The Journal of Experimental Biology 214, 3843-3849

doi:10.1242/jeb.062430

RESEARCH ARTICLE

Dopamine as an anorectic neuromodulator in the cockroach *Rhyparobia maderae*

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Accepted 24 August 2011

SUMMARY

Insects, including cockroaches, self-select a balanced diet when faced with different nutrient choices. For self-selection to be carried out effectively, insects possess neuroregulatory systems to control their food intake. In the present study, we examined the role of the neurotransmitter dopamine (DA) in the feeding regulation of the Madeira cockroach (*Rhyparobia maderae*). When *R. maderae* nymphs were injected with 20 μl of 100 mmol l^{-1} DA, they showed an 83.3% reduction in sucrose intake and a 78.9% reduction in total intake compared with saline-injected controls. The DA agonist, 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (6,7-ADTN) (100 mmol l^{-1} in 1 μl), caused a significant reduction in sucrose feeding, reducing feeding by 47.3% compared with saline-injected controls. Protein feeding was also significantly reduced by 6,7-ADTN to 62%. *Rhyparobia maderae* nymphs injected with the DA antagonist chlorpromazine (100 mmol l^{-1} in 1 μl) did not differ significantly from control nymphs in their feeding behavior. Interestingly, *R. maderae* nymphs injected with 2 μl or 5 μl chlorpromazine (100 mmol l^{-1}) showed significantly increased mortality rates of 47.5% or 66.7%, respectively. The DA antagonist, spiperone (100 mmol l^{-1} in 1 μl), caused a significant feeding response, showing an increase in feeding in both sucrose (310.6%) and total intake (236.3%). Casein feeding in *R. maderae* nymphs was also elevated (70.8%) but this was not statistically significant. The experiments with DA, the DA agonist 6,7-ADTN and the DA antagonist spiperone strongly suggest that the neurotransmitter DA is involved in regulating feeding in the cockroach *R. maderae*.

Key words: dopamine, diet-mixing, 6,7-ADTN, spiperone.

INTRODUCTION

Considerable advancement has been made over the past several decades in understanding the mechanisms behind hunger, satiation and the physiological controls of food intake in animals (Edgecomb et al., 1994; Wei et al., 2000; Stoffolano et al., 2007). Animals, including insects, have the ability to obtain an optimal diet when offered a choice of different foods; this behavior is known as 'nutrient self-selection' or 'diet-mixing' (Cohen, 2001; Cohen et al., 2002). The criteria for nutrient self-selection are that: (1) there is non-randomness of choice; (2) a uniform group of individuals tend to select major nutrients in consistent proportions; and (3) individuals that have a choice to self-select do as well or better than if self-selection were not possible (Nation, 2002). To reach their nutritional target, insects combine two or more foods or ingest different foods in a consistent ratio, and changing from one food to another often helps an insect reach this target more easily (Waldbauer et al., 1984; Raubenheimer and Simpson, 2003). For example, research from our laboratory has shown that cockroaches are able to diet-mix. When faced with a wide array of nutrients, nymphs of the Madeira cockroach *Rhyparobia maderae* maintained consistent protein:carbohydrate intake ratios (*ca.* 25:75) (Cohen, 2001).

Nutrient self-selection occurs by a constant physiological regulation of the food ingested. This regulation involves frequent switching of foods in order to achieve an optimum nutrient balance through non-random choices that ultimately benefits the animal (Waldbauer and Friedman, 1991). In nature, many foods are normally nutritionally complete, differing only quantitatively (Cohen, 2001). Thus, diet-mixing is an opportunity for an animal, such as an insect, to obtain an optimal balance of a suite of nutrients (i.e. protein, carbohydrate, fat) based on its nutritional need at the time (Cohen, 2001; Behmer, 2009). For nutrient self-selection to operate proficiently, a free-ranging animal, such as a cockroach, must have an internal system to precisely regulate its nutrient intake (Cohen et al., 2002). Many regulatory hypotheses regarding nutrient self-selection have been proposed but there are two main hypotheses that seem to fully explain the regulation of this feeding behavior. The first hypothesis states that diet-mixing relies mostly on the activation of gustatory (taste) receptors (Bernays and Bright, 1993). The alteration of the gustatory receptor response has been directly correlated with hemolymph nutrient composition in insects (Simpson et al., 1991).

The second hypothesis regarding nutrient self-selection suggests that changes in the central nervous system (CNS), such as changes in the levels of neurotransmitters or neuromodulators, regulate feeding behavior in conjunction with the nutritional value of a food (Leibowitz and Stanley, 1986). Biogenic amines [octopamine (OA), serotonin (5-HT) and dopamine (DA)] regulate various functions in the CNS of invertebrates, serving as neurotransmitters and neuromodulators (Walz et al., 2006). In the cockroach *R. maderae*, injections of OA or its agonist synephrine caused a significant increase in both protein and carbohydrate feeding (Cohen et al., 2002). Conversely, the absence of OA signaling (from injections with the antagonist phentolamine) blocked the feeding response on both protein and carbohydrate in *R. maderae* nymphs (Cohen et al., 2002). Alternatively, 5-HT appears to regulate carbohydrate feeding in insects (Cohen, 2001). Cockroach nymphs (*R. maderae*) injected with 5-HT ate less sucrose than control nymphs that were injected

with insect saline. When the cockroach nymphs were injected with the 5-HT antagonist methyltryptophan, the nymphs increased sucrose feeding compared with the controls (Cohen, 2001).

The biogenic amine DA has been shown to be in high amounts in the CNS of insects (Vieillemaringe et al., 1984). DA is synthesized from the amino acid tyrosine by the sequential actions of tyrosine hydroxylase (TH), which generates L-dihydroxyphenylalanine (L-DOPA), and L-aromatic amino acid decarboxylase (AADC), which helps produce DA (Zhou and Palmiter, 1995; Monastirioti, 1999). Recent cloning and expression studies revealed that DA receptors are localized in the mushroom bodies, the major brain center for learning and memory processing. In addition, pharmacological studies in bee brains suggested possible effects of DA on the conditioning of olfactory responses (Monastirioti, 1999). Indeed, pharmacological data from crickets suggests that dopaminergic neurons participate in aversive olfactory and visual learning (Unoki et al., 2006).

DA has also been shown to be involved in sucrose responsiveness in the honeybee (Scheiner et al., 2002). When honeybees were injected with DA or the DA receptor agonist 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (6,7-ADTN), there was a reduction in their responsiveness to sucrose, suggesting that dopaminergic pathways are involved in regulating gustation in bees (Scheiner et al., 2002). Sucrose responsiveness is an excellent indicator of the behavioral state of a bee because it correlates with a number of distinctive behaviors. For example, honeybees that forage for pollen are more responsive to sucrose than nectar foragers (Page et al., 1998).

The animal of interest in this study is the Madeira cockroach, *R. maderae*. Cockroaches are excellent models to study feeding behavior because of their capability to consume a variety of food sources during their different growth phases. While there are several papers on the effect of DA on vertebrate feeding, there are few describing the role of DA in insect feeding. In our present study, we examined the role of DA (by using various dopaminergic agonists and antagonists) in cockroach food consumption and diet-mixing. Understanding the invertebrate feeding system allows us to make better comparisons with the regulatory pathways that control feeding.

MATERIALS AND METHODS

Insect rearing

The insect used in the following experiments was the Madeira cockroach, *Rhyparobia maderae* (Fabricius). The colony was kept in plastic storage containers inside an incubator at 27°C with a 12 h:12 h light:dark cycle, where the cockroaches had *ad libitum* access to rat chow and water. Cockroaches were removed from the colony and used in experiments during their last instar. Seven days prior to each experiment, experimental nymphs (mean wet mass ± s.e.m.=512±7.5 mg) were collected and placed in a large glass bowl lined with petroleum jelly to prevent escapes. To normalize feeding histories, these nymphs were left in this bowl for seven days, given water *ad libitum* but no food.

Diets

The food used in all experiments was a lyophilized, agar-based diet. The diets contained essential nutrients, including vitamins, minerals and lipids, lacking either carbohydrate but not protein (casein cube) or protein but not carbohydrate (sucrose cube) (Cohen, 2001). The diets were stored in plastic Petri dishes at –20°C until use. Before being given to the nymphs, the diets were cut into ~1×1×1 cm blocks and then dried in a 60°C oven for 2 h. At the end of the experiments, the remaining food was collected and placed in a 60°C oven for 24 h. After 24 h, the final dry mass of all of the food was recorded. To reduce any errors that may result from humidity, all dry masses were measured on the same day within a 1 h period.

Drugs

The drugs used were DA (100 mmol l^{-1} in 10–20 µl volumes in 0.7% NaCl), the DA agonist 6,7-ADTN (100 mmol l^{-1} in 1 µl in 0.7% NaCl), the DA antagonist chlorpromazine (100 mmol l^{-1} in 1–5 µl in 0.7% NaCl), the DA antagonist spiperone (100 mmol l^{-1} in 1 µl of 100% ethanol) or vehicle [insect saline (0.7% NaCl in 1 µl) or 100% ethanol in 1 µl]. Two experiments were conducted using different DA antagonists due to the observation that chlorpromazine resulted in significantly high mortality rates (see Fig. 2). All drugs were stored at 4°C until use. All drugs were purchased from Sigma Chemical Co. (St Louis, MO, USA).

Experimental design

After the seven-day starvation period, *R. maderae* nymphs were randomly selected and divided into the appropriate groups for each experiment. Experimental nymphs were anesthetized with CO_2, and neuroactive drugs or vehicle were injected into the insect (between the second or third abdominal sternite) using a 10 µl or 100 µl Hamilton syringe (Reno, NV, USA). An injection was deemed successful if no hemolymph was observed leaking from where the needle punctured the abdominal wall. If an injection was considered unsuccessful, that nymph was allowed to recover for 25 min before receiving a second injection. If the second injection was considered unsuccessful, the nymphs were discarded and removed from the experimental pool. After receiving the appropriate injection, all nymphs were allowed a final 25 min recovery period. Following this recovery period, nymphs were placed in individual 10 cm Petri dishes containing separate casein and sucrose cubes, and water (sealed in a 10×30 mm plastic Petri dish with a wick of cotton). The cockroaches were allowed to feed on the protein and carbohydrate cubes for 24 h in an incubator set at 27°C with a 12 h:12 h light:dark cycle. The Petri dishes were positioned randomly inside the incubator to control for any differences in temperature or light. When the 24 h feeding experiment ended, the remaining food from each dish was placed in a 60°C oven to dry for 24 h. The amount of food consumed by each nymph was calculated by taking the dry mass of food prior to any feeding minus the dry mass of the food after the 24 h feeding assay. Any nymph that died or molted during the experiment was not used in the final analysis of the data.

Pharmacology experiments

First, we tested DA and the DA antagonist chlorpromazine at various doses on *R. maderae* diet-mixing. For the DA injections, nymphs were randomly divided into three groups: a control group injected with insect saline (1 µl of 0.7% NaCl; N=30); a group that received 10 µl of 100 mmol l^{-1} DA (N=26); and a group that received 20 µl of 100 mmol l^{-1} DA (N=19). For the chlorpromazine injections, experimental nymphs were randomly divided into four groups: the control group received a 1 µl injection of 0.7% NaCl (N=60); the other three groups were randomly divided and received 100 mmol l^{-1} chlorpromazine in volumes of 1 µl (N=54), 2 µl (N=59) or 5 µl (N=60).

Second, we examined the role of the DA agonist 6,7-ADTN and the DA antagonist spiperone in *R. maderae* diet-mixing using single injection protocol. For 6,7-ADTN injections, the experimental nymphs were divided into two groups. The control group received a 1 µl injection of 0.7% NaCl, and the second group received a 1 µl injection of 100 mmol l^{-1} DA agonist 6,7-ADTN. Both groups were allowed to feed for 24 h, and after 24 h, remaining food cubes were collected, dried and weighed.

In the spiperone experiment, there were also two groups: a control vehicle group; and a group injected with 1 µl (100 mmol l^{-1}) of spiperone. Spiperone has extremely low solubility in water; thus, in order to get the drug to dissolve, 1 µl of 100% ethanol was used as the vehicle.

Motor activity test for the effects of ethanol

As described above, in the spiperone experiment, 100% ethanol was used as the vehicle. As ethanol has been shown to have depressant effects on insects (Maze et al., 2006), we assayed if the ethanol vehicle (used in the spiperone experiment) had any effect on the motor ability of the *R. maderae* nymphs, thereby affecting feeding behavior. In this experiment, there were two groups: the control group was injected with 1 µl of insect saline (0.7% NaCl); and the other group was injected with 1 µl of 100% ethanol (the same volumes used above). Each *R. maderae* nymph was housed in 10 cm individual Petri dishes with *ad libitum* access to water (sealed in a 10×30 mm plastic Petri dish with a wick of cotton) after receiving injections. Motor abilities were measured post-injection at four times intervals: 30 min, 1 h, 5 h, and 24 h. Between trials, cockroaches were placed in individual Petri dishes and kept inside a 27°C incubator.

The motor activity of the *R. maderae* nymphs was tested on a laboratory bench. One at a time, a nymph was handed to a second researcher, who was blind to the treatment that the nymph received. The second person placed the nymph on a start line, and a timer was started. The timer ran for 5 s. At the end of 5 s, a mark was made at the caudal end of the nymph. The distance from the start line to the ending mark was measured. If a nymph did not move in the 5 s period, the distance was recorded as zero. No help was given to the nymphs to help them initiate movement. Any nymph that died, molted or was injured (missing an appendage) during the entire 24 h experiment was not used in the final analysis of the data.

Data analysis

Values are presented as means±s.e.m. Statistical significance was set at $P<0.05$. In the dose–response experiments, a one-way ANOVA was used to test for differences among treatments, and Tukey's pairwise comparisons were used to determine significance of differences between the means. In the single injection experiments, significant differences were determined by *t*-tests. In the motor ability test, a repeated-measures ANOVA was used to test for differences between treatments and time after injection, and also to see if individuals changed over the testing period. To test for significance in percentage mortality, a G^2 test of independence was used, and Tukey-like pairwise comparisons were used to determine significance in percentage mortality between groups.

RESULTS

Pharmacology experiments: dose–response

These experiments were performed in order to determine if chlorpromazine (DA antagonist) and the neurotransmitter DA had any effect on the feeding behavior in *R. maderae* nymphs and, if so, what the most effective dose was. Nymphs were offered both a carbohydrate (sucrose) and protein (casein) food source and were allowed to self-select between the sucrose and casein blocks for 24 h.

Treatment with DA had a significant effect on sucrose feeding ($F=3.33$, d.f.=2, 72, $P<0.05$; Fig. 1). Nymphs treated with 20 µl of DA consumed the lowest amount of sucrose (2.8±1.1 mg), where they showed a significant ($P<0.05$) reduction (83.3%) compared with saline-injected controls (17.1±4.5 mg) (Fig. 1). Although nymphs injected with 10 µl of DA consumed 50.2% less sucrose (8.5±3.5 mg) than the controls, this was not significantly different ($P>0.05$; Fig. 1). Also, the nymphs injected with 20 µl of DA consumed 66.5% less sucrose than those injected with 10 µl of DA, but this difference was not significant ($P>0.05$; Fig. 1). DA treatment also had a significant effect on the total amount eaten ($F=3.57$, d.f.=2, 72, $P<0.05$; Fig. 1). Nymphs injected with 20 µl of DA showed the lowest overall intake (3.9±1.1 mg), and this was a significant reduction (78.9%) compared with the saline-injected controls' overall intake (18.4±4.5 mg) ($P<0.05$; Fig. 1). The total intake of nymphs injected with 10 µl of DA (9.6±3.6 mg) was reduced by 47.6% compared with controls, but this was not significant ($P>0.05$; Fig. 1). Although the nymphs injected with 20 µl of DA had a 59.6% reduction in total intake compared with the 10 µl dose, these two doses did not differ significantly from each other ($P>0.05$; Fig. 1). There was a non-significant trend ($F=0.63$, d.f.=2, 72, $P>0.05$) for those nymphs injected with DA to consume less casein than their saline-injected counterparts (1.4±0.2 mg). The nymphs injected with 10 µl showed a 15.5% reduction in casein consumption compared with vehicle (1.1±0.2 mg), while those nymphs injected with 20 µl of DA showed the largest reduction in casein consumed (1.0±0.1 mg, a 23% reduction compared with controls; Fig. 1). Thus, 20 µl appears to be an effective dose at eliciting a depressed response in the feeding behavior in *R. maderae* nymphs. A one-way ANOVA showed that DA injection did not have a significant effect on percentage casein intake out of the total intake in *R. maderae* nymphs ($F=0.81$, d.f.=2, 72, $P>0.05$). The mean percentage casein intake of control nymphs selecting between a protein cube and a carbohydrate cube was 29.4%; in other words, their mean percentage sucrose intake was 70.6%. Nymphs injected with 10 µl of DA showed a mean percentage casein intake of 31.8% and a mean sucrose intake of 68.2%. *Rhyparobia maderae* nymphs injected with 20 µl of DA produced a mean percentage casein intake of 39.2% and a mean percentage sucrose intake of 60.8%.

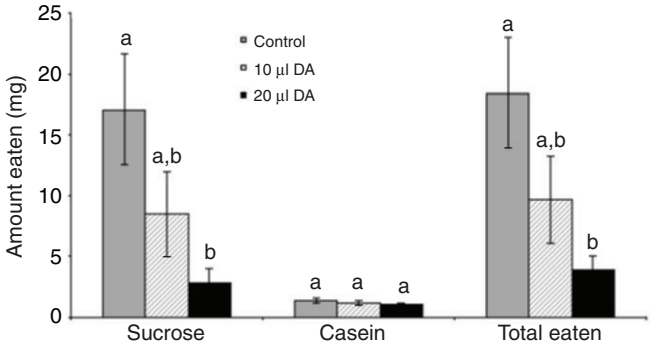

Fig. 1. Dopamine (DA) dose–response experiment. The effects of 100 mmol l^{-1} DA or vehicle (0.7% NaCl) on *Rhyparobia maderae* nymph nutrient self-selection behavior. The neurotransmitter DA was administered in the following volumes: 10 µl (N=26), and 20 µl (N=19). Insect saline was administered at a dose of 1 µl (N=30). Shown is the amount of sucrose and casein consumed by the cockroach nymphs with each treatment in addition to the total amount of food eaten. All values are means ± s.e.m. Within a food category, same letters indicate that means were not significantly different from one another ($P>0.05$) by Tukey's pairwise comparisons.

Chlorpromazine dose–response experiments showed that dosage had a highly significant effect on mortality rate in *R. maderae* nymphs ($G^2=62.10$, $P<0.001$; Fig. 2). The saline-injected controls had a significantly lower percentage mortality (11.7% died) compared with those nymphs that were injected with 2 µl (47.5%) and 5 µl (66.7%) ($P<0.05$; Fig. 2). However, the mortality rate of

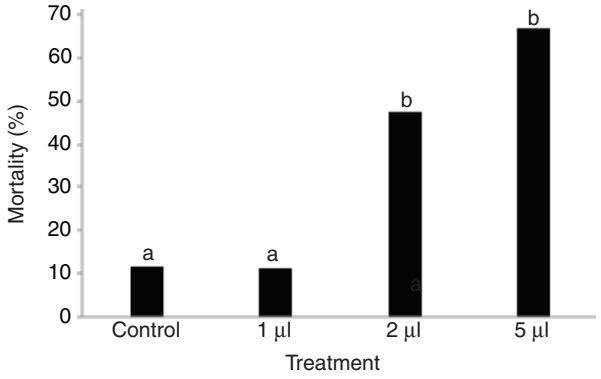

Fig. 2. Mortality of cockroach nymphs due to chlorpromazine dose. Percentage of *Rhyparobia maderae* nymphs that died following injection of various dosages of chlorpromazine (100 mmol l^{-1} in 1 µl, 100 mmol l^{-1} in 2 µl, and 100 mmol l^{-1} in 5 µl) or insect saline (1 µl in 0.7% NaCl). Same letters indicate that percentages were not significantly different by Tukey-like pairwise comparisons. *N*=60 for controls; *N*=54 for 1 µl; *N*=59 for 2 µl; *N*=60 for 5 µl.

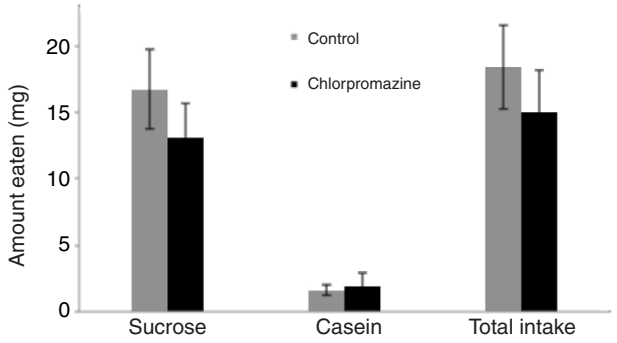

Fig. 3. Chlorpromazine experiment (1 µl). Effects of the dopamine antagonist chlorpromazine (100 mmol l^{-1} in 1 µl) or vehicle control (1 µl in 0.7% NaCl) on nutrient self-selection feeding behavior in *Rhyparobia maderae* nymphs. Shown is the amount of sucrose and casein consumed by the cockroach nymphs with each treatment in addition to the total amount of food eaten. All values are means±s.e.m. No statistically significant differences were observed between chlorpromazine (1 µl) and saline controls (*t*-test; *P*>0.05). *N*=51 for saline-injected (1 µl in 0.7% NaCl) cockroaches and *N*=48 for chlorpromazine-treated cockroaches.

the controls did not differ significantly ($P>0.05$) from the mortality rate of nymphs injected with 1 µl of chlorpromazine (11.1%). Also, percentage mortality did not differ between the 2 µl and 5 µl doses ($P>0.05$; Fig. 2).

As the 1 µl dose of chlorpromazine did not show a significant difference from the controls for percentage mortality, this was the dose used in the subsequent chlorpromazine experiment. The amount of sucrose consumed by the nymphs injected with 1 µl of chlorpromazine (13.1±2.6 mg) did not differ significantly from those nymphs injected with 1 µl in 0.7% NaCl (16.7±3.0 mg) ($t=0.92$, $P>0.05$; Fig. 3). There was also no significant difference ($t=0.29$, $P>0.05$) in casein consumption between the saline-injected controls (1.6±0.4 mg) and those nymphs injected with 1 µl of chlorpromazine (1.9±1.0 mg) (Fig. 3). Total intake (sucrose + casein) did not differ significantly between vehicle (18.4±3.1 mg) and chlorpromazine-injected nymphs (15.0±3.2 mg) ($t=0.75$, $P>0.05$; Fig. 3). Finally, control nymphs showed a mean percentage casein intake of 27.4% or, in other words, a mean percentage sucrose intake of 72.6%, and these values were statistically similar ($t=0.43$, $P>0.05$) to the mean percentage casein intake (24.6%) and mean percentage sucrose intake (75.4%) of nymphs injected with 1 µl of chlorpromazine.

Pharmacology experiments: single injection

These experiments were performed to examine the effects of two dopaminergic drugs (6,7-ADTN and spiperone) on *R. maderae* nymphs offered a carbohydrate (sucrose) and protein (casein) source. Nymphs injected with 1 µl of the DA agonist 6,7-ADTN showed a 47.3% reduction in carbohydrate feeding (9.5±2.0 mg) compared with controls injected with 1 µl of insect saline (18.0±2.8 mg), and this decrease was significant ($t=2.47$, $P<0.05$; Fig. 4). Nymphs treated with 6,7-ADTN ate significantly less from their casein diet cubes (0.5±0.1 mg; 62% reduction) than saline controls (1.4±0.4 mg) after 24 h ($t=2.20$, $P<0.05$; Fig. 4). Finally, injection of 6,7-ADTN caused a 48.3% decrease in overall feeding (10.0±2.0 mg), and this measurement was significantly different ($t=2.64$, $P<0.01$) from the total intake of controls (19.4±2.9 mg) (Fig. 4). Control nymphs selecting between a protein cube and a carbohydrate cube showed a mean percentage casein intake of 19.0% or, in other words, a mean percentage sucrose intake of 81.0%, and these values did not differ significantly ($t=0.21$, $P>0.05$) from the mean percentage casein intake (19.8%) and mean percentage sucrose intake (80.2%) of nymphs injected with 1 µl of 6,7-ADTN.

The effect of the DA antagonist spiperone proved opposite to the effects of DA and its agonist 6,7-ADTN. Nymphs injected with 1 µl of the DA antagonist spiperone fed significantly more ($t=3.52$, $P<0.001$) from their sucrose diet cubes (15.1±3.1 mg; 310.6% increase) compared with ethanol-injected controls (3.7±1.0 mg) (Fig. 5). Despite a 70.8% increase in protein feeding (2.8±0.8 mg) seen in nymphs injected with spiperone, this increase was not significant ($t=1.23$, $P>0.05$) compared with the amount of casein consumed by ethanol-injected controls (1.6±0.4 mg) (Fig. 5). Total food eaten was significantly greater ($t=3.56$, $P<0.001$) for those nymphs injected with spiperone (17.9±3.2 mg; 236.3% increase) compared with control nymphs that received ethanol (5.3±1.0 mg) (Fig. 5). Ethanol-injected control nymphs selecting between a protein cube and a carbohydrate cube showed a mean percentage casein intake of 46.2% and a mean percentage sucrose intake of 53.8%, and these values were significantly different ($t=2.44$, $P<0.05$) from the mean percentage casein intake (31.9%) and mean percentage sucrose intake (68.1%) of nymphs injected with 1 µl of spiperone.

Motor activity experiment for the effects of ethanol

As control nymphs in the spiperone experiment received injections of ethanol (as opposed to the standard insect saline), this experiment was performed to determine if 100% ethanol had any depressive effects on the motor ability of *R. maderae* nymphs. Nymphs were treated with either 1 µl of 100% ethanol or 1 µl of insect saline, and their motor activity was tested at various time intervals after injection. A repeated-measures ANOVA showed that there was not a significant interaction between time category and treatment ($F=1.17$, d.f.=3, 84, $P>0.05$; Fig. 6), indicating that ethanol had no effect on the relationship between time after injection and the nymphs' willingness to move. Also, individual nymphs did not change over the 24 h testing period in their motor behavior ($F=1.01$, d.f.=3,84, $P>0.05$; Fig. 6). Treatment with ethanol did not have an effect on the motor ability of the *R. maderae* nymphs ($F=1.19$, d.f.=1, 28, $P>0.05$; Fig. 6). Thirty minutes post-injection, nymphs treated with ethanol moved a mean distance of 25.3±6.3 cm compared with those nymphs injected with 0.7% saline who moved 16.4±3.2 cm. After 1 h, nymphs injected with ethanol moved a mean distance of

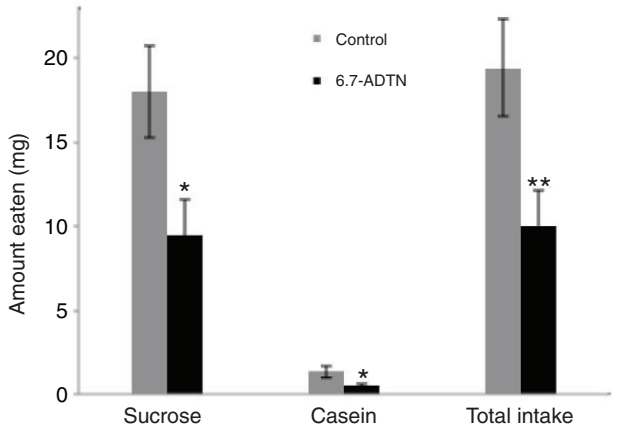

Fig. 4. 2-Amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (6,7-ADTN) experiment. Effects of the dopamine agonist 6,7-ADTN injection (100 mmol l^{-1} in 1 µl) or vehicle control (1 µl in 0.7% NaCl) on *Rhyparobia maderae* nymph nutrient self-selection feeding behavior. Shown is the amount of sucrose and casein consumed by the cockroach nymphs with each treatment in addition to the total amount of food eaten. All values are means ± s.e.m. Significant differences between the treated group and controls are indicated by asterisks. **P*<0.05; ***P*<0.01 (*t*-test); *N*=58 for both the saline-injected and the 6,7-ADTN-treated cockroaches.

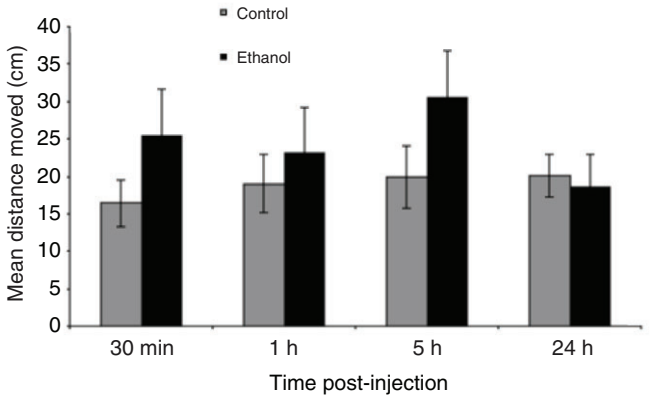

Fig. 6. Motor activity experiment. Effects of 1 µl of 100% ethanol or control (1 µl of 0.7% NaCl) on motor activity in *Rhyparobia maderae* nymphs. Shown is the mean distance moved in 5 s (cm) at four time intervals. All values are means ± s.e.m. No statistically significant differences were observed between ethanol treatment and saline controls (repeated-measures ANOVA, *P*>0.05) or between the various time intervals (repeated-measures ANOVA, *P*>0.05). *N*=16 for saline-injected controls and *N*=14 for ethanol-injected nymphs.

23.0±6.2 cm, and those injected with insect saline moved 19.0±3.9 cm. Nymphs that received 1 µl of ethanol traveled 30.5±6.3 cm after 5 h, and nymphs that received 0.7% NaCl traveled a mean distance of 19.9±4.2 cm. Twenty-four hours after treatment, ethanol-treated nymphs traveled slightly less (18.6±4.3 cm) than control nymphs (20.0±2.8 cm).

DISCUSSION

Animals, including insects, regulate their food intake to optimize growth and performance, and this regulation ensures that an animal consumes an optimal mixture of the required nutrients – the 'nutrient target' (Raubenheimer and Simpson, 1997; Raubenheimer and Simpson, 1999). In many insects, food selection involves detecting a desired food, initiating ingestion of that food, consuming the food and terminating the meal (Edgecomb et al., 1994). The selection of a particular food is a physiological complex process, influenced by sensory information (i.e. sight, smell and taste), previous ingestion experience, feedback from peripheral systems, such as stretch receptors in the gut, hormonal signals, blood composition and brain neurotransmitters, such as DA (Meguid et al., 2000; Wei et al., 2000).

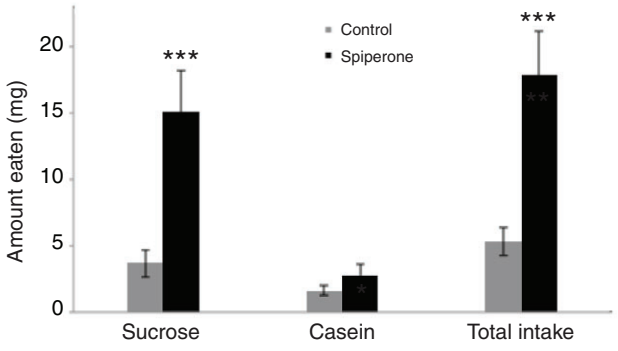

Fig. 5. Spiperone experiment. Effects of the dopamine antagonist spiperone (100 mmol l^{-1} in 1 µl of 100% ethanol) or control (1 µl of 100% ethanol) on *Rhyparobia maderae* nymph nutrient self-selection feeding behavior. Shown is the amount of sucrose and casein consumed by the cockroach nymphs with each treatment in addition to the total amount of food eaten. All values are means ± s.e.m. Significant differences between the treated group and the controls are indicated by asterisks: ****P*<0.001 (*t*-test); *N*=44 for the spiperone-injected cockroaches and *N*=40 for the ethanol-injected controls.

In this study, when *R. maderae* nymphs were injected with 20 µl of 100 mmol l^{-1} DA, they showed a significant reduction in sucrose (83.3%) and total intake (78.9%) compared with saline-injected controls (Fig. 1). Although, not significant, nymphs injected with 20 µl of DA showed a 23% reduction in casein feeding compared with controls (Fig. 1). What was interesting was that administration of the DA receptor agonist 6,7-ADTN resulted in a significant feeding response at a lower dose than DA itself. *Rhyparobia maderae* nymphs injected with 1 µl of 100 mmol l^{-1} 6,7-ADTN showed a significant reduction in sucrose (47.3%), casein (62%) and total food (48.3%) intake (Fig. 4). Neither DA nor 6,7-ADTN injections significantly affected percentage casein intake in *R. maderae* nymphs, providing evidence that the feeding reductions seen in nymphs injected with either DA or 6,7-ADTN occur for total food intake because casein:sucrose ratios were not affected. The balance of the macronutrients seems relatively stable in the face of changes in appetite in *R. maderae* nymphs.

So, why did 6,7-ADTN cause a significant response at a lower dose in our experiment? Experiments have shown that in the honeybee (Mustard et al., 2003) and cockroach brains (Orr et al., 1987), 6,7-ADTN actually stimulated DA receptors (as measured by cAMP production) at a level higher than that caused by DA when both were applied at the same concentration, suggesting that 6,7-ADTN is more potent at insect DA receptors than DA. The fact that 6,7-ADTN appears to be a more potent ligand at the insect DA receptor may explain why a higher dose of DA was needed to see significant feeding responses in our experiment.

The experiment with the DA antagonist chlorpromazine was conducted to determine an effective dose at eliciting a feeding response in *R. maderae* nymphs. Chlorpromazine was originally selected in this experiment because it is a known DA antagonist in

vertebrates, and in the cockroach *Nauphoeta cinerea* chlorpromazine was found to be the most potent antagonist at DA receptors on salivary gland cells (Evans and Green, 1990). In addition, the lipophilic structure of chlorpromazine allows it to cross the blood–brain barrier easily (Martel et al., 1996). Surprisingly, higher doses of chlorpromazine (2 µl and 5 µl) in our experiment were lethal to *R. maderae* nymphs (Fig. 2). In contrast to the higher doses, nymphs injected with 1 µl of chlorpromazine did not differ significantly from controls in percentage mortality. Subsequently, this dose was used to determine if chlorpromazine had any effect on feeding behavior. Nymphs injected with 1 µl did not differ from controls in their sucrose, casein and total intake (Fig. 3). The two questions, then, are why did chlorpromazine cause mortality at higher doses, and why at the 1 µl dose was there no significant feeding response?

The simplest explanation is that chlorpromazine has been shown to bind to more receptor types than just DA. In insects, for example, chlorpromazine has been shown to bind to tyramine receptors, and has also been shown to act as an antagonist at insect OA receptors (Vanden Broeck et al., 1995; Blenau and Erber, 1998). In the salivary glands of the cockroach, *Periplaneta americana*, the 5-HT-induced secretory response was significantly reduced after treatment with chlorpromazine, suggesting that chlorpromazine may also act as a 5-HT receptor antagonist (Marg et al., 2004). The fact that chlorpromazine has the potential to inhibit so many receptor types, suggests that, at the higher doses used, more receptors were blocked in the nervous system of the *R. maderae* nymphs. Antagonizing multiple receptor types could have been the cause for the mortality in the groups that received the higher doses of chlorpromazine.

Although few *R. maderae* nymphs died from the 1 µl chlorpromazine injection, the drug still did not show any significant feeding response. It is possible that the group of nymphs injected with 1 µl also had more than one receptor type blocked (i.e. DA, OA, 5-HT); however, as the dose was lower, not as many receptors may have been occupied by chlorpromazine, thus causing the lower percentage mortality. This may also explain why there was not a significant feeding response seen with the 1 µl dose because blocking more than one type of receptor (i.e. OA, DA) could have caused all of these receptor systems to counteract each other. For example, OA antagonists have been shown to decrease feeding in *R. maderae* nymphs (Cohen et al., 2002) whereas in our experiments with the DA antagonist spiperone, *R. maderae* nymphs increased feeding (Fig. 5). Casein:sucrose ratios were statistically similar between the control nymphs and nymphs injected with 1 µl of chlorpromazine, suggesting that low doses of chlorpromazine do not affect *R. maderae*'s ability to diet-mix.

In contrast to the chlorpromazine experiment, the DA antagonist spiperone caused a significant increase in feeding in both sucrose (75.6%) and total intake (70.3%) (Fig. 5). Casein feeding in *R. maderae* nymphs was also elevated (41.4%) but this was not statistically significant. The DA receptor antagonist spiperone has a higher affinity for the DA receptor compared with chlorpromazine (Degen et al., 2000). As spiperone has been shown to bind with higher affinity to DA receptors only, this may explain why spiperone showed a significant response where chlorpromazine did not. Both DA and the DA agonist 6,7-ADTN resulted in reduced feeding in *R. maderae* nymphs whereas the DA antagonist spiperone produced an increase in consumption, pointing to a role for DA in regulating feeding behavior in the *R. maderae* cockroach.

Another interesting observation in the spiperone experiment was that the *R. maderae* control nymphs injected with 100% ethanol consumed a much lower amount of sucrose compared with the control nymphs (injected with insect saline) in the other experiments. Control nymphs in the other experiments consumed ~17 mg of sucrose whereas in this experiment, *R. maderae* consumed less than 4 mg. Total feeding in the ethanol-injected control nymphs was much lower (~5 mg) compared with the saline-injected control nymphs in the other experiments (~18 mg). In addition, ethanol appeared to affect the ability of *R. maderae* nymphs to diet-mix. For example, percentage sucrose intake was significantly less in the ethanol-treated controls (53.8%) compared with nymphs injected with spiperone (68.1%) or, in other words, percentage casein intake was significantly higher in ethanol-injected controls (46.2%) compared with nymphs injected with spiperone (31.9%). The ethanol appeared to cause a reduction in feeding in the control nymphs in this experiment; and, based on the percentages listed above, the majority of this reduction is a result of decreased sucrose feeding. Consistent with these results, when honeybees were fed with 10% ethanol, after 24 h, they showed a significant decrease in the amount of sucrose eaten compared with control bees (Mustard et al., 2008). In this experiment, ethanol may be affecting the perceived state of hunger. The DA antagonist spiperone was dissolved in 100% ethanol, and spiperone completely reversed the effect ethanol had on satiety. Interestingly, ethanol consumption in vertebrates has been shown to lead to the release of DA (Tupala and Tiihonen, 2004). Experiments on *R. maderae* nymphs have confirmed that DA causes a reduction in feeding. If DA is potentially released by the presence of ethanol, it may explain the decrease in sucrose consumption in control nymphs injected with ethanol and the ability of spiperone as a DA antagonist to reverse the anorectic effect of ethanol on *R. maderae* nymphs.

To rule out the possibility that the low consumption values shown by the control nymphs in the spiperone experiment were not due to motor depression caused by ethanol, a motor activity test was conducted. A previous study has shown that ethanol consumption in insects can have negative effects on locomotion, such as a reduction in walking behavior and the loss of the righting reflex (Maze et al., 2006). Results from this experiment showed that ethanol did not affect locomotion in *R. maderae* nymphs (Fig. 6). As ethanol hemolymph levels have shown time-dependent changes after ingestion in the honeybee (Mustard et al., 2008), *R. maderae* nymphs were tested at four time intervals. Results on *R. maderae* showed that time post-injection had no affect on locomotion (Fig. 6). These results suggest that because locomotion was not impaired in nymphs injected with ethanol, the low values consumed in the spiperone experiment by nymphs injected with ethanol were probably not due to motor deficits.

In summary, the experiments with DA, the DA agonist 6,7-ADTN and the DA antagonist spiperone strongly suggest that the neurotransmitter DA is involved in regulating feeding in the cockroach *R. maderae*. If DA is in fact controlling feeding behavior in insects, then questions remain as to where the proposed regulation occurs (i.e. CNS, peripheral nervous system or a combination of both). DA is found in high amounts in the insect nervous system. DA receptors have been identified in the brain of the cockroach *P. americana* (Orr et al., 1987) and also in motorneurons of the prothoracic ganglion of its ventral nerve cord (Davis and Pitman, 1991). DA receptors have also been localized in the brains of many insects. Honeybees (*Apis mellifera*) expressed DA receptor mRNA in all regions of the brain, including cells scattered around the antennal and optic lobes and in Kenyon cells within the mushroom bodies (Beggs et al., 2005). In *Drosophila melanogaster*, both larvae and adults showed strong DA receptor expression in the mushroom

bodies and ventral nerve cord (thoracic and abdominal ganglia); adults also showed expression in the central complex (the brain structure controlling higher-order motor control in insects), near the outer edge of the optic lobe and near the antennal lobe (Kim et al., 2003; Draper et al., 2007). The wide distribution of DA receptors in the insect CNS suggests that the effect of DA on diet regulation may in fact be acting centrally.

At this point in our laboratory, we have now described many neural signals that appear to regulate feeding behavior in the *R. maderae* cockroach. These experiments have shown that DA appears to be a signal in terminating the current meal in order to avoid hyperphagia. In contrast, OA appears to be a signal for initiating food intake in order to prevent starvation, as OA and OA agonists caused an increase in both carbohydrate and protein intake (Cohen et al., 2002). 5-HT seems to be essential for macronutrient selection, as experiments with *R. maderae* nymphs showed that increased levels of 5-HT caused a reduction in carbohydrate feeding only (Cohen, 2001). Thus, DA and OA seem to be more involved in controlling aspects of meal size, while 5-HT seems to be involved in appetite by regulating nutrient type. All of this feeding data provide useful insight into the regulatory pathways (i.e. dopaminergic, octopaminergic, serotonergic) that may control dietary behavior in all insect species. In addition, one key objective of animal research is to generate theories about the mechanisms of human biology. Thus, an ideal model subject should be simple enough to study yet display similarities to the mammalian physiology. Insects possess simpler nervous systems than mammals but display many complex behaviors, allowing them to be useful models to understand the various mechanisms in the mammalian feeding system (Mustard et al., 2005). Knowing that insects may be viable models to study the regulatory pathways that control feeding in mammals, insects can potentially be used to examine the effects of anti-obesity drugs or anti-anorectic drugs, hopefully providing treatment one day for life-threatening conditions, such as obesity and anorexia nervosa.

ACKNOWLEDGEMENTS

We would like to thank Dr Paul Wilson for statistical advice and Dr Dan Odom for reading the manuscript. We would also like to thank Patty Villa for help with the project.

FUNDING

These experiments were supported by a Student project grant from The University Corporation at California State University Northridge and a Graduate Studies Research Grant from the Graduate Studies Office at California State University Northridge.

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