

Maxillectomy Does Not Disrupt Self-Selection by Larvae of *Manduca sexta* (Lepidoptera: Sphingidae)

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ABSTRACT *Manduca sexta* (Johannson) larvae were maxillectomized early in the third instar, and as newly emerged fourth instars were permitted to feed by self-selection (i.e., offered the opportunity to feed selectively on a defined sucrose diet [lacking only protein], or a defined protein diet [lacking only sucrose]) through the entire stadium. They did not differ significantly in their self-selected protein/carbohydrate ratio from sham-operated control larvae. Further experiments were done in which similarly operated larvae and controls were preconditioned for 15 h on diets lacking either sucrose or protein, starved for 4 h, placed in a self-selection environment, and observed every 10 min for 5 h of a 7-h period as they made their choices between sucrose and protein diets. Larvae that had been preconditioned on a protein-free diet tended to associate with the protein diet, whereas those preconditioned on a sucrose-free diet spent their early hours on the sucrose diet. The behavior of maxillectomized larvae differed only in minor ways from that of the controls.

KEY WORDS *Manduca sexta*, behavior, maxillectomy

RECENT WORK SHOWS that insects of various orders are capable of self-selecting a nutritionally favorable balance of nutrients when the nutrients are presented to them separately (see the reviews by Simpson & Simpson [1990] and Waldbauer & Friedman [1991]). The mechanisms that control self-selection are not well understood either in insects or vertebrates. Both metabolic feedback and chemosensory cues are certainly involved, but how these two factors interact to govern self-selection is by no means clear (Cohen et al. 1987, Abisgold & Simpson 1988, Simpson & White 1990; Friedman et al. 1991).

The sensitivity of peripheral receptors to gustatory stimuli may vary for several reasons, including the recent feeding history and nutritional state of the insect (Blaney et al. 1986). It has been shown that the sensitivity of the receptors on the maxillary palps of *Locusta migratoria* (L.) varies with the level of protein in the diet and free amino acids in the hemolymph. Feeding on a low-protein diet increases maxillary sensitivity to certain amino acids; feeding on a high-protein diet or injecting amino acids into the hemolymph decreases maxillary sensitivity to these amino acids (Abisgold & Simpson 1987, 1988). Simpson et al. (1990) showed that variations in the sensitivity of maxillary receptors to amino acids and sucrose correlate with changing patterns of protein and sucrose ingestion during the somatic growth phase of adult *Locusta*. Schoonhoven et al. (1991) found that changes in

the sensitivity of the maxillary sensilla styloconica of the larvae of the lepidopteran *Spodoptera littoralis* (Boisduval) also accompany changes in feeding behavior.

On the basis of these findings and others, it has been postulated that self-selection is governed by changes in peripheral gustatory sensitivity (Simpson & Simpson 1990, Schoonhoven et al. 1991). Abisgold & Simpson (1987, 1988) and Simpson & Simpson (1990) proposed that, in *Locusta*, self-selection for protein may be directly controlled by the changing sensitivity of chemoreceptors on the maxillary palps as sensitivity varies with diet-induced fluctuations in the amino acid content of storage tissues such as the hemolymph. Although no direct evidence is available, these authors suggested that a similar peripheral mechanism may control self-selection for carbohydrates. And, inferring a commonality between *Locusta* and *Spodoptera* based upon the similarity of changes in maxillary sensilla sensitivity in response to similar feeding stimuli, Simpson & Simpson (1990) also conjectured that peripheral sensitivity may control self-selection by *Spodoptera* and other Lepidoptera.

Ongoing investigations in our laboratory (unpublished data) have shown that, just as in other Lepidoptera, fourth instars of *Manduca sexta* (Johannson) self-select from artificial diets to obtain a favorable balance of protein and sucrose. The maxillae of this insect are already known to play an important role in host plant selection, in that total maxillectomy (Waldbauer & Fraenkel

1961), or the removal of specific portions of the larval maxilla (de Boer 1991), result in a marked reduction in the ability to discriminate among host plants. This insect should, therefore, provide a good model upon which to frame the question of whether the maxillae are, indeed, a necessary component of the system involved with the ability of the animal to satisfy its metabolic needs through self-selection. This article reports the effect of ablating the maxillary sensilla on the ability of *Manduca* larvae to self-select.

Materials and Methods

The *Manduca* larvae used in these experiments came from a culture maintained on a wheat germ diet in our laboratory (Ahmad et al. 1989). No voucher specimens were retained. Maxillectomy was carried out as described in detail by Waldbauer & Fraenkel (1961). The larvae were anesthetized on ice, and the distal two-thirds of the maxilla was pinched off with a sharpened no. 5 watchmaker's forceps. This removed all of the maxillary chemoreceptors, both sensilla styloconica and sensilla basiconica. Control larvae (sham-operated) were similarly anesthetized, and the anal horn was pinched off. All larvae were operated on early in the third stadium. After the operation they were replaced on wheat germ diet and allowed to feed and recover until they were ready to molt to the fourth instar, as signaled by the cessation of feeding and forward slippage of the head capsule. Larvae that had been traumatized more than usually by the operation were eliminated automatically because they either died or grew more slowly than the main group. The larvae to be used in experiments as unfed fourth instars were then placed individually in clean 5-cm petri dishes, each lined with a disk of moist filter paper but containing no food, and allowed to molt. No regeneration of maxillae was noted after the molt.

Experiment 1 determined the long-run effects of maxillectomy by allowing larvae to self-select from the beginning to the end of the fourth stadium. In this experiment, we used controls from which the anal horn had been ablated and larvae from which only the left maxilla, only the right maxilla, or both maxillae had been ablated. Shortly after they molted to the fourth instar, larvae of each treatment (see Table 1 for *n* values) were placed in self-selection arenas, one larva in each arena. The arenas were 15-cm petri dishes lined with a disk of moist filter paper. Two cylindrical pieces (2.3 cm³) of defined diet (Ahmad et al. 1989) were placed 1 cm apart on an aluminum foil slip. One of them, the protein diet, lacked sucrose but contained casein and all of the other required nutrients. The other, the sucrose diet, lacked casein but contained sucrose and all of the other required nutrients. In each of these incomplete diets, the missing nutrient was

replaced with an equivalent weight of the other nutrient. Thus, each of the two incomplete diets contained a weight of sucrose or casein equal to the combined weights of sucrose and casein in the complete defined diet. Dishes containing larvae were placed on a table in the laboratory at 25 ± 2°C under constant illumination. At the end of the fourth stadium, marked by the forward slippage of the head capsule, the amount eaten from each diet piece and the conventional utilization parameters (Scriber & Slansky 1981) were determined by the dry-weight method of Waldbauer (1968): CR, consumption rate (mg eaten/d); GR, growth rate (mg body weight gained/d); ECI, efficiency of conversion of ingested food to biomass; AD, approximate digestibility; and ECD, efficiency of conversion of digested (assimilated) food to biomass.

Experiment 2 examined in greater detail the short-run effect of maxillectomy on self-selection for sugar and protein. Newly molted, unfed, maxillectomized and control larvae were preconditioned in 5-cm petri dishes using defined diets in three regimens: (1) 15 h on a sucrose diet, (2) 15 h on a protein diet, or (3) 15 h on a complete defined diet with an 80:20 protein/sucrose ratio. All treatments were followed by 4 h of starvation.

At all stages of experiment 2, larvae were individually confined in 5-cm petri dishes lined with moist disks of filter paper. All diet pieces were 1.9-cm³ cylinders and were placed on slips of aluminum foil. Larvae were transferred to clean petri dishes lined with moist filter paper for the 4-h starvation period. During preconditioning and the following observation periods, the dishes containing larvae were kept on a table in the laboratory under constant illumination at a temperature of 25 ± 2°C.

Twenty intact and 20 maxillectomized larvae were taken for use from each of the preconditioning treatments. Immediately after the starvation period, each larva was transferred to a separate self-selection arena containing one piece of protein diet and one piece of sucrose diet placed 1 cm apart on a slip of aluminum foil. Preliminary experiments with intact fourth-instar *Manduca* showed no significant differences in the self-selected protein/sucrose ratio when diets were placed 1–8 cm apart. Larvae in these arenas were observed and scored once every 10 min for the first 2 h following starvation, left unwatched for the next 2 h, then again scored once every 10 min for the following 3 h. They were scored as being in association with a diet only if the mouthparts were actually touching the diet.

The data obtained in the second experiment are complex in that they involve elements of partial nonindependence. Adequate statistical compensation for these elements is achieved if the actual counts of larvae are used only to indicate the rank orderings of treatment responses in a randomized-block analysis of rankings fol-

Table 1. Effect of various maxillary ablations on self-selection for protein and sucrose by fourth-instar *M. sexta*

Treatments ^a	Total intake, mg	Intake from each diet, mg	
		Protein diet	Sucrose diet
CT	376 ^a ± 14	287 ^a ± 15 (76.3 ± 1.9)	89 ^a ± 11 (23.7 ± 2.7)
BM	404 ^a ± 18	257 ^a ± 10 (65.7 ± 3.6)	147 ^a ± 21 (34.3 ± 3.6)
RM	379 ^a ± 12	281 ^a ± 13 (75.2 ± 3.4)	98 ^a ± 15 (24.8 ± 3.4)
LM	393 ^a ± 15	280 ^a ± 13 (72.4 ± 3.5)	113 ^a ± 17 (27.5 ± 3.5)

n = 20 except for BM, for which n = 19. All values are means ± SE. Means within a column followed by the same letter are not significantly different (ANOVA, P > 0.05). Values in parentheses are percentage intakes.

^a CT, control (anal horn ablated); BM, bilateral ablation; RM and LM, unilateral ablation of right and left maxilla, respectively.

lowed by Tukey's multiple-comparison procedure (Zar 1984).

Results and Discussion

Larvae that had been unilaterally maxillectomized (UM) showed only slight and statistically insignificant differences from the sham-operated control larvae (CT) in the total intake of food and the proportions of protein and sucrose that were self-selected during the fourth stadium. The bilaterally maxillectomized (BM) larvae selected a smaller proportion of protein than did the others, but the difference is not statistically significant (Table 1). Furthermore, as Table 2 shows, the self-selected protein/sucrose ratio of the BM larvae fell within the presumably optimal range. The food utilization parameters of the BM larvae did not differ from those of the larvae in any of the other treatments except for a small but statistically significant decrease in digestibility (AD) compared with the CT larvae. However, this was balanced by an insignificant increase in ECD, resulting in a GR, CR, and ECI that did not differ significantly from those of the CT larvae.

Although we did not record feeding per se in experiment 2, the disappearance of diet and production of feces made it obvious that a great deal of feeding had occurred during the observation

period, and that the most feeding had occurred on the diet with which the larvae were most often in contact.

As expected from previous work with *Spodoptera* (Simpson et al. 1988) and *Helicoverpa zea* (Boddie) (Friedman et al. 1991), intact *Manduca* larvae were markedly affected by preconditioning (Fig. 1). During the first 2 h of the 7-h self-selection period, intact (control) larvae that had been preconditioned on the complete defined diet were found associated with both the protein and sucrose diets but showed a marked preference for the protein diet. Intact larvae that had been preconditioned on a sucrose diet showed a strong tendency to associate with the protein diet, whereas those that had been previously conditioned on a protein diet showed a strong tendency to associate with the sucrose diet. During the last 3 h of the self-selection period, the intact (control) larvae that had been preconditioned on protein or sucrose behaved essentially as did those that had been preconditioned on the complete defined diet. Sucrose-conditioned controls continued to associate mainly with the protein diet, but members of the protein-conditioned group abandoned their initial preference for sucrose and were more often found associated with the protein diet.

There were no statistically significant differences in self-selection patterns between intact and maxillectomized larvae among the three preconditioned groups during the first 2 h of the 7-h self-selection period (Table 3). During the last 3 h of this period, there was one significant difference between intact and maxillectomized larvae: within the group preconditioned on sucrose, there was a small difference between the number of intact and maxillectomized larvae found on protein (Table 3; Fig. 1).

The general lack of significant differences between groups of intact and maxillectomized larvae during the first 2 h of the self-selection period, notwithstanding the one small difference during the last 3 h, indicates that changes in the sensitivity of the maxillary receptors cannot be the sole mechanism mediating self-selection. Our argument is supported by the results of experiment 1, in which maxillectomy did not affect

Table 2. Duration, growth, and utilization parameters of intact and maxillectomized fourth-instar *M. sexta* larvae during the entire stadium when given a choice of two defined diets, one lacking sucrose and the other lacking protein

Treatments	Duration, h	Weight gain, mg	CR, mg/d	GR, mg/d	AD, %	ECI, %	ECD, %
CT	82.80 ^a ± 2.11	95 ^a ± 3	110 ^a ± 5	28 ^a ± 1	49.53 ^a ± 1.06	25.33 ^a ± 0.45	51.47 ^a ± 1.12
BM	98.31 ^a ± 2.77	104 ^a ± 3	100 ^a ± 5	26 ^a ± 1	46.26 ^b ± 0.57	26.29 ^a ± 0.90	56.89 ^a ± 1.91
RM	92.40 ^a ± 3.10	103 ^a ± 3	100 ^a ± 4	27 ^a ± 1	48.03 ^{ab} ± 0.65	27.35 ^a ± 0.62	57.08 ^a ± 1.36
LM	92.20 ^a ± 3.39	102 ^a ± 4	102 ^a ± 4	27 ^a ± 1	48.21 ^{ab} ± 0.66	26.38 ^a ± 0.71	54.94 ^a ± 1.69

n = 20 except for BM, in which N = 19. CR, consumption rate; GR, growth rate; AD, approximate digestibility; EC, efficiency of conversion of ingested food; ECD, efficiency of conversion of digested food. All values are means ± SE. Means within a column followed by the same letter are not significantly different (ANOVA followed by Tukey's HSD test, P < 0.05).

^a CT, control (anal horn ablated); BM, bilateral ablation; RM and LM, unilateral ablation of right and left maxilla, respectively.

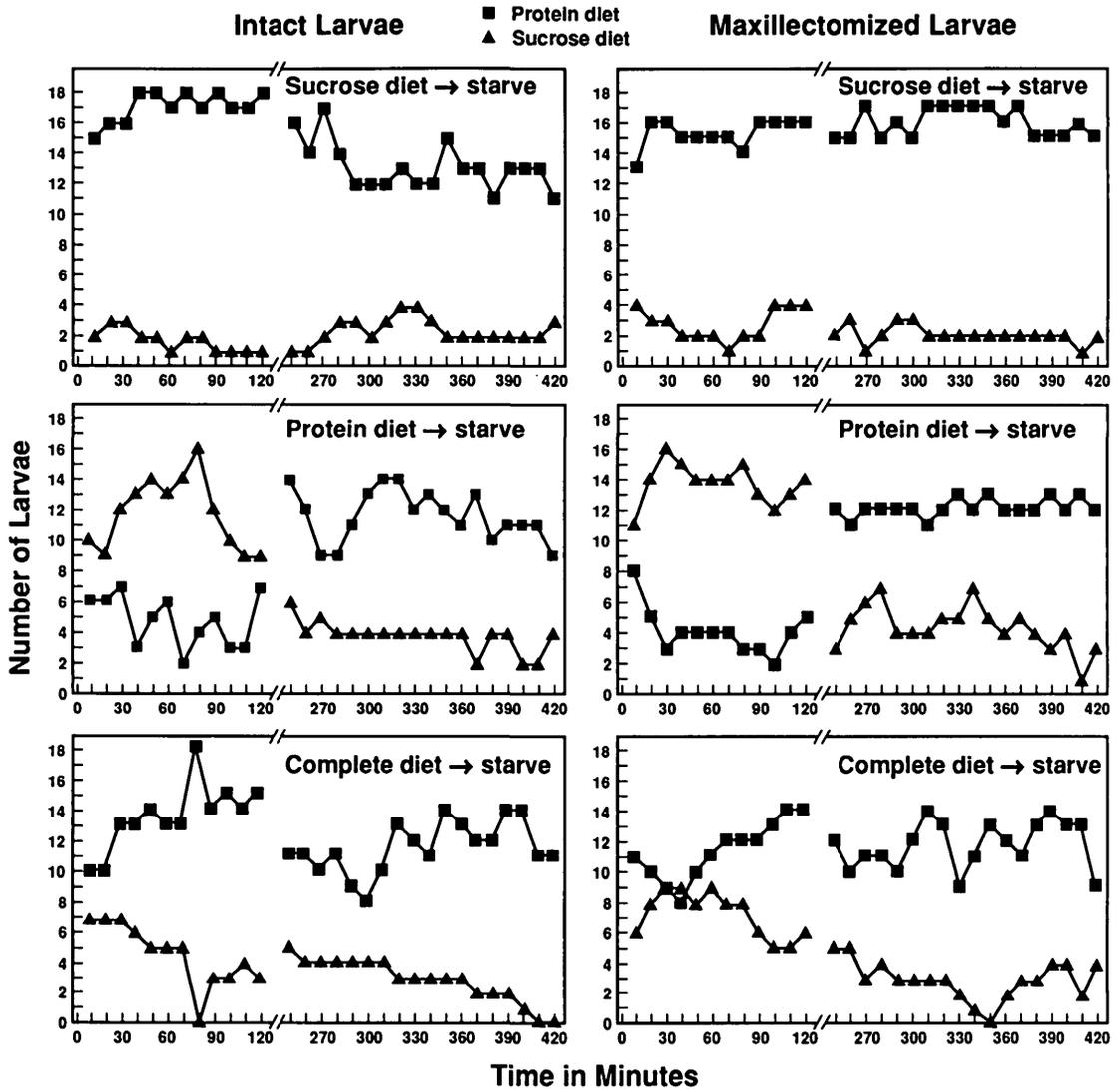


Fig. 1. Numbers of intact and maxillectomized *M. sexta* larvae associated with a sucrose or a protein diet at 10-min intervals during the first 2h and the last 3h of the 7-h period immediately following the preconditioning regimen indicated on each graph. Larvae not accounted for in the curves did not have their heads in contact with either of the two diets. $n = 20$ larvae per each of the six treatments.

the self-selected protein/carbohydrate ratio of larvae that were allowed to feed throughout the fourth stadium. (In this connection, it is worth noting that Miller & Teates (1986) showed that total gustatory deafferentation did not prevent rats from self-selecting a nutritionally satisfactory diet.) It seems unlikely, although not certain, that any meaningful perturbation would have been seen during the 2 h that the larvae were not being observed between the end of the first 2 h of observation and the first observation of the last 3 h of the self-selection period because one would expect changes in peripheral sensitivity to be effective immediately rather than to take hours to develop. Further, we cannot exclude the

possibility that some temporary perturbation of self-selection might have occurred during the 10 min that elapsed from the end of the starvation period to the first observation of the self-selection period. It is also possible that maxillectomy caused changes in feeding behavior that are not revealed by our methods. The point is, however, that any such changes that might have occurred are either peripheral to self-selection or are too slight to affect the ultimate result of selection, which is the chosen protein/sucrose ratio.

It is well established that the gustatory sensitivity of maxillary receptors varies with recent feeding history, having been reported for both *L. migratoria* and *S. littoralis*. Our results, how-

Table 3. Average ranks and significance patterns indicated by Tukey's multiple-comparison procedure following a randomized-block analysis of rankings of diet use during a self-selection experiment using fourth-instar *M. sexta*

Diet chosen during self-selection		Type of operation and preconditioning				
		Hours 1 and 2				
Protein	Protein	Protein	Complete	Complete	Sucrose	Sucrose
Avg rank	-MX	+MX	-MX	+MX	-MX	+MX
	1.33	1.67	3.17	4.00	5.00	5.83
Sucrose	Sucrose	Sucrose	Complete	Complete	Protein	Protein
Avg rank	+MX	-MX	+MX	-MX	+MX	-MX
	1.37	2.00	2.71	3.92	5.17	5.83
Hours 4-7						
Protein	Protein	Complete	Complete	Protein	Sucrose	Sucrose
Avg rank	+MX	-MX	+MX	-MX	+MX	-MX
	2.67	2.69	2.64	3.14	3.94	5.92
Sucrose	Sucrose	Sucrose	Complete	Complete	Protein	Protein
Avg rank	-MX	+MX	+MX	-MX	+MX	-MX
	1.92	2.58	3.19	3.47	4.72	5.11

+MX, sham-operated; -MX, maxilla oblated. Treatments sharing a common underline do not differ at the 0.05 level of significance.

ever, show that self-selection proceeds in a normal or near normal manner after ablation of the maxillae in at least one species, *M. sexta*. How, then, are these seemingly contradictory results to be interpreted?

There appears to be general agreement among investigators that metabolic feedback is essential to the control of dietary self-selection. There also seems to be general agreement that metabolic feedback could govern self-selection by the indirect route of associative learning, such feedback acting as the unconditioned stimulus. Our experiments were developed to evaluate whether or not metabolic feedback governs self-selection via its direct effect on the sensitivity to nutrients of the maxillary chemoreceptors. With these experiments, we have shown that the ablation of all of the chemoreceptors on the maxillae of *M. sexta* does not significantly perturb its ability to self-select. This does not prove that the maxillae do not play some role when they are present and does not, of course, eliminate from consideration a hypothesis of self-selection that is based in changes in peripheral sensitivity. However, at present it does leave such a hypothesis without experimental support.

Do changes in peripheral sensitivity actually control self-selection? Chemosensory sensilla in the preoral cavity or on structures other than the maxillae (perhaps the epipharynx) may also be caused by metabolic feedback to vary in their sensitivity to nutrients. It remains to be seen, however, whether or not peripheral sensilla other than those on the maxillae actually do vary in their sensitivity to nutrients with changes in diet, and whether or not these sensilla will actually substitute for the ablated maxillary chemoreceptors.

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