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# The Role Of Octopamine In Syllable-Period Selective Phonotaxis In Female Cricket Acheta Domesticus

Darley Magno
*Andrews University*, darley@andrews.edu

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Andrews University

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# The role of octopamine in syllable-period selective phonotaxis in female cricket *Acheta domesticus*

Darley Magno
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Research Advisor: Dr. Benjamin Navia

Primary Advisor Signature: ____________________

Department: Biology

## ABSTRACT

Female crickets respond phonotactically to the calls of conspecific males. Females' phonotaxis has been reported to be variable, ranging from unselective to selective in response to calls with varying syllable periods (30–90 ms). Octopamine, an invertebrate neurotransmitter, has been reported to increase aggressive behavior in crickets, (Stevenson et al. 2005) but the effects of octopamine on behaviors such as phonotaxis have not been investigated. The goal of this study is to determine the effects of octopamine on the syllable-period selective phonotactic response of females. Results suggest a decrease in phonotactic responsiveness shown by 5-10 day-olds after prothoracic nanoinjection of octopamine.

## INTRODUCTION

In nature, various species of animals rely on acoustic communication in order to undergo activities that meet the basic needs for survival, such as mating and reproduction. Through observing and studying such behaviors, it brings about the question of the neural mechanisms that control these behaviors. Cricket phonotaxis is a good model to study how simple neural circuits influence behavior.

Female crickets exhibit phonotaxis, or the orientated movement towards an auditory stimulus, specifically to the call of a potential mate. Such call is composed of chirps, each containing three syllables with a defined time interval between each syllable (termed syllable period, SP; Figure 2). For *Acheta domesticus,* the most attractive SP range has been reported to be 50-70 ms (Stout et al. 1997). The SP is an important parameter for which females differentiate between calls of different species. The cricket's phonotactic behavior has been reported to be variable. In response to model calls with varying SPs ranging from 30-90 ms, some females are unselective (respond to more than 5 SPs) while others are selective (respond to 5 or less SPs). Younger females (5-10 day old) are more likely to be selective than older females (Stout et. al 2010).

Few neuromodulators have been reported to be influential in modifying behavioral responses when injected into the prothoracic ganglion (Atkins et al. 2008). Specifically, juvenile hormone III (JHIII), picrotoxin, and pyrilamine have been shown to increase SP selective phonotaxis in female *A. domesticus* (Atkins et al. 2008 & Yoon et al. 2011). The prothoracic ganglion (Figure 1) contains auditory interneurons (ON1, L1, L3) that have been proposed to play a role in influencing phonotactic behavior (Atkins et al. 1989, Atkins et al. 2011, Stout et al. 1991). Octopamine is an essential neuromodulator that is a monohydroxylic analog of norepinephrine found in both vertebrates and invertebrates. However, it has not yet been evaluated as a possible neuromodulator for phonotactic behavior. In other insects, such as the firefly, it governs endocrine gland activity that contributes to emission of light (Farooqui 2007, 2011). In other cricket species such as in *Gryllus bimaculatus*, octopamine plays an important role in aggression, flying and courting behaviors of the male (Adamo et al. 1995 & Stevenson et al. 2005). These findings of the role of octopamine in the behavior of different species lead to the main goal of this research to determine whether or not octopamine has an effect on SP selective phonotaxis in female *A. domesticus.*

## METHODS

### *Cricket care*

Four-week-old nymphs of *A. domesticus* were purchased from Fluker's Cricket Farm (Baton Rouge, Louisiana) and were maintained in 16-L or 100-L containers. Cricket food (Fluker's Cricket Farm), water and egg cartons were provided in each container. Containers that housed the nymphs were checked daily for adults, which were removed. Newly-moulted females (0 days old) were transferred to 16-L plastic containers. Newly-moulted males were discarded. Temperatures of colony chamber were maintained at 21 – 23 °C and under 12:12 light/dark photoperiod (lights on 06.00 h).

### *Behavioral testing*

Young (5-10 day old) females were pre-tested in a circular arena (1.52 meters in diameter; Figure 2) with a speaker at the center of the arena projecting sound at 360° (Atkins et al. 2008). The crickets were given a 5-minute period to familiarize itself in the arena. Computer generated calling songs (CS) which match those of the conspecific male (frequency of 5kHz) with various SPs ranging from 30-90 milliseconds (ms) were played for 5 minutes in a non-sequential order (50, 90, 70, 30, 60, 80, 40 ms), with a 2 minute resting period in-between. The sound intensity was recorded as 85 dB around the midpoint between the speaker and the edge of the arena. The pathways of the females were then observed and video-recorded. The temperature and humidity of the arena were also recorded. Positive phonotaxis was determined as the cricket walked from the edge of the arena and continuously approached the speaker within one body length of the speaker. Cricket movement towards the speaker at angles greater than 60° relative to the speaker were not considered as positive phonotaxis (Atkins et al. 2008).

*Nanoinjection*

Following pre-test, crickets were mounted ventral side up on a wax block in order to perform incisions to expose the prothoracic ganglion. A nanoinjector (Drummond Nanoinjector II; Drummond Scientific Co., Broomall, PA) was used to inject 9.2 nL of $10^{-6}$M octopamine in saline solution into the prothoracic ganglion. After a 10-minute recovery period, phonotactic behavior was evaluated in the same manner as the pre-tests. Crickets were classified as either selective or unselective following the criteria established by Stout et al. (2010).

Females in the control group were injected with 9.2 nL of saline solution in the prothoracic ganglion and phonotactic behavior was measured in the same manner described above for the experimental group.

*Statistical analysis*

McNemar's Test was used to determine significance in phonotactic behavior before and after injection of octopamine in the experimental group. The same statistical analysis test was used to determine significance in phonotactic behavior before and after injection of saline in the control group.

## RESULTS

*Experimental group*

Twelve crickets were injected with 9.2 nl of $10^{-6}$M solution of octopamine (Table 1). According to the results of the pre-test, six crickets responded selectively to the preferred range (50-70 ms). Four of the six crickets also responded to CSs with a SP of 90 ms. Two of the six responded to CSs with a SP of 30 ms. One cricket responded to every other SP ranging from 30-90 ms. Six crickets responded with positive phonotaxis to six or all SPs presented. The number of SPs that elicited positive phonotaxis significantly decreased post-injection of octopamine (McNemar's Test, $P < 0.001$; Figure 3). Overall, crickets' responsiveness was decreased (Figure 5). Of the twelve crickets injected, three did not respond to any SP. Six responded to only one syllable period with no clear preference to a specific SP. Two crickets responded to two SPs, but with no clear trend for any specific SP. One cricket responded to all SPs except to a 90 ms call. In terms of phonotactic selectivity, before injection, crickets were attracted to 70 and 90 ms SP CSs. Post-injection, the SPs that were most attractive were 30 and 60 ms SP CSs. Overall, the trend in behavioral response as indicated by the SPs they responded to pre and post injection was similar, except for the response exhibited at 90 ms SP (Figure 5).

*Control group*

Twelve crickets were injected with saline solution in the control group. Seven of the twelve crickets that were pre-tested responded selectively. Five crickets responded unselectively, and all crickets responded within the attractive range of 50-70 ms SPs.

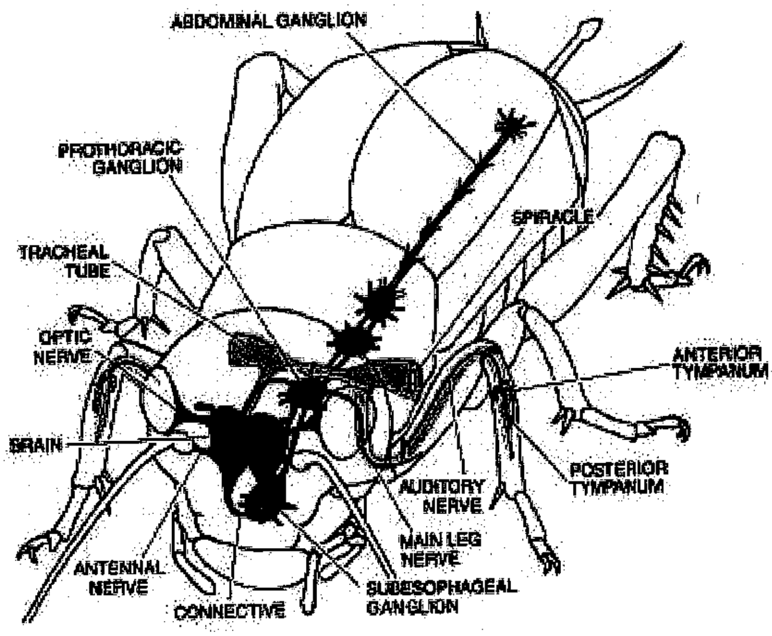


**Figure 1.** The figure above shows the neural circuit of the *A. domesticus,* starting from the brain to the abdominal ganglion. Of particular interest is the prothoracic ganglion which contains neurons which influence phonotaxis in crickets. Diagram provided by Dr. Navia.

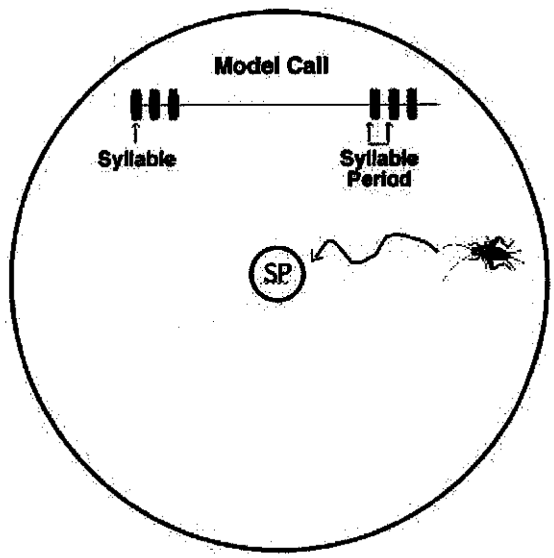


**Figure 2.** The figure above represents an example of a circular arena and a computer generated model call. Diagram provided by Dr. Navia.

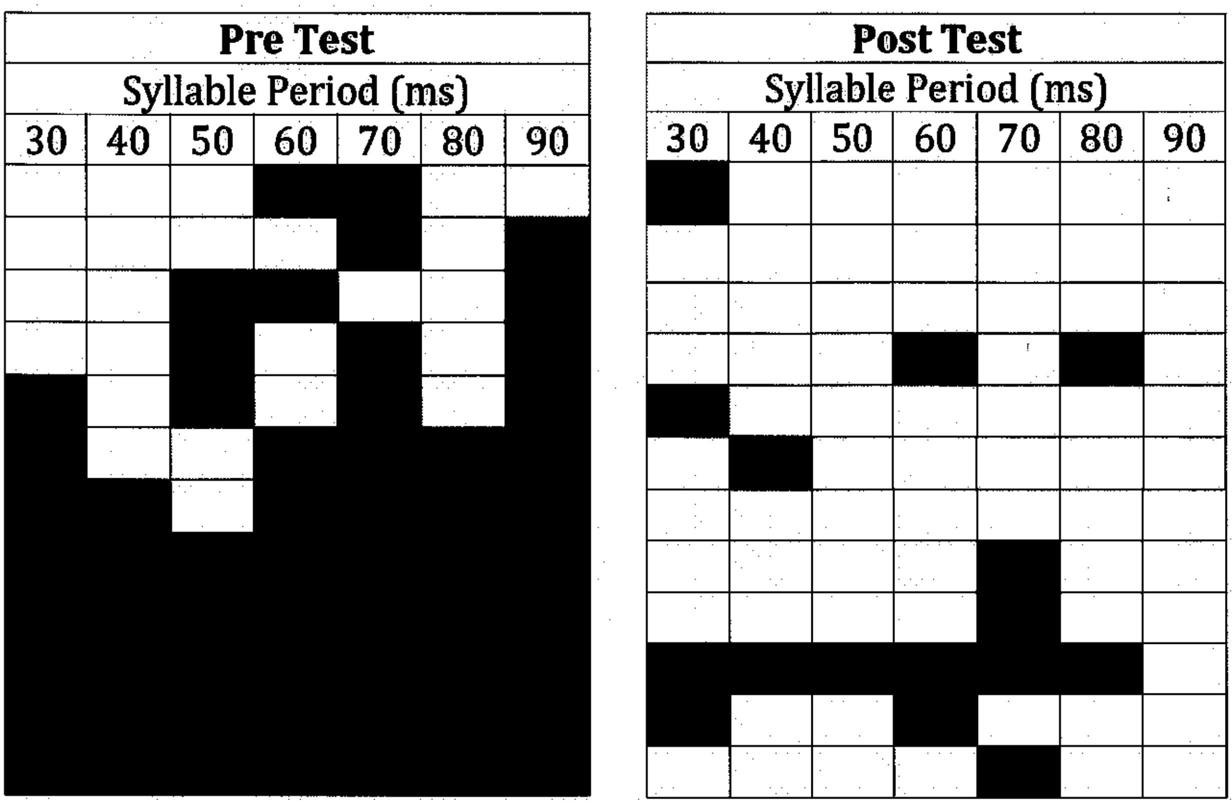


**Figure 3**: Tables above show the phonotaxis of female *A. domesticus* (n=12) in response to model calls with SPs ranging from 30-90 ms. Each row represents the phonotactic behavior of a single female cricket before and after injection of octopamine into the prothoracic ganglion. The shaded boxes indicate the SPs the females responded to. Results show (McNemar's Test) a significant reduction in the number of SPs that females prefer following injection of octopamine. ($P < 0.001$)

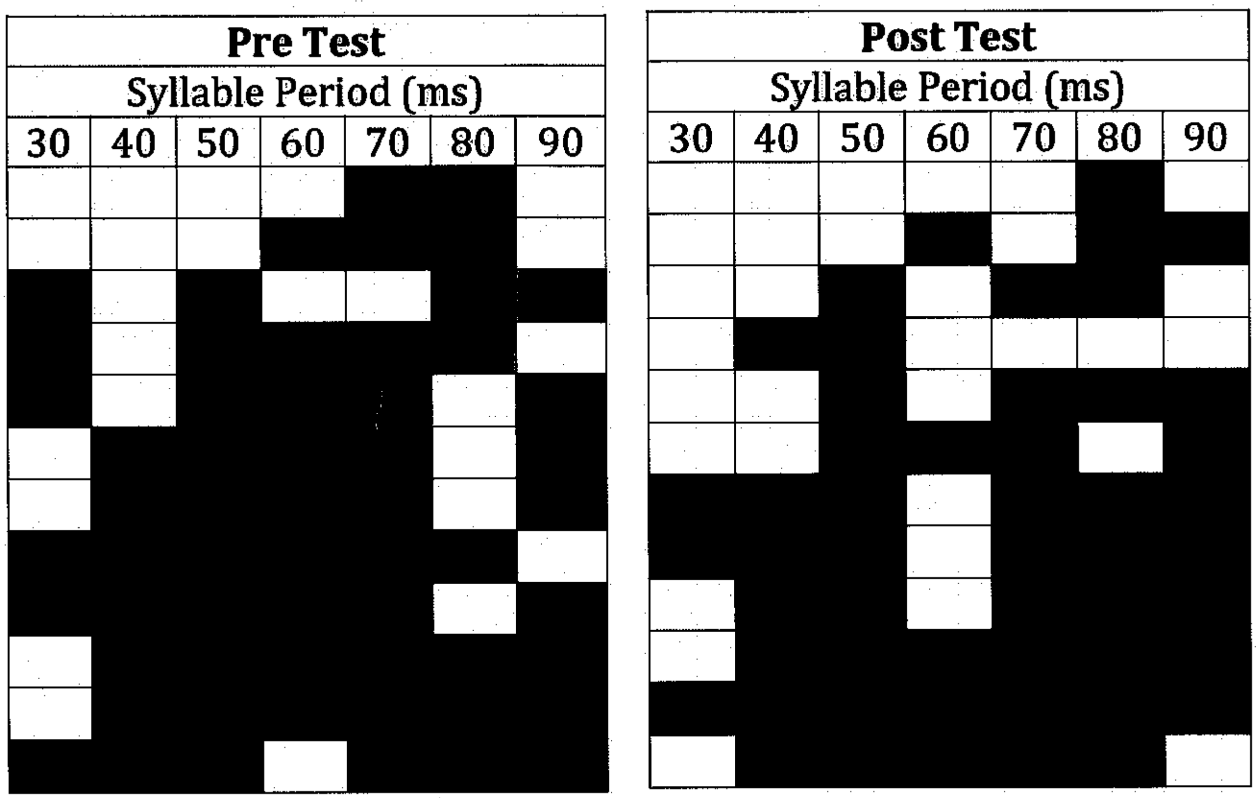


**Figure 4:** Tables above show phonotaxis of female *A. domesticus* (n=12) in response to model calls with SPs ranging from 30-90 ms. Each row represents the phonotactic behavior of a single female cricket in the control group. Results show (McNemar's Test) no significant difference in the number of SPs that females responded to before and after when undergone dissection only or when injected with a saline solution. (P = 0.170)

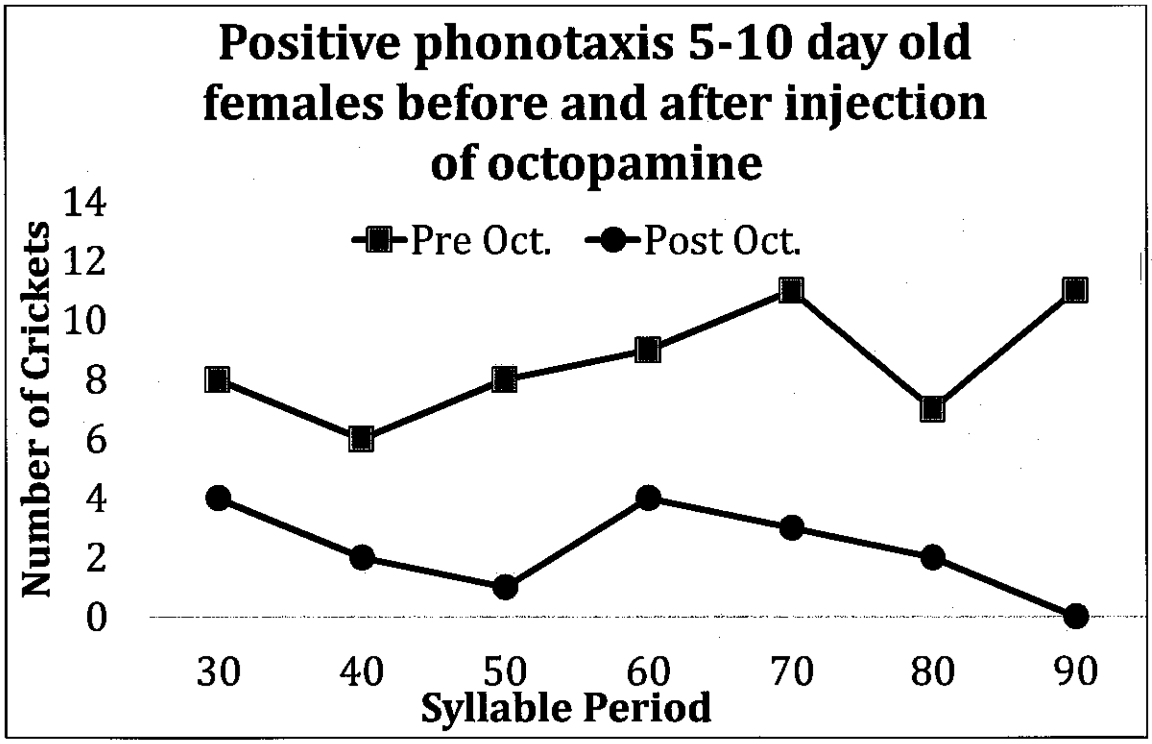


**Figure 5:** Graph above shows the phonotaxis of female *A. domesticus* (n=12) in response to SPs ranging from 30-90 ms. Each line represents the number of females that exhibited positive phonotaxis in response to a specific SP before and after injection of octopamine. Results illustrate the overall trend that octopamine reduces the likelihood of females respond phonotactically to each SP. The greatest reduction occurred in response to 50, 70 and 90 ms.

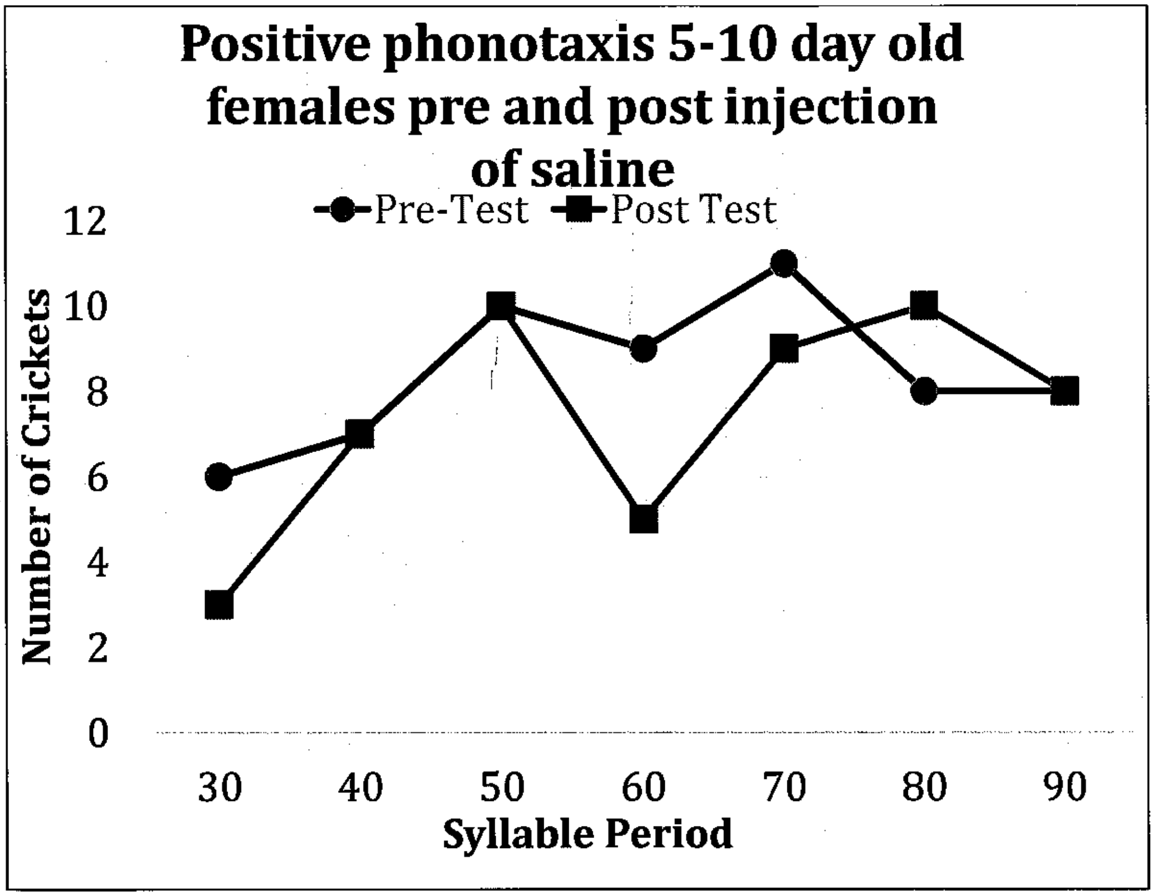


**Figure 6**: Graph above shows the phonotaxis of female *A. domesticus* (n=12) in response to SPs ranging from 30-90 ms. Each line represents the number of females that exhibited positive phonotaxis in response to a specific SP before and after injection of saline. Results illustrate the overall trend that the phonotactic behavior females do not change after injection of saline.