Acoustic Attraction of the Parasitoid Fly *Ormia ochracea* to the Song of its Host

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Summary: The parasitoid fly *O. ochracea* locates its cricket host by acoustically detecting its calling song. The phonotactic abilities of the fly's auditory system were investigated behaviorally in a large (2.5x4.5x3m), sound-proofed, flight cage. Simulated cricket calls with different carrier frequencies including the standard song (4.8kHz, 45pps, 13 ms pulse length) were played back to attract the flies. Behavioral threshold curves were established by varying the sound pressure level (SPL) for songs with different frequencies (3.5 to 6.0kHz). Minimum thresholds were observed to be around 40 dB SPL at frequencies 4.5 to 5.2 kHz. In the same frequency range, choice experiments with 2 loudspeakers revealed that the flies discriminate between frequencies differing by only 300 Hz. Additionally signal detection at 4.8 kHz was shown to be least affected by masking noise. Further, masking experiments showed that a 60 dB SPL signal is masked by noise levels above 44 dB SPL.

INTRODUCTION

O. ochracea (Diptera: Tachinidae) is a parasitoid fly which uses acoustic cues to find its cricket host (1). For this purpose it produces a remarkable behavior: when an appropriate acoustic signal mimicking the cricket host is broadcast to a naive, resting gravid female fly, it will fly directly to the loudspeaker. Also, in the absence of an acoustic signal, the fly is never observed to approach a silent loudspeaker. This phonotactic behavior provides us with a rather unique opportunity to test the auditory capabilities in a free flying insect. This study investigates the phonotactic behavioral threshold and tests some of the frequency discrimination abilities of free flying female flies in acoustically controlled conditions.

METHODS

The experiments were performed in a large (width 2.5 m x length 4.5 m x height 3.0 m) flight cage completely covered by a sound absorbing foam to minimize sound reflection at frequencies of interest for the experiments. Its temperature was held at 27°C with a relative humidity of 50 % on average. Environmental parameters were shown to be critical for phonotaxis to occur (2).

The experimental setup consisted of 2 loudspeakers connected to the amplifier and computer, through which artificially synthesized cricket songs were broadcast. In dim conditions, the fly was positioned on a platform at 2.5 m from the loudspeakers and at a height of 1.5 m. It stayed there, until it was attracted to the loudspeakers by a proper acoustic signal. The fly's behavior was noted as positive phonotaxis only when it landed on the loudspeaker, which meant that it was able to detect and locate the signal.

The artificial cricket song was derived from a sample song of *Gryllus rubens*, a natural host of the fly (4.8 kHz carrier frequency). Its pulse consisted of a 13 ms long tone followed by a 9.2 ms pause (45 pulses per second). The tone part had an onset ramping of 2 ms and an offset of 5 ms. Prior to the experiments, the signal accuracy was tested and key areas of the sound field were measured. SPL was always measured at the flies' release platform.

RESULTS

The behavioral threshold was established by attracting the fly to a single loudspeaker broadcasting cricket songs with different carrier frequencies. At each frequency, the sound pressure level (SPL) was decreased until phonotaxis failed to occur. This threshold was only validated if the fly subsequently reacted to the next higher SPL in the intensity series.

The typical V-shape of the behavioral threshold reflects that of the physiological thresholds (RMS values gained by extracellular recordings from the neck connectives of the flies (3)).

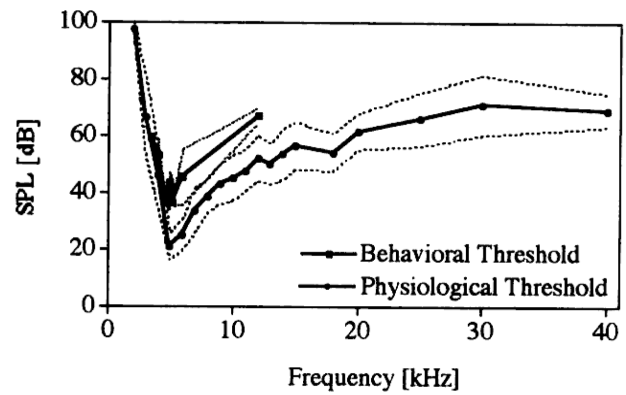

FIGURE 1: Behavioral (squares; n=5, except n=3 for 12 kHz; total individual flies N=21) and physiological (circles; n=7) threshold. T-lines are ± standard deviation.

Figure 2 shows a zoomed-in view of the frequency range from 3 to 6 kHz in figure 1. Between 4.5 and 5.2 kHz the thresholds were measured every 100 Hz. The threshold within this section remains approximately at the same average level of 38.9 dB (sd=2.3).

The masking threshold in figure 3 was gained by attracting the fly to the 60 dB SPL song overlaid with increasing levels of constant broadband noise until reaching the noise level at which the flies stopped reacting, which is to say, until the signal was masked by the noise. The behavior is least affected by noise at 4.8 kHz, where successful phonotaxis occurs up to a masking noise level of 45.1 dB SPL. There is a significant sensitivity difference in terms of masking between 4.8 kHz and its tested neighbor frequencies. The difference is 6.2 dB compared to 5.0 kHz and 7.2 dB compared to 4.6 kHz.

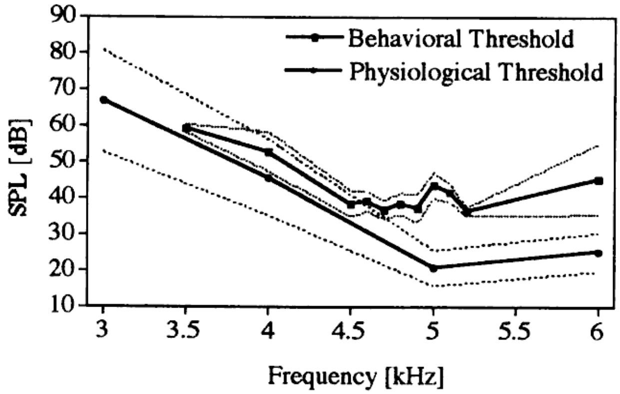

FIGURE 2: Behavioral (squares; n=5) and physiological (circles; n=7) threshold of *O. ochracea*. Zoomed in picture of the frequency range between 3 and 6 kHz in figure 1

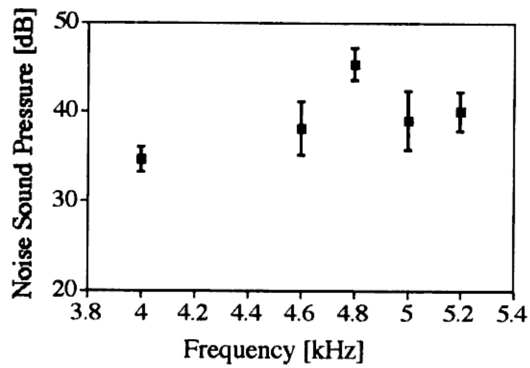

FIGURE 3: Masking threshold of *O. ochracea*. Squares represent the mean threshold value, T-lines are ± standard deviation. For each frequency n=10. total number of individual flies N=50.

Choice experiments were performed to investigate the frequency resolution ability of the fly's auditory system. The alternative frequencies (4.0, 4.5, 4.6, 4.7, 4.9, 5.0, 5.1, 5.2 and 9.6 kHz) were tested against the standard frequency 4.8 kHz. The difference in phonotactic choice is statistically significant for the trials where the standard 4.8 kHz was tested against 4.0, 4.5, 5.1 and 9.6 kHz (Fig. 4, asterisks on X-axis). This means that 300 Hz differences are discriminable in a significant way by the fly's auditory system in this range of frequencies.

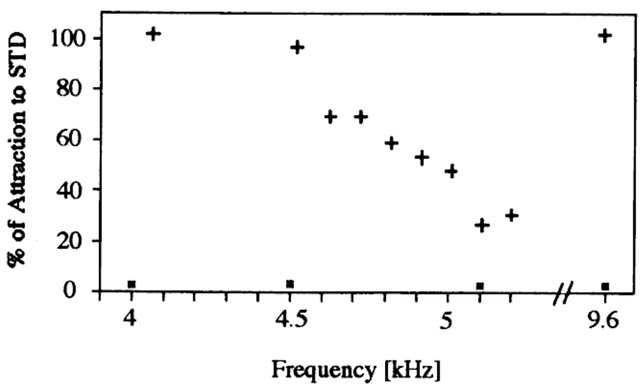

FIGURE 4: Percent of attraction to the standard signal 4.8 kHz (STD) when tested against alternative frequencies. Each cross represents the percentage out of 19 flies (n=10 for 5.2 kHz and n=14 for 4.0 and 9.6 kHz) that were attracted to the standard signal. Asterisks mark alternative frequencies which elicit statistically significant difference in attraction when tested against STD.

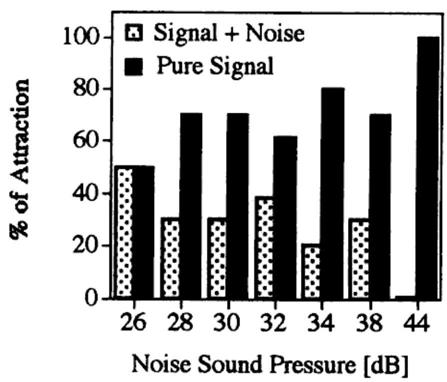

FIGURE 5: Masking. Choice experiment between STD signal of 60 dB at release site, and the same signal masked with different noise levels. n=10 for each noise level (for 32 dB noise level n=3). The data of N=15 flies was evaluated, in total 40 flies were tested.

Discrimination within noise (Fig. 5) was investigated in a second set of choice experiments. A 4.8 kHz standard signal at 60 dB SPL was tested against the same signal but overlaid with increasing levels of noise. At noise levels of 26 dB SPL the flies were attracted equally to both loudspeakers. At noise levels between 28 and 36 dB SPL they were attracted 70 % in average to the pure signal. At 44 dB SPL of overlaid noise they were attracted entirely to the pure signal.

DISCUSSION

Frequency choice experiments show that the flies discriminate between frequencies differing by only 300 Hz. They also are more attracted to frequencies higher than the STD (4.8 kHz), which represents the peak frequency of their host's calling song. This result is supported by the behavioral threshold values, which show best sensitivity at higher frequencies than the STD. Best frequency discrimination is in the same range as best detection and localization seen in the behavioral threshold, constituting consistent results. On the other hand some neighboring frequencies possess almost the same threshold, but are perfectly well discriminated. It can also be seen that the typical V shape of the behavioral threshold curve reflects that of the physiological thresholds. The masking threshold reveals that the behavior of the flies is least affected in the same frequency range in which they display best auditory sensitivity. This fact may point out the increasing sharpness of the sensory cells' filter characteristic when approaching the frequencies of best sensitivity. The behavior in the discrimination experiments within noise was only affected above noise levels of 26 dB SPL. This result can be used as a value gained by a "biological microphone" for an approximation of the background noise level in the flight room. As noise was added stepwise to the pure signal, the energy content of the overall signal increased gradually. Even so the flies were always more often attracted to the energetically lower, but pure signal, thus using frequency and temporal cues for phonotactic orientation, and not the energy content.

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