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ORIGINAL PAPER

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Effect of pheromones, hormones, and handling on sucrose response thresholds of honey bees (*Apis mellifera* L.)

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Abstract The responsiveness of bees to sucrose is an important indicator of honey bee foraging decisions. Correlated with sucrose responsiveness is forage choice behavior, age of first foraging, and conditioned learning response. Pheromones and hormones are significant components in social insect systems associated with the regulation of colony-level and individual foraging behavior. Bees were treated to different exposure regimes of queen and brood pheromones and their sucrose responsiveness measured. Bees reared with queen or brood pheromone were less responsive than controls. Our results suggest responsiveness to sucrose is a physiologically, neuronally mediated response. Orally administered octopamine significantly reduced sucrose response thresholds. Change in response to octopamine was on a time scale of minutes. The greatest separation between octopamine treated and control bees occurred 30 min after feeding. There was no significant sucrose response difference to doses ranging from 0.2 μg to 20 μg of octopamine. Topically applied methoprene significantly increased sucrose responsiveness. Handling method significantly affected sucrose responsiveness. Bees that were anesthetized by chilling or CO_2 treatment were significantly more responsive than control bees 30 min after handling. Sixty minutes after handling there were no significant treatment differences. We concluded that putative stress effects of handling were blocked by anesthetic.

Keywords Honeybee · Octopamine · Pharmacology Pheromone · Response threshold

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Abbreviations BP brood pheromone · JH juvenile hormone · OA octopamine · PER proboscis extension response · PER-RT proboscis extension response threshold · QMP queen mandibular pheromone

Introduction

A dominant view of animal behavior is the response threshold model where individuals interact with stimuli that release or prime behavioral responses. Neurosensory responses play an important role in the social organization of colonies, affecting response thresholds for foraging behaviors, such as age of first foraging and forage choice-specific behaviors. Honey bees respond reflexively by extending the proboscis when a drop of sucrose solution is applied to the antennae. This is called the proboscis extension response (PER) to sucrose. When bees are presented with an ascending concentration series of sucrose solutions, the response threshold (PER-RT) of an individual can be determined as the lowest concentration that elicits proboscis extension (Page et al. 1998; Pankiw and Page 1999; Ben-Shahar and Robinson 2001). The response threshold of an individual is a window into the neural system that is correlated with foraging behavior, and changes with age (Pankiw and Page 1999).

Modulators of response thresholds are particularly interesting for their potential to change individual behaviors and consequentially change colony-level organization. Two honey bee primer pheromones, queen mandibular and brood semiochemicals are known to affect age of first foraging (Pankiw et al. 1998a; LeConte et al. 2001). Brood pheromone (BP) also releases pollen foraging specific behavior and modulates sucrose response thresholds (Pankiw et al. 1998b; Pankiw and Page 2001a; Pankiw and Rubink 2002). Variation in brain titers of the biogenic amine, octopamine, have been correlated to colony environment, season, worker age, experience, foraging ontogeny, and stress (Harris and Woodring 1992; Bozic and Woodring 1998; Schulz

and Robinson 1999; Wagener-Hulme et al. 1999; Schulz and Robinson 2001). Two studies have examined the relationship between orally administer octopamine (OA) and the onset of foraging in honey bees (Schulz and Robinson 2001; Barron et al. 2002). Behavioral changes correspond with shifts in age-related endocrine gland activity, specifically the corpora allata secretion of juvenile hormone III (JH). Generally, JH titers in honey bee hemolymph increase as workers age (Sasagawa et al. 1989; Huang et al. 1991; Huang and Robinson 1995). Treatment with JH mimic, JH, or the JH analogue methoprene, induces precocious foraging (Robinson 1985; Robinson and Ratnieks 1987; Sasagawa et al. 1989; Huang et al. 1991,1994; Huang and Robinson 1995). Here we examine the modulatory effect of (1) handling method, (2) queen mandibular pheromone (QMP) and BP, as well as (3) OA and (4) methoprene on honey bee PER to sucrose.

Materials and methods

PER assay

The PER assay was used to test each bee's sensitivity to an ascending concentration series of sucrose solutions. Bees were secured with tape into modified Eppendorf tubes (2 ml) that restrained body movement but allowed free movement of the antennae and mouthparts (Bitterman et al. 1983). All bees were first tested for their response to water. Any bees responding to water were allowed to imbibe water until they no longer responded to water stimulation. In this way we controlled for the confounding effect of thirst on sucrose sensitivity (Edgecomb et al. 1994; Pankiw and Page 2001a). The PER assay sucrose solutions were based on a $\log_{10}$ series of −1.0, −0.5, 0.0, 0.5, 1.0, and 1.5 corresponding to the following sucrose concentrations: 0.1%, 0.3%, 1%, 3%, 10%, and 30% (w/v). Sucrose solutions were prepared using distilled and Millipore-filtered water as the solvent for Sigma sucrose (99.5% purity). A droplet of solution was expressed from the tip of a 27-gauge needle and touched to each antenna. An inter-trial interval of 5 min was maintained to reduce sensitization (Menzel et al. 1999). Positive and negative proboscis extension response to each sucrose solution was recorded.

Experiment 1: effect of handling methods on proboscis extension response to sucrose

Laboratory environment can confound behavioral assays. Recently it was demonstrated that even when test protocols are rigorously controlled and inbred strains of test animals are used, significant inter-laboratory effects are observed (Crabbe et al. 1999). Pre-test conditioning of animals to a test condition such as light, reduces intra-laboratory behavioral variation (Walker and Davis 2002). We begin this series of experiments by examining the effects of different intra-laboratory pre-test handling methods on sucrose response thresholds.

The purpose of this experiment was to determine whether different handling methods affected the PER assay responses of 3-day old worker bees. Three hundred newly emerged bees from at least three different sources of wild-type, open-mated queens were placed in Plexiglass/wire-mesh cages (15 cm×8 cm×5 cm). Bees were provided 30% sucrose solution (w/v) and water ad libitum, and reared in an incubator maintained at 33°C, 55% RH, and 24-h light cycle. Bees were handled using three different methods and two different recovery times. Groups of five workers were placed into glass vials and anesthetized by chilling for approximately 4 min at 5°C. Bees were allowed to recover for 30 ($n=59$) and 60 ($n=60$) minutes prior to conducting the PER assay. In the second method bees were mounted in the tubes without anesthesia and allowed recovery times of 30 ($n=60$) and 60 ($n=60$) minutes. The third method anesthetized groups of five bees in 1 l of CO_2 contained in a sealed Ziploc plastic bag for 15 s, and allowed to recover for 30 min ($n=59$) and 60 min ($n=60$) prior to testing.

Experiment 2: queen and brood pheromone effects on PER to sucrose

Experiment 2 tests our prediction that bees reared with QMP will have lower response thresholds than control bees reared without QMP. This prediction is based on the following observations: (1) QMP delays age of first foraging when bees are reared with supplemental amounts of the pheromone Pankiw et al. (1998a), (2) the low-pollen-hoarding strain of bees consistently forages at older ages than the high-pollen-hoarding strain (Calderone and Page 1988, 1996; Calderone 1993; Pankiw and Page 1999, 2001b; Pankiw et al. 2002), and 3) the low strain has characteristically lower response thresholds to sucrose than the high strain (Page et al. 1998; Pankiw and Page 1999, 2001b; Pankiw et al. 2002). As primers we expect larval and queen pheromones to change individuals. If primer pheromones cause physiological change then we expect that individuals withdrawn from or exposed to primer pheromones will exhibit incomplete modulation of sucrose response thresholds compared to individuals reared continuously with or without primer pheromones. Experiment 2 tests this prediction for larval and queen pheromones.

Three hundred newly emerged bees were placed into cages provided with 30% sucrose solution and water ad libitum, and reared in incubator conditions described above. Sucrose and water consumption were measured daily. One cage received a pheromone treatment (QMP or BP) and another cage received a control treatment for 6 days. On the 7th day entire cages of bees were immobilized by chilling at 5°C for approximately 8 min. Half the bees from the pheromone treatment were withdrawn from pheromone treatment for 24 h (QMP + control or BP + control) and half the bees in the control treatment were exposed to pheromone for 24 h (control + QMP or control + BP). The remaining bees continued in a control or pheromone treatment for an additional 24 h. Bees were handled without immobilization and allowed to recover for at least 1 h prior to testing. The PER assay was conducted as described above.

QMP was applied using Bee Boost (PheroTech). Bee Boost is designed to deliver approximately one queen equivalent of QMP per day for about 30 days. One queen equivalent is the average amount of pheromone found in a pair of queen mandibular glands: 200 μg 9-keto-2-(*E*)-decenoic acid, 100 μg 9-hydroxy-2-(*E*)-decenoic acid [88% R-(−) and 12% S-(+)], 20 μg methyl *p*-hydroxybenzoate, and 2 μg 4-hydroxy-3-methoxyphenylethanol (Melathopoulos et al. 1996). The control consisted of blank Bee Boost polymer of equivalent size. The QMP experiment was replicated three times and at least a total of 167 bees per treatment were tested in the PER assay.

A ten-component blend of synthetic BP at a dose of 10 larval equivalents per bee was applied to a glass plate (7 cm×8 cm) and replaced daily (Pankiw and Page 2001a). One larval equivalent of the BP blend used here contained; 5.6 ng ethyl linoleate, 72.8 ng ethyl linolenate, 44.8 ng ethyl oleate, 16.8 ng ethyl palmitate, 39.2 ng ethyl stearate, 11.2 ng methyl linoleate, 117.6 ng methyl linolenate, 140.0 ng methyl oleate, 16.8 ng methyl palmitate, and 95.2 ng methyl stearate. The control consisted of an equivalent volume of hexane applied to a glass plate. Hexane was allowed to evaporation prior to introduction to cages. The BP experiment was replicated four times and at least a total of 130 bees per treatment were tested in the PER assay.

Experiment 3: effect of OA on PER to sucrose

Experiment 3 examines dose- and time-dependent components of orally administered OA on sucrose response threshold modulation.

Hormone treatments PER assay

The assay was modified for these experiments. Bees were not satiated with water prior to testing because sensitivity to water is a potential effect of hormone treatment. Response to water was tested prior to each sucrose concentration tested. There were six water trials and the sucrose concentration series was the same as above.

Effect of time after feeding

Newly emerged bees were reared in cages as described above. At 3 days of age bees were placed in holders without immobilization. Four hours later bees were fed 20 µg OA in 10 µl 30% sucrose solution (Schulz and Robinson 2001) or a control solution of 10 µl 30% sucrose. Orally administered OA is known to increase OA titers in the brain and antennal lobes of honey bees (Schulz and Robinson 1999, 2001; Schulz et al. 2002). A Drummond digital micropipette with a glass tip was used to dispense the solutions. The antennae of each bee were touched with the pipette to stimulate proboscis extension. Nearly all bees imbibed the 10-µl solutions; any that didn't were eliminated from the experiment. Bees were tested using the PER assay 15, 30, and 60 min after feeding. The experiment was replicated three times. Number of bees tested were as follows: OA, 15 min ($n=60$), 30 min ($n=64$), 60 min ($n=77$); control, 15 min ($n=59$), 30 min ($n=67$), 60 ($n=78$).

Dose of OA

Bees were reared in cages and handled as described above. Two hours after placement in holders bees were fed 10 µl of the following treatments: (1) 30% sucrose solution control, (2) 0.2 µg OA in 30% sucrose solution, (3) 2.0 µg OA in 30% sucrose, and (4) 20 µg OA in 30% sucrose solution. At least 50 bees per dose were tested.

Experiment 4: effect of methoprene on PER to sucrose

Newly emerged bees were treated with 5 µl acetone, 200 µg methoprene (Robinson 1987), and 200 µg methoprene acid applied to the abdomen. Methoprene was dissolved in acetone to a total volume of 5 µl. Bees were paint-marked to distinguish treatment and reared in a common cage in an incubator as described above. At 3 and 8 days of age a sub-sample of bees from each treatment were tested in the PER assay. Number of bees tested were as follows: 3 days, control $n=58$, methoprene $n=61$; 8 days, control $n=54$, methoprene $n=43$.

Statistics

Logistic regression model analysis was used to render visual displays and to perform statistical analyses. Because individuals were repeatedly tested for their responses to sucrose and water, responses were not independent following the first solution in the PER assay. The repeated measures and dependent options were used in the PROC GENMOD procedure of SAS (Allison 1999; SAS 2000; Ben-Shahar and Robinson 2001; Hartz et al. 2001). PROC GENMOD produced generalized estimating equations (GEE) for main effects and their interaction. PROC GENMOD also calculated a logistic regression line that best fitted each response curve and tested differences between slopes. Results for slope difference analyses are presented as the delta (Δ) regression coefficient ($\pm$ SE). Additional slope analyses were the result of user-defined contrasts. Where the Δ regression coefficient was significant, slopes were significantly different. Sucrose and water consumption data were analyzed using ANOVA.

Results

Experiment 1: effect of handling methods on PER score

A saturated logistic regression analysis including handling method, time of recovery prior to testing, and their interaction indicated handling method significantly affected responsiveness to sucrose (GEE $X^2=26.3$, $df=2$, $P<0.0001$; Fig. 1a, b). Recovery time was not significant (GEE $X^2=1.0$, $df=1$, $P>0.05$); however, there was a significant interaction of handling method×recovery time (GEE $X^2=27.1$, $df=2$, $P<0.0001$). The control treatment, bees mounted without anesthesia, with a recovery of 30 min singularly contributed to the significant interaction (GEE $X^2=36.1$, $df=2$, $P<0.0001$; Fig. 1a). Bees tested 60 min after handling showed no significant differences between treatments (GEE $X^2=0.03$, $df=2$, $P>0.05$; Fig. 1b). Consequently, in subsequent testing, bees were allowed to recover for at least 60 min prior to testing.

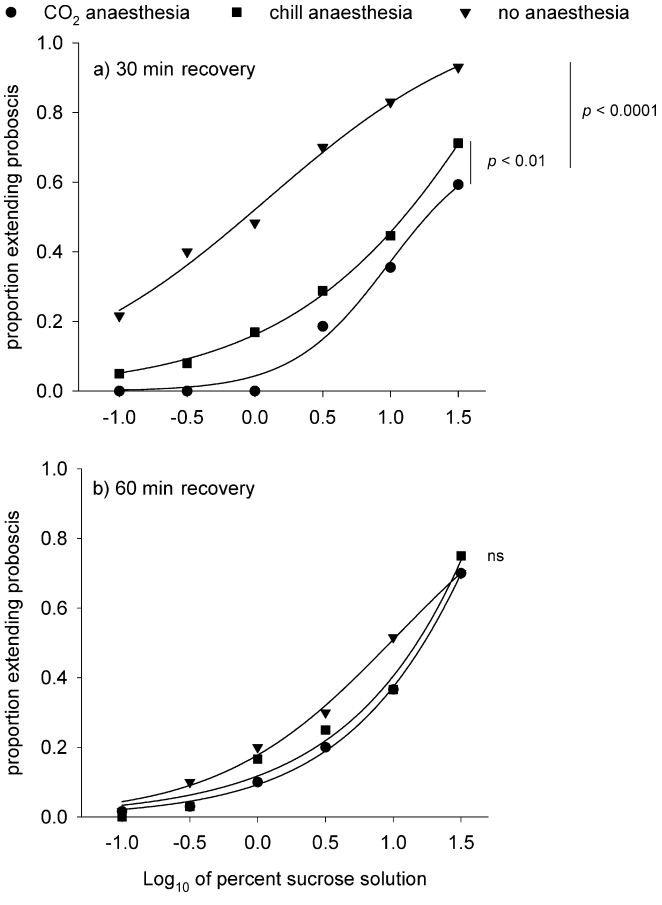

Fig. 1a, b Effect of handling methods on sucrose responsiveness. Bees were anesthetized with CO_2 or chilling or not anesthetized prior to placement into Eppendorf holding tubes. **a** 30 min and **b** 60 min after recovery bees were tested for their responses to sucrose in the proboscis extension response (PER) assay

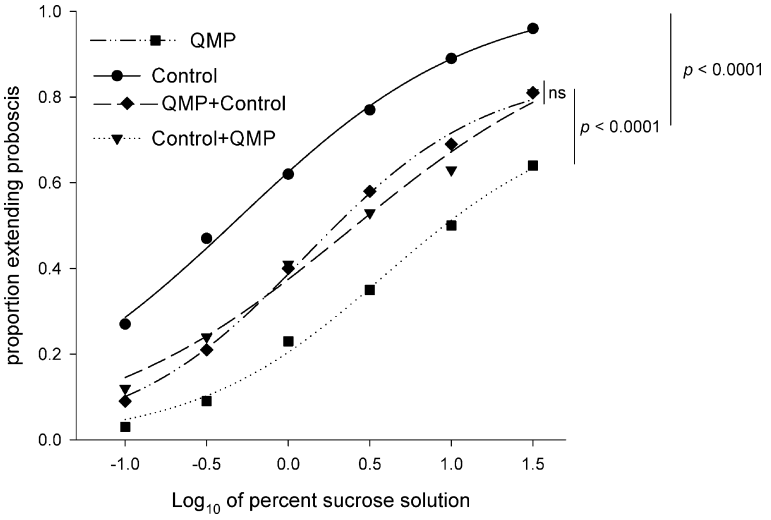

Fig. 2 Sucrose responsiveness of bees reared with queen mandibular pheromone (*QMP*) or no pheromone (*Control*) for 7 days. On the 6th day half of the bees from the QMP treatment were withdrawn from the pheromone for 24 h (*QMP* + *Control*), and half of the control bees were reared with QMP for 24 h (*Control* + *QMP*)

Experiment 2: QMP and BP effects on PER to sucrose

In summary, QMP and BP modulated PER scores over days and hours. QMP modulated the sucrose responses of bees (GEE $X^2 = 80.4$, $df = 3$, $P < 0.001$; Fig. 2). Control bees were significantly more responsive to sucrose than all other treatments (QMP: Δ regression coefficient = 1.2 ± 0.1, $P < 0.0001$; QMP + control: Δ regression coefficient = 0.70 ± 0.1, $P < 0.0001$; control + QMP: Δ regression coefficient = 0.67 ± 0.1, $P < 0.0001$). Bees withdrawn from QMP for 24 h were significantly more responsive than the subset that continued with QMP (Δ regression coefficient = −0.53 ± 0.1, $P < 0.0001$). Bees receiving 24-h QMP exposure or 24-h QMP withdrawal were not significantly different (Δ regression coefficient = −0.01 ± 0.1, $P > 0.05$; Fig. 2).

Amounts of sucrose and water consumed were analyzed using replicate and treatment as main effects in ANOVA. There were no significant replicate effects on the consumption of sucrose (ANOVA, $F = 0.8$, $df = 2$, $P > 0.05$) and water (ANOVA, $F = 0.7$, $df = 2$, $P > 0.05$). There was a significant treatment effect on consumption of sucrose (ANOVA, $F = 36.6$, $df = 3$, $P < 0.0001$) and water (ANOVA, $F = 21.8$, $df = 2$, $P < 0.01$). QMP-treated bees consumed an average of 12.8 ± 0.3 ml sucrose and 5.2 ± 0.7 ml water. Bees withdrawn from QMP for 24 h consumed 6.2 ± 0.1 ml sucrose and 2.5 ± 0.3 ml water. Control bees consumed 12.4 ± 0.5 ml sucrose and 6.3 ± 0.6 ml water. Bees exposed to QMP for 24 h consumed 6.2 ± 0.1 ml sucrose and 2.2 ± 0.5 water. The significant treatment effect was likely due to reducing the number of bees per cage for only one 24-h period. This can be demonstrated by truncating the consumption data to include only control and QMP treatments in ANOVA; sucrose ($F = 0.2$, $df = 1$, $P > 0.05$) and water ($F = 1.0$, $df = 1$, $P > 0.05$). An analysis that included only QMP + control and control + QMP in the ANOVA demonstrated no significant consumption differences for sucrose ($F = 0.04$, $df = 1$, $P > 0.05$) and water ($F = 0.5$, $df = 1$, $P > 0.05$).

BP modulated sucrose sensitivity over days and hours (GEE $X^2 = 19.5$, $P < 0.001$; Fig. 3). In general any treatment with BP significantly decreased responsiveness to sucrose compared to the control (BP: Δ regression coef-

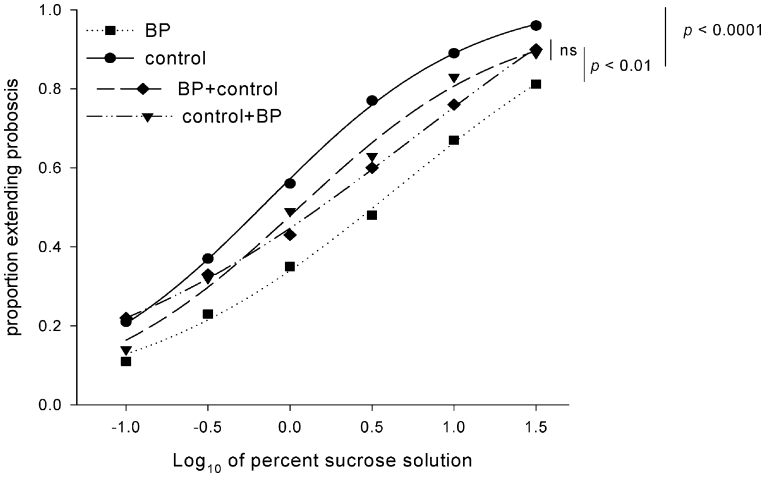

Fig. 3 Sucrose response curves of bees reared with brood pheromone (*BP*) or no pheromone (*Control*) for seven days. On the 6th day half of the bees reared with BP were withdrawn from the treatment for 24 h (*BP* + *Control*), and half of the control bees were reared with BP for 24 h (*Control* + *BP*)

ficient = 0.74 ± 0.1, $P < 0.0001$; BP + control: Δ regression coefficient = 0.50 ± 0.1, $P < 0.0001$; control + BP: Δ regression coefficient = 0.30 ± 0.1, $P < 0.001$). Sucrose responsiveness was significantly lower in bees reared with BP compared to those withdrawn from BP for 24 h (Δ regression coefficient = 0.34 ± 0.1, $P < 0.01$). Bees withdrawn from BP and those exposed to BP for 24 h were not significantly different (Δ regression coefficient = 0.17 ± 0.1, $P > 0.05$).

There was no significant replicate effect on sucrose (ANOVA, $F = 0.3$, $df = 3$, $P > 0.05$) and water consumption (ANOVA, $F = 2.3$, $df = 3$, $P > 0.05$). Treatment significantly affected consumption of sucrose (ANOVA, $F = 5.6$, $df = 3$, $P < 0.01$) and water (ANOVA, $F = 3.1$, $df = 3$, $P < 0.05$). Again, the treatment effect was most likely due to reducing by half the number of bees per cage as part of the pheromone manipulation. This can be seen in the mean volumes of sucrose consumed by treatment: BP (12.5 ± 0.6 ml) versus control (12.1 ± 0.7 ml), ANOVA, $F = 0.2$, $df = 1$, $P > 0.05$; BP + control (6.8 ± 0.2 ml) versus control + BP (7.3 ± 0.1 ml) ANOVA, F = 2.3, $df = 1$, $P > 0.05$. There were no significant differences in water consumption for bees reared for days with or without pheromone; BP (4.3 ± 0.4 ml) versus control (4.3 ± 0.4 ml), ANOVA $F = 0.01$, $df = 1$, $P > 0.05$. However, bees receiving 24-h pheromone manipulation consumed significantly different volumes of water: BP + control (2.0 ± 0.1 ml) versus control + BP (7.3 ± 0.1 ml), ANOVA, $F = 11.8$, $df = 1$, $P < 0.05$).

Experiment 3: effect of OA on PER to sucrose

Effect of time after feeding

Oral octopamine significantly increased sucrose responsiveness of 3-day-old bees (GEE, $X^2 = 23.5$, $df = 1$, $P < 0.0001$; Fig. 4). Time also significantly influenced sucrose responsiveness (GEE, $X^2 = 15.0$, $df = 2$, $P < 0.001$). There was no interaction of treatment×time (GEE, $X^2 = 2.1$, $df = 2$, $P > 0.05$). OA-treated bees tested 30 min after feeding showed the greatest effect and the most significant difference from the control in sucrose responsiveness (Δ regression coefficient = 0.9 ± 0.2, $P < 0.0001$). Significant sucrose response differences between OA-treated groups were; 15 versus 30 min (Δ regression coefficient = 0.4 ± 0.2, $P < 0.05$), and 15 versus 60 min (Δ regression coefficient = 0.6 ± 0.2, $P < 0.001$). Significant sucrose response differences between groups fed the 30% sucrose control were: 15 versus 60 min (Δ regression coefficient 0.7 ± 0.2, $P < 0.001$), and 30 versus 60 min (Δ regression coefficient = 0.6 ± 0.2, $P < 0.001$).

Responsiveness to water was not significantly affected by treatment (GEE, $X^2 = 0.2$, $df = 2$, $P > 0.05$), but time after feeding significantly increased responsiveness to water (GEE, $X^2 = 8.0$, $df = 2$, $P < 0.05$). There was no significant interaction of treatment×time on responsiveness to water (GEE, $X^2 = 3.8$, $df = 2$, $P > 0.05$), meaning that treatment did not differentially affect water responsiveness over time.

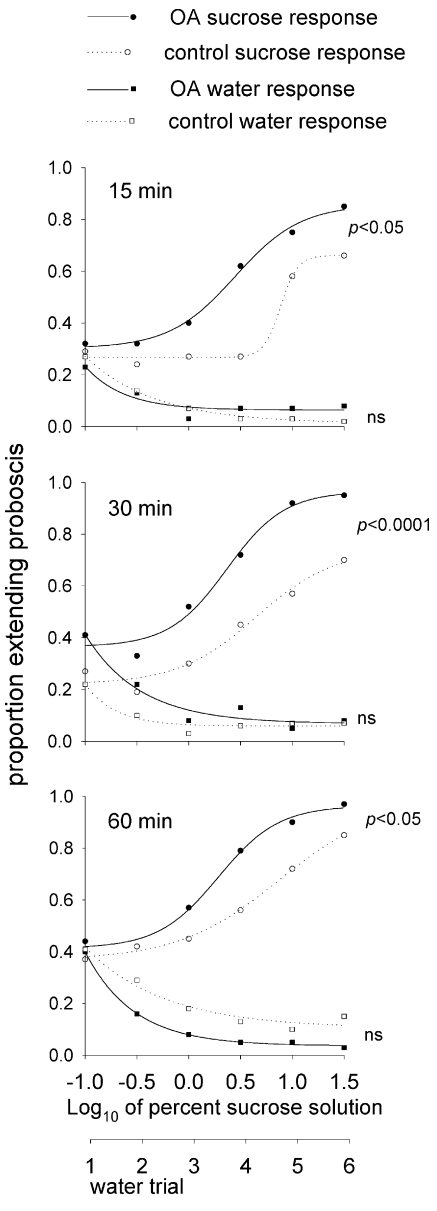

Fig. 4 Effect of time after oral treatment with 10 µl of 10^{-2} mol l^{-1} octopamine in a 30% sucrose solution or a control solution of 10 µl 30% sucrose on PERs to sucrose and water

Dose of OA

In general, all doses of OA significantly increased responsiveness to sucrose in the PER assay (GEE, $X^2 = 11.0$, $df = 3$, $P < 0.05$), but not the water responses (GEE, $X^2 = 4.8$, $df = 3$, $P > 0.05$). Increasing the dose of OA increased the difference in the responsiveness between control and OA-treated bees as demonstrated by the increasing Δ regression coefficient with increasing

Table 1 Logistic regression analysis of sucrose and water responsiveness of bees fed increasing doses of octopamine (OA) in sucrose solution versus bees fed a control solution of sucrose

Parameter	Sucrose response			Water response		
	Δ Regression coefficient	SE	*P*	Δ Regression coefficient	SE	*P*
Contrasts						
OA 20 μg	−0.70	0.25	**	0.54	0.35	ns
OA 2 μg	−0.87	0.28	**	0.71	0.34	*
OA 0.2 μg	−0.94	0.31	**	0.26	0.33	ns
Control	E_{max}	E_{max}	E_{max}	E_{max}	E_{max}	E_{max}
OA 20 μg versus 2 μg	0.19	0.27	ns	−0.16	0.35	ns
OA 2 μg versus 0.2 μg	0.24	0.30	ns	0.30	0.34	ns
OA 3 μg versus 0.2 μg	0.08	0.30	ns	0.40	0.34	ns

*$P < 0.05$;
**$P < 0.01$; *ns* not significant ($P > 0.05$)

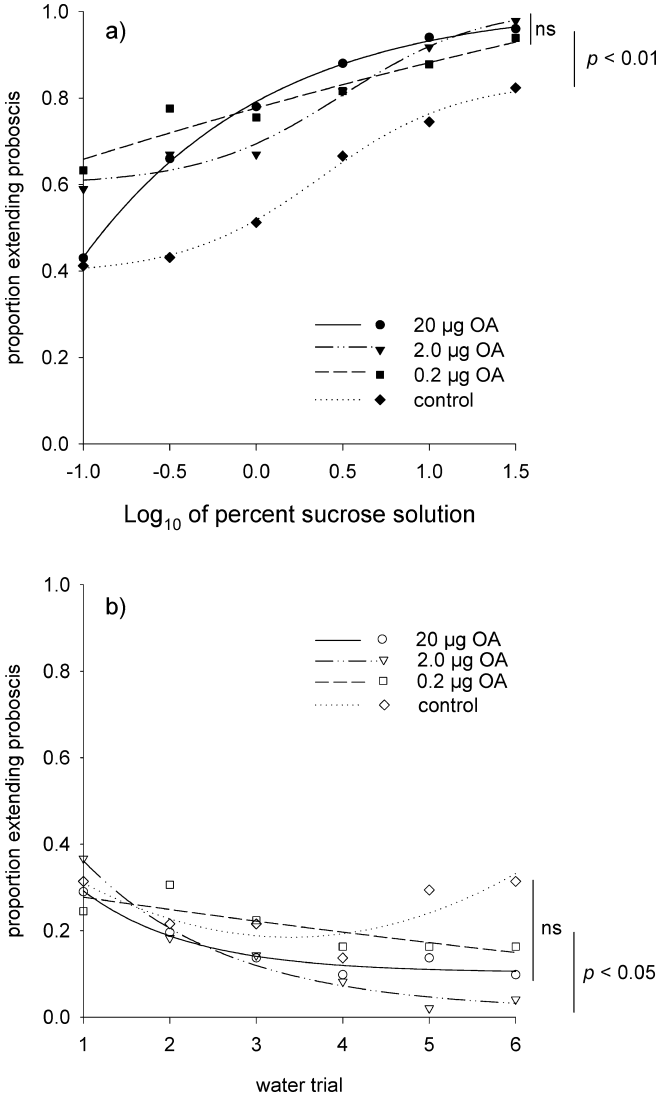

Fig. 5a, b Effect of increasing concentration of oral OA in 10 μl of a 30% sucrose solution and a control solution of 10 μl 30% sucrose on proboscis extension responses to **a** sucrose and **b** water

dose (see parameter in Table 1; Fig. 5). There were no significant differences between the doses of OA for sucrose or water responsiveness (see contrasts in Table 1).

Experiment 4: effect of methoprene on proboscis extension response to sucrose

Methoprene significantly decreased the sucrose response thresholds of bees (GEE, $X^2 = 9.76$, $df = 2$, $P < 0.01$; Fig. 6). Age significantly affected responses to sucrose such that 8-day-old bees were more responsive than 3-day-old bees (Δ regression coefficient $= -0.71 \pm 0.2$, $P < 0.0001$). There was a significant interaction of age by treatment on sucrose responsiveness (GEE, $X^2 = 13$, $df = 2$, $P < 0.01$), due only to the differential responses of acetone treated bees at 3 and 8 days of age (Δ regression coefficient $= -0.6 \pm 0.2$, $P < 0.01$).

The main effect of treatment on responses to sucrose was examined by age. Among 3-day-old bees there was no significant effect of treatment (GEE, $X^2 = 1.6$, $df = 2$, $P > 0.05$). There was a significant treatment effect among 8-day-old bees (GEE, $X^2 = 11.8$, $df = 2$, $P < 0.01$). Compared to acetone treated bees, methoprene-treated bees were significantly more responsive (Δ regression coefficient $= -0.6$, $P < 0.01$).

There was a significant effect of age on responses to water such that 8-day-old bees were significantly more responsive than 3 day-old bees (GEE, $X^2 = 8.2$, $df = 1$, $P < 0.01$; Fig. 6). Treatment did not affect responses to water (GEE, $X^2 = 0.07$, $df = 2$, $P > 0.05$). There was no significant interaction of age by treatment on response to water (GEE, $X^2 = 2.1$, $df = 2$, $P > 0.05$).

Discussion

The response threshold model for division of labor states that the probability of task performance increases when the stimulus associated with that task exceeds an individual's response threshold. This series of experiments demonstrated that changes in pheromone, hormone and, handling environments can change sucrose response thresholds. We show that pheromone and hormone environments modulate the neural systems of bees and can result in the modulation of a neuronal reflex, the PER to sucrose. We assume that changes in PER response thresholds to sucrose are indicators of broader neuronal changes that result in changes in response thresholds to other behaviorally relevant stimuli.

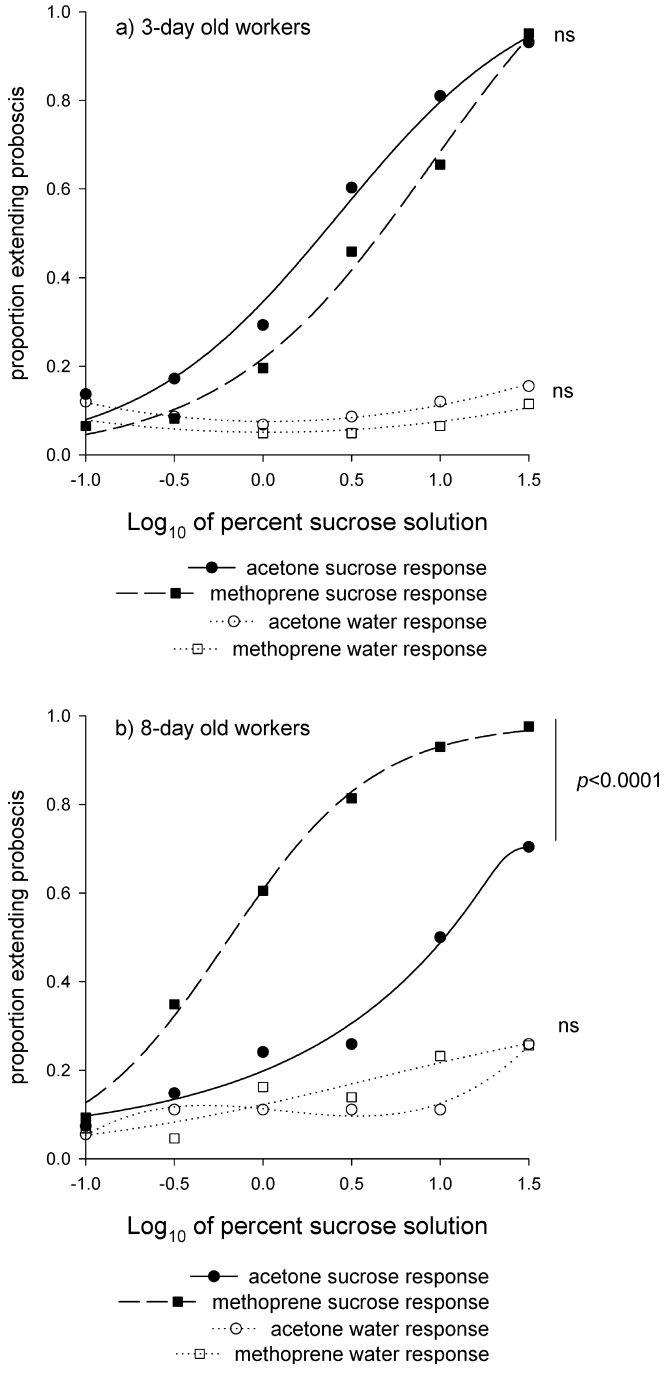

Fig. 6 Newly emerged bees were treated with acetone and methoprene. PER curves to increasing concentrations of sucrose solutions and water at **a** 3 days and **b** 8 days old

Effect of handling method

Handling-related variation in a behavioral assay is a serious consideration that is given little attention. Caution must be exercised when making inferences based on inter- and intra-laboratory experimental results. Handling method significantly affected honey bee sensitivity to sucrose. Bees anesthetized by chilling or CO_2 did not demonstrate time-dependent changes in sucrose responsiveness. The differential PER scores associated with time-dependent sucrose sensitivity in non-anesthetized bees suggests handling stress. Harris and Woodring (1992) stressed workers by grasping one leg with forceps. Octopamine levels peaked at 10 min and reduced to pre-stress levels by 20 min. Putative stress caused by securing bees in Eppendorf tubes required 60 min to decrease to pre-disturbance levels. Handling stress as measured in the PER assay appears to be a transient state changing in minutes. Anesthetizing with CO_2 and chilling may mask, block, or bypass the putative effects of handling stress. It is well known that long-term stress affects the physiology and ontogeny of invertebrates and vertebrates (Hoffmann and Parsons 1994). Less well known are physiological and ontogenetic effects of transient stress events. Concentrated CO_2 anesthetizes bees in the short-term. Long-term exposure results in tissue anoxia, asphyxiation. Learning and memory are impaired in bees when the process of learning is interrupted by CO_2 anesthesia (Erber 1975a, 1975b). It is not known whether CO_2 anesthesia prior to learning impairs subsequent learning and memory. Learning and memory are impaired when these processes are interrupted by chilling in honey bees (Erber et al. 1980) and *Drosophila* (Quinn and Dudai 1976). Cooling of the brain results in reduced amplitude of action potentials and conduction velocity (Winter 1973). Cooling effects on memory were time dependent in the above studies, such that there is an inverse relationship between time to cold treatment after conditioning and degree of memory impairment.

QMP and BP effects on PER to sucrose

QMP and BP significantly modulated worker sucrose responsiveness. It was predictable that bees reared with QMP would have higher response. The prediction was based on previous results that QMP delayed the onset of foraging (Pankiw et al. 1998a) and that the low-pollen-hoarding strain of bees with characteristically high response thresholds, forage later in life than the high-pollen-hoarding strain with characteristically lower response thresholds to sucrose (Page and Erber 2002). The causal relationship between the effects of the pheromone on sucrose response threshold and age of first foraging may be direct or indirect. This remains to be demonstrated.

The incomplete modulation of PER scores in bees withdrawn or exposed to pheromones for 24 h suggests that modulation was a primer response, at least for young bees. BP has dose-dependent effects on foraging ontogeny (Hunt et al. 1999), variably inhibits worker ovary development in queenless colonies (Mohammedi et al. 1998), and stimulates hypopharyngeal gland development (Mohammedi et al. 1996). The modulation of PER to sucrose with primer pheromones suggests that sucrose sensitivity in honey bees is a physiological, neuronally mediated response.

The dose of BP used in this experiment and by Pankiw and Page (2001a) modulated PER scores in opposite directions. These two experiments were conducted in different laboratories using different honey bee stocks from central Texas and northern California, respectively. Differential behavioral responses may be observed from different laboratories even when test apparatus, protocols and environmental conditions are rigorously controlled (Crabbe et al. 1999). Genotypic variation for releaser and primer pheromone responses are not surprising for honey bees (Pankiw et al. 1994, 2000; Hunt et al. 1999), and are documented in other insects (Cowan and Rogoff 1968; Roelofs et al. 1987; Collins and Cardé 1989; Löfstedt et al. 1989; Berisford et al. 1990). Twenty-four-hour exposure or withdrawal demonstrates phenotypic plasticity in response to environmental change. Previous pheromone environment constrained modulation, suggesting neural or physiological change; a primer response.

Effect of OA on PER to sucrose

We predicted that oral treatment of OA significantly increased PER-RT based on observations that (1) OA brain titers are higher in foragers compared to pre-foragers (Schulz and Robinson 1999), and (2) orally administered OA significantly decreased age of first foraging (Schulz and Robinson 2001; Barron et al. 2002). OA has also been implicated as a modulator of pheromone response in division of labor (Barron et al. 2002). The interaction of OA and BP significantly increased pollen foraging activity. It would appear that these substances act synergistically to concurrently decrease response thresholds and stimulus environment.

Oral administration of OA is a recently developed methodology. Previously, it was believed that only injection of OA would affect behavior. However, it has been demonstrated in at least two separate studies that orally administered OA results in elevated brain OA titers (Schulz and Robinson 2001; Barron et al. 2002) A non-surgical method of introducing OA likely eliminated stress effects associated with wounding. Our method of dosing individuals with precise amounts of oral OA solution reduced inter-individual feeding variation cited by Schulz and Robinson (2001) as a source of variation in OA brain levels in their whole-colony feeding method. Our objective was to measure the endogenous effect of OA on sucrose response thresholds of bees. Methodological integration of invasive and non-invasive procedures is likely to emerge.

The time-dependent experiment of OA on sucrose responsiveness demonstrated that oral ingestion of OA lead to a rapid response as measured by increased sucrose responsiveness compared to the control. Time played a role in increasing the separation between control and treated bees. This suggests physiological or molecular processes are involved to effect increased sucrose sensitivity. Dose-dependent responses to OA in the PER assay were not clearly resolved in this study. The highest dose used here was most similar to that used by Schulz and Robinson (2001), and the lowest dose was approximately three orders of magnitude higher than that injected into the brain by Hildebrandt and Mueller (1995). Bees treated with the intermediate dose of OA were significantly less likely to respond to water (Fig. 5). The role of OA in the modulation of sucrose response thresholds and regulation of foraging behavior awaits a better understanding of the neurological levels at which OA functions.

Effect of methoprene on PER to sucrose

Methoprene significantly reduced sucrose response thresholds. Topical application of methoprene has repeatedly been demonstrated to result in precocious foraging (reviewed in Robinson (1992). Selection for the amount of pollen that colonies store in combs resulted in strains of bees that vary in their sucrose response thresholds and rates of development. Five different studies have been conducted constituting 13 different trials under different treatment conditions (Calderone and Page 1988, 1996; Pankiw and Page 2001b; Pankiw et al. 2002). In 11 cases the differences in foraging age were statistically different between the strains with high strain bees foraging at younger ages. The results from this study on the modulation of sucrose response thresholds with methoprene combined with the above studies suggests that low response thresholds to sucrose are associated with reduced age of first foraging.

Methoprene modulated sucrose response thresholds offers one explanation for the mature short-term memory demonstrated by Maleszka and Helliwell (2001) in young, JH-treated bees. Clearly methoprene decreased sucrose response thresholds in this study. Bees with low sucrose response thresholds have repeatedly been demonstrated to have acquisition and extinction curves that are higher and lower in amplitude, respectively, compared to bees with high response thresholds (Scheiner et al. 1999, 2001). A low response threshold to sucrose may occur through several mechanisms: genotype, modulation through feeding and foraging experience, and treatment with pheromones and hormones. Results from this study suggest learning behavior competence may be conferred by individual sucrose response threshold independent of time-dependent nervous system maturation.

Conclusion

We demonstrate how changes in pheromone and hormone environment modulated a neuro-sensory response to sucrose associated with honey bee foraging behavior. Phenotypic plasticity is a dynamic process in which exocrine and endocrine systems link changing environmental cues with individual physiological

behavioral responses. Environmentally induced changes in physiology are among the underlying mechanisms of phenotypic plasticity. Flexible phenotypes occupy developmental trajectories whose directions may be fine-tuned over multiple time-scales in response to environmental information. A division of labor emerges when individuals have different response thresholds to stimuli that release behavioral responses. Colonies may respond to transient changes in environment through flexible phenotypes.

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