretests and the posttests.

Pretest



Posttest

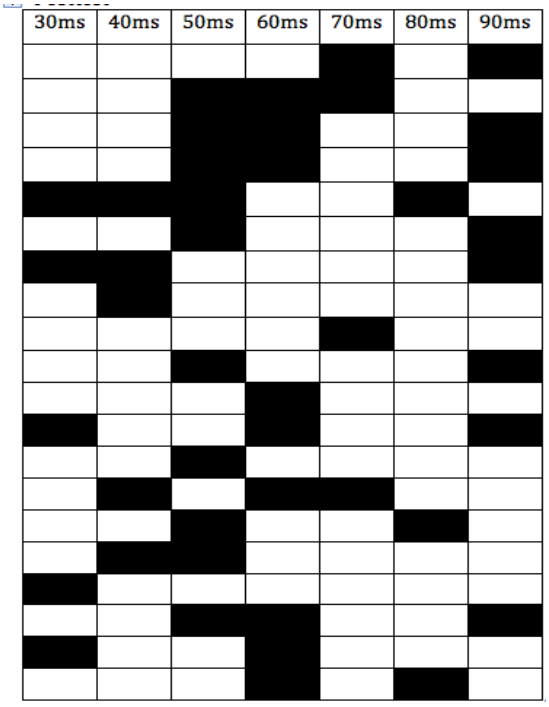


Figure 2: Latrunculin B and Juvenile Hormone III mix (Experimental) data, with the pre and post test comparison of the phonotactic response of the female crickets to the difference syllable periods of the male calling songs. Each row indicates a different cricket. White boxes indicate no phonotaxis was recorded, while the black boxes indicate that phonotaxis occurred, the same 20 crickets conducted both the pretests and the posttests.

Pretest Posttest

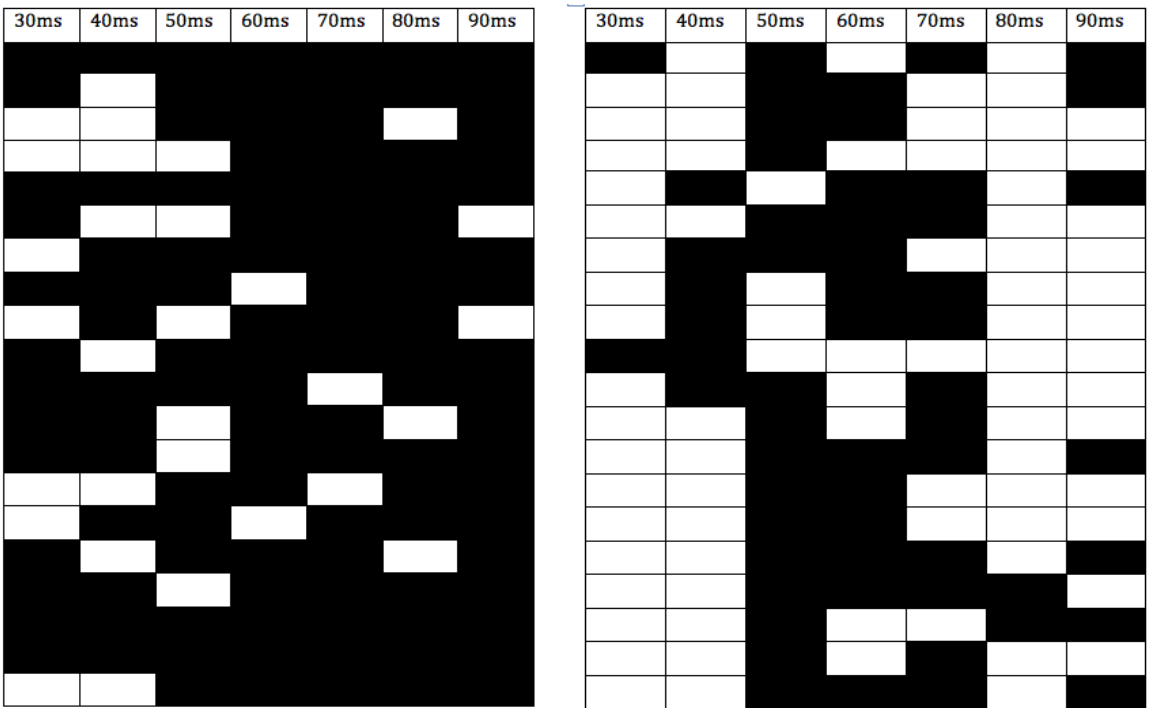


Figure 3: Graphical representation of the comparison for the pre and posttest showing the frequency of cricket responses to the different syllable periods. (20 Crickets)

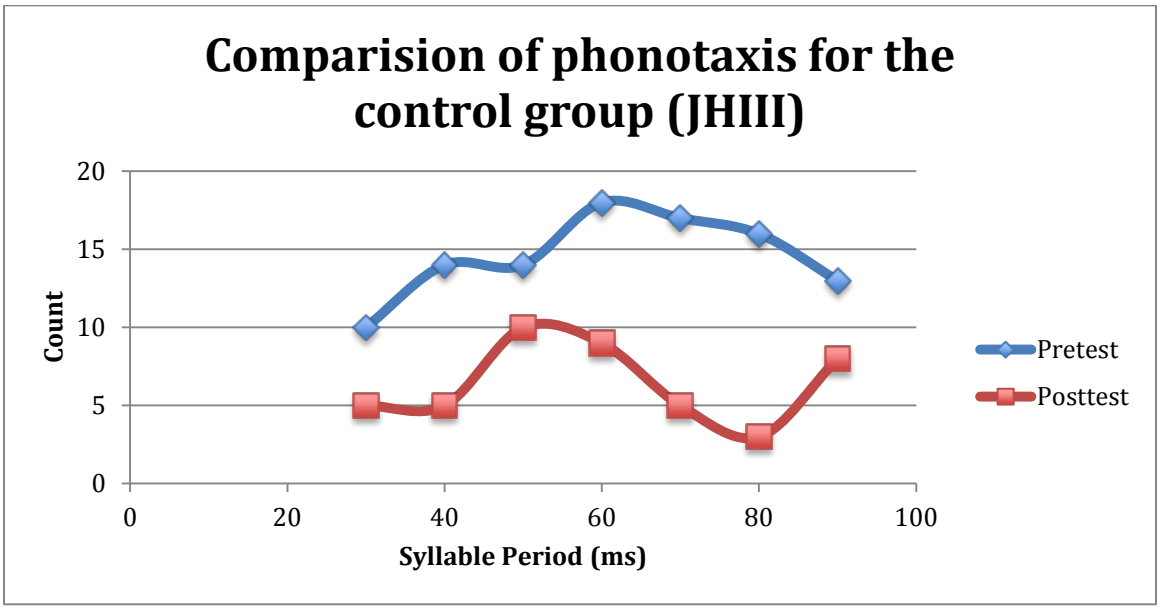
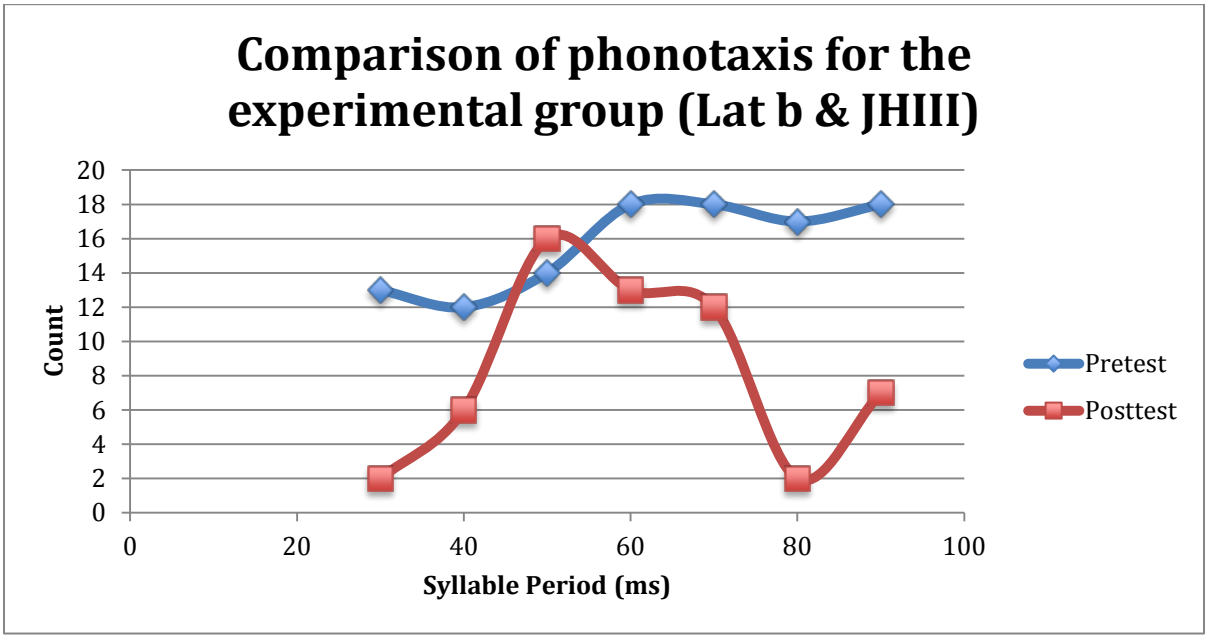


Figure 4: Graphical representation of the pre and posttest of the experimental group showing the frequency of cricket responses to the different syllable periods (20 crickets).



Discussion

In the control experiment we concluded in terms of phonotaxis, JHIII increased the selectivity of crickets. We rejected our null hypothesis, based on the p value of 1.302772e-8 (Figure 1), because a change was observed as a result of JHIII treatments. This is consistent with past studies which showed that JHIII increased the selectivity of crickets that were previously unselective (Henley, 1991). We also observed an increase in the selectivity of the crickets in the experimental group treated with a mixture of JHIII and Latrunculin B. Based on a p value of 1.99712e-09 when we compared the pretest and the posttest of the experimental group we were able to reject the null hypothesis that states there was no significant difference in phonotaxis as a result of the Latrunculin B mixed with JHIII treatment, because selectivity increased after the treatment (Figure 2).

We then compared the control and the experimental groups in order to determine if the effects of the two treatments were identical, or if there was a measurable difference. We observed that there was no statistical difference of phonotaxis in the pretest of the control group compared to the pretest of the experimental group, based on a p value of 0.289999709. However, we observed that there was a significant difference in the posttest of the experimental group compared to the posttest of the control group (Fig. 2); this divergence in comparative significance in the pretests and the posttests indicated that Latrunculin B at least partially affected the action of JHIII within the prothoracic ganglion. These results indicated that Latrunculin B had a partial inhibitory affect on the action of JHIII, since there was a decrease in the selectivity of crickets in the experimental group as compared to the control group. Previous research indicated that

Latrunculin B acted as an actin Kinase inhibitor (Moscatelli 2012),; therefore, allowing us to accept that synaptic growth was at least a partial mechanism for JHIII induced up-regulation of the Omega neuron on the L3 neurons since adding Latrunculin B reduced JHIII effects.

Future research into this topic could test the effects of altering the concentrations of the two drugs. Perhaps the effect of Latrunculin B could be amplified by increasing the concentration of Latrunculin B injected into the ganglion. Or perhaps through the pretreatment of Latrunculin B on the prothoracic ganglion prior to the injection of JHIII, because the effect may have been diminished due to the timing of drug introductions.

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