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Light Intensity and Time of Day Influence Female Acheta Domesticus Phonotaxis

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Abstract

Positive phonotaxis occurs when a female cricket walks toward the source of an auditory stimulus. This study tested the effects of light intensity and time of day on phonotaxis of female *Acheta domesticus*. First, females were tested at four times of day; second, under three light intensities; and third, under four combinations of light intensity and time of day. Our results show that these two factors combined exert a more pronounced effect on female *A. domesticus* selectivity than either factor individually, and subsequent research will test these factors against the current neuronal model for SP-selective phonotaxis.

Introduction

Positive phonotaxis occurs when a female cricket locates and walks toward the source of an auditory stimulus that has the characteristics of the conspecific male's call. Years of research on the phonotactic behavior of female *Acheta domesticus* crickets has culminated in a model for syllable period (SP)-selective phonotaxis that posits the L3 auditory neuron as the selective filter in the prothoracic ganglion (Atkins *et al.*, in preparation).

Two L3 neurons reside in the prothoracic ganglion. Application of juvenile hormone III (JHIII) onto the surface of the prothoracic ganglion was associated with an increase in phonotactic selectivity amongst female *A. domesticus* (Stout *et al.* 2002); photoinactivation of one of the L3 neurons resulted in errors in angular orientation during female phonotaxis (Atkins *et al.,* 1992); and injection of picrotoxin (PTX) into the prothoracic ganglion resulted in increased phonotactic selectivity similar to that of JHIII injection (Atkins *et al.*, 2008). All of these experiments manipulated endogenous factors in an attempt to modify the behavior of the cricket in order to test the model proposed by Atkins *et al.* (in preparation).

To facilitate a similar investigation with novel exogenous factors, the present study investigated the effects of light intensity and time of day on *A. domesticus* female phonotaxis. Our results provide evidence demonstrating that these two factors significantly affect female selectivity. Our experiments indicate that variation in time of day, independent of light intensity, does not significantly influence female selectivity; however, variation in light intensity alone does elicit a significant change in female selectivity. Furthermore, by combining light intensity and time of day, we have demonstrated that the two factors together exert a more pronounced effect on female selectivity than either factor does individually.

Methodology

Cricket care

Five-week-old nymphs of *A. domesticus* were purchased from Fluker's Cricket Farm (Baton Rouge, LA). Males and females were raised together as nymphs in 16-L plastic containers at 22-24 °C in the same room. Egg cartons were included for shelter, and cricket chow (Fluker's Cricket Farm, Baton Rouge, LA) and water were supplied daily. The nymphs were raised in artificial lighting that maintained a 12:12 hr light:dark cycle; the exact hours for the light:dark cycle were determined based on amenability to testing. The crickets were sorted daily. Adult females were removed from the nymphs and housed in separate 16-L plastic containers (maximum of 30/container) supplied with food, water and shelter as described above, and adult males were discarded. All crickets were housed in the same room as the nymphs.

Phonotaxis testing

Adult virgin female crickets of known ages were tested for phonotactic selectivity in a circular sand-bottomed arena (152 cm in diameter) with a centrally located, omnidirectional speaker facing upwards (Atkins, *et al.,* 2008; Stout *et al.*, 2010). The light intensity in the arena varied depending on the test being conducted as described above. The arena was located in an insulated box (183 cm long x 183 cm wide x 124 cm high), which was contained in a walk-in climate-controlled chamber. The edge of the arena was surrounded by a 10-cm transparent plastic border that was inclined towards the center. A camera fixed above the arena allowed for visualization of the crickets' path. The video image was displayed on a computer screen, from which the pathways were traced onto clear plastic sheets. Seven computer-generated model calling songs, which had a SP ranging from 30-90 ms in increments of 10 ms, were played from the centrally located speaker in standard, non-sequential order. The calling songs had three syllables per chirp, a carrier frequency of 5 kHz, a pulse duration of 25 ms (5 ms rise and fall times), and a chirp period of 666 ms (Atkins *et*

al., 2008; Stout *et al.*, 2010). The sound intensity was calibrated 10 cm in from the edge of the arena at 85 dB sound pressure level (SPL) and did not vary more than ± 2 dB along the edge. Each calling song played for a maximum of 5 minutes; however, and the calling song was terminated earlier if the cricket reached within one body length from the speaker before 5 minutes had passed. Each bout of calling song was followed by 3 minutes of silence. Positive phonotaxis was defined as movement from the edge of the arena approaching one body length of the speaker, not walking at angles greater than 90° relative to the speaker (Atkins *et al.,* 2008; Stout *et al.,* 2010). Up to four crickets were tested simultaneously (Atkins *et al.,* 2008; Stout *et al.,* 2010).

Experimental conditions

My project consisted of three distinct experiments that related to each other in a sequential fashion.

1. Experimental conditions for testing different times of day

First, I addressed the effect of four different times of day on phonotactic selectivity. The four times of day were as follows: dawn, noon, dusk, and midnight. All of these time periods related to the 12:12 light:dark cycle. Dawn included the first three hours after the lights turned on; noon included 4.5-7.5 hours after the lights turned on; dusk included the first three hours after the lights turned off; and midnight included 4.5-7.5 hours after the lights turned off. Tests were conducted within a 3 hour interval for each of the four time periods in the arena under standard light intensity (i.e. 38 lux). The sample size for each group was at least 20 crickets.

2. Experimental conditions for testing different light intensities

Second, I investigated the effect of three different light intensities on phonotactic selectivity. The three different light intensities were as follows: bright, normal, and red. Bright light measured at 510 lux; normal light measured at 38 lux; and red light measured at 3 lux. In a study conducted by Shaefer and Wilkinson (2004), *A. domesticus* did belong to any of the insect orders that were

identified as having red-light sensitive receptors, and no evidence yet exists that *A. domesticus* can detect red light. Hence, red light simulated dark conditions for *A. domesticus*, while still allowing for visualization of the cricket. On average, the crickets were tested at 09.30h±2. The sample size for each group was 20 crickets.

3. Experimental conditions for testing the combination of different times of day with different light intensities

Third, I tested the effect of bright and red light intensities on phonotaxis at noon (±1.5 hours) and midnight (±1.5 hours). Both the light intensities and times of day were described above. Noon and midnight represented the extremes for time of day, and bright and red light represented the extremes for light intensities. In essence, I tested two types of conditions. The first condition examined phonotaxis under the expected light intensity at a particular time of day (i.e. bright light at noon and red light at midnight). The second condition examined phonotaxis under a light intensity that was opposite of the expected for a particular time of day (e.g. bright light at midnight and red light at noon). The sample size for each group will be 20 crickets.

Statistical Analysis

For each of the three pats of this experiment, I ran an analysis of variance (ANOVA) test to determine if there were significant changes in phonotactic behaviour. For the second and third parts of this experiment (i.e. combination of time of day and light intensity), I ran post hoc two-tailed paired T-tests based on the significant results from the ANOVA.

Results

Tests for Time of Day

For each of the four times of day, 20-21 females (6-7 days old) were tested. These females demonstrated different levels of selectivity to male SPs—ranging from females that responded to no SPs (Dusk; Fig. 1c, top three rows) to females that responded to all seven SPs (Dusk; Fig. 1c, bottom two rows). On average, females tested at dawn responded to 4.43 SPs; females tested at noon responded to 4.09 SPs; females tested at dusk responded to 3.62 SPs; and females tested at midnight responded to 4.65 SPs. In other words, female selectively increased on average by 1.03 SPs between midnight and dusk; 0.81 SPs between dawn and dusk; and 0.47 SPs between noon and dusk. For all four time groups, SPs within a range of 50-70 ms were generally chosen most frequently by females (Fig. 1e); the one exception was the group of crickets tested at dawn that responded more frequently to the SP of 90 ms than the SP of 50 ms. Overall, the female selectivity among the four time groups did not differ significantly (ANOVA, $p= 0.307$).

Tests for Light Intensity

For each of the three light intensities, 20 females (3-5 days old) were tested. SPs within a range of 50-70 were chosen most frequently by females for all three light intensity groups (Fig. 2d); however, females tested in the normal light intensity responded more frequently to the 80 ms SP than to the 50 ms or 60 ms SP. Furthermore, the 70 ms SP was chosen most frequently for all groups, and the SP of 60 ms was chosen least frequently within the 50-70 ms range. On average, the females tested in the red light intensity responded to 3.6 SPs; the females tested in the normal light intensity responded to 4.95 SPs; and the females tested in the bright light intensity responded to 4.55 SPs.

The selectivity of females in the three light intensity groups differed significantly (ANOVA, $P= 0.049322$). The difference between the selectivity of females tested in the red light intensity and

of females tested in the normal light intensity was significant (paired t-test, p=.015). Conversely, the difference between the selectivity of females tested in the red light intensity and of females tested in the bright light intensity was not significant (paired t-test, p=.09). Additionally, the difference between the selectivity of females tested in the normal light intensity and of females tested in the bright light intensity was not significant (paired t-test, p=.49).

Tests for Combination of Time of Day and Light Intensity

Twenty females (3-5 days old) were tested for each of the four groups. SPs within a range of 50-70 were chosen most frequently by females for all three light intensity groups (Fig. 3e). Also, the SP of 60 ms was chosen least frequently within the 50-70 ms range. On average, the females that were tested at noon in the bright light intensity responded to 5.3 SPs; the females that were tested at noon in the red light intensity responded to 3.6 SPs; the females that were tested at midnight in the bright light intensity responded to 5.15 SPs; and the females that were tested at midnight in the red light intensity responded to 4.25 SPs.

The selectivity of females in the four groups differed significantly (ANOVA, P = 0.000835). More specifically, the results of the six possible post-hoc t-tests are as follows. The selectivity of females that were tested at noon in the bright light intensity and of females that were tested at noon in the red light intensity differed significantly (paired t-test, p=0.0002). More specifically, the females that were tested at noon in the bright light intensity were less selective than the females that were tested at noon in the red light intensity. Conversely, the selectivity of females that were tested at midnight in the bright light intensity and of females that were tested at midnight in the red light intensity did not differ significantly (paired t-test, p=0.076). However, the relationship between these two groups mirrored the relationship between the two groups at noon because the females that were tested at midnight in the bright light intensity responded to more syllables than the females that were tested at midnight in the red light intensity. The selectivity of females tested at noon in the bright

light intensity did not differ significantly (paired t-test, p=0.689) from the selectivity of females tested at midnight in the bright light intensity. The selectivity of females that were tested at noon in the red light intensity and of females that were tested at midnight in the red light intensity did not differ significantly (paired t-test, p=0.223). The selectivity of females that were tested at noon in the bright light intensity and of females that were tested at midnight in the red light intensity differed significantly (paired t-test, p=.028). Also, the selectivity of females that were tested at noon in the red light intensity and of females that were tested at midnight in the bright light intensity differed significantly (paired t-test, p=.001).

Discussion

The results indicate that the combination of the exogenous factors of light intensity and time of day affect female *A. domesticus* selectivity to a greater degree than either one of these two factors alone. Our experiments demonstrated that female selectivity can change significantly depending on light intensity (Fig. 2a-c), and our post-hoc T-tests further indicate that the degree of brightness in a lit environment may influence female selectivity. Furthermore, cricket phonotaxis is affected by varying light intensity during daytime but not during night time (Fig. 3a-d). Given that typical light conditions at night do not naturally vary as widely as they can during the day, a possible explanation for our results is that the circadian rhythm of *A. domesticus* females allows for a more variable response during the day than at night. Since the light intensity can vary during daytime depending on various factors such as the location of the female and the weather conditions, the females' responses might be programmed for greater variation during daylight hours.

Our results carry several implications for past research on *A. domesticus*. First, variability in the testing times between and within different research projects should not significantly alter results (Fig. 1a-d). Nevertheless, because our data did vary between different testing times, past researchers who performed experiments at different times of day may have experienced noise in their results, and future researchers might observe more accurate data if they perform tests at standard times. Second, because research in the arena takes place at the same light intensity for all experiments, our results do not invalidate the results of results from past arena testing (Fig. 2a-c). Furthermore, while we recommend that future researchers choose a standard time for testing, our results indicate that researchers could theoretically perform identical tests at midnight and at noon and obtain yield results that are not significantly different in the arena. At the same time, the resolution of our results is not very precise because we only tested two opposing light intensities at two opposite times of day (Fig. 3a-d). Thus, future researchers should determine how much the light intensity can vary at noon

without causing significantly different results. Also, although running identical tests at different times of day did not produce significantly different results, future research should work to increase the resolution of our current results by testing times between noon and midnight at the bright and dark light intensities.

Our results appear to contradict the results of Stout et al. (1987), but these contradictions most likely stem from differences in experimental methods. First, our tests occurred in the arena, while Stout *et al.* (1987) conducted their tests solely in the compensatory treadmill. Also, Stout *et al.* (1987) noted that the range of attractive SPs for females responding on the treadmill in the dark was more than double the attractive SP range in the arena Stout *et al.* (1983). This observation suggests that the visual environments of the lit arena and lit treadmill affect female selectivity in divergent manners. Second, the arena is visually-homogenous, while the treadmill used by Stout *et al.* (1987) contained structures that might inadvertently affect cricket phonotaxis. These structures may not have necessarily appeared attractive to the females; nonetheless, a study by Atkins *et al.* (1987) demonstrated that unattractive targets in a treadmill cause *A. domesticus* females to orient randomly with no net directionality in their scototactic response. Thus, in the study by Stout *et al.* (1987), the scototactic response of the females in the lit treadmill may have artificially made them appear more selective in comparison to females on the dark treadmill. Third, our tests controlled for light intensity and time of day, while Stout *et al.* (1987) did not explicitly control these two exogenous factors. Assuming that most of the testing by Stout *et al.* (1987) occurred during the day, our results suggest that a significant result will emerge based solely on differences in the combinations of light intensity and time of day between the arena and treadmill(Fig. 3a-d).

Overall, these results highlight two exogenous factors to consider when designing future projects involving *A. domesticus*. Our results carry implications for projects that utilize both the arena and compensatory treadmill. Typically, the arena is lit, while the compensatory treadmill is dark.

Thus, the results from these two testing procedures for the same experiment should differ significantly (Fig. 3a-d). However, in a comparison of a lit arena and an unlit treadmill, Stout *et al.* (2010) suggested that the results from both testing procedures were similar enough to conclude that *A. domesticus* selectivity is not significantly affected by differences in the experimental conditions of the arena and treadmill. This discrepancy between the indirect implications of our results and the direct observations of Stout *et al.* (2010) may exist due to the resolution of our results; Stout *et al.* (2010) tested a SP range of 30-90 ms at 20 ms increments, while we tested that same range at 10 ms increments. To minimize the risk of erroneous results, future researchers should consider the lighting intensity and time of day when comparing, modelling, or testing phonotactic selectivity in the arena and treadmill.

Also, continued investigation into the effect of light intensity and time of day on female selectivity should further our understanding of the model for syllable period-selective phonotaxis as proposed by Atkins *et al.* (in preparation). In this model, the L3 auditory neuron in the prothoracic ganglion functions in SP recognition. More specifically, the level of SP-selective decrement in the L3 correlates with the level of female selectivity, and the identity of the neurons that influence the decrement in L3 is not yet known. Also, this model identifies the ON1 neuron as an inhibitor of the L3. Although Atkins *et al.* (in preparation) based this model predominantly on research that examined the effect of endogenous factors (e.g. levels of JHIII inside the female) on female phonotaxis, recent research indicates that this model also accurately predicts the effect of exogenous factors on female phonotaxis. For example, Navia *et al.* (submitted) measured the effect of temperature on female phonotaxis and on the auditory responses of the L3 neuron, and it was discovered that variation in temperature changed the peak in selectivity of the L3's response and shifted the SPs that elicited positive phonotaxis in a parallel manner. Our results indicate that both a combination of these factors and light intensity, alone, should affect the response of L3 in a way that

parallels changes to female phonotactic selectivity. Thus, the effect of the exogenous factors of time of day and light intensity can test the current model further.

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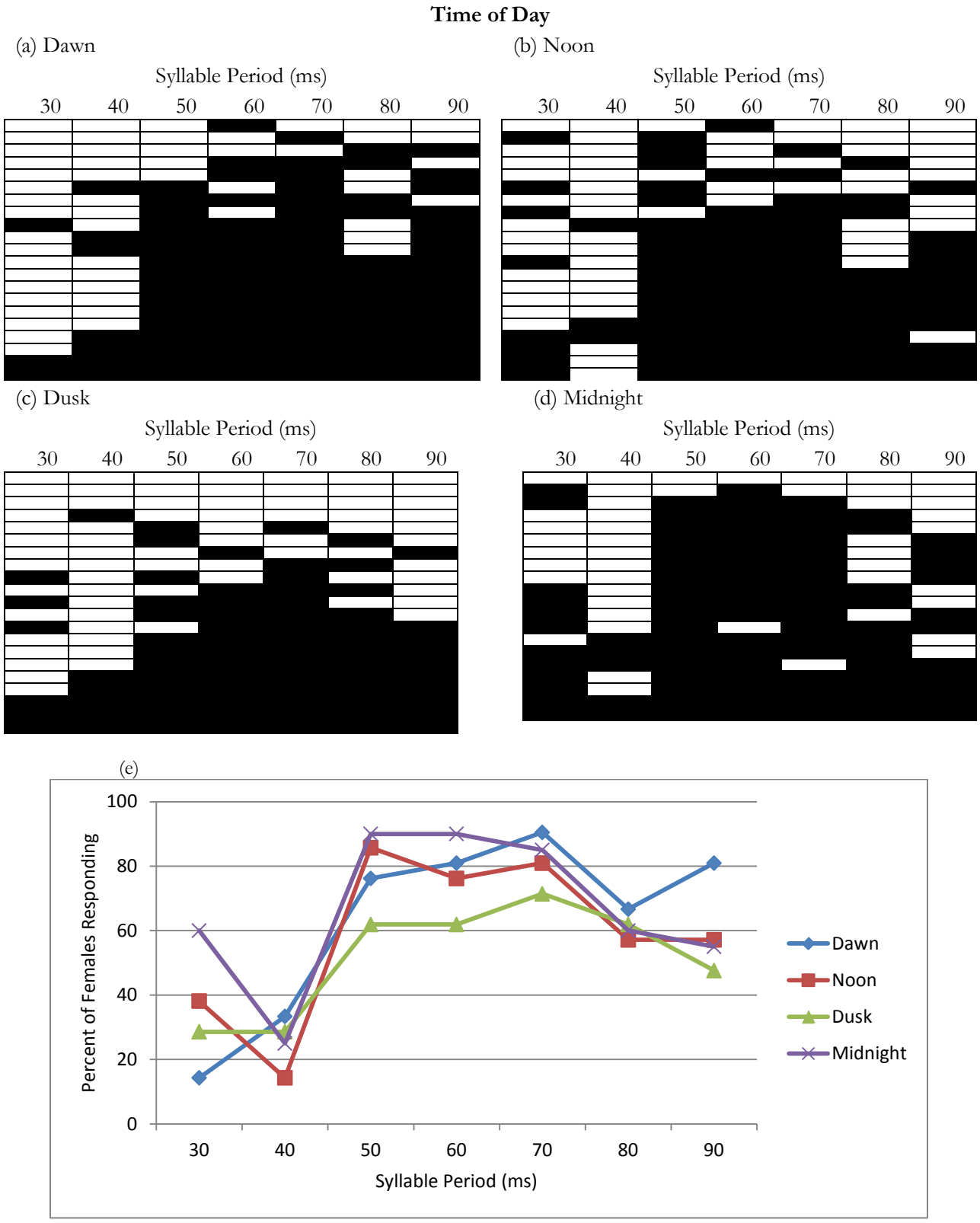

Figure 1. Effect of time of day on phonotaxis. (a-d) Tables showing positive phonotaxis (shaded boxes) of 20-21 females in response to syllable periods (SPs) that range from 30 to 90 ms. Each column represents a SP, and each row represents a female cricket. (e) Graphs showing the percentage of females that responded for each time of day.

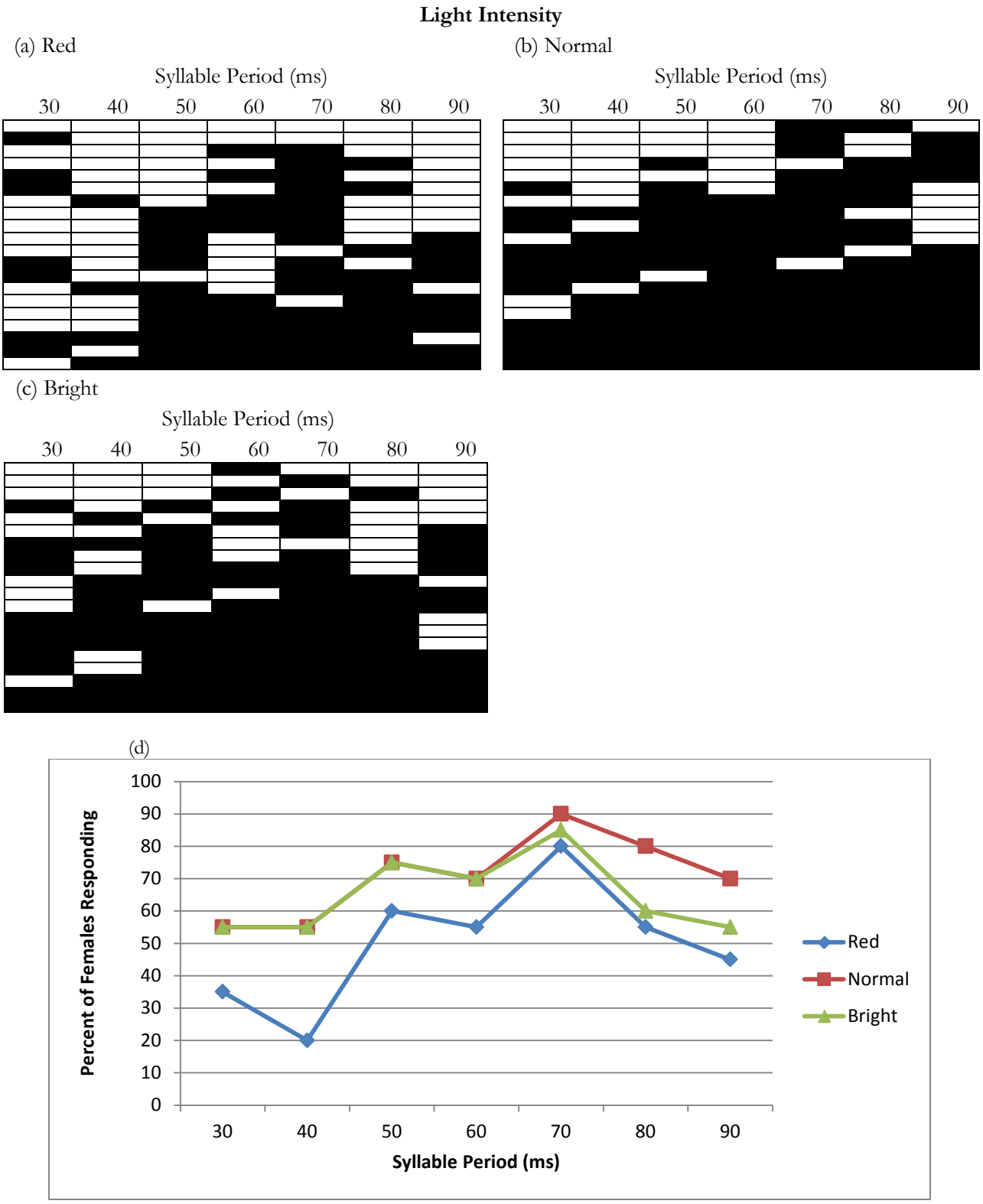

Figure 2. Effect of light intensity on phonotaxis. (a-c) Tables showing positive phonotaxis (shaded boxes) of 20-21 females in response to syllable periods (SPs) that range from 30 to 90 ms. Each column represents a SP, and each row represents a female cricket. (d) Graphs showing the percentage of females that responded for each time of day.

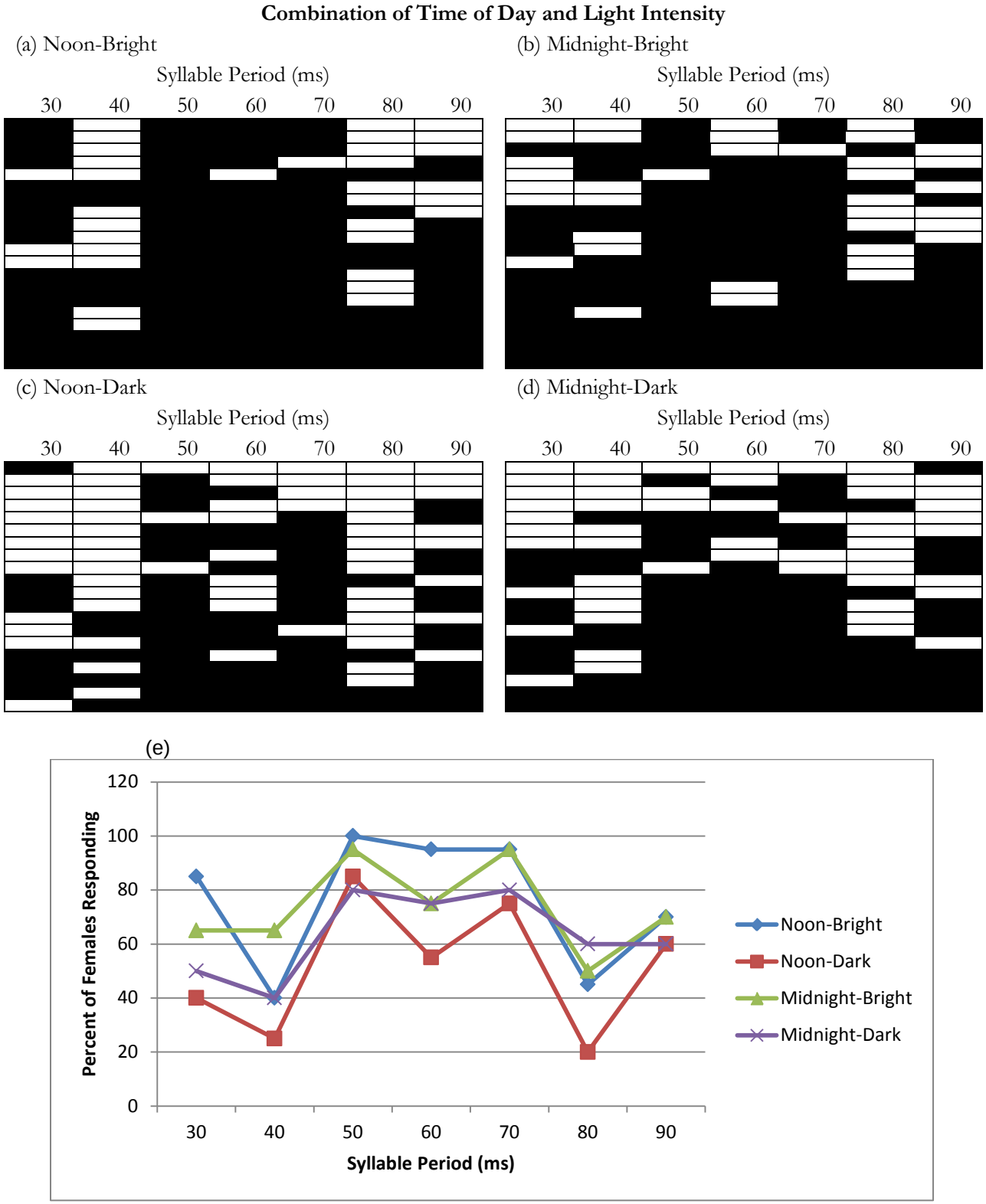

Figure 3. Effect of light intensity and time of day on phonotaxis. (a-d) Tables showing positive phonotaxis (shaded boxes) of 20-21 females in response to syllable periods (SPs) that range from 30 to 90 ms. Each column represents a SP, and each row represents a female. (e) Graphs showing the percentage of females that responded for each time of day.