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Reduced ability of ethanol drinkers for social communication in honeybees (*Apis mellifera carnica* Poll.)

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Abstract

Foraging behavior was evaluated in honeybees trained to fly to a feeder containing sucrose only, 1% ethanol, 5% ethanol, or 10% ethanol. The results indicated that exposure to ethanol disrupted several types of honeybee social behavior within the hive. Consumption of ethanol at the feeding site reduced waggle dance activity in foraging bees and increased occurrence of tremble dance, food exchange, and self-cleaning behavior. These ethanol-induced changes in behavior may reflect effects on the central nervous system similar to the previously observed effects of food poisoning with sublethal doses of insecticides.

Keywords: Honeybee; Waggle dance; Tremble dance; *Apis mellifera*; Social behavior; Ethanol

1. Introduction

This is the sixth in a series of behavioral experiments testing the suitability of honeybees (*Apis mellifera* L.) as an animal model for the study of alcoholism. Previous results from this laboratory have shown several alcohol-related effects in bees that share properties in common with similar effects in humans. These include self-administration, disruption of learning, locomotion, and decision making, preferences for commercially available alcoholic beverages, the ability of an emetic to limit consumption of ethanol, and an increase in aggression (Abramson et al., 2000, 2003, 2004a, 2004b, 2005).

This paper continues the search for common behavioral effects of ethanol on human and honeybee behavior by asking the question whether ethanol consumption influences the foraging honeybee behavior. It is known that consumption of ethanol in humans can cause cognitive dysfunction, aggression, and other abnormal social behavior (see recent papers Giancola, 2004; Herzog, 1999; Peirce et al., 2000). In the interest of further developing our social insect model, this study evaluated the influence of ethanol consumption on behavior of the foraging bee inside of the hive. The effects of ethanol consumption on the waggle dance and other behaviors of foraging bees were assessed after they returned to the hive following a foraging trip.

After a foraging trip, a honeybee shares collected nectar among nestmates by mouth-to-mouth food exchange, also called trophalaxis (Winston, 1987). Excited upon finding a nectar source, the foraging bee performs a waggle dance on the comb inside of the hive. This dance contains information about the location of a food source (von Frisch, 1965). Bees that follow the dancer are in the best position to pick up dance information (Bozic & Abramson, 2003). It is also known that bees that are disrupted at a feeding site will emit a tremble dance rather than a waggle dance. The tremble dance consists of irregular movements in all directions (Seeley, 1992). We expected that ethanol will affect waggle dance behavior and other related behavior inside of the hive during foraging activity.

2. Materials and methods

2.1. Procedure

Honeybees (*Apis mellifera carnica* Poll.) were reared in a two frame observation hives during spring 2003. For 2 days, we individually marked potential foragers at the hive entrance using numbered tags. After tagging, we trained bees to forage to a feeder located 250 m from the hive. The feeders were custom made from six, 200 ml plastic

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cups. This training took 1 day. The following morning we started the first day of actual experimental trials.

In the morning of the first experimental day, bees were offered a 1.5 M sucrose solution. After a minimum of 10 marked bees were consistently returning to the feeder for 90 min, we switched the 1.5 M solution to a solution containing 10% (v/v) ethanol. The 10% solution remained in place until no marked bees appeared for 15 min. After this 15-min period, the 10% solution was removed and the feeder remained empty for 4 h. The 10% solution reduced the number of foraging visits so dramatically in a half an hour (last recorded visit of the marked bee in 25th min) that we were not able to continue with 10% test solution (no marked bee in next 15 min). The rationale behind the 4-h time period was to ensure that bees were not suffering aftereffects from the 10% solution when we switched to a new ethanol solution. Following the 4-h period, we renewed the feeder with 1.5 M sucrose and within 30 min the 10 marked bees were returning. The sucrose-only feeder remained in place for 60 min at which time we replaced the sucrose-only feeder with one containing 1% (v/v) ethanol. The 1% ethanol feeder remained in place for 90 min—until 30 min before sundown. The experiment was continued the following day. On the second day of experiment, we started with a 1% ethanol solution for 60 min, then switched to a 5% (v/v) solution for 60 min, and finally returned to a sucrose-only solution.

At the feeding site, we tried to remove all unmarked bees on the first day. On the second day, we could not do this because of the large number of unmarked foragers. The solution in the feeder was replaced as needed. At the feeding site, we recorded the time of arrival of any marked bee. Once inside the observation hive, we recorded the behavior of the marked bees with a Cannon MV600i miniDV camcorder.

2.2. Data analysis

We calculated the number of visits, number of returns, and duration of returns for each feeder solution. For all marked bees, which were observed at the feeder and inside of the hive, we counted the number of trophalaxis encounters, waggle dances, tremble dances, self-cleaning, attending and following of the waggle dancers, and walking or resting on the comb inside of the hive in 30-min time periods. Return times between successive treatments at the feeding station were tested with one-way analysis of variance with post hoc multiple Tukey comparison at 0.05 significance level. The number of visits at the feeder between successive 30-min time periods was tested with χ^2 goodness-of-fit test. We compared counts of behavior inside of the hive with the number of visits at the feeder during the same time periods. Our hypothesis was that occurrence of observed behaviors inside of the hive was under influence of foraging visits. Possible independence of in-hive behavior counts was tested with two by two Fisher's exact test, because many observed frequencies were less than five. The same test was used to evaluate independence of occurrence of waggle dance to other hive behavior. The waggle dance behavior is the only behavior that can be clearly related to the foraging visits between observed behaviors inside of the hive, and therefore was chosen for comparison with other behavior on the comb. All statistics were applied using SPSS for Windows 12.0.

3. Results

3.1. Foraging activity

Foraging activity was significantly affected by the presence of ethanol in the feeding solution. Return time of the foragers (Fig. 1) was significantly affected by exposure to the ethanol solution in the feeder [$F(6, 853) = 7.33$, $P < .001$], even by the 1% ethanol solution (Tukey multiple comparison test, $P < .05$). Ethanol exposure significantly increased return time of foragers when exposed to 1% ethanol solution at the feeder compared to solution containing no ethanol, and also when exposed to 5% ethanol solution at the feeder compared to 1% ethanol solution or to the solution containing no ethanol (Fig. 1). We were not able to show significantly longer time for 10% ethanol solution because only 10 bees returned for a total of 17 times to the feeder. We also observed that when exposed to 10% ethanol solution, some bees actually were not able to fly back into the hive.

In contrast, foraging visits were affected only by 5% and 10% ethanol solution (Fig. 2). Significant decreases in number of visits were observed in 10% ethanol exposure on the first day (goodness-of-fit test, $\chi^2 = 16.3$, $df = 1$, $P < .001$) and 5% ethanol on the next day (goodness-of-fit test, $\chi^2 = 23.4$, $df = 1$, $P < .001$). We observed 55 visits in 30 min of feeding on sugar solution containing no ethanol before bees were exposed to 10% ethanol solution. We counted only 20 visits during feeding on ethanol solution in next 30 min. Actually, most visits (12 out of 20) occurred in the first 10 min. Due to the low frequency of visits, we stopped exposure to 10% ethanol after 30 min of data collection.

When the feeder was removed for 4 h and subsequently filled with sucrose only, we counted 36 visits in next 30 min of data collection. This was significantly higher than that when the animals were exposed to the 10% ethanol solution (goodness-of-fit test, $\chi^2 = 4.57$, $df = 1$, $P = .033$; Fig. 2). In the next 30 min, we observed a significant increase of foraging activity, up to 72 visits (goodness-of-fit test, $\chi^2 = 16.08$, $df = 1$, $P < .001$).

On the next day, we observed a significant drop in visits—from 36 in the first 30 min of foraging on 5% ethanol solution to only five visits in the next 30 min of feeding on 5% ethanol solution (goodness-of-fit test, $\chi^2 = 23.4$, $df = 1$, $P < .001$). We were not able to observe any additional