

Hearing in honeybees: localization of the auditory sense organ

C. Dreller, W.H. Kirchner

Theodor-Boveri-Institut für Biowissenschaften der Universität, Lehrstuhl für Verhaltensphysiologie und Soziobiologie, Am Hubland, D-97074 Würzburg, Germany

Accepted: 23 April 1993

Abstract. Airborne sound signals emitted by dancing honeybees (*Apis mellifera*) contain information about the locations of food sources. Honeybees can perceive these near field sounds and rely on them to decode the messages of the dance language. The dance sound is characterized by rhythmical air particle movement of high velocity amplitudes. The aim of the present study was to identify the sensory structures used to detect near field sound signals. In an operant conditioning experiment, bees were trained to respond to sound. Ablation experiments with these trained bees revealed that neither mechanosensory hairs on the antennae or head nor bristle fields at the joints of the antenna, but Johnston's organ, a chordotonal organ in the pedicel of the antenna, is used to detect near field sound in honeybees.

Key words: *Apis mellifera* – Auditory sense – Near field sound – Johnston's organ – Dance language

Introduction

The dance language of the honeybee is one of the most complex communication processes in the animal kingdom. A dancing bee informs the follower bees about distance, direction and profitability of a food source or a nesting site (von Frisch 1965). While the "sender" in this information process has been studied extensively, very little is known about the behaviour of the "receivers", i. e. the nestmates following the dances. The followers have to decode the message of the dance in the darkness of the beehive. Therefore visual cues cannot be used in *Apis mellifera*. One sensory channel available for the follower bee to get the information of the dance is the acoustical one. Dance sounds produced by the dancing bee during the figure-eight wagging run were discovered independently by Esch (1961) and Wenner (1962). These acousti-

cal signals provide information about the location (Esch 1964; Kirchner et al. 1988) and profitability (Spangler 1991; Waddington and Kirchner 1992) of a food source. For a long time thought to be deaf, bees are in fact capable of detecting such airborne sound (Towne and Kirchner 1989; Kirchner et al. 1991). By means of a mechanical dancer, Michelsen et al. (1992) demonstrated that the sound is indeed necessary for successful dance communication in *A. mellifera*.

The sounds of a dancing bee with a carrier frequency of about 260 Hz are generated by wing vibrations: close to the abdomen, the sound pressures in the air space above and below the plane of the wings are totally out of phase, causing air movements with velocity amplitudes of up to 1 m/s (Michelsen et al. 1987). The pressure gradient decreases rapidly with increasing distance from the wings. No substrateborne vibrations are induced during sound production (Michelsen et al. 1986). Thus, bees have to somehow perceive near field sound to extract information from the dance.

Mechanosensory hairs responding to rhythmical displacement of the surrounding air are one type of sense organs detecting near field sound. For instance, caterpillars of a noctuid moth, *Barathra brassicae*, possess sensory hairs on the surface of the body which detect the flight-generated air vibration of approaching parasitic wasps (Markl and Tautz 1975). Similarly, trichobothria in spiders play a role for the localization of flying prey (Görner and Andrews 1969).

Like in most other insects, the cuticle of honeybees is covered with hairs, many of which presumably have mechanosensory functions. Bristles on the eyes, for example, are innervated (Neese 1965) and could serve as sound receivers. Mechanosensory hairs can also be found on the antennae. Although they are short (< 35 µm, Esslen and Kaissling 1976), one cannot rule out their involvement in the perception of near field sound.

Furthermore, the antennae of many insects respond to airborne vibrations. In Diptera, the antennae are the principal organs of hearing. Females of *Drosophila* perceive the courtship songs produced by the males' wings

(Manning 1967; Ewing 1978), and in male mosquitos the antenna is most sensitive to air movements at the wing-beat frequency of the female (Tischner 1953). The resonance frequency of the antennal flagellum in honeybees is about 260–280 Hz (Heran 1959; Dreller 1990). The antennae may therefore well be involved in the detection of dance sounds.

At least two different sensory structures may perceive the rhythmical movements of the flagellum: mechanosensory bristles located dorsally and ventrally at the proximal joint of the pedicel (Markl 1962) and Johnston's organ, a chordotonal organ in the antennal pedicel (Snodgrass 1956), which is used for sound detection in flies and mosquitos (Belton 1961). Johnston's organ consists of an array of scolopidial mechanoreceptors that respond to movements of the antennal flagellum relative to the pedicel (Belton 1989).

The present study used operant conditioning to show, which sensory structures are involved in the perception of near field sound in the honeybee.

Methods

All experiments were performed during the summers of 1991 and 1992 at the bee research station of the University of Würzburg, Germany. Colonies were housed in a two-frame observation hive.

An operant conditioning procedure was used to train bees to associate sound signals with a sucrose reward. The experimental set-up and the conditioning paradigm were recently used to determine the frequency range and thresholds of hearing in honeybees (for details see Kirchner et al. 1991).

Individually marked forager bees were trained to visit a feeding station at a distance of 3 m from the hive. They had to walk into a Y-shaped gallery to find a small droplet of sucrose solution (2 M) at the end of either the left or right arm of the Y-maze. Simultaneously a sound stimulus of 265 Hz similar to the near field sound of a dancing bee was presented at the same side. The sound stimulus was generated as described by Towne and Kirchner (1989). The velocity amplitude of the stimulus was about 600 mm/s (peak-to-peak), which corresponds to the natural signal and is well above the threshold of hearing in honeybees (Kirchner et al. 1991). Unlike the natural signal, the conditioned stimulus was not pulsed. The bee received only a small reward of 5 µl sucrose solution per trial, hence she made up to 10 decisions before returning to the hive to unload. The side on which reward and sound stimulus were presented was changed randomly for each trial.

A training procedure was considered successful when at least 73.3% out of 30 successive trials were performed correctly. This is significantly above the random level of 50% ($\chi^2 = 3.455$, $P < 0.05$). As soon as the bees fulfilled this criterion, different ablations of potential sensory organs (see below) were carried out. The following 30–60 decisions of the manipulated bees in the Y-maze were recorded. During these tests, as during the training period, correct choices continued to be rewarded. The number of correct choices before and after treatment were compared by Mann-Whitney-Wilcoxon-tests.

Manipulations of trained bees. Immediately after being trained, bees were captured at the feeder and restrained in a small plastic tube (Eppendorf). To test whether hair receptors serve as auditory sense organs, all hairs on the head and the eye bristles were removed under a stereomicroscope using a battery shaver (Sanyo SV-M730) and a piece of razor blade.

In order to investigate the role of antennae in hearing, one or both antennae of another group of bees were cut at the base of the scape with a fine pair of scissors. To find out whether the hairs on

the antennae or sensory organs located on the pedicel are involved in hearing, a small cuff made of silicone tubing (length 1 mm, inner diameter 0.25 mm) was fixed around the pedicel of one antenna to immobilize the proximal and distal joints of the pedicel. A smaller cuff (length 0.5 mm) was attached to immobilize the scape-pedicel joint only, leaving the distal part of the pedicel and the entire flagellum free. The use of cuffs instead of glue not only prevented the antennal nerve from being affected by heat or poisonous substances, but also allowed us to check whether the immobilization of the joints was effective. The joints remained completely immobile as long as the silicone tubing was attached. In order to attach the cuffs, bees were kept under a continuous CO₂ stream. The silicone tubing was drawn over an injection needle and the antenna was slid carefully into the cannula. Then the cuff was slipped off and fastened by a droplet of liquid wound spray (Flint, Tegal). As soon as the bees resumed their foraging activities, normally after 1–2 h, they were tested in the Y-maze.

A group of manipulated bees – previously trained to 265 Hz – was also tested at 10 Hz, in order to investigate the perception of low frequencies.

Results

The bees did associate the sound stimulus with the reward and, after 40–100 trials, they preferred the side on which the sound was presented. The average number of correct decisions during the last 30 trials before manipulation is given as control (white bars in Figs. 1, 2, 3).

Figure 1 shows the results for the experiments testing whether mechanosensory hairs or the antennae serve as auditory sense organs. Removing all hairs on the head as well as the eye bristles had little or no effect on the bee's

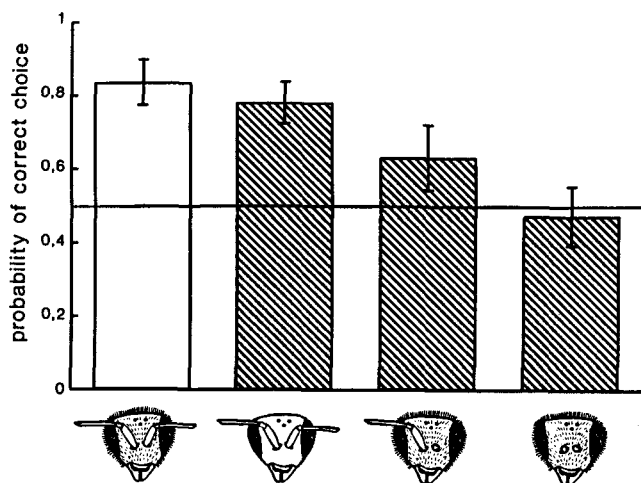


Fig. 1. Learning and ablation experiments to determine whether the antennae or sensory hairs are involved in sound reception. The probability of correct decisions in the Y-maze (mean ± SD) is shown for trained intact bees (white bar) and bees which were manipulated after training and tested again (hatched bars). Drawings below the bars indicate different treatment: control (1st bar), hairs removed (2nd bar), one (3rd bar) or both (4th bar) antennae ablated. The random level of 50% is indicated. Intact bees significantly prefer the side with sound ($N=22$ bees, $n=883$ decisions). After removing all hairs and bristles on the head no differences in performance are found ($N=4$, $n=184$, $P=0.6$). Removing one antenna leads to a significant decrease in the probability of correct choices ($N=18$, $n=792$, $P<0.001$) compared to intact bees. Bees without antennae choose significantly less frequently the side with sound than the control group ($N=6$, $n=238$, $P<0.001$); there is no difference to the random level ($P=0.7$).