Diet Balancing in the Cockroach *Rhyparobia madera:* Does Serotonin Regulate this Behavior?

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Animals, including insects, have the ability to self-select an optimal diet from a choice of two or more incomplete diets that lack an essential nutrient. This paper demonstrates that nymphs of the cockroach Rhyparobia madera *also have this ability. The nymphs chose a protein:carbohydrate (P:C) ratio of approximately 25:75 when faced with a choice between one cube of protein (casein) and another of carbohydrate (sucrose). This self-selected ratio was shown to promote growth as well or better than other diets tested. When given a wide range of P:C choices, the* R. madera *nymphs consistently selected a P:C ratio of approximately 25:75, suggesting that they have the ability to diet-balance. Finally, injections of various serotonergic drugs into self-selecting nymphs influenced their choice of diets. Serotonin promoted a decrease in carbohydrate feeding, while injection of the antagonist α-methyltryptophan caused the nymphs to overfeed on carbohydrate. The results suggest that serotonin may help alter the carbohydrate feeding response in cockroaches.*

INTRODUCTION

Some animals, including humans, when offered an array of different foods, can combine two or more of them to obtain a nutritious diet. This behavior, termed "nutrient self-selection" (Richter *et al.*, 1938), has been extensively studied in mammals (Rozin, 1976) but observed in only a few arthropods,

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including spiders (Greenstone, 1979), beetles (Waldbauer and Bhattacharya, 1973), caterpillars (Waldbauer *et al.*, 1984), cockroaches (Cohen *et al.*, 1987), and grasshoppers (Bernays and Bright, 1993).

To date, insect feeding studies have used nutritionally incomplete foods (Cohen *et al.*, 1987) or whole pieces of different food stuffs to demonstrate self-selection (Waldbauer and Bhattacharya, 1973). In nature, however, foods are normally nutritionally complete, varying in quantitative differences. Thus, a foraging insect must make dietary choices based on a combination of the total nutrients contained in available foods and its nutritional need at the time. Not only must an insect select an optimal diet, but also it must strive to maintain a balanced intake ratio of the major nutrients (i.e., protein, carbohydrate, lipid) regardless of any food encountered. This special aspect of self-selection, known as "nutritional wisdom" (Overmann, 1976) or "diet balancing" (Castonguay and Collier, 1986), has been fully explored in mammals. Rats fed one nutritionally complete diet and two or more dietary supplements adjusted their intake to compose an overall diet that was uniform in composition throughout the experiment (Castonguay and Collier, 1986). Wong (1986) showed that hamsters and gerbils maintained the same caloric intake even though they were provided with varying concentrations of a sucrose solution and standard lab rat chow.

Thus, a question remains whether insects can "diet balance" in nature. The phenomenon of compensatory feeding has been known to occur in insects given some type of diluent in their diets. Slansky and Wheeler (1989) showed that velvetbean larvae (*Anticarsia gemmatalis*) increased their feeding consumption on water-diluted diets. These compensatory feeding larvae ultimately achieved similar levels of growth and feeding efficiencies compared to those larvae on normal diets. Chambers *et al.* (1995) looked at the feeding behavior of *Locusta migratoria* nymphs in a diet-mixing situation faced with a wide variety of foods. They found that the nymphs changed the amount of food consumed by extending their feeding bouts on various diets, resulting in similar proportions of protein and carbohydrates consumed. Cohen *et al.* (1987) showed that nymphs of the cockroach *Supella longipalpa* feeding on individual diet blocks of either protein (casein) or carbohydrate (sucrose) can adjust their intake so that they normally choose a diet containing 16% casein and 84% sucrose. This mixture was shown to be benefical for growth and survival. Interestingly, this ratio appeared to vary depending on the cockroach stage (Cohen *et al.*, 1987). Using the adult german cockroaches (*Blattella germanica*), Hamilton and Schal (1988) examined protein compensation and its effects on reproduction. Female cockroaches increased feeding when faced with low protein levels, producing similar number of eggs per oötheca. Recently, Hagele and Rowell-Rahier (1999) showed that three

generalist herbivores grew better on a mixture of various plants than on any single plant.

The above research suggests that insects can compensate for missing or reduced nutrients. Questions remain as to whether insects can continually adjust their intake by mixing diets given the choice among a wide concentration range of nutrients. Not only can they survive, but they can maintain an optimal balance of essential nutrients. This article demonstrates that diet balancing occurs in the nymphs of the cockroach, *Rhyparobia madera*. Here, nymphs, encountering diets with an array of different blends of nutrients, can maintain an optimal ratio that has been shown best to support their growth. Also, a possible neurophysiological mechanism regulating diet balancing is suggested by showing a link between carbohydrate feeding and the neurotransmitter serotonin.

MATERIALS AND METHODS

The insect used in the following experiments is the cockroach, *Rhyparobia* (*Leucophaea*) *madera* (Dictyoptera: Blaberidae). Experimental *R. madera* nymphs were collected from a colony housed in a 30-gal plastic trash can. The inside rim of the can was smeared with Vaseline to prevent escapes. Squares of wood were stacked upon one another (using U-nails as feet) to provide shelter. The cockroaches had free access to food (rat chow) and water. The colony was kept inside a 26°C incubator, and a light source maintained a 12:12 light:dark cycle.

One week before an experiment began, similarly sized, fourth-instar nymphs (100- to 150-mg dry weight) were placed in a large glass bowl and given water *ad libitum*. All experimental nymphs were also given one diet cake of casein-only and one of sucrose-only diets to condition the cockroaches to the novel diets. (Previous experiments have shown that the nymphs will spend much of their first week eating strictly the sucrose-only diet; the rat chow that they normally eat has a much lower percentage of sugar than they require.) Following a week of conditioning to food, the nymphs were divided randomly into the various experimental groups and housed singly in individual feeding arenas (20 × 100-mm glass petri dishes). Each arena contained water (sealed 10 × 30-mm plastic petri dishes with a wick of cotton sticking out of a hole in the top) and food.

The food used throughout these experiments is a lyophilized, defined diet containing all essential nutrients (Table I) (Cohen *et al.*, 1987). To prepare the food, the agar was melted in half the water volume and allowed to cool to 60°C or less. The other portion of water and all dry ingredients were added and blended thoroughly before the mixture was poured into

Table I. Ingredient List for the Diets Used in the Experiment[a]

Ingredient	Amount
Water	400.0 ml
Agar	12.0 g
Casein	8.72 g
Sucrose	34.88 g
Wesson's salt mix	2.4 g
Cholesterol	0.6 g
Inositol	0.02 g
Ascorbic acid	0.2 g
Choline chloride	0.2 g
Ca pantothenate	0.004 g
Niacinamide	0.004 g
Riboflavin	0.002 g
Pyridoxine HCl	0.001 g
Thiamine HCl	0.001 g
Biotin	0.00008 g
Vitamin B$_{12}$	0.000008 g
Folic acid	0.001 g
α-Tocopherol	33 μl
Wheat germ oil	4 ml

[a] The recipe is for a 20:80 (casein:sucrose) diet. Other diets differ
only by their casein:sucrose ratios.

9-cm plastic petri dishes. The contents were allowed to solidify at room temperature before being placed in a −20°C freezer overnight. The diets were then placed in a lyophilizer and allowed to freeze dry for at least 48 h. These diets were then stored at −20°C until use. Before being given to the nymphs, the lyophilized diets were cut into food blocks and dried in a 60°C oven for 3 h. This oven-drying step insured complete desiccation prior to the initial weighing of the diet blocks.

Experiment 1. In this self-selection experiment, the *R. madera* nymphs ($n = 20$ per group) were given a choice between two diet cubes: one cube had all nutrients except it lacked carbohydrate (sucrose) and had twice the normal amount of protein (casein) and the other cube also had all nutrients except it lacked protein and had twice the usual amount of carbohydrate. These nymphs were compared with four groups of nymphs that received a single diet block that varied in its protein:carbohydrate ratio: 100:0, 80:20, 50:50, 20:80, or 0:100 cubes. Nymphs were allowed to feed for 2 weeks in a 26°C incubator. Feeding arenas were positioned randomly inside the incubator to control for any temperature or light differences.

Evaluation of nymph performance on each diet type was based on food consumption, nymphal growth, and food utilization parameters (Waldbauer, 1968). All parameters were measured on a dry weight basis as described by Cohen *et al.* (1987). Briefly, initial weights of the nymphs were estimated

by recording each nymph's fresh weight and then determining its estimated dry weight by multiplying the percentage dry mass from cohort nymphs (at least 10) from the same group. Left-over food, feces, and nymphs were dried in a 60°C oven for 48 h before final weights were determined. To reduce possible errors because of daily humidity differences, all dry weighings from each experiment were conducted on the same day and within 2 h. Previously, I have determined that this method reduces any rehydration errors to less than 1% of the total dry weight of food, feces, or carcasses.

Final utilization parameters were determined from the methods of Waldbauer (1968) and Scriber and Slansky (1981): ECI, efficiency of conversion of ingested food to biomass; ECD, efficiency of conversion of digested food to biomass; AD, approximate digestibility; RCR, relative consumption rate; and RGR, relative growth rate. ANOVAs were employed to check for significant differences among treatments. A Student–Newman–Keuls (SNK) test was utilized to determine differences among means.

Experiment 2. In this diet-balancing experiment, the cockroach nymphs ($n = 20$ per group) were given a choice between two diets that differed in protein:carbohydrate (casein:sucrose) concentrations. Four experimental groups were selected, with the following choices: Group 1, 100:0 and 0:100; Group 2, 100:0 and 20:80; Group 3, 80:20 and 0:100; and Group 4, 20:80 and 80:20. The diet performance was analyzed as described above.

Experiment 3. In this pharmacological experiment, alteration of *Rhyparobia madera* feeding behavior caused by injection of various serotonergic drugs was studied. Nymphs (100–150 mg) were anesthetized with CO_2 and then injected (up to 10-μl volumes) with either 2.5 mg/kg serotonin (agonist), 2.5 mg/kg α-methyltryptophan (antagonist), or vehicle (0.7% saline). These doses were originally selected from a series of preliminary dose–response experiments (data not shown). All injections were made between the second and the third sternite. A successful injection was determined if no hemolymph was observed exuding from the needle puncture. Nymphs were then allowed to recover for 30 min. Recovered nymphs ($n = 15$ per group) were allowed to feed in a diet-mixing situation (100:0—all casein and 0:100—all sucrose) for 24 h. Afterward their diet cubes were oven-dried (60°C oven for 48 h) and analyzed for amount eaten per cube. This experiment was repeated with a second cohort of nymphs ($n = 15$ per group). A two-way ANOVA was used to test for differences among treatments and to compare results between cohorts.

RESULTS

Table II shows that *R. madera* nymphs have the capacity to diet-mix a proper meal for themselves. The "diet mixers" (those receiving a choice

Table II. Biomass Gained and Dietary Efficiencies of *Rhyparobia madera* Nymphs Given Different Single Cubes Containing Casein and Sucrose at Various Ratios or Given One Protein (Casein) Cube and One Carbohydrate (Sucrose) Cube in a Diet-Mixing Situation[a]

Diet choice (casein:sucrose)	Weight gain (mg)	ECI	ECD	AD	RCR	RGR
100:0	5.3 ± 1.1^b	—	—	—	—	—
80:20	$30.3 \pm 6.9^{a,*}$	18.4 ± 1.0^a	26.8 ± 1.0^b	68.6 ± 1.0^a	$0.057 \pm .004^a$	$0.010 \pm .001^{a,b}$
50:50	$47.2 \pm 9.1^{a,b}$	$20.7 \pm 1.1^{a,b}$	30.1 ± 1.2^c	69.1 ± 0.9^a	$0.080 \pm .005^b$	$0.016 \pm .002^{b,c}$
20:80	67.0 ± 11.9^b	22.4 ± 1.4^b	$28.4 \pm 1.4^{b,c}$	78.9 ± 1.2^b	$0.098 \pm .005^{b,c}$	$0.022 \pm .001^{c,d}$
0:100	16.8 ± 4.9^a	11.7 ± 1.6^c	14.7 ± 1.9^a	79.1 ± 0.9^b	$0.050 \pm .005^a$	$0.006 \pm .001^a$
Diet mixers	72.6 ± 7.6^b	23.3 ± 1.1^b	31.5 ± 1.6^c	74.0 ± 0.9^b	$0.106 \pm .004^c$	$0.024 \pm .002^d$
ANOVA (*F*)	17.48	5.24	5.36	3.37	6.15	5.88

[a]The experiment lasted 2 weeks. $N = 20$ for all groups. All values are $\bar{X} \pm$ SE. ECI, efficiency of conversion of ingested food to biomass; ECD, efficiency of conversion of digested food to biomass; AD, approximate digestibility; RCR, relative consumption rate; RGR, relative growth rate.

[b]Only a few nymphs fed during the experiment.

*Within a column, values followed by the same superscript are not significantly different from each other, $P > 0.05$, SNK procedure. ANOVA values (*F* statistics) are all $P < 0.05$.

between a casein cube and a sucrose cube) selected a protein:carbohydrate ratio of $23.7 \pm 2.0:76.3 \pm 2.0$ (this ratio was derived by dividing the amount eaten in each cube by the total amount consumed). These diet mixers grew at efficiencies and rates that were among the highest for all groups studied. To no surprise, the nymphs feeding on 20:80 single cubes (approaching the natural mixing ratio) were also provided a nutritious meal as indicated by dietary efficiency measures (Table II). All efficiencies from nymphs feeding on 20:80 cubes were statistically similar to those obtained by the diet mixers. The 50:50 feeders did fairly well on their diets. However, the high protein concentration of these cubes was reflected in a lower digestibility (AD), lower consumption rate (RCR), and lower growth rate (RGR) of nymphs feeding on this diet compared to the diet mixers. The effects of high protein in the diet were even more severe to the 80:20 feeders: statistically lower figures in weight gain and in all efficiencies tested. Nymphs feeding on all-carbohydrate diets (0:100) ate substantially (high AD, relatively high RCR) but had very low growth rates and growth efficiencies (ECI, ECD). Nymphs feeding on the all-protein diets (100:0) fared the worst. Only a few (40%) actually fed on the diet cubes provided, and four of the nymphs died during the experiment (no nymphs in the other treatments died during the 2-week period). Because of the extremely low rate of feeding, the various efficiencies and rates from these nymphs were not provided in Table II.

Table III shows that *R. madera* nymphs are capable of diet balancing when offered two diet cubes that widely varied in protein:carbohydrate ratios. Group 1 nymphs, selecting between a protein cube (100:0) and a carbohydrate cube (0:100), fed at a ratio of 24.6:75.4 (protein:carbohydrate). This consumption ratio was very similar to that obtained previous by nymphs (23.7:76.3) in the same situation (Table II). Group 2 nymphs, given the choice between the 20:80 cubes and a protein cube (100:0), ate mostly from the sugar diet. However, they occasionally fed on the protein cube, producing a mean protein:carbohydrate ratio of 27.5:72.5, which was statistically similar ($P > 0.10$) to that of all other groups. Nymphs from Group 3 fed primarily from the all-sucrose cube yet also fed on the 80:20 cube. Their mean protein:carbohydrate ratio was 24.6:75.4, exactly the same as that of Group 1. Finally, Group 4 (20:80 and 80:20 cubes) achieved a ratio of 27.3:72.7 by mixing their consumption of both cubes. Again, this ratio was statistically similar to that of all other groups ($P > 0.10$). I also measured the various dietary efficiencies and rates for all groups (data not shown). There were no statistical differences in any measure among each group. ANOVA results were as follows: weight gain, $F = 0.42$; ECI, $F = 0.02$; ECD, $F = 0.21$; AD, $F = 1.46$; RCR, $F = 0.79$; and RGR, $F = 0.47$.

The last experiment looked at the possible neurochemical regulation of feeding, by examining the role of the neurotransmitter 5-hydroxytryptamine

Table III. Mean Amount of Diets Eaten by *Rhyparobia madera* Nymphs Given Two Cubes Containing Protein (Casein) and Carbohydrate (Sucrose) at Various Ratios in a Diet-Mixing Situation[a]

Diet choice (casein:sucrose)	Group 1	Group 2	Group 3	Group 4
0:100	184 ± 23 (75.4%)	—	162 ± 18 (69.2%)	—
20:80	—	223 ± 29 (90.7)	—	180 ± 17 (87.8%)
80:20	—	23 ± 6 (9.3%)	72 ± 8 (30.8%)	25 ± 6 (12.2%)
100:0	60 ± 10 (24.6%)	—	—	—
% protein	24.6	27.5	24.6	27.3
% carbohydrate	75.4	72.5	75.4	72.7

[a]Choices included the following: Group 1, 0:100 (casein:sucrose) and 100:0; Group 2, 20:80 and 100:0; Group 3, 0:100 and 80:20; and Group 4, 20:80 and 80:20. Numbers in parentheses represent the mean percentage feeding for each diet per group, and the percentages at the bottom represent the total protein or carbohydrate consumed for each group. The experiment lasted 2 weeks. $N = 20$ for all groups. Values are $\bar{X} \pm SE$.

(serotonin) in a diet-mixing situation. This experiment was replicated with two cohorts of nymphs. The diet-mixing results of both experiments were similar (both control groups fed at a protein:carbohydrate ratio of approximately 22:78; $P > 0.10$), although the saline-injected controls for the two groups differed in how much total food they ate (Cohort 1 controls ate 8.2 ± 1.5; Cohort 2 controls ate 12.1 ± 2.0). Thus, I combined the data from both experiments by comparing intakes based on group control feeding (Fig. 1). Injection of serotonin caused a 9.7% reduction in carbohydrate feeding and a concomitant 8.3% reduction in overall feeding. Both measures were significantly different ($P < 0.05$) compared to those of control nymphs injected with saline. Nymphs injected with the serotonin receptor antagonist α-methyltryptophan showed opposite results: a 7.1% increase in carbohydrate feeding and a small, 2.9% increase in overall feeding. Only the carbohydrate increase was statistically significant compared to that of controls. Interestingly, protein feeding,
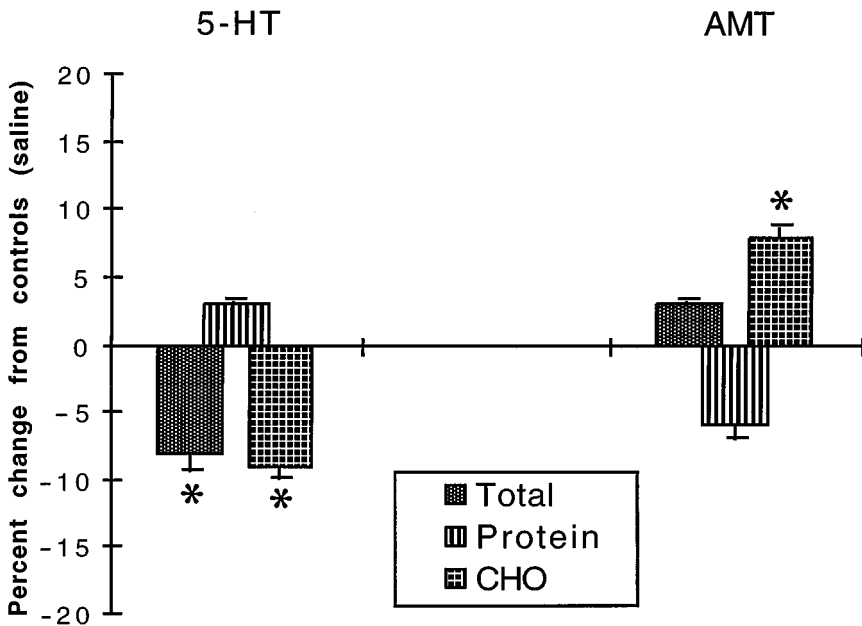

Fig. 1. Alteration of *Rhyparobia madera* nymph feeding behavior caused by injection of various serotonergic drugs. Nymphs were injected with either 2.5 mg/kg serotonin (5-HT group), 2.5 mg/kg α-methyltryptophan (AMT group), or vehicle (saline) and fed a choice between a 100:0 all-casein cube (protein) and a 0:100 all-sucrose cube (CHO) for 24 h. The total and amount from each cube eaten were compared to those for the saline-injected controls. Results were pooled from two experiments ($n = 15$ per group for each). Bars represent SE. *Statistically different compared to saline-injected nymphs.

although not significantly different from that of controls, showed opposite results to carbohydrate feeding.

DISCUSSION

The results confirmed that the cockroach *R. madera* has the ability to select a nutritious diet when faced with single cubes of protein and carbohydrate. The diet mixing ratio of 24:76 was judged the best possible choice based upon a wide variety of rates that measure food consumption as it relates to growth. This is now the second cockroach species that has been shown to diet-mix, as *Supella longipalpa* also showed the ability to self-select an optimal diet (Cohen *et al.*, 1987). Interestingly, both cockroach species selected similar high carbohydrate ratios when given a choice between protein and sucrose cubes. Both species grew very well on low-protein, high-carbohydrate diets, perhaps reflecting the need for a higher energy intake at the cost of a lower growth rate.

Interestingly, the *R. madera* nymphs (like *S. longipalpa* nymphs) given mixtures of nutrients showed that their growth efficiency is positively correlated with the amount of carbohydrate in their diet and negatively correlated with the amount of protein. Nymphs feeding on cubes containing mostly sugar had dietary efficiencies that were shown to be best to support growth. In addition, nymphs feeding on carbohydrate-only (0:100) cubes survived and, surprisingly, added biomass. I have preliminary data that show that *R. madera* nymphs feeding on the 0:100 cubes had similar consumption rates (RCR) compared to 20:80 feeders for the first 2 days of an experiment.

On the other hand, too much protein is either physiologically harmful or simply distasteful as shown by the nymphs given a protein-only diet cube. Thus to survive, the *R. madera* nymphs feed almost-exclusively on carbohydrate and yet must feed from the "harmful" protein cube occasionally.

Not only were the *R. madera* nymphs capable of dietary self-selection, but also they showed that they can diet-balance when faced with a wide range of the nutrients, protein and carbohydrate. Regardless of the variety of diet choices, the nymphs maintained similar protein:carbohydrate intake ratios (ca. 25:75). This was especially interesting in the treatment involving 20:80 feeders. In Experiment 1 (Table II), the 20:80 single-cube feeders had statistically similar values for every parameter measured compared to the diet mixers (protein vs carbohydrate), suggesting that this diet (20:80) was as nutritious as any self-selected one. Yet in Experiment 2 (Table III), nymphs choosing between a 20:80 cube and a cube rich in protein (Group 2, 100:0; Group 4, 80:20) always ate a little from the casein cubes, suggesting that a 20:80 diet is slightly suboptimal in protein levels.

Additionally, it is known that cockroaches change their protein:carbohydrate "optimal mixture" as they mature (Cohen *et al.*, 1987). What might be best for them one week may be unnecessary the next. The 2-week span that I used to monitor feeding must have included periods when the 25:75 ratio changed because of physiological needs (i.e., ecdysis). Also, it is probable that the nymphs self-selecting between two extreme diets move frequently between diet cubes, constantly adjusting their intake ratio to match the nutrient demand.

Questions remain regarding the physiological controls of self-selection and diet balancing in insects. Based upon the findings in this paper, the regulatory processes governing selection of the proper nutrient ratio must be quite precise. Numerous regulatory hypotheses have been advanced, including control by chemosensory receptors (Simpson and Simpson, 1990), blood sugar levels (Friedman *et al.*, 1991) and neurotransmitters (Cohen *et al.*, 1988).

In mammals, alterations in brain neurotransmitter levels have been shown to regulate nutrient intake. While many neurotransmitters, neuropeptides, and neuromodulators have been linked to changes in feeding behavior, important to my study is the historical correlation of serotonin and carbohydrate intake. First described 30 years ago by Fernstrom and Wurtman (1971) and implicated in recent human diet pill controversies, this association implies nutritional control over neurotransmitter synthesis: an increased amount of carbohydrate intake leads to an increased rate of serotonin synthesis. This increase in serotonin synthesis then alters the feeding behavior by reducing carbohydrate feeding (Thibault and Booth, 1999).

Cohen *et al.* (1988) showed that the serotonin–carbohydrate relationship may occur in insects. They showed that by pharmacologically altering brain serotonin levels in the lepidopteran larvae *Helicoverpa* (*Heliothis*) *zea*, it was possible to manipulate the overall carbohydrate intake. Specifically, decreasing brain serotonin results in an increase in carbohydrate consumption, while artificially increasing brain serotonin caused the larvae to feed on less carbohydrate.

The Experiment 3 results suggest that this phenomenon is widespread in insects. By manipulating serotonin concentrations, cockroach nymphs change their feeding behavior (Fig. 1). Like the *H. zea* larvae, decreased brain serotonin led to an increase in carbohydrate feeding by *R. madera* nymphs; increased brain serotonin leads to a decrease in carbohydrate feeding. Thus, serotonin appears to manipulate carbohydrate selection in *R. madera* nymphs, implying a major regulatory role in diet balancing as well. Future experiments using HPLC and immunohistochemistry should be done to determine whether this phenomenon is localized in the central nervous system or isolated in peripheral nerves.

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