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Phonotactic Tuning in Female Cricket Acheta Domesticus

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Abstract

Phonotactic behavior of female cricket *Acheta domesticus* has been shown to vary among individuals. While some females are finely tuned to calls with syllable periods in the natural range of conspecific males, others respond phonotactically to a wider range of syllable periods and therefore lack the ability to discriminate between attractive and unattractive calls. When females are exposed to males but prevented from mating, their ability to discriminate attractive calls is reduced, suggesting that factors other than mating alter phonotactic behavior. This study evaluated the effect of male exposure on females' phonotactic tuning and responsiveness in relation to underlying neural elements. Results appear to contradict past findings that male exposure reduces females' ability to discriminate attractive calls but are consistent with previous research on the tuning of phonotaxis. Male-exposed females and unexposed females also differed in their phonotactic tuning at different sound intensities, suggesting that electrophysiological testing of the relevant neural elements may be employed for future studies investigating male exposure.

Introduction

Acoustic information is utilized by many members of the animal kingdom, such as marine mammals, bats, and frogs for a variety of purposes that includes sensing the environment, avoiding predators, and attracting mates (Hügel et al., 2017; Johnson et al., 2009; Gerhardt, 1994). Crickets, which have been used as models to study behavior and underlying neural mechanisms in sexual communication systems, use chirps to mate. In order to attract female crickets, male crickets create chirps by rubbing their wings together (Jacob & Hedwig, 2016). Female crickets then move towards or away from the sound in a process known as phonotaxis, with movement towards the source of sound classified as positive phonotaxis and movement away from the source of sound called negative phonotaxis (Doherty et al., 1985).

One of the main features of male cricket mating calls is the syllable period, which is the amount of time between the beginning of a sound pulse within a chirp and the consecutive sound pulse. Female crickets will exhibit positive phonotaxis and head towards the source of sound if they find a call with a certain syllable period attractive. Otherwise, they will not head towards the source of sound. In general, females prefer the syllable periods that conspecific males naturally produce and show selectivity, or discrimination, towards which syllable periods they respond to. In the house cricket *Acheta domesticus*, the most attractive range of syllable periods is 50 to 70 ms (Stout et al., 1983). When tested in the lab, females of this species show individual variability in the range and number of syllable periods towards which they exhibit positive phonotaxis. Internal and external variables also affect selectivity, including levels of Juvenile Hormone III (Atkins et al., 2008), age (Stout et al., 2010), and temperature (Navia et al., 2015).

At the neural level, certain neurons of the auditory system, such as the L1 and L3 interneurons, play a key role in phonotactic behavior. Most notably, the L3 neuron, which is located in the prothoracic ganglion and projects axons to the brain, has a unique pattern of response that also provides information about the quality of the male call (Atkins et al., 1989). At sound intensities below L3's threshold of 65 to 75 dB at 5 kHz (Atkins et al., 1992) but above L1's threshold of 45 to 50 dB at 5 kHz, L3 is not recruited and phonotaxis occurs without being particularly tuned to the most attractive range of syllable periods. At intensities above L3's threshold, phonotaxis appears to be tuned towards more attractive syllable periods.

Previous research by An (2021) on the effect of male exposure on female *A. domesticus* (where females and males were kept in close proximity to each other but prevented from mating) suggested that male exposure makes young females less selective compared to unexposed virgin females. Females were considered to be selective or unselective, with selective crickets showing positive phonotaxis to five or less syllable periods, as defined by Stout et al. (2010). This study also investigates the effect of male exposure on young female crickets (5 to 10 days old), while additionally evaluating phonotaxis at sound intensities above and below L3's threshold. The hypothesis that male exposure makes young females less selective, or rather, more likely to show positive phonotaxis to a greater number of syllable periods will be examined. This study also explores whether L3's threshold is affected by male exposure, by using behavioral testing at different sound intensities and comparing patterns of phonotactic responses at these decibel levels.

Materials and Methods

Cricket care

Each group of 250 four-week-old *A. domesticus* nymphs obtained from Fluker Farms, Inc (Baton Rouge, LA) was placed in 100-L containers along with cricket feed, water, and egg cartons. These containers were stored in a growth chamber at 22-24 ºC in a 12-hour light-dark cycle (with lights on at 06:00 hr). Crickets were sorted regularly for males and females, with females and males placed in 16-L containers and in separate growth chambers.

Exposing female crickets to males

Young adult crickets were placed in a plastic container with a perforated divider in between females and males, so that females were exposed to males through pheromones and chirps but prevented from mating. Females were exposed to males for 3 to 4 days, so that they were 5 to 10 days old by the time of testing phonotactic behavior.

Behavioral testing

The setup for testing phonotaxis used by Samuel et al. (2013) was utilized in this study. Female crickets were first attached to metal pins with melted wax on the pronotum. Then, they were mounted onto a metal rod that held them in place on top of a non-compensating, spherical Styrofoam ball. Air underneath the ball allowed the treadmill to move freely as crickets moved, with two optical mice tracking the movement. The speaker and treadmill setup were kept in a dark, soundproof box at a temperature of 20-23 ºC.

Before testing, crickets were given 5 minutes to adjust to testing conditions. Then, computer-generated model calls of syllable periods ranging from 30 to 90 ms were played non-sequentially using the computer program Audacity. The model calls consisted of three 25-ms sound pulses at 5 kHz and played at 60 dB and 85 dB for each cricket, with the order in which the different sound intensities were played being balanced. The calls were also calibrated so that the sound intensities were 60 and 85 dB at the location of the cricket on the treadmill, rather than the speaker. Each cricket's movement was recorded for 5 minutes during a call, with 3 minutes of rest in between syllable periods. All syllable periods were tested at one sound intensity before switching to the other sound intensity, with a 10-minute break between the two sets of calls at different sound intensities. Positive phonotaxis towards a call with its corresponding syllable period was classified as having an average angular orientation ranging from -60 to 60 along with path length towards the speaker that was at least twice the path length away from the speaker (Samuel et al., 2013). These measurements were taken using the program Optical Kugel.

Statistical analysis

When analyzing data, the number of syllable periods to which crickets showed positive phonotaxis was compared. A Mann-Whitney U test was used to compare differences between exposed and unexposed groups in how many syllable periods the cricket responded positively to, at the same sound intensity, as well as a Wilcoxon Signed-Rank test to compare differences between decibel levels, within the same group. A Mann-Whitney test will also be used to determine whether the order in which the sound intensities were changed had an effect on the number of syllable periods responded positively to.

Results

A total of 40 crickets were tested, but crickets that did not respond phonotactically to any syllable period at both sound intensities were excluded from analysis. This resulted in 37 crickets (5 to 10 days old) that were used for analysis, with 19 unexposed females and 18 male-exposed females. Individual females varied in the number of syllable periods that elicited positive phonotaxis, ranging from zero to all seven syllable periods (Figure 1). A Mann-Whitney U test showed no significant difference between unexposed (*Md* = 3, n = 19) and exposed females (*Md* = 5, n =18) at the 60 dB level, $U = 235.000$, $z = 1.965$, $p = .053$, $r = .323$. There was also no significant difference between unexposed (*Md* = 4, n = 19) and exposed (*Md* = 4, n =18) females at the 85 dB level, $U = 192.000$, $z = .646$, $p = .538$, $r = .106$. Overall, there was no significant difference in the number of syllable periods that elicited positive phonotaxis between unexposed and male-exposed females, at either sound intensity.

When the number of syllable periods responded to positively was compared between 60 dB (*Md* = 3) and 85 dB (*Md* = 4) for unexposed females (n = 19) using a Wilcoxon Signed-Rank test, there was no significant difference, $z = .885$, $p = .376$, $r = .144$. Similarly, for exposed females (n = 18), there was no significant difference in responses, $z = -1.224$, $p = .221$, $r = .204$ between 60 dB (*Md* = 5) and 85 dB (*Md* = 4). The number of crickets that displayed positive phonotaxis at each syllable period are shown in Figures 2 and 3. Figure 2 shows the pattern of responses for unexposed females, which had the highest number of crickets with positive phonotaxis for model calls at 60 ms and 60 dB. At the 85 dB level, model calls with a syllable period of 50 ms most prompted positive phonotaxis for unexposed females. While there was a marked preference for the model calls of a 50-ms syllable period at 85 dB, the preference for syllable periods ranging from 50 to 90 ms was similar at 60 dB. Overall, there appeared to be a tuning of phonotaxis towards the 50 ms syllable period above the threshold of L3. For male-exposed females, 70 ms elicited the most positive phonotactic responses at 85 dB, while there was a preference for 30 ms at 60 dB (Figure 3). The most attractive range of syllable periods was 60 to 80 ms at 85 dB, with the 50 ms syllable period at a close fourth in order of preference. At 60 dB, there appeared to be no particular preference for a specific range of syllable periods; in general, phonotaxis seemed to be tuned towards the 60 to 80 ms range above the L3's threshold (Figure 3).

Additionally, there was no significant difference between playing decibel levels in the order of 60 to 85 dB (*Md* = -.5, n =18) and 85 to 60 dB (*Md* = 0, n = 19) in the change in number of syllable periods responded to, $U = 168.500$, $z = -.077$, $p = .940$, $r = .013$. This indicated that the order in which the sound intensities were tested did not influence the results.

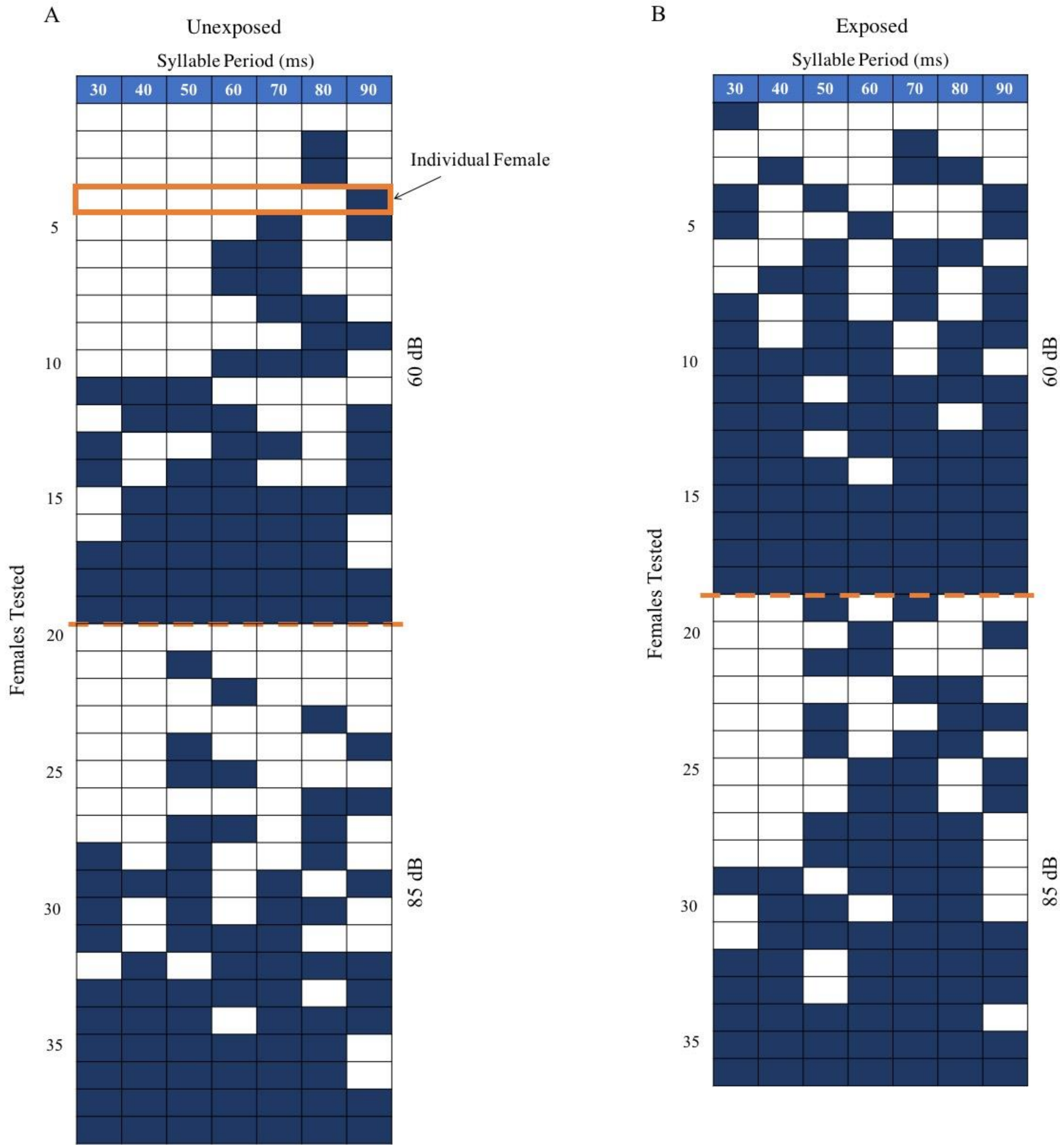

Figure 1. Phonotactic Choices of Unexposed and Exposed Female Crickets at 60 dB and 85 dB. Tables A and B show the phonotactic responses of 37 females, with 19 unexposed crickets (A) and 18 exposed crickets (B). Each column refers to the syllable period of the model call, and each row represents an individual cricket. Colored cells indicate positive phonotaxis for the model call at the specific syllable period. Rows above the orange line represent crickets tested at 60 dB, while rows below the line represent crickets tested at 85 dB.

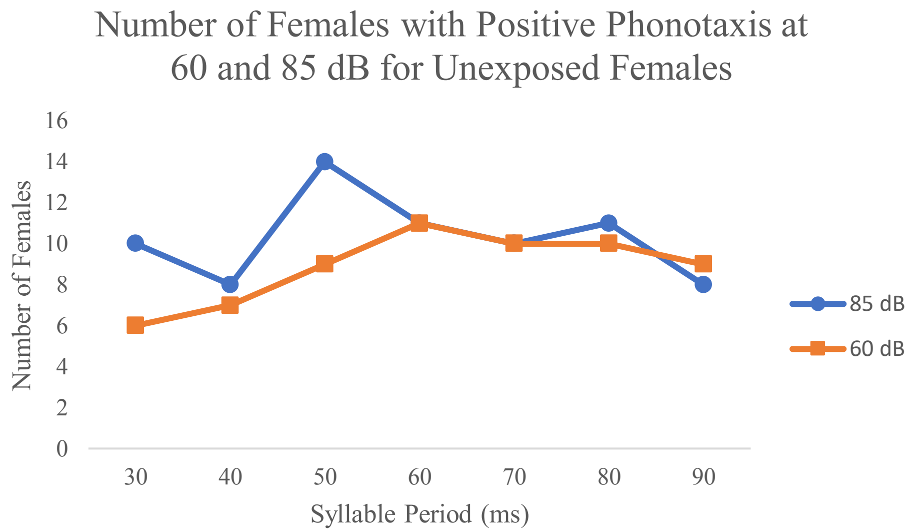

Figure 2. Phonotactic Tuning at 60 dB and 85 dB for Unexposed Females. The most unexposed females responded to model calls with a 60 ms syllable period at 60 dB, while they were particularly tuned to 50-ms model calls at 85 dB.

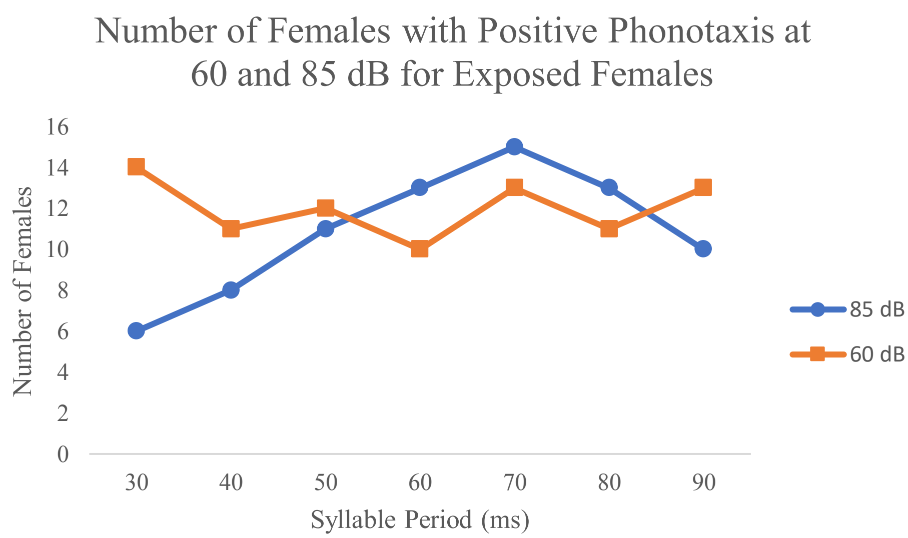

Figure 3. Phonotactic Tuning at 60 dB and 85 dB for Male-exposed Females. The most male-exposed females responded to 30-ms model calls at 60 dB, while they were particularly tuned to the range of 60 to 80 ms at 85 dB.

Discussion

The lack of significant difference between unexposed and exposed females in the number of syllable periods towards which they exhibited positive phonotaxis, at both 60 dB and 85 dB, disagrees with previous findings that male-exposed females are less selective (responds positively to more than 5 syllable periods) than unexposed females (An, 2021). This discrepancy may be due to utilizing the number of syllable periods that females responded positively to, rather than categorizing females as selective or unselective, and choosing an appropriately different statistical test (a Mann-Whitney U test in this study). The lack of significance ($p = .053$) between unexposed and exposed females at 60 dB is also likely due to the small sample size used in this study. There was, however, a medium effect size ($r = .323$) at the 60 dB level. This was also greater than the small effect size when unexposed and exposed females were compared at 85 dB ($r = .106$), which suggests that male exposure may affect phonotaxis at 60 dB more than it does at 85 dB.

Moreover, females did not respond more to one decibel level over the other during testing, showing that there were no changes in responsiveness whether females were tested below or above L3's threshold. This suggests that recruiting the L3 does not modify overall responsiveness to calls. However, when examining the preference of certain syllable periods by counting the number of females that responded to calls of each syllable period, unexposed females showed a marked tuning towards 50 ms at 85 dB and a relatively stable level of preference for the 50 to 90 ms range. This appears to be consistent with the expectation that females become more tuned to the 50 to 70 ms range as the sound intensity exceeds L3's threshold (Atkins et al., 1992). For exposed females, there is no particular pattern of preference at 60 dB, although the syllable period that was most responded to was 30 ms. Preference for the

60 to 80 ms range at 85 dB was also observed, which is also consistent with expectations for the tuning of phonotaxis towards more attractive syllable periods at 85 dB. Most importantly, since the patterns of response to certain syllable periods do not appear to be the same for unexposed and exposed females, it is unclear whether male exposure directly impacts L3's threshold. Because we cannot rule out the possibility that L3's threshold is affected by male exposure, future investigations utilizing electrophysiological testing is recommended.

This study provides further clarity on the observations of An (2021) by showing that male exposure does not affect the number of syllable periods that females respond to. By using a Mann-Whitney U test to compare means between unexposed and exposed groups, differences in responsiveness to calls may be more apparent, in comparison to using a Chi-squared test with fixed, dichotomous classifications for selectivity. To elucidate whether the findings in this study is consistent with previous research, access to the data for females tested in An (2021) and statistical analysis using a Mann-Whitney U test would be required. Since this study also tested crickets at 60 dB, there may be other factors that contribute to deviations from An's data. These include possible fatigue due to longer periods of testing and the effect of testing at 60 dB first on data for 85 dB. However, the effects of longer testing was attempted to be mitigated by the 10 minutes of rest in between decibel levels. The order in which decibel levels were tested did not influence the number of responses to different calls. Temperature was also held relatively constant and within a similar range, at 20 to 23 ºC when testing. One notable difference, however, is the length of exposure and the timing of exposure, as the previous study by An exposed females to males for three or more days, starting when females were one to two days old. In this study, females were consistently exposed for 3 to 4 days, but the timing of exposure varied, since females were exposed until they were anywhere from 5 to 10 days old. Since the condition of male exposure is a relatively new focus of study, the length and timing of exposure has not been yet standardized or investigated. Future studies may look at these parameters and determine the length and timing of exposure that results in observable changes in phonotaxis, as well as whether length or timing is more influential than the other.

Since females were subjected to other factors such as pheromones and sound through male exposure, future research may also aim to isolate these factors and quantify their effect on phonotaxis. The effect of age, which inevitably increases during the period of exposure and has been shown to influence phonotactic selectivity, may also be controlled. Lastly, other obtained data such as the length of the path females walk towards the source of sound may be statistically analyzed to provide further insight and detail as to how the responsiveness of females towards syllable periods is modulated by factors such as male exposure.

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