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Chelerythrine Chloride And Its Effects On Phonotactic Behavior In Female Crickets Acheta Domesticus

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Honors Thesis

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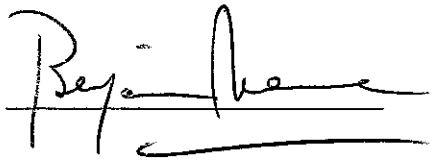
Primary Advisor Signature: 

Department of Biology

Abstract

Selective phonotaxis by female crickets has been shown to be variable. Mechanisms which underlie such behavioral variability are being studied. Juvenile Hormone III is a neuromodulator that has been shown to increase selectivity in phonotactic behavior of female crickets. In an attempt to further explore its effects on the behavioral responses of female crickets, experiments have been performed with Chelerythrine Chloride that is known to have an opposite effect of Juvenile Hormone III. Chelerythrine Chloride, a potent protein kinase C blocker, has been hypothesized to block the effect of Juvenile Hormone III which acts through a protein kinase C pathway, leading to a reduced selectiveness in the female's behavioral response.

List of Abbreviations

CS	Calling Song
SP	Syllable Period
PTG	Prothoracic Ganglion
JHIII	Juvenile Hormone III
CC	Chelerythrine Chloride

Introduction

Phonotaxis is defined as the movement of an organism in relation to a sound source. This movement may be explained by females responding to the courtship song of a potential mate or animals that may flee from the sound of a predator ("phonotaxis"). This study looks at the phonotactic behavior of the female cricket *Acheta domesticus* in response to the male crickets' calling songs. The calling song (CS) of male *A. domesticus* is characterized by its syllable period (SP). A CS consists of chirps with three sound pulses. The SP indicates the period between the beginning of one sound pulse and the beginning of the next sound pulse in one chirp. Female crickets' phonotactic behavior was studied by looking at how females respond to different SPs. It has been shown that females are most likely to respond positively to SPs in the range of 50ms to 70ms which resembles the CS of the male in nature (Stout *et al.,* 1983).

In a previous study that evaluated the behavioral responses of a large number of female crickets of different species, Stout et al. (2010), classified female as either selective or unselective according to the number of SPs they responded to. Selective females were defined as those that responded to 5 or fewer SPs of the 7 presented, and unselective females responded to 6 or more (Stout *et al.,* 2010). It has been concluded that phonotaxis is not a fixed, automatic reaction but, instead, a rather complex behavior that can be modified by internal and external conditions (Stout et al., 2010; Choi et al., 2012; Navia et al., 2015). Such internal condition can be modified by different neuromodulators. Neuromodulators are substances released by a neuron which transmit information to other neurons and can have an underlying effect to altering the dynamics of the neural circuit that could lead to a change in a specific behavior. Auditory neurons have been studied seeking to understand the variable selectivity reported in the behavioral response. One of the neuromodulators used has been Juvenile Hormone (JH) III that

has been correlated with female's varying selectivity with its age. Female crickets of 3-5 days old are more likely to exhibit selectiveness and females are likely to become less selective as they get older. Stout *et al.,* (1976) reports that some of the phonotactic plasticity observed can be attributed to JHIII. Juvenile Hormone peaked at its younger age (3-5days) when the females were most selective and Juvenile Hormone declined as females got older which could explain their reduced selectiveness.

Atkins *et al.* (2008) report that SP-selective phonotaxis by female *A.domesticus* is influenced by an auditory circuitry in the prothoracic ganglion (PTG). The PTG is located between the two front legs of the cricket on its ventral side. Several identified auditory interneurons—L3, L1, ON1—which have been proposed to be involved in phonotactic behavior are located in the PTG. An experimental approach which involves a nanoinjection of a specific chemical have been carried out into the PTG in order to study how behavior can be modified and its link to neural circuits that control such behaviors. Choi *et al.* performed nanoinjections of JHIII into the PTG of females (2012). After JHIII injection, the recipient females were attracted to a significantly narrower range of SPs that are typically focused on calls with SPs of 50ms - 70ms. Furthermore, Lynch (2015) looked at neuronal response to the injection of CC into the PTG. CC, a potent protein kinase C blocker, has been hypothesized to block the effect of JH III which act through a protein kinase C pathway leading to a reduced selectiveness in the female's behavioral response (Chao *et al.*, 1998; Liu *et al.*, 2015). Her results showed that CC injection led to a more uniform, consistent physiological response which can be paralleled with reduced phonotactic selectiveness. Accordingly, it has been hypothesized that CC injection into the PTG will lead to a reduced selectiveness in female's behavioral response.

Materials and Methodology

Cricket Care

Acheta domesticus nymphs were obtained from Fluker Farms, Inc. and raised in 100-L plastic containers with temperature ranging between 22-24 °C in a 12-hour light-dark cycle beginning at 06:00 hrs. Crickets were given cricket chow, water and egg cartons for shelter. Upon molting, adult crickets were separated from the nymphs and adult females were kept separately in 16-L plastic containers to prevent exposure to adult males.

Evaluating Behavioral Response

Young female crickets (3-7 days) were used for this study. Phonotaxis was tested on a noncompensating, spherical treadmill (Choi *et al.* 2012). Computer generated model calling songs (85dB, 5kHz) were composed of three syllable chirps with SPs of 30, 40, 50, 60, 70, 80, and 90 ms, and were played in a standard non-sequential order (50, 90, 70, 30, 60, 80, and 40 ms) from a speaker positioned 30 cm away from the cricket. The cricket's directional movements were recorded on an Apple Mac Book Pro computer with Optical Kugel application (2008: Version 0.5 beta) and evaluated for positive phonotaxis. Calling songs were played for 5 min each, with 2 min of silence between tests. Sound intensities (85 $\pm$ 1 dB) were calibrated at the position of the cricket on top of the treadmill. During testing, a calling song was considered attractive if the mean angular orientation was $\pm$60° toward the speaker and if the distance walked toward the speaker was at least twice the distance walked away from the speaker.

Nanoinjection of Chelerythrine Chloride

Female crickets that had been pre-tested on the treadmill were mounted ventral side up to a wax block and were immobilized. A small incision was made to expose the PTG without damaging surrounding acoustic tracheae. Saline was used to keep the tissues moist throughout

the procedure. A Drummond Nanoinject II nanoinjector (Drummond Scientific Co., Broomall, Pennsylvania) was used to inject 9.2nL of 10^{-5} M CC (Sigma) in saline. After injection, the specimen was removed from the block and inspected for any physical damage and set aside for a 15-20 min recovery period. Post-injection testing was conducted in a manner identical to the pre-injection tests. Number of SPs attractive to each subject was noted for analysis.

Statistical Analysis

The change in average number of SPs females responded to in pre-injection test and post-injection test were analyzed by applying Paired t-test and McNemar's test.

Results

Twenty-two females were pre-tested and were phonotactically attracted to an average of 4.5 of the seven SPs presented. These females exhibited various levels of selectiveness to SP, rangining from zero SP (pre-injection test; Figure 1 Top row) to those that were attracted to all seven SPs presented (Figure 1, bottom six rows). Average number of SPs crickets responded to were analyzed within a specific range: 50-70ms, 30-40ms, and 80-90ms. Females responded to an average of 2 SPs out of the three most attractive SPs (50,60,70ms). Females responded to an average of 1.3 SPs in the 30-40ms range and an average of 1.2 SPs in the 80-90ms range. Post-injection tests revealed that females were attracted to an average of 3.2 SPs, which is significantly smaller than the average number of SPs females responded to in the pre-injection test according to the Paired t-test ($p=0.015$) and McNemar's test ($p<0.001$). After CC-injection, females responded to an average of 1.5 SPs out of the three most attractive SPs (50,60,70ms) which shows no significant change from the average number of SPs females responded to in the pre-injection test ($p=0.061$). Females responded to an average of 0.86 SPs in 30-40ms range and

an average of 0.82 SPs in 80-90ms range. Such results show that the number of SPs females responded to both in 30-40ms range and 80-90ms range decreased significantly ($p=0.038$, $p=0.042$)

Control incisions or injections of 9.2 nL of saline into the ventral portion of the PTG did not significantly change the selectiveness of the 9 females tested (Figure 2) in terms of the average number of SPs responded to (5.1 SPs before injection; 3.7 SPs after incision or injection) according to Paired t-test. McNemar's test showed significant change under p value of 0.01 compared to the experimental group that showed significant change under p value of 0.001.

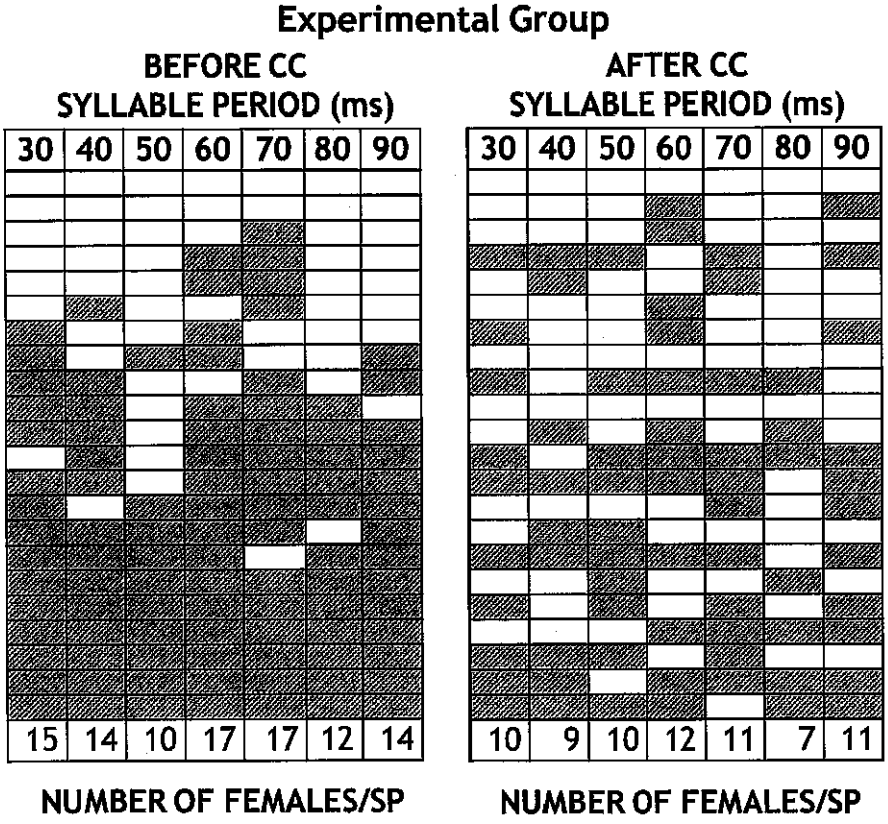


Figure 1. Effects of Chelerythrine chloride (CC) on phonotactic behavior. Each row represents the phonotactic behavior of a single female cricket before and after injection. The shaded boxes indicate the SPs the females responded to. Before injection, the females (n=22) responded to an average of 4.5 SPs and, after injection, they responded to an average of 3.2 SPs, resulting in significant differences in the number of SPs responded to (paired t-test, $p=0.0152$; McNemar's test, $p<0.001$).

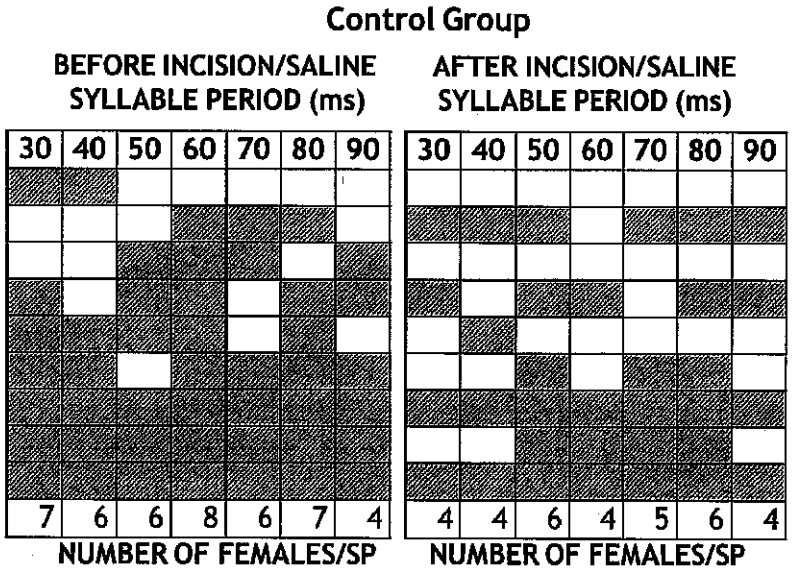


Figure 2. Effects of making incisions on the ventral portion of the prothoracic ganglion on phonotaxis. Before incision, the females (n=9) responded to an average of 5.1 SPs and, after incision, they responded to an average of 3.7 SPs, resulting in not significant differences in the number of SPs responded to (paired t-test, $p=0.102$; McNemar's test, $p<0.01$).

Discussion

The results rejected the original hypothesis that CC injection would lead to less selectiveness of females with larger number of SPs responded to. However, the results show that the number of SPs females were attracted to decreased significantly. Unlike the selectiveness trend of the young crickets suggested by Stout *et al.,* (1976), these crickets were random in number of SPs they responded to. In order to determine if the reduction of average number of SPs females responded to signifies more selectiveness or less responsiveness, change in average number of SPs attracted to in the range of 50-70ms was determined. Despite the reduction in average number of SPs females responded to, females did not necessarily 'tune' to the range of 50-70ms, which characterize the calling songs of conspecific *A. domesticus* males. Rather, crickets' response had no significant change in the 50-70ms range ($p=0.061$). This result does not signify more selectiveness of the females because they did not necessarily respond more to a specific 50-70ms range, but rather responded to random SPs. Such less responsiveness of females could possibly be explained by the characteristic of CC which was injected into these crickets. It is possible that CC, a potent Protein Kinase C blocker, in the process of blocking the effect of JH III could have made the crickets less responsive to any calling songs. This result may point toward an importance of the neuromodulator, JH III, as part of the neural circuit that controls the behavioral response.

Additionally, pre-injection females were grouped into two separate groups: selective and unselective crickets. If females responded to 5 or fewer SPs of 7 presented, they were grouped as being selective and if crickets reponded to 6 or more SPs, they were grouped as being unselective. Selective group showed no significant change in the average number of SPs they responded to ($p=0.807$) compared to unselective group that significantly decreased in the average

number of SPs they responded to ($p=0.00126$). This analysis showed that selective crickets remained "selective" and unselective crickets became "selective". This result was especially interesting regarding the results of a previous study (Lynch, 2015). Lynch recorded the neural responses to the injection of CC into the PTG (2015). Her results showed that CC injection led to a more uniform, consistent physiological response which can be paralleled with reduced phonotactic selectiveness. Similarly, the results reported here show a more uniform, consistent behavioral responses after the CC injection despite the different levels of responsiveness of crickets in the pre-injection test.

This study is unique in its choice of statistical analysis test. Past related studies that involved JH III injection and CC injection into the PTG employed paired t-test in determining the significance of post test results. However, for this research, choice of statistical analysis test was made by carefully considering the characteristic of the data, average number of SPs responded to. McNemar's test that is a statistical test used on paired nominal data was chosen to be the most optimal statistical analysis test for this study. In future similar studies, instead of using the nominal data, the number of SPs females responded to, quantitative data provided by the Optical Kugel (length toward the speaker and average angular orientation) could possibly be put into test to interpret the change in the females' selectiveness in pre-injection test and post-injection test.

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