Pergamon

Journal of Insect Physiology 46 (2000) 793–801

Journal of Insect Physiology

www.elsevier.com/locate/jinsphys

Effect of an amino acid on feeding preferences and learning behavior in the honey bee, *Apis mellifera*

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Received 29 January 1999; accepted 18 August 1999

Abstract

Amino acids are common constituents of floral nectars and can be critical components in the diets of insect pollinators. Yet the means through which insects detect amino acids can be complex and arise from pre- and post-ingestive mechanisms. Furthermore, the response to an amino acid can change depending on an insect's nutritional status. Here we use a sensitive feeding assay and Proboscis Extension Response (PER) conditioning in the honey bee to assay the effect of glycine, which is a common constituent of nectars and pollens. Subjects preferred to feed on a sucrose stimulus that contained glycine, and the highest relative preference was recorded for the highest concentration of glycine. However, the highest response rate occurred at lower than maximal concentrations and differed depending on the physiological status of the subjects. These results are consistent with a model in which subjects attempt to maintain a physiological target amount of glycine/amino acid relative to other nutrients. All concentrations of glycine enhanced the rate and magnitude of a conditioned response to an odor in the PER assay, which demonstrates that animals can learn to modify their responses to an odor conditioned stimulus based on the presence of amino acid. This capability would enhance a honey bee's ability to evaluate the quality of floral nectars, which are associated with, among other things, odor cues given off by flowers. In future studies these techniques will allow us to evaluate the physiological roles that amino acids play in honey bee diet and choice behavior.

Keywords: Honey bee; Sucrose; Amino acid; Taste; Conditioning

1. Introduction

Amino acids are common constituents of floral nectars of insect pollinated plants (Baker and Baker, 1973a,b; Baker, 1977a,b). The presence of amino acids presumably affects the attractiveness of nectar to pollinators (Baker, 1977a,b). Yet only recently have experimental approaches been applied to begin to elucidate the role of amino acids in nectar. For example, both amino acids and sugar content affect the recruitment of fire ants (*Solenopsis geminata* and *S. invicta*) to plants (Lanza et al., 1993). Other studies have found preferences in butterflies and honey bees for nectars that contain specific amino acids (Inouye and Waller, 1984; Alm et al., 1990). The general conclusion from these studies is that, within certain concentration ranges, insects prefer solutions that contain some kinds of amino acids. Furthermore, at least for honey bees (Inouye and Waller, 1984), high concentrations of some amino acids are repulsive.

Any further interpretation of the role that amino acids play in the regulation of pollinator behavior would necessitate a more detailed understanding of their sensory, nutritional and physiological functions. Indeed there are many such functions, which might make any such interpretation very complex. Amino acids contribute to the intake of dietary nitrogen and are precursors to proteins, and some amino acids are essential components of insect diets (Chen, 1986). In addition, amino acids occur in high concentration in the insect nervous systems (Bicker, 1991), where they act as important neuromodulators. Amino acids have also been implicated in the process of memory consolidation in crickets (Jaffe et al., 1992), which is important given that learning and memory are involved in the nectar foraging behavior of many nectivorous insects (Lewis and Papaj, 1993).

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0022-1910/00/$ - see front matter
PII: S0022-1910(99)00168-7

These different functions can arise from a variety of pre- and post-ingestive effects on behavior. Nutritional and neuromodulatory functions are primarily post-ingestional effects. That is, the amino acids would first pass through the gut (Neal, 1996) and into the hemolymph of the insect before having an effect. For example, a modulatory peptide such as glycine, or a derivatized version of it, would have to circulate in the hemolymph to target tissues in the CNS. Post-ingestive effects would most likely have a time course on the order of minutes to hours. Several insects also have sensory receptors that are sensitive to amino acids (Simpson et al., 1991; Mullin et al., 1994). Therefore, the detection of and behavioral responses to an amino acid in food could in part be driven by pre-ingestional sensory inputs, which might be expected to have much shorter time courses, i.e., on the order of seconds.

Because of the complex nature of pre- and post-ingestive effects, it would be necessary to study the behavioral effects of amino acids in an animal in which the time course for measuring behavior can be precisely controlled. The honey bee (*Apis mellifera*) is ideal for this type of research. First, sucrose solutions spiked with an amino acid elicit reactions from freely flying forager honey bees that depend on the specific amino acid (Inouye and Waller, 1984; Alm et al., 1990). Some amino acids elicit no change in foraging effort relative to a control sucrose solution, whereas others produce an increase or a decrease in preference. Second, individual honey bee workers can be behaviorally tested using a controlled procedure, such as Proboscis Extension Response conditioning (PER; Menzel and Bitterman, 1983; Menzel, 1990). Subjects are conditioned to respond to an odorant by associating it with sucrose reinforcement. This procedure allows for the precise control of exposure to stimulation and subsequent testing of behavioral responses.

In this work we have used several behavioral assays, including PER, to determine the effect of glycine on feeding behavior. We chose glycine because it is a constituent of some nectars (Baker and Baker, 1973a,b; Baker, 1977a,b), and it is also common in pollen (Barbier, 1971; Mullin et al., 1993). Furthermore, although it has not yet been shown for honey bees, at least some insects possess sensory receptors that respond to glycine and GABA (Mullin et al., 1994). Inouye and Waller (1984) have shown that honey bees are repelled by unnaturally high concentrations of glycine, while at natural concentrations glycine had little effect on feeding preference. Using the latter range of concentrations, we show that glycine can have a variety of effects that are dependent on the behavioral response measure, concentration and physiological status of test animals. These data provide some insight into the processes that control amino acid preference behavior and complement assays under field conditions (Inouye and Waller, 1984).

2. Methods

Worker honey bees were collected individually in small glass jars either as they departed from a colony maintained out of doors or as they flew within a flight cage in an indoor flight room. Each experiment used exclusively bees from one or the other source. Subjects used for PER were then cooled to 5°C in an ice–water bath. Immediately after they ceased moving they were restrained in small harnesses and fastened by a strip of tape (placed between the head and thorax). They were then left for 2 h at room temperature to acclimate to the conditioning situation. In one experimental series, which involved tests for feeding preferences, subjects were collected and placed directly into a small test arena without cooling.

2.1. Feeding preferences

Feeding preferences for glycine+sucrose versus sucrose were assayed using a proboscis print assay (R. Edgecomb, personal communication). Five subjects were collected each day from the entrance to an outside colony or from an indoor flight room and confined to a small ($17\times11\times16$ cm^3) box for 1 h. The box contained two microscope cover slips placed side by side on the floor of the box. One side of the box was made of glass to allow the entry of light from the room. One (*reference*) cover slip had been dipped into a 1% agar (Sigma Chemical Co.) solution containing sucrose at 0.5 M concentration. The remaining (*treatment*) cover slip contained the same sucrose concentration together with glycine at one of the following amounts per 50 ml: 100 mg, 1 mg, 0.01 mg, 0.0001 mg, or 0 mg (this last group served as a reference group for side preferences). Cover slips were allowed to dry for 2 h at room temperature.

When walking on a cover slip subjects would extend their proboscides to make contact with, and presumably feed on, the material deposited in the agar. When the proboscis was withdrawn a small print was left behind, which could clearly be recognized as a proboscis print. After a trial, the number of prints on each side was counted. The summed number of prints on both sides and the ratio of prints on the treatment versus reference cover slips were used as response measures. For the latter measure, a ratio significantly higher than 0.5 would indicate a preference for the glycine-treated side.

2.2. Proboscis extension response conditioning

PER conditioning procedures were run according to established protocols (Menzel and Bitterman, 1983; Menzel, 1990; http://iris.biosci.ohio-state.edu/honeybee). After they had been set up in restraining harnesses, subjects were fed approximately 1 μl of 1.5 M sucrose solution. They were then left on the countertop for 1 h prior

to the start of a conditioning procedure. Subjects were tested for motivational state prior to conditioning by touching one antenna with the 1.5-M sucrose solution without subsequent feeding. If a subject extended its proboscis, as most did, it was selected for use in the conditioning procedure.

The odorant Conditioned Stimulus (CS) was prepared by placing 3 μl of pure odorant onto a strip of filter paper that was inserted into a 1-cc glass syringe. Geraniol and 1-hexanol counterbalanced so that approximately the same number of subjects were conditioned to each. The rubber seal of the plunger, through which a small opening was cut, restricted the opening of the syringe. The ground glass fitting on the syringe was used to connect the syringe to a small valve. When the valve was opened by a signal from a computer, air was shunted through the syringe and over the subject for 4 s.

Three seconds after onset of the odor CS the solution used as the Unconditioned Stimulus (US) was first applied to the antennae and then to the extended proboscis for feeding. A 1-μl droplet was used, and subjects always consumed the entire droplet within the allotted 2-s time-span. The base solution used as the US was always 0.5 M sucrose. Such a low concentration should still support learning (Smith, 1997). However, because the response levels might be lower than would be expected from the use of higher sucrose concentrations as the US, there would be enough flexibility to determine increases or decreases in response levels due to amino acid content.

One control group always received the 0.5-M sucrose solution as the US. Other groups received this same sucrose concentration, but the US solution also contained one of the following amounts of glycine added to 50 ml: 100 mg, 1 mg, 0.01 mg, or 0.0001 mg. The concentration series was made via serial dilution of a glycine stock solution into the stock sucrose solution.

One training trial consisted of the presentation of the CS for 4 s and partially overlapping it with the presentation of the US until the droplet was consumed, which always occurred within 2 s. Eight such trials were presented to each subject. A trial began with placement of a subject into a training arena through which a constant exhaust stream of air was drawn. Approximately 20 s after placement, the CS and US were delivered, shortly after which the subject was moved to a holding tray and replaced with the next subject. Each trial lasted for a total of 1 min. The intertrial interval was constant and set by the number of subjects conditioned per day. One subject per treatment group was conditioned each day. Thus, if six treatment groups were used for an experiment, the intertrial interval was 6 min.

Extinction trials were performed after the fourth and the eighth conditioning trials and consisted of the presentation of only the odorant CS, as described above. During these trials the responses to CS presentation were videotaped. Off-line analysis of the videotaped responses allowed for the calculation of response duration using frame codes placed on each video frame. Duration is defined as the cumulative time the proboscis was extended beyond the opened mandibles during a given trial Smith, 1997).

2.3. Ingestion of amino acid solutions

For some experiments, honey bees were fed a treatment solution prior to a behavioral assay that was designed to evaluate the rate of freely moving activity. Behavioral assays with these subjects began 30 min after treatment. In order to test for non-specific changes in activity levels, three groups were assayed for line crossing activity for 5 min in a 9-cm diameter Petrie dish. Two perpendicular lines were drawn on the top of each dish, and each time a subject crossed one of these lines a counter was incremented.

2.4. Statistical analyses

Statistical analyses were performed according to the procedures in Sokal and Rohlf (1995). For data that met the appropriate assumptions, an appropriate parametric ANOVA was performed. The raw data were either arcsin (ratio data) or log transformed (duration scores from PER studies) prior to use in a two-way ANOVA.

3. Results

3.1. Effect of glycine on feeding preferences

The effect of glycine on feeding preferences of freely moving honey bees was complex, with at least three significant treatment effects that depended on the response measure. Figs. 1 and 2 summarize the data for the ratio of proboscis prints on the treatment versus control sides and for the summed number of prints. The data are further subdivided according to whether subjects were collected from a field colony or from a colony maintained in a flight room.

The ratio measure clearly shows a preference for the treatment slip when that slip contained the highest (100 mg/50 ml) amount of glycine (Fig. 1). The ratio of treatment to reference sides was significantly higher than the respective ratios for the remaining groups. The ratios for groups that were given glycine-coated treatment slips that contained 1 mg of glycine, or less, per 50 ml did not differ markedly from the ratio for the control group, which received no glycine on either slip. This effect of concentration led to a significant glycine-side effect in the ANOVA (F=5.3; df=4; $p<0.001$). Furthermore, the ratio for the high concentration of glycine was significantly higher in subjects collected from the flight room

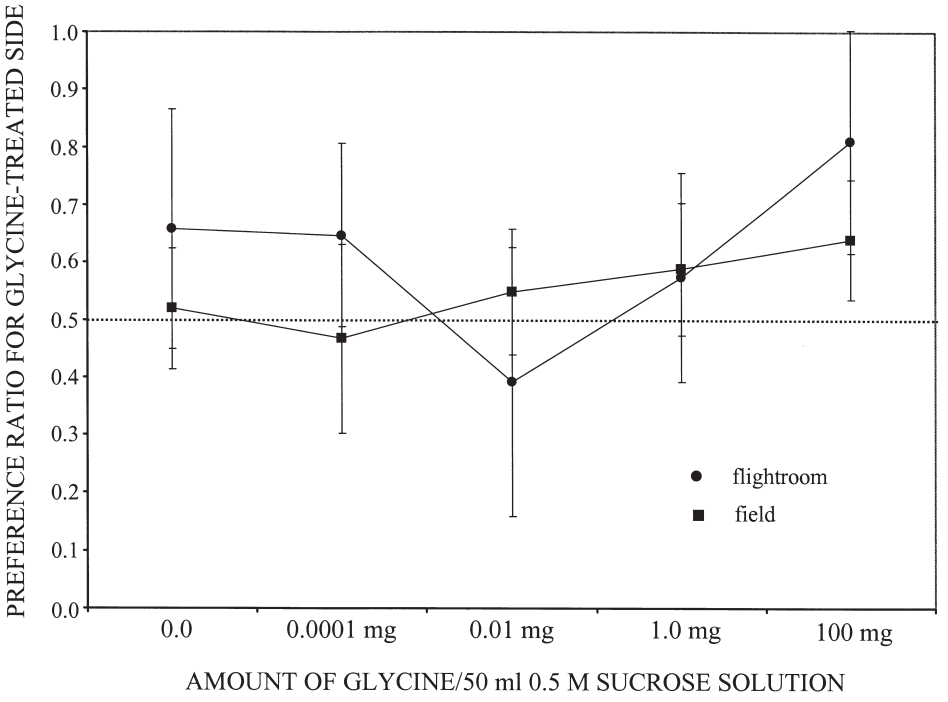

Fig. 1. Preference ratio for feeding from slides coated with sucrose or sucrose+glycine in the proboscis print assay. Honey bee workers were collected as they flew in an indoor flightroom (●) or as they departed from a colony maintained outdoors (■). Each point represents the mean of seven to 10 independent groups of five subjects that were confined to a small box and given a choice between two cover slips. One slip was coated with a solution that contained 0.5 M sucrose. The other slip was coated with sucrose that contained glycine added in the amount indicated on the abscissa. The preference ratio was calculated by dividing the number of prints on the glycine slip by the total number of prints on both slips. In the case of the control group, which was presented with two sucrose-coated slips, one slip was chosen at random as the 'treatment' slip. Vertical bars represent standard errors.

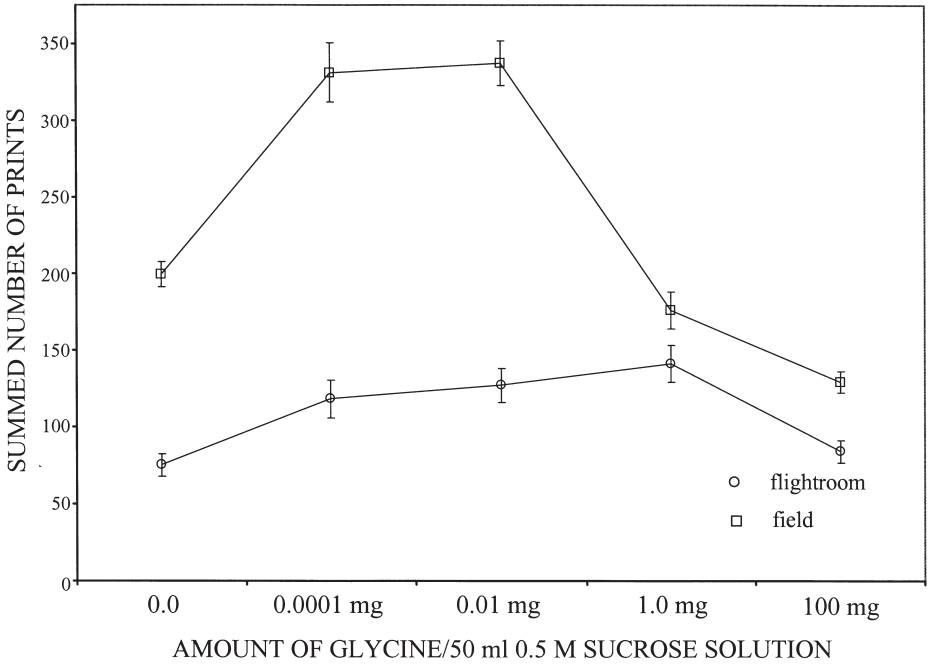

Fig. 2. Total number of proboscis prints left while feeding from slides coated with sucrose or sucrose+glycine in the proboscis print assay. Honey bee workers were collected as indicated in Fig. 1. Each point represents the mean of seven to 10 independent groups of five subjects that were confined to a small box and given a choice between two cover slips. One slip was coated with a solution that contained 0.5 M sucrose. The other slip was coated with sucrose that contained glycine added in the amount indicated on the abscissa. The total number was counted across both slips. Vertical bars represent standard errors.

than in subjects collected from field colonies (i.e., 0.81 versus 0.64, respectively), which yielded a significant collection site effect (F=6.9; df=1; $p<0.05$). Finally, the relative change in preference for the highest concentration of glycine was greater for flight room bees, which was reflected in the significant glycine×collection site interaction term (F=3.2; df=4; $p<0.05$).

The summed number of proboscis prints on each cover slip provides a measure of the rate of feeding activity during the 1-h period during which subjects were exposed to the cover slips. In contrast to the effects revealed by the ratio measure, the rate of feeding activity decreased as the concentration of glycine increased across the range used in the present study (Fig. 2). Thus, a significant effect of concentration was evident (F=28.3; df=4; $p<0.01$). As with the ratio measure, the magnitude of the effect differed according to the collection site (F=4.3; df=1; $p<0.01$): subjects collected from a field colony showed on average higher rates of feeding activity than subjects collected from the flight room colony. The fact that different glycine concentrations elicited the highest activity levels between the two collection sites yielded a significant interaction term (F=2.67; df=4; $p<0.05$). For collected–collected bees the highest activity level was observed when the treated slide was coated with an intermediate glycine concentration (1 mg glycine/50 ml), whereas for field–collected bees the highest activity levels were observed for the two lowest glycine concentrations (i.e., 0.01 and 0.0001 mg glycine/50 ml).

3.2. Effect of glycine on learning performance in PER conditioning

The addition of glycine to the US had a significant effect on learning performance. Fig. 3 shows the percentage of subjects in each treatment group that responded to the odor prior to the presentation of the US during acquisition trials. In the group that received 0.5 M sucrose as the US (filled circles), response levels reached an asymptotic level of 40–60% by trial 4. This level of responding is lower than that typically observed in PER conditioning of honey bees, perhaps because of the lower concentration of sucrose used as a reinforcement in the present experiment. As for trial 2, the response levels in all groups in which glycine supplemented the 0.5-M sucrose US were higher than the sucrose-only control group.

These differences were manifested during the extinction (odor-only) trials performed after acquisition trials 4 and 8 (Fig. 4). These trials were inserted to assay performance at set points during acquisition, at which subjects were tested under a common set of conditions. The general pattern was qualitatively the same at both test points. There was a significant effect due to whether or not glycine was present in the US (F=3.9; df=4; $p<0.01$). As expected from the lower response levels during acquisition (Fig. 3), the group that received sucrose-only as the US had lower mean duration of response scores at both test points. Groups that received a glycine supplement to the US, regardless of the concentration, had significantly higher mean duration scores. There was no effect of trial (F=0.2; df=4; ns) or trial×glycine interaction (F=0.3; df=4; ns). The lack of significance in the latter two terms indicates that asymptotic levels of the conditioned response were achieved by trial 4 and that all of the glycine groups performed comparably in spite of the slightly lower response levels for the intermediate glycine concentrations at trial 8.

3.3. Effect of glycine on rate of activity

The effects of glycine described above could be attributable to an increased rate of activity in groups that were fed glycine solutions. An increase in the rate of activity via glycine treatment would have the effect of increasing rate of feeding, and it might also increase the rate of response in the PER assay. However, glycine failed to affect rate of line crossing activity when it was fed to subjects 30 min prior to a line crossing assay (F=0.3; ns). Mean numbers of line crosses during the assay, in which subjects were confined to a Petrie dish, did not differ across treatment groups fed 0.5 M sucrose (mean=97.0; n=9) or sucrose that contained either of two amounts of glycine (means=84.3 and 87.7 for 0.001 and 0.1 mg/50 ml, respectively; n=9 in each case).

4. Discussion

The general conclusion from this series of experiments is that glycine can modulate feeding preferences and learning performance in honey bees. In contrast to Inouye and Waller (1984), who reported that glycine only inhibited feeding at high concentrations, we have found conditions under which glycine potentiates those responses. As reviewed below, we feel that the discrepancy between the studies could reflect the maintenance of a physiological set point for the amino acid that the animals are attempting to maintain. The effects we found were dependent on a number of factors, which included concentration of glycine in the 0.5-M stock sucrose solution, source of the subjects, and the behavioral measure. The first experiment revealed that subjects prefer a sucrose solution that contained glycine. At higher glycine concentrations, subjects fed more often from the glycine treatment slip than from a sucrose-only control slip. At lower glycine concentrations the pattern of choice was random, as subjects did not prefer one slide over the other. Interestingly, the overall rate of feeding activity increased at lower glycine concentrations. This increase in feeding activity was not simply due to an

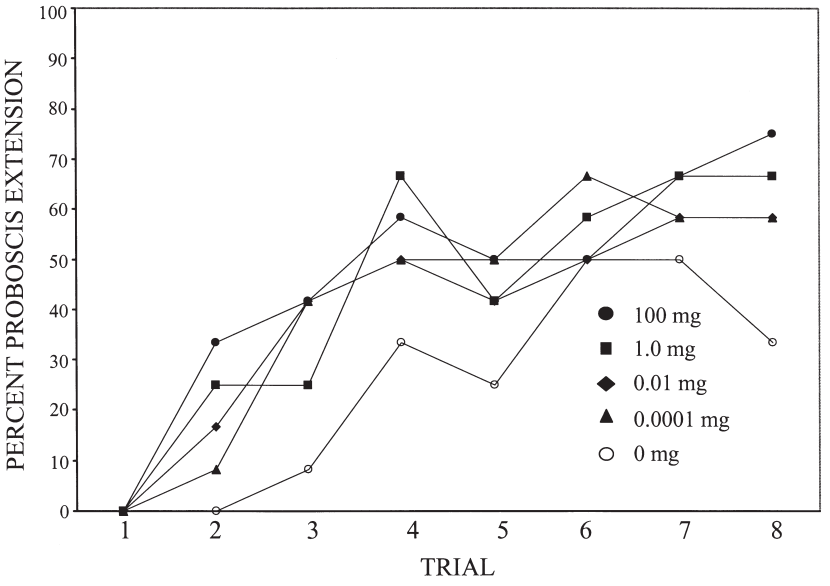

Fig. 3. Percentage of subjects that responded to the odor conditioned stimulus for each of the eight conditioning trials. For each trial the odor was associated with a 0.5-M sucrose solution that contained the indicated amount of glycine/50 ml. There were 12 subjects in each group. 1-hexanol and geraniol were counterbalanced as the conditioned stimulus. Note that the reinforcement was omitted on trials 4 and 8 in order to calculate the response duration from videotape recordings (see Fig. 4).

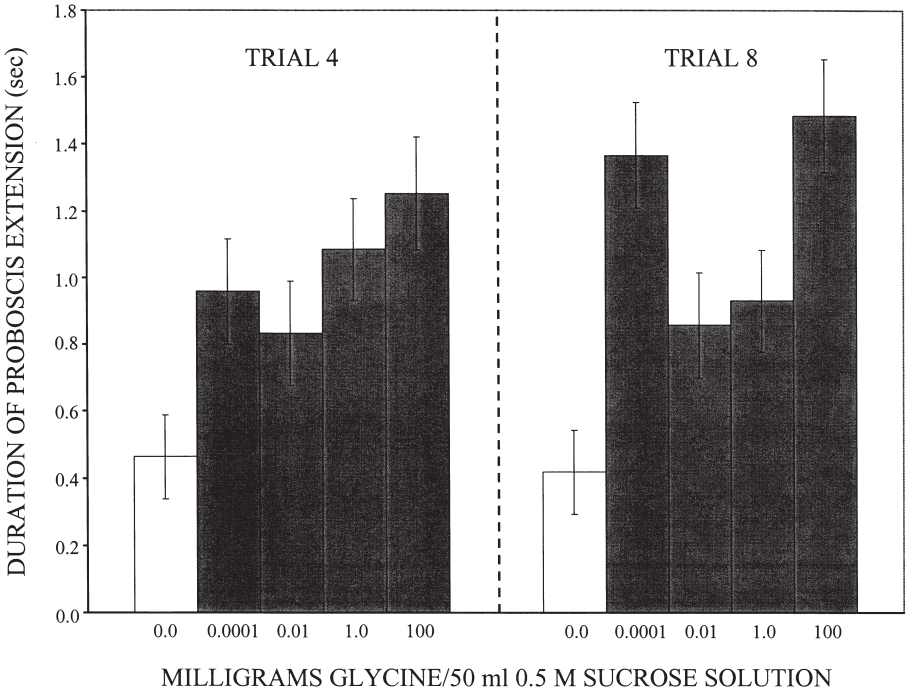

Fig. 4. Mean response durations during trials 4 and 8 for groups conditioned to 0.5 M sucrose solution that contained the indicated amount of glycine. *N*=12 for each of the five groups. Vertical bars represent standard errors.

increase in the rate of locomotor activity, because subjects fed glycine failed to show higher rates of activity than those fed only a sucrose solution. Finally, when glycine was added to a sucrose solution used to reinforce the odor in a Pavlovian conditioning paradigm, all concentrations of glycine increased the rate and magnitude of the conditioned response.

The preferences for glycine in the first experiment could have arisen if subjects were attempting to maintain a specific nutrient target (Simpson and Raubenheimer, 1993a, 1995; Raubenheimer and Simpson, 1994). Insects modify their feeding patterns on carbohydrate and protein diets depending on the levels of both in the hemolymph (Simpson and Raubenheimer, 1993b). Animals

prefer a protein diet if they have been fed on a carbohydrate diet that lacked protein, and they will prefer a carbohydrate diet if they have been fed on a protein diet that lacked carbohydrate (Simpson et al., 1988, 1990). In each case, the lack of an essential diet component would have pushed the animals away from the set point for that nutrient. The preference for the missing nutrient should persist until the desired target is reestablished. This mechanism for the regulation of feeding behavior can result in inverted-U shaped or negatively sloping preference functions across increasing concentrations of the missing nutrient (Raubenheimer and Simpson, 1993). These types of functions arise because nutrient-deprived animals must feed on a high concentration of that nutrient for less time, or at a lower rate, than on a lower concentration in order to reestablish the target for hemolymph amino acid levels. If the amino acid concentration in the food approaches zero, or the limits of detectability, the preference for that diet would drop off once again. Also the amino acid target level can change depending on the level of a second nutrient (Raubenheimer and Simpson, 1993). In our case, a change in sucrose concentration from the standard 0.5-M level that we adopted might be expected to change the slope or the shape of responses to different concentrations of glycine.

The shapes of the amino acid preference functions (Figs. 1 and 2) could be consistent with a model that involves the maintenance of a set point for two or more nutrients, in which the value of one nutrient depends on the value of another. At higher glycine concentrations subjects might attain the target rapidly with a few feeding bouts on the glycine-treated slip. This would result in a preference for glycine and a low rate of feeding activity. At lower concentrations, attainment of the target might require prolonged high rates of activity in groups in which one slip was treated with glycine. The higher rate could cause animals to respond less specifically to the glycine-treated slip. The drop off in activity at the lower end occurred in groups in which neither slip was treated with glycine.

The effect of collection site on feeding preferences for amino acids could arise from two causes. First, subjects collected in a flight room could be maintaining different protein/amino acid targets than subjects collected as they departed from field colonies. Alternatively, they could be maintaining the same target from different starting points in a multidimensional nutrient space. Simpson and Raubenheimer (1996) described a similar set of curves in a model of the relative phagostimulatory power of different concentrations of two nutrients. In our case, the level of carbohydrate was fixed. It could be that this level was near optimal for field bees, which in the model would be sufficient to reveal differential sensitivity to the amino acid. But the sucrose concentration may have been either too high or too low for flight room bees given the range of amino acids tested. In that case, the model would predict the flatter curve that we observed.

It is reasonable to assume that field and flight room bees are different in terms of their metabolisms. Honey bees would normally be in an over wintering mode during winter months, which might entail the alteration of their basic metabolism. We maintain these bees at higher rates of activity in the flight room by placing sugar-water and pollen into the colony. We have observed that workers collected while flying in the flight room have more pollen in their hindguts than foragers collected from field colonies in the summer. It is therefore possible that, under these conditions, flight room bees would differ from field collected bees, which for the most part would be foragers collected as they depart on foraging flights. At present we cannot resolve this issue. But the existence of this effect indicates that a honey bee's nutrient space might be manipulable in future experiments.

Glycine also potentiated the conditioned response when it was fed as part of the reinforcement in a PER conditioning paradigm. Studies of PER conditioning in honey bees traditionally use sucrose reinforcement of odor conditioned stimuli (Menzel and Bitterman, 1983; Menzel, 1990). Under normal circumstances, animals increase their response rates and magnitudes as the concentration of sucrose reinforcement is increased (Smith, 1997). This increase could be attributable to sucrose-sensitive taste receptors on the antennae and/or mouthparts (Whitehead, 1978). These receptors feed into the subesophageal ganglion of the brain, where they interact with brain interneurons that relay the sensory information to other brain centers that mediate the association of sensory traces from olfactory centers (Hammer, 1993; Hammer and Menzel, 1995). Amino acids could have an effect on sensory coding via two mechanisms. First, it is possible that taste cells that are responsive to amino acids, which have been described for other insect species (Mullin et al., 1994), exist in the honey bee. These cells might mediate excitatory associations with olfactory pathways in much the same manner as the sucrose receptors. Second, amino acids such as glycine may synergize the response of sucrose sensitive cells to sucrose. Sensory cell recordings would be required to resolve this issue.

Alternatively, glycine could exert its effect post-ingestively via the hemolymph. Once ingested, amino acids are passed to the midgut where they are transported by proteins across the brush border membrane (Neal, 1996). If enough glycine is transported into the hemolymph, then it is possible that its effect on learning could arise from its post-ingestive effect on target organs via hemolymph transport. Locusts adjust feeding responses to protein based on levels of protein/amino acid in the hemolymph (Abisgold and Simpson, 1987), and it is common for the sensitivity of taste receptors to vary with recent feeding history (Simpson et al., 1991). Simpson

and Simpson (1992) have demonstrated that amino acids in the hemolymph influence the responsiveness of taste receptors, perhaps by reaching the sensillum liquor that bathes receptors. This would be a post-ingestive means through which the sensitivity of the sensory cells is regulated.

Regardless of the locus of the effect, it is clear that honey bees can learn to modify their behavior based on the detection of amino acids in a feeding stimulus. This ability would certainly help them to evaluate the quality of floral nectars, at least with regard to their value in reaching and maintaining a desired nutrient target. However, we have not yet demonstrated whether the effect on learning is due to associative mechanisms involved in linking the sensory representations of an odor with specific reinforcement pathways in the brain, as has been done for sucrose (Hammer and Menzel, 1995). It could be, for example, that the presence of amino acid sensitizes honey bees such that they non-specifically heighten their responsiveness to odor, as can occur with sucrose stimulation (Hammer et al., 1994).

The effect of amino acids on learning, which we have identified, parallels a similar process in locusts. Simpson and White (1990) conditioned locusts to associate one odor with a protein-rich diet and a second odor with a carbohydrate-rich diet. They then deprived the locusts of one of the two nutrients. When given a choice between the two odors, locusts that had been deprived of protein for as little as 4 h chose the odor associated with protein, perhaps by being repelled less by that odor than by the other. No such effect was apparent for the odor associated with carbohydrate. Furthermore, Raubenheimer and Tucker (1997) found that visual cues could be associated with both protein and carbohydrate diets.

In conclusion, we have demonstrated that honey bees can detect and respond to an amino acid in a sucrose solution that they are fed on. The effect is complex in that it depends on a number of different variables. Most likely though, they are all affected by the physiological/metabolic status of bees as they attempt to maintain nutrient balance. That these results are consistent with models that balance several nutrients (Simpson and Raubenheimer, 1995) indicates that studies of amino acid preferences in field conditions (Inouye and Waller, 1984) need to take these factors into consideration in the future. The sign of the response (excitatory/inhibitory) to a particular amino acid might easily reverse under different nutrient conditions. Further work, particularly regarding sensory processing, will also be necessary to reveal to what extent the effect results from sensory or post-ingestive mechanisms, and to what extent the effect on learning results from non-associative and/or associative mechanisms.

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