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Detection of odor perception in Asiatic honeybee (*Apis cerana* Fabricius, 1793) workers by changing membrane potential of the antennal sensilla

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ABSTRACT

The role of honeybee mandibular gland compounds is poorly understood, although they may act as alarm pheromones. We measured forager and guard bee antennal responses evoked by two major components of mandibular gland secretions of the Asiatic honeybee, *Apis cerana.* Membrane potentials of antennal sensilla were measured after exposure to three concentrations of the synthetic alarm pheromones 2-heptanone and (Z)-11-eicosen-1-ol using a potentiostat (EA161) connected to an e-corder (ED401) with microelectrodes. The resting membrane potential of *A. cerana* foragers and guards was -55.23 ± 1.44 and -56.41 ± 1.21 mV, respectively. The membrane potential of foragers after exposure to 1.0, 5.0 and 10.0% 2-heptanone was -5.32 ± 0.46, -8.41 ± 1.33 and -11.53 ± 2.16 mV, respectively. The membrane potential of guards was -5.49 ± 1.66, -8.46 ± 1.32 and -7.31 ± 3.46 mV, respectively. Exposure of foragers to 1.0, 5.0 and 10.0% (Z)-11-eicosen-1-ol induced membrane potentials of -24.00 ± 6.56, -36.36 ± 5.18 and -14.60 ± 8.20 mV, respectively; for guards they were -47.62 ± 1.46, -46.08 ± 0.87 and -9.35 ± 1.96 mV, respectively. The highest membrane potential was found in foragers exposed to 1.0% 2-heptanone. The membrane potentials of foragers were higher than that of guards except at the highest concentration (10.0%) of both pheromones. These findings suggest that antennal sensory receptors of foragers may have higher specific thresholds than those of guards.

Introduction

Honeybees perceive pheromones with sensory receptors located on the antennae comprising eight types of sensilla: they are sensilla ampullacae (a receptor for carbondioxide), sensilla basiconica (unknown), sensilla campaniforme (a mechanoreceptor), sensilla placodae (an odor receptor), sensilla trichodae type A (unknown), B, C (mechanoreceptor) and D (a gustatory receptor) (Agren, 1977). The main olfactory sensilla are sensilla placodae which are abundant over the last segment of the antenna. This sensilla type is innervated by 15 to 30 neurons which respond to flower odors and honeybee pheromones (Claudia et al., 2002). 2-heptanone, the major component of the mandibular glands of honey bees, is an alarm pheromone and has repellent properties affecting foraging bees (Shearer and Boch, 1965; Reith et al., 1986; Yokoi and Fujisaki, 2007). This pheromone may be repellent at high concentrations and is probably deposited when a bee visits flowers which signals other bees of nectar depleted flowers (Boch and Shearer, 1971; Crew and Hasting, 1976; Balerrama et al., 1996; Gawleta et al., 2005). However, it can also be an attractant at low concentrations (Shearer and Boch, 1965; Boch and Shearer, 1971; Kerr et al., 1974; Koeniger et al., 1979; Vallet et al., 1991).

The pheromone concentration secreted by workers of different ages varies. Younger bees produce low or undetectable levels of 2-heptanone and production increases with age (Ferguson and Free, 1979; Lensky, 1985; Sakamoto et al., 1990; Pankiw, 2004). Release of alarm pheromones by guard bees alerts others workers to a source of potential danger (Maschwitz, 1964). Within the sting apparatus of honeybee workers, low concentrations of (Z)-11-eicosen-1-ol were found to repel worker bees, but at high concentrations it did not (Pickett et al., 1982; Free et al., 1983, 1988). It acts similar to isopentyl acetate as an alarm pheromone from the sting of *A. mellifera*; however, it is the main secretion of mandibular glands of four native honeybee species of Thailand (Pickett et al., 1982; Suwannapong et al., submitted for publication).

The response of honeybees to specific pheromone concentrations is still unclear and not well understood. In the present study, we measured changing antennal sensilla membrane potential of *Apis cerana* foragers and guards in response to different concentrations of 2-heptanone and (Z)-11-eicosen-1-ol dissolved in isomolar bee saline. The objective was

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to understand the effect of different concentrations of each pheromone on honeybee response.

Materials and methods

To study the pheromone sensing in *A. cerana*, we used a potentiostat (EA161) connected to an e-corder (ED401) with microelectrodes to measure changing membrane potentials of antennal sensilla responding to different concentrations of 2-heptanone and (Z)-11-eicosen-1-ol (Sigma, USA). Pheromone concentrations included 0.0 (the control membrane potential or resting membrane potential), 1.0, 5.0 and 10.0% (v/v) in 10^{-5} M bee saline (15.66 g NaCl, 0.238 g KCl, 0.177 g $CaCl_2$, 2.033 g MgCl, 2.093 $C_7H_{15}NO_4S$ (Mops) per liter aqua dest) (Pribbenow and Erber, 1996).

Honeybees

In early summer 2007, a total of 160 adult *A. cerana* workers (80 foragers and 80 guards) were caught directly over the nest from queen right colony located at Burapha University, Chon Buri, Thailand. Foragers had pollen loads in their pollen baskets and guards stood in front of the nest entrance and displayed aggressive and defensive behaviors. Bees were fed 54% sucrose solution and kept in an incubator at 29 ± 2 °C and 70% ± 5 relative humidity until they were immobilized by cooling. They were then mounted in individual silicone rubber tubes for electrophysiological measurements.

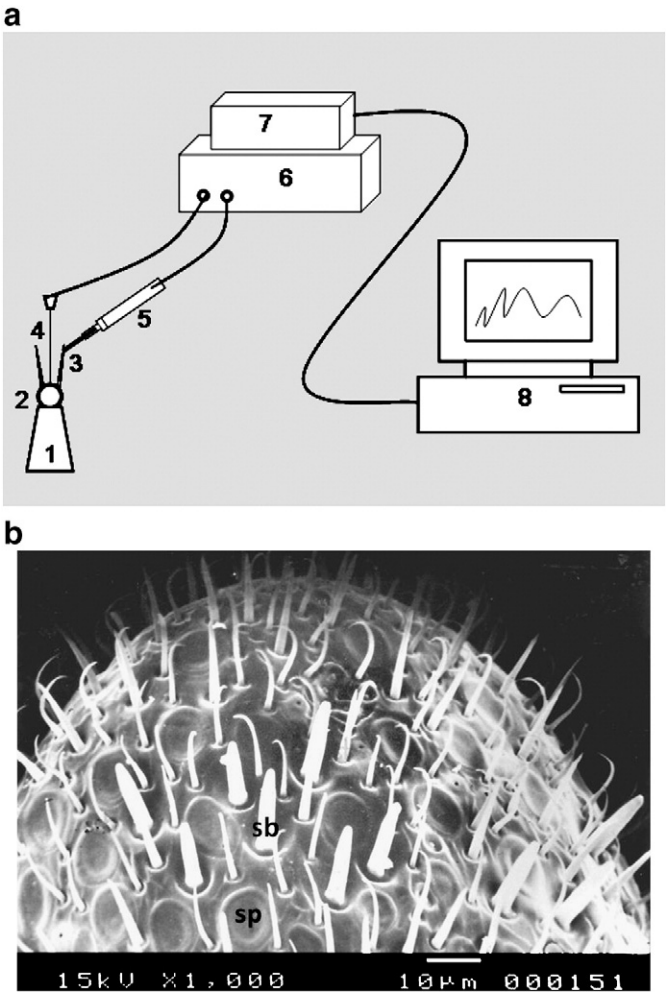

Fig. 1. (a) Schematic drawing of the pheromone exposure system for honeybee antennal sensilla. Silicone rubber tube (1), bee head (2), antenna (3), reference microelectrode (4), recording microelectrode (5), potentiosat (EA161) (6), e-corder (ED 401) (7), and monitor (8). (b) A scanning electron micrograph of the 10th segment of a *A. cerana* worker antenna showing the distribution of sensilla types. Abbreviations: sb, sensilla basiconica; sp, sensilla placodae.

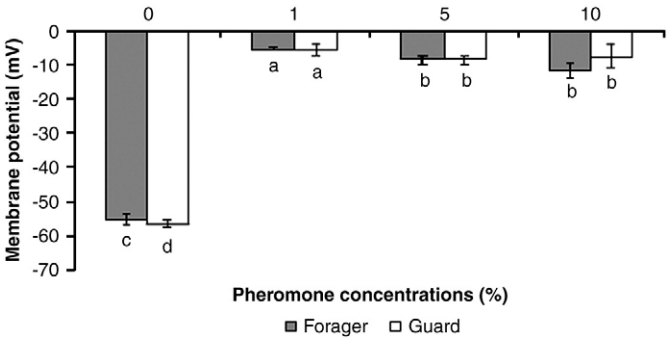

Fig. 2. Membrane potentials of antennal sensillae of *A. cerana* foragers and guards responding to 0.0 (resting potential), 1.0, 5.0 and 10.0% 2-heptanone in bee saline. Means ± SD followed by different letters show significant differences (ANOVA - Duncan's Multiple Range Test, $F = 485.66$, $df = 7$, $P < 0.0001$; Caste, $F = 0.97$, $df = 1$, $P > 0.331$; Caste*Dose, $F = 2.80$, $df = 3$, $P > 0.560$).

Electrophysiological measurements

The head of each bee was fixed with wax. The reference electrode, 0.25 mm diameter copper wire which was connected to a potentiostat (EA161) and an e-corder (ED401) was inserted between the median ocellus and the base of the antenna. One antenna was immobilized with a metal hook for membrane potential measurements. The recording microelectrode, a 30 µm diameter tungsten wire, was connected with a potentiostat (EA161) and e-corder (ED 401) to the antennal sensilla between the tenth segment of the flagellum (Fig. 1a), its tip etched to 0.5 µm diameter was inserted into the antennal sensillar hemolymph at the base of sensilla placodae (Fig. 1b). The head was kept wet at all times with bee physiological saline solution. All measurements were recorded in 5 min intervals and recordings were done in trigger mode using BNC connector. Data were digitized using chart and scope software (Edaq Pty Ltd., UK).

Odor exposure

2-heptanone and (Z)-11-eicosen-1-ol at 0.0, 1.0, 5.0 and 10.0% in bee saline were used as odor stimulants. Each odorant was mixed with solvent and then gently warmed at 70 °C to bring the solids into solution. Both pheromones were 99% pure and purchased from Sigma. Solutions were vortexed then 1 µl was transferred using a micropipette (Socorex) over the antennal sensilla on the tip of the flagellum. Ten foragers and guards were used for each pheromone concentration.

Table 1
Membrane potentials of antennae of *A. cerana* foragers and guards responding to 0.0 (resting potential), 1.0, 5.0 and 10.0% 2-heptanone and (Z)-11-eicosen-1-ol.

Pheromone concentrations (%)	Membrane potential (mV)			
	Foragers		Guards	
	2-heptanone	11-eicosen-1-ol	2-heptanone	11-eicosen-1-ol
0	−55.23 ± 1.44f	−55.23 ± 1.44f	−56.41 ± 1.21g	−56.41 ± 1.21g
1	−5.32±0.46a	−24.00 ± 6.56c	−5.49±1.66a	−47.62 ± 1.46e
5	−8.41±1.33b	−36.36 ± 5.18d	−8.46±1.32b	−46.08 ± 0.87e
10	−11.53 ± 2.16b	−14.60 ± 8.20b	−7.31±3.46b	−9.35±1.96b

Means ± SD followed by different letters show significant difference (ANOVA - Duncan's Multiple Range Test, $n = 160$, $F = 223.03$, $df = 15$, $P < 0.0001$; Pheromones * Doses * castes, $F = 19.75$, $df = 3$, $P < 0.0001$).

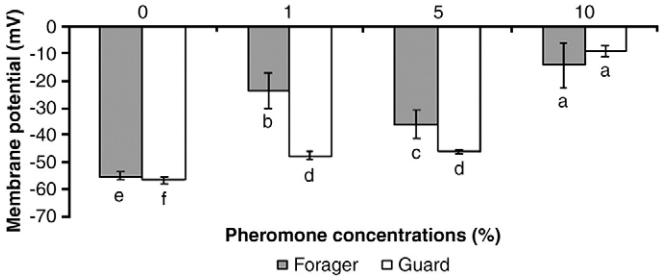

Fig. 3. Membrane potentials of antennal sensilla of *A. cerana* foragers and guards responding to 0.0 (resting potential), 1.0, 5.0 and 10.0% (Z)-11-eicosen-1-ol in bee saline. Means ± SD followed by different letters show significant differences (ANOVA - Duncan's Multiple Range Test, $F=88.68$, $df=7$, $P<0.0001$; Caste, $F=28.47$, $df=1$, $P<0.0001$, Caste*Dose, $F=20.70$, $df=3$, $P<0.0001$,).

Data analysis

ANOVA with Duncan's Multiple Range Test (at $P<0.05$) was used to compare the differences of membrane potential within bee groups (guards and foragers), pheromone types and concentrations.

Results

The resting membrane potentials of *A. cerana* foragers and guards were significantly different ($F=4.68$, $df=1$, $P<0.0407$). Overall, the membrane potentials of foragers exposed to 2-heptanone were not significantly different from those of guards ($F=2.38$, $df=1$, $P>0.1144$ and $F=2.80$, $df=3$, $P>0.560$) (Fig. 2). The membrane potentials of both guards and foragers differed significantly between low concentrations of 2-heptanone (1.0%) and higher concentrations (5.0 and 10.0%). There was no difference between 5.0 and 10.0% ($F=485.66$, $df=7$, $P<0.0001$ and $F=2.80$, $df=3$, $P<0.0510$) (Table 1). The highest membrane potential was in foragers exposed to 1.0% 2-heptanone.

The membrane potentials of foragers exposed to (Z)-11-eicosen-1-ol were significantly different from those of guards ($F=27.08$, $df=1$, $P<0.0001$ and $F=88.68$, $df=7$, $P<0.0001$). The exposure of foragers to the three different concentrations of (Z)-11-eicosen-1-ol showed significantly different membrane potentials among all doses ($F=223.03$, $df=15$, $P<0.0001$ and $F=28.63$, $df=1$, $P<0.0001$) and the same as guards, except between concentrations of 1.0 and 5.0 % which showed no statistically significantly difference ($P>0.05$) (Fig. 3). Bees exposed to 10.0% pheromone solutions had significantly higher membrane potentials than bees exposed to 1.0% or 5.0% pheromone solutions. In general, except for the highest concentration (10.0%) of both pheromones, the membrane potentials of foragers exposed to pheromones were higher than those of guards (Table 1). The membrane potentials of both guards and foragers were significantly greater in response to 2-heptanone than to (Z)-11-eicosen-1-ol ($F=507.41$, $df=1$, $P<0.0001$ and $F=24.19$, $df=1$, $P<0.0001$) (Table 1).

Discussion

The resting membrane potentials of *A. cerana* foragers (−55.23 ± 1.44 mV) were significantly higher than those of guards (−56.41 ± 1.21 mV) ($F=28.63$, $df=1$, $P<0.0001$).

The changes in membrane potential of guards responding to low concentrations (1.0 and 5.0%) of 2-heptanone were significantly different from that of (Z)-11-eicosen-1-ol while it was not significantly different at the 10.0% concentration. This result corresponds to that of foragers responding to these two pheromones where membrane potentials exposed to 2-heptanone were significantly higher than for (Z)-11-eicosen-1-ol (Table 1). The highest membrane potential was found in foragers responding to 1.0% 2-heptanone while the lowest membrane potential was found in guards responding to 1.0% (Z)-11-eicosen-1-ol. This suggests that foragers of this species might have a lower concentration threshold than that of guards. The finding also indicates that increasing concentrations of (Z)-11-eicosen-1-ol may lead to increasing membrane potentials in both guards and foragers. In contrast, membrane potentials decreased with increasing of 2-heptanone concentrations. The changes in membrane potentials of both guards and foragers to these two chemicals suggest that antennal sensilla of this species have different concentration threshold sensitivities to each chemical. It seems likely that variable pheromone concentrations could lead to differences of membrane potential changes during depolarization that are essential for honeybee responses to stimuli (Suzuki and Tateda, 1974; Homberge, 1984).

Because the membrane potentials of both forager and guard bees exposed to the lower concentrations of 2-heptanone were higher than those exposed to higher concentrations, *A. cerana* workers may respond to lower concentrations more quickly than to higher concentrations, although this should be tested with appropriate behavioral bioassays. It is possible that low concentrations of the alarm pheromone attract honeybee workers since they demonstrated high membrane potential changes when exposed to high concentrations. Due to the function of 2-heptanone, which may be a repellent at high concentrations, but an attractant at low concentrations (Maschwitz, 1964; Shearer and Boch, 1965; Boch and Shearer, 1971; Vallet et al., 1991), we assume that low concentration leads to a passage for molecules of 2-heptanone to a specific type of sensory receptor that represents as an attractant. In contrast, at high concentrations it might lead to a passage for molecule of 2-heptanone to other types of antennal sensilla distributed over the tip of the flagellum, resulting in acting as a repellent (Fig. 1b).

There were significant differences between the antennal responses of foragers and guards upon exposure to 1% and 5% concentrations of (Z)-11-eicosen-1-ol. At both concentrations, the response of foragers was significantly greater than that of guards. This may be due to inherent chemosensory differences in response to this compound. However, it may also arise from the higher resting membrane potentials of guards (Fig. 3). Moreover, foragers were more sensitive to the lower concentration (1%) because they exhibited a stronger response to 1% compared to 5%. There was no significant difference between the antennal responses of guard bees at these two concentrations. Thus, (Z)-11-eicosen-1-ol may be more biologically relevant to foragers than guards. It is interesting that foragers were more sensitive to the lowest concentration than guards, and future studies should examine the natural context of this sensitivity in the field on floral resources. The response of guards to high concentrations of (Z)-11-eicosen-1-ol corresponds to its function as an alarm pheromone which alerts guards when they are exposed to colony enemies (Boch and Shearer, 1971; Free et al., 1982, 1988). These findings suggest that honeybee antennal sensory receptors might have specific thresholds to concentrations of different chemicals (Akers and Getz, 1993).

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