

The ability of the parasitoid fly *Ormia ochracea* to distinguish sounds in the vertical plane^{a)}

Ben J. Arthur^{b)} and Ronald R. Hoy

Department of Neurobiology and Behavior Mudd Hall, Cornell University, Ithaca, New York, 14853

(Received 31 January 2006; revised 22 May 2006; accepted 21 June 2006)

The parasitic fly *Ormia ochracea* localizes its host, field crickets, by homing in on their calling song. Previous phonotactic studies indicate that their sound localization ability in azimuth is extraordinarily acute, but the fly's ability to localize the elevation of sound sources has not been tested to date. Here we show that in a freely-walking closed-loop Y-maze task elevational performance is well above chance, but slightly below the fly's performance in azimuth. Immobilizing the head or the halteres (sensory organs of balance) slightly lowered elevational discrimination, but performance was still well above chance. Because ormiine ears are thought to be symmetric and the pure-tone models of a cricket's call used in these experiments contained little to no spectral bandwidth, additional studies will be needed to elucidate the underlying cues. Nonetheless, it is clear that while walking, *Ormia* flies are capable of distinguishing sound sources that differ only in elevation. © 2006 Acoustical Society of America. [DOI: 10.1121/1.2225936]

PACS number(s): 43.66.Qp, 43.66.Gf, 43.64.Bt, 43.64.Ha [JAS]

Pages: 1546–1549

I. INTRODUCTION

Ormia ochracea is an acoustic parasitoid of field crickets. Gravid female flies use calling male crickets as hosts for larvae, finding them in the first place by listening for their mating songs (Cade, 1975). The azimuthal sound localization acuity of tethered female *Ormia* has been demonstrated to be two degrees when walking on a trackball (Mason *et al.*, 2001), which matches both humans (Hartmann and Rakerd, 1989) and barn owls (Knudsen *et al.*, 1979). Such an acuity is surprising given the 0.5 mm distance between the ears, but can be explained through a tympanal hearing organ (Robert *et al.*, 1992; Lakes-Harlan and Heller, 1992) in which the two tympana are mechanically coupled (Miles *et al.*, 1995; Robert *et al.*, 1996). To date, however, little data exist concerning elevational ability. One would think that such an ability might be well developed since flying *Ormia* can localize singing crickets, both in nature (Walker, 1993) and in experimental flight rooms (Ramasauer and Robert, 2000; Müller and Robert, 2001). Moreover, in the frequent case that a gravid *Ormia* alights near the cricket instead of landing directly on it, she is confronted with the need to navigate through a complex microterrain, a task for which elevational discrimination might be essential. This study addresses the elevational ability of *Ormia* through the use of a vertical Y-maze in which flies freely walk towards sound sources varying in elevation.

II. METHODS

Females were tested on both horizontal (azimuthal) and vertical (elevational) Y-mazes for which the angle between the branches was about 85° (Fig. 1). The mazes were con-

structed of metal window screen mesh and suspended in the air with dental floss and steel weights in a 0.5 m³ box lined with sound-absorbing foam (Sonex, Illbruck Acoustic, Minneapolis, MN). All three branches of both maze types were 1 cm wide, just wide enough to accommodate the leg span of *Ormia*, and 9 cm long, making the total path length from the base through the bifurcation to the speaker 18 cm. Artificial cricket songs (4800 Hz carrier, 13 ms pulses at 45 Hz, 2 ms rise and 5 ms fall times) were synthesized (Matlab 7.0.1, The MathWorks, Natick, MA) by a computer (Dimension 8200, Dell, Round Rock, TX; 2.4 GHz Pentium 4, Intel, Santa Clara, CA; Windows XP, Microsoft, Redmond, WA) and played (RP2.1, PA5, System 3, Tucker Davis Technologies, Alachua, FL) from one of two miniature speakers (MDR-ED228, Sony; PM655, Harmon Kardon) placed at the end of each branch. The sound level was adjusted to be 80 dB SPL (re. 20 μPa) at the base of the Y and checked to make sure it was free of any harmonic distortion (4134 1/2 in. condenser microphone, 2608 measuring amplifier, 4220 pistonphone, Brüel and Kjær, Nærum, Denmark). Gravid flies were taken from our laboratory colony that originated from wild flies caught in Bradenton, Florida (Wineriter and Walker, 1990) and allowed to acclimate to the box for 5 min before testing began. Prior to this acclimatization period two of the five experimental groups had either the heads or halteres immobilized with wax under cold anaesthesia (5 min on ice; 5 min recovery). Several test trials were run in white light to show flies that there was a path on which they could walk towards the cricket song. For these trials the placement of the flies on the maze was initially on the arms of the Y near the speakers and then over the course of several trials the placement was gradually moved back through the bifurcation towards the base of the Y. Twenty real trials were then performed in red light (590–838 nm, Sylvania, Danvers, MA) to quantify the phonotactic ability of the fly. Two scoring methods were used: a strict criterion counted as correct only those trials for

^{a)}This work was presented in "Sound localization ability of the parasitoid fly *Ormia ochracea* in the elevational plane," International Congress of Neuroethology, Nyborg, Denmark, August 2004.

^{b)}Electronic mail: bja28@cornell.edu

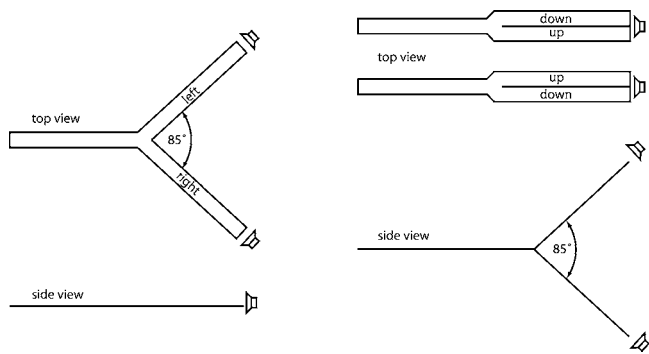


FIG. 1. The three mazes used in these experiments. (Left) The azimuthal maze. (Right) The two elevational mazes. Note that elevational mazes were *not* simply azimuthal mazes rotated 90°. Rather the walking surface of the initial branch of both maze types was normal to gravity. Whereas the two bifurcating branches for azimuthal mazes were splayed 85° in the horizontal plane, in the elevational mazes these two branches were side-by-side when viewed from above and spread 85° in the vertical plane. Two chiral elevational mazes were used to control for the confound between azimuth and elevation due to the asymmetry at the bifurcation. Speakers for the elevational mazes were placed on the midline so as to minimize any azimuthal cues.

which the flies went straight to the correct speaker, stepping for no more than one body length on the wrong branch; a more relaxed criterion counted as correct the additional trials for which the flies initially chose the wrong branch at the bifurcation in the Y and then, before reaching the terminus, turned around and went toward the correct speaker. Flies that were unresponsive to cricket song, repeatedly flew off the maze, or showed a consistent bias towards one side were not included in the analysis. Performance differences between groups were quantified by a binomial ANOVA done in R (R Foundation for Statistical Computing, Vienna, Austria). The null hypothesis that all groups had equal means was compared to a model in which the two chiral elevational mazes were combined but the others were left to vary, as well as one in which all groups could vary. For both the strict and relaxed criteria, the null hypothesis was rejected ($p < 1e-8$) and treating the two elevational groups separately did not account for significantly more of the variance ($p > 0.4$). Comparisons with chance were then calculated using the combined model and omitting the intercept term, and comparisons between groups by including it, with the resulting p values being reported in the next section. A small subset of flies, not included in the analysis of performance, was videotaped using either conventional (DCR-TRV38, Sony) or high-speed (250 fps, MotionScope HR1000, Redlake Camera) cameras. Taping was not done routinely because of concerns about the sound field, the limited angular resolution of the information gained, and increased experimental difficulty due to the flies' tendency to hide in the many facets of the camera if they flew off the maze.

III. RESULTS

The data presented here consist of the five following experimental conditions: (1) an azimuthal maze (L/R); (2+3) two chirally nonequivalent elevational mazes, a left-handed (D/U) and right-handed (U/D) form; (4) an elevational maze with the fly's head waxed to its thorax (Head); and (5) an

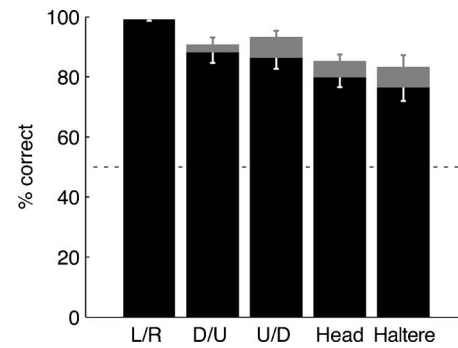


FIG. 2. Localization performance in five experimental conditions using two scoring methods. Results for the azimuthal maze are shown in the left bar, and for the elevational mazes in the four bars on the right. Black indicates the mean percentage of correct trials as judged by the strict criterion, and grey indicates the additional trials scored as correct by the more relaxed criterion (see Methods). Horizontal dashed line indicates the chance level at 50%. All conditions were significantly above chance; the D/U and U/D elevational conditions were not significantly different than each other; azimuthal performance was significantly above performance in the combined D/U and U/D elevational conditions; and the Head and Haltere conditions were on the borderline of being significantly different from the combined D/U and U/D conditions. Ten separate flies were used in each condition with 15–20 trials for each fly. Percent correct was computed for each fly, and then averaged across flies to get the mean and standard error bars.

elevational maze with the fly's halteres waxed to the calypters (Haltere). The azimuthal task was performed to compare our current experimental paradigm with previous work (Mason *et al.*, 2001), and the latter two conditions served to search for potential mechanisms by which *Ormia* might detect elevational cues. The heads were immobilized specifically because ormiine ears are located on the thorax in the narrow cleft separating it from the head, and so even small movements of the head could effect the sound field surrounding the ears. The halteres were immobilized because they were observed to oscillate in response to auditory stimulation in tethered walking flies (personal observation; data not shown), and it was desired to see if this was necessary for successful discrimination.

Ten separate flies were used in each of the five conditions (50 flies total) and 15–20 trials were run on each fly (992 trials total). As judged by the strict criterion, performance was significantly above chance in all conditions (Fig. 2; $p < 0.00001$). While performance for the azimuthal maze was near perfect at $99 \pm 0.6\%$, the average for the two elevational mazes, which were not significantly different than each other ($p > 0.5$), dropped significantly compared to azimuthal performance to $87 \pm 1.9\%$ ($p < 0.005$). Immobilizing the head lowered elevational performance to $80 \pm 3.2\%$, but not significantly ($p > 0.05$). Immobilizing the halteres significantly lowered elevational performance to $76 \pm 3.5\%$ ($p < 0.01$).

In the azimuthal trials, flies typically oriented (yawed) their body towards the sound as they ran along the base of the Y towards the bifurcation, indicating that the location of the sound was perceived well before a decision had to be made. A similar change in the angular pitch of the thorax was sometimes observed in elevational trials, although this observation was far less robust. High-speed video was used to ascertain whether flies might rotate their bodies to convert

world-centered elevation into body-centered azimuth, or rotate their heads relative to their thorax to induce a change in the sound field near the tympana on the thorax. While small rolls of the thorax were observed to naturally occur during the normal walking gate, no pronounced or obviously intentional rolls of a great magnitude were observed. Nor was the head ever observed to make large rolls independently of the thorax except while grooming between trials. This last observation is consistent with the results described above of only a minimal decrement in performance when the heads were waxed to the thorax.

When tested in each of the four elevational conditions, a portion of the initial wrong decisions made by the fly at the bifurcation were corrected before the terminus of the incorrect branch was reached. Even when these trials are scored as correct using the more relaxed criterion, the results presented above are similar: (a) performance is significantly above chance for all conditions ($p < 0.00001$), (b) azimuthal performance ($99 \pm 0.6\%$) is slightly greater than elevational performance ($92 \pm 1.5\%$; $p = 0.021$), and (c) immobilizing the head or the halteres slightly lowered elevational performance ($85 \pm 2.8\%$, $p = 0.042$ and $83 \pm 3.0\%$, $p = 0.013$, respectively). Note that points (b) and (c) are on the borderline of being significant, having p values between 0.01 and 0.05.

IV. DISCUSSION

The data presented here show that phonotaxis towards synthetic cricket song by freely walking flies is at performance levels well above chance in both the elevational and azimuthal planes. Azimuthal performance was significantly, albeit slightly, better than elevational performance, possibly due to a left/right asymmetry in the design of the vertical Y mazes: whereas the flies' tendency to rotate their bodies towards the speaker naturally guided them through the bifurcation in the azimuthal task, the vertical mazes did not permit this because they had the up and down branches to the left and right of each other. The ability of flies to localize in elevation at all is intriguing because ormiine ears are thought to be symmetric and the artificial cricket song used here was tonal. Hence neither the interaural intensity difference cues used by barn owls due to their asymmetric ears (Kaup, 1862; Payne, 1971; Olsen *et al.*, 1989) nor the monaural spectral cues used by humans for broadband noise stimuli (Angell and Fite, 1901; Roffler and Butler, 1967) are available to flies as elevational cues.

To investigate one possible strategy that might enable *Ormia* to localize elevationally distinct sources, an experimental manipulation of waxing the head to the thorax was performed. Given that ormiine ears are in the cleft separating the head from the thorax, head movements relative to the thorax could affect the sound field. It has long been known that flies move their heads during tethered visual fixation tasks (Land, 1973; Geiger and Poggio, 1977), and recently head movements have also been shown to occur in free-flight during body saccades (Schilstra and van Hateren, 1998). The purpose is thought to be the stabilization of gaze to minimize visual blur and enhance object recognition. Head movements can be stimulated by other modalities as well, including tac-

tile stimulation of the halteres (Sandeman and Markl, 1980) and auditory stimulation (personal observation). The latter was observed during presentation of synthetic cricket calls while tethered *Ormia* were walking on a trackball. Though modifying the sound field through head movements could be one strategy *Ormia* use to overcome the limitations of symmetric ears and a tonal stimulus, the data here show that they are not necessary, as elevational performance is only slightly degraded when such movements are prevented.

A second manipulation performed here was preventing the movement of the halteres by waxing them to the calypters. The halteres are a pair of oscillating pendulums protruding from the thorax just posterior to the wings which are thought to act as sensory gyroscopes to stabilize flight (Derham, 1713; Fraenkel and Pringle, 1938). Halteres also flap when not in flight however (Fraenkel, 1939; Schneider, 1953; and personal observation in *Ormia*), and it has been hypothesized that such oscillations sense angular accelerations of the surface that flies are walking or resting on (Miller, 1977). The data of Sandeman and Markl (1980) address this point by showing that ablating the halteres has a significant effect on a walking fly's ability to compensate for rotations of teeter-totters and turntables. The effect was so "slight," however, that the authors conclude that sensory input from haltere oscillations are predominantly used during flight. Because it is still essentially an open question then as to why halteres oscillate while flies are not flying, we immobilized them during a portion of our elevational trials. The results reported here confirm that halteres are not necessary for normal walking behavior in that the effect on discrimination performance was small. It seems likely then that the slight decrement in performance both in our data, as well as in Sandeman and Markl (1980), is perhaps merely due to the short-term effects associated with haltere immobilization or removal (e.g., novelty of immobility, surgical trauma, cold anaesthesia). Were this true, one would expect performance difficulties to be abated if more time were allotted to the recovery period, as Fraenkel (1939) found for haltereless flight endurance, spontaneous take off, and sensitivity to startle.

In summary, the Y-maze data presented here show successful phonotaxis towards sounds in the elevational plane by freely-walking *Ormia ochracea* even when their head and halteres are immobilized. Further experiments will be necessary, however, to determine the underlying cues used. Open-loop behavioral studies, head-related transfer function (HRTF) measurements, and dichotic stimuli would in particular facilitate a direct comparison with strategies used by other species.

ACKNOWLEDGMENTS

Thanks to James Booth for help with the statistics, to Cole Gilbert for help with the haltere literature, to Bob Wytenbach and one anonymous reviewer for commenting on the manuscript, and to the National Institute on Deafness and Other Communication Disorders (NIDCD) for funding.

- Angell, J. R., and Fite, W. (1901). "The monaural localization of sound," *Psychol. Rev.* **8**, 225–245.
- Cade, W. (1975). "Acoustically orienting parasitoids: Fly phonotaxis to cricket song," *Science* **190**, 1312–1313.
- Derham, W. (1713). *Physico-Theology*, 1st ed. (W. Innys, London), p. 406.
- Fraenkel, G. (1939). "The function of the halteres of flies (diptera)," *Proc. Zool. Soc. A* **109**, 69–78.
- Fraenkel, G., and Pringle, J. W. S. (1938). "Halteres of flies as gyroscopic organs of equilibrium," *Nature (London)* **141**, 919–920.
- Geiger, G., and Poggio, T. (1977). "On head and body movements of flying flies," *Biol. Cybern.* **25**, 177–180.
- Hartmann, W. M., and Rakerd, B. (1989). "On the minimum audible angle: A decision theory approach," *J. Acoust. Soc. Am.* **85**, 2031–2041.
- Kaup, J. J. (1862). "Monograph of the strigidae," *Trans. Zool. Soc. Lond.* **4**, 201–260.
- Knudsen, E. I., Blasdel, G. G., and Konishi, M. (1979). "Sound localization by the barn owl (*Tyto alba*) measured with the search coil technique," *J. Comp. Physiol. [A]* **133**, 1–11.
- Lakes-Harlan, R., and Heller, K. G. (1992). "Ultrasound-sensitive ears in a parasitoid fly," *Naturwiss.* **79**, 224–226.
- Land, M. F. (1973). "Head movement of flies during visually guided flight," *Nature (London)* **243**, 299–300.
- Mason, A. C., Oshinsky, M. L., and Hoy, R. R. (2001). "Hyperacute directional hearing in a microscale auditory system," *Nature (London)* **410**, 686–690.
- Miles, R. N., Robert, D., and Hoy, R. R. (1995). "Mechanically coupled ears for directional hearing in the parasitoid fly *Ormia ochracea*," *J. Acoust. Soc. Am.* **98**, 3059–3069.
- Miller, P. L. (1977). "Haltere activity in a flightless hippoboscid fly, *Craetarina pallida*," *J. Insect Physiol.* **23**, 855–860.
- Müller, P., and Robert, D. (2001). "A shot in the dark: The silent quest of a free-flying phonotactic fly," *J. Exp. Biol.* **204**, 1039–1052.
- Olsen, J. F., Knudsen, E. I., and Esterly, S. D. (1989). "Neural maps of interaural time and intensity differences in the optic tectum of the barn owl," *J. Neurosci.* **9**, 2591–2605.
- Payne, R. S. (1971). "Acoustic location of prey by barn owls (*Tyto alba*)," *J. Exp. Biol.* **54**, 535–573.
- Ramsauer, N., and Robert, D. (2000). "Free-flight phonotaxis in a parasitoid fly: Behavioural thresholds, relative attraction and susceptibility to noise," *Naturwiss.* **87**, 315–319.
- Robert, D., Amoroso, J., and Hoy, R. R. (1992). "The evolutionary convergence of hearing in a parasitoid fly and its cricket host," *Science* **258**, 1135–1137.
- Robert, D., Miles, R. N., and Hoy, R. R. (1996). "Directional hearing by mechanical coupling in the parasitoid fly *Ormia ochracea*," *J. Comp. Physiol. [A]* **179**, 29–44.
- Roffler, S. K., and Butler, R. A. (1967). "Factors that influence the localization of sound in the vertical plane," *J. Acoust. Soc. Am.* **43**, 1255–1259.
- Sandeman, D. C., and Markl, H. (1980). "Head movements in flies (*Calliphora*) produced by deflexion of the halteres," *J. Exp. Biol.* **85**, 43–60.
- Schilstra, C., and van Hateren, J. H. (1998). "Stabilizing gaze in flying blowflies," *Nature (London)* **395**, 654.
- Schneider, G. (1953). "Die halteren der schmeissfliege (*Calliphora*) als sinnesorgane und als mechanische flugstabilisatoren," *Zeitschrift für Vergleichende Physiologie* **35**, 416–458.
- Walker, T. J. (1993). "Phonotaxis in female *Ormia ochracea* (diptera: Tachinidae), a parasitoid of field crickets," *J. Insect Behav.* **6**, 389–410.
- Wineriter, S. A., and Walker, T. J. (1990). "Rearing phonotactic parasitoid flies [*Diptera: Tachinidae, Ormiini, Ormia spp.*]," *Entomophaga* **35**, 621–632.