

DIETARY SELECTION BEHAVIOUR IN *SPODOPTERA LITTORALIS*: THE EFFECTS OF CONDITIONING DIET AND CONDITIONING PERIOD ON NEURAL RESPONSIVENESS AND SELECTION BEHAVIOUR

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Summary

1. This paper forms part of our study of the behavioural and neural mechanisms involved in dietary selection behaviour of larvae of the polyphagous noctuid *Spodoptera littoralis* (Boisduval).

2. Larvae were conditioned on one of four chemically defined artificial diets for 4, 8 or 12 h. Diets differed in their proportions of protein and digestible carbohydrate: the PC diet contained 20 % protein and 10 % carbohydrate; the P diet contained 20 % protein but the 10 % carbohydrate was replaced by indigestible cellulose; the C diet contained 10 % digestible carbohydrate and the protein was replaced by cellulose, and the 0 diet lacked both protein and carbohydrate. After conditioning, the larvae were exposed to either behavioural or electrophysiological experiments.

3. In the behavioural choice experiment, larvae were provided with both P and C diets for 9 h. The conditioning diets P and 0 influenced which of the two choice diets was first contacted; significantly higher proportions of the larvae conditioned on the P diet initially contacted the C choice diet and significantly higher proportions of larvae conditioned on the 0 diet initially contacted the P choice diet. Larvae fed on the diet they first contacted and the probability of stopping the first meal was greater when that diet was P, regardless of the conditioning diet. The duration of the first meal on the C choice diet was significantly longer for larvae conditioned on diets lacking digestible carbohydrate (P and 0) than for those conditioned on either PC or C diets.

4. Larvae were observed to compensate for a deficiency in protein by increasing the frequency of feeding contacts with the P choice diet but not the duration of these contacts. To compensate for a deficiency in digestible carbohydrate, the larvae increased the duration of their feeding contacts with the C choice diet.

5. In the electrophysiological experiment, the maxillary styloconic sensilla were stimulated with an amino acid mixture and sucrose. Neural responses were recorded from two groups of larvae, one after they had been conditioned on one of the four diets for 4, 8 or 12 h and the other after they had subsequently been

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exposed to the choice diets for 9 h. Larvae in the first group that had been conditioned on diets lacking sucrose (P and 0) were more responsive to sucrose than larvae conditioned on diets containing sucrose (PC and C). The reverse occurred with the response to the amino acid mixture, with larvae conditioned on diets containing protein (PC and P) being less responsive to the amino acid mixture than those conditioned on diets lacking protein (C and 0). After the 9 h choice period, the responsiveness of sensilla was no longer related to the conditioning diet.

Introduction

Recently there has been considerable interest in 'dietary self selection' in insects (McFarland and Thorsteinson, 1980; Waldbauer *et al.* 1984; Cohen *et al.* 1987, 1988; Waldbauer and Friedman, 1988; S. J. Simpson *et al.* 1988, 1990, 1991; Simpson and Simpson, 1990; Chyb and Simpson, 1990). Certain insects have been shown to select, from available foods, a mixture that supports growth and development better than any single food alone (see reviews by Waldbauer and Friedman, 1988; Simpson and Simpson, 1990).

Cohen *et al.* (1988), investigating dietary selection behaviour in *Heliothis zea*, showed that larvae offered a choice of two diets, one containing protein and the other digestible carbohydrate, switched between the two types of diet. Over the whole stadium they spent more time on the protein diet than on the carbohydrate diet. This corresponded well with the total amounts eaten (Waldbauer *et al.* 1984). S. J. Simpson *et al.* (1988, 1990) demonstrated that larvae of *Spodoptera littoralis* exhibited compensatory selection behaviour. They conditioned larvae, for different periods, on one of four artificial diets that differed in their nutrient composition. When conditioned on a diet lacking digestible carbohydrate and subsequently given a choice of diets containing either protein or digestible carbohydrate, larvae ate more of the diet containing digestible carbohydrate during the initial hour of a 9 h experiment. Larvae conditioned on diets lacking protein took longer to show compensation behaviour (Simpson *et al.* 1988). The detailed patterning of feeding was not reported, however.

In this study we examine in detail the compensatory feeding behaviour of *S. littoralis* larvae during a 9 h choice period after being exposed to diets lacking protein and or digestible carbohydrate for 4, 8 or 12 h.

We also investigate the role that changing the responsiveness of peripheral taste neurones can have in dietary selection behaviour. Previous studies with caterpillars have shown that chemosensory neurones in the maxillary styloconic sensilla play a central role in the initiation of feeding (Schoonhoven, 1987; Blaney and Simmonds, 1988; Blaney *et al.* 1987). The responsiveness of these neurones is not constant and can be manipulated by rearing larvae on diets that differ in their nutrient composition. Simmonds *et al.* (1991) showed that the responses of the sensilla to sucrose were correlated positively with feeding behaviour, so that an increase in neural activity was related to an increase in food intake. However, they did not investigate the mechanisms underlying these changes in neural responsive-

ness. The present study examines the effect that nutrient deprivation can have on the responsiveness of neurones in the gustatory sensilla and investigates the relationship between the neural activity of these neurones and feeding behaviour.

Materials and methods

Insects

Larvae of *Spodoptera littoralis* (Lepidoptera: Noctuidae) were obtained from a culture at the Department of Biology, Birkbeck College, London. Female larvae, freshly ecdysed to the final (sixth) stadium, were placed individually in Petri dishes (9 cm diameter) containing two cylindrical blocks of PC diet (2 cm long, 0.75 cm diameter).

Diets

PC diet contained 20 % protein (casein), 10 % digestible carbohydrate (sucrose) and other ingredients as listed in Simpson *et al.* (1988); P diet contained 20 % protein but with the digestible carbohydrate component replaced by indigestible cellulose; C diet contained 10 % digestible carbohydrate with the protein component replaced by cellulose; 0 diet had both the protein and carbohydrate components replaced by cellulose. The diets were presented to the insects wet.

Experimental protocol

The conditioning period

Larvae, which had been given access for 30–36 h to the PC diet, were observed until they had taken a meal. The larvae were then placed individually in Petri dishes containing two weighed blocks of a diet, PC, P, C or 0, both blocks being of the same diet. The larvae were left in these dishes for one of the conditioning periods, 4, 8 or 12 h (± 10 min). The diets were then dried and reweighed and the amount eaten was calculated. There were 30 replicates for each of the three conditioning periods on the four conditioning diets, involving a total of 360 larvae.

The choice periods

After the conditioning period, larvae were transferred individually to Petri dishes containing two blocks of weighed choice diet, one of P and the other of C. The behaviour (see below) of the larvae was discontinuously recorded at 30 s intervals for 1 h and the data were fed directly into a computer. The larvae were then transferred to another set of Petri dishes containing preweighed blocks of one of each of the P and C diets. The behaviour of the larvae was recorded on video for the next 8 h and subsequently analysed by entering into a computer the behaviour of each larva discontinuously at 1 min intervals. The diets from both choice periods were then removed, weighed and dried before being reweighed and the amount eaten calculated (see Simpson *et al.* 1988, for details of calculations).

Categories of behaviour

The following categories of behaviour were recorded in the first hour of the choice period.

(1) *Feeding*: moving the head from side to side and vertically while the mandibles remove pieces of food to be ingested.

(2) *Contacting*: typically starts with the larva raising its head into a characteristic elongated position and palpating the food with the maxillary palps, whilst the body is either on or off the food.

(3) *Moving*: crawling around the container or on the food without feeding or palpating.

(4) *Resting*: remaining completely still, either on or off the food.

In the subsequent 8 h of the choice period, the categories feeding, contacting and resting on the diet were combined, because it was not always possible to verify whether a larva was feeding when in contact with a diet. In the first hour of the choice period a meal was deemed to have started after 60 s of feeding and terminated after at least 2 min without feeding. These criteria were based on log-survivorship curves (M. S. J. Simmonds and W. M. Blaney, unpublished results, based on the methods used by Simpson, 1982).

Neural responses of the maxillary styloconica

The procedures for the conditioning and choice periods used in the neurophysiological investigation were similar to those used in the behavioural study. Electrophysiological recordings were obtained from two separate groups of larvae; recordings from group A were obtained after the conditioning period on one of the four diets; recordings from group B were obtained after the 9 h choice period. The start of the conditioning period was staggered for both experimental groups so that larvae were available for the electrophysiological recordings from 17:00 h to 22:00 h. The time interval between the end of either the conditioning period or choice period and the electrophysiological experiments was never greater than 15 min and the electrophysiological stimulations took 15–20 min. These precautions to standardise the experimental protocol, especially the time of day when the electrophysiological studies were undertaken, were to avoid fluctuations in neural responses that occur in experiments undertaken in the morning and afternoon (Simmonds *et al.* 1991). Standard tip-recording techniques (Hodgson *et al.* 1955) were used to investigate the responsiveness of the lateral and medial maxillary styloconic sensilla to different concentrations of sucrose and an amino acid mixture. Both sensilla were stimulated once each with seven solutions: 0.05 mol l⁻¹ potassium chloride; 0.01, 0.05 and 0.1 mol l⁻¹ sucrose; and 0.01, 0.05 and 0.1 mol l⁻¹ amino acid mixture (see below). All solutions were made up in the electrolyte potassium chloride (0.05 mol l⁻¹). The amino acid mixture contained a 47:40:30:40:58:45:20:50 ratio of the L-amino acids leucine:glutamine:serine:methionine:phenylalanine:lysine:valine:alanine. The composition of the amino acid mixture is similar to that used by Abisgold and Simpson (1988). Each sensillum

was first stimulated with the electrolyte, then with either the amino acid or sucrose solutions used in ascending concentration. Successive stimulations of a sensillum (1.25–1.5 s) were never separated by less than 2 min. Recordings were obtained from a total of 240 larvae, 120 in each group with 10 replicates per conditioning period on each conditioning diet.

Recordings were stored on magnetic tape and hard copies of the recordings were taken from the tapes using an oscillograph. The total number of action potentials elicited in the first second of stimulation and the number of neurones responding were determined by visual inspection. The accuracy of this assessment was monitored by digitizing (Metrabyte DAS-16 A/D card) recordings for computer analysis (see Frazier and Hanson, 1986; Smith *et al.* 1990).

Results

Behaviour

Those larvae that did not feed for longer than 2 min during either the conditioning period or the observation periods were not included in the analysis (72 out of 360 larvae). Overall, the conditioning diet influenced the larval behaviour more than did the conditioning period (Table 1).

The initial choice of diet

The majority of larvae contacted a diet within the first 10 min of the choice period. Table 2 shows that the larvae conditioned on diets containing digestible carbohydrate (PC and C) did not discriminate between the choice diets before contacting them, whereas a high percentage of those conditioned on P chose the C choice diet (66 %) and a high percentage of those conditioned on 0 chose the P choice diet (62 %).

Table 1. *F-ratios from the analysis of variance (ANOVA) of the behavioural data from Spodoptera littoralis larvae conditioned on one of four artificial diets (PC, P, C or 0) for 4, 8 or 12 h and then given a choice between P and C diets for 1 h*

Main variables	d.f.	<i>F</i> -values Proportion of time spent			Duration of intervals between contacts with choice diets
		Contacting	Resting	Moving	
Conditioning diet	3	18.4**	6.3**	7.4**	8.5**
Conditioning period	2	1.3	2.2	1.7	1.8
Interactions between variables					
Conditioning diet× Conditioning period	6	0.3	1.3	1.2	1.9
Total	297				

 ** *P*<0.001.

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Table 2. *Effect of conditioning diet (conditioning period data pooled) on the percentage of insects contacting and feeding on each of the choice diets*

Percentage of larvae contacting the choice diets												
Conditioning diet	PC		P		C		0					
Choice diet	P	C	P	C	P	C	P	C	P	C	P	C
First contact*	54	46	34	66	53	47	62	38				
Choice diet	P	C	P	C	P	C	P	C	P	C	P	C
First meal†	100	0	0	100	100	0	0	100	100	0	0	100
For example, of those larvae conditioned on the PC diet 32 (54 %) made their first contact with the P choice diet and all these larvae 32 (i.e. 100 %) went on to feed on this diet.												
* Percentage of total number of insects conditioned on each conditioning diet that made their initial contact with either the P or C choice diet.												
† Percentage of those insects contacting the choice diets that went on to have their first meal on that diet.												

All the larvae fed on the diet initially contacted (Table 2). Overall, the duration of the first meal on the choice diets was affected by the nature of the conditioning diet (analysis of variable, ANOVA, $F=33.4$, $P<0.001$) and by the duration of the time spent on the conditioning diet (ANOVA, $F=15.3$, $P<0.001$). The nature of the conditioning diet affected the duration of the first meal on the C choice diet (ANOVA, $F=32.4$, $P<0.001$) more than that on the P choice diet (ANOVA, $F=4.1$, $P>0.05$) (Fig. 1).

The frequency with which choice diets were contacted

Larvae conditioned on the PC diet contacted the choice diets less frequently during the 9 h choice period than those conditioned on the nutrient-deficient diets (P,C and 0) (Table 3A). Larvae conditioned on the C diet contacted the P choice diet nearly twice as frequently as they contacted the C choice diet. Larvae conditioned on the P and 0 diets made a similar number of contacts with the P and C choice diets, although larvae conditioned on the P diet made fewer contacts with either of the choice diets compared to larvae conditioned on the 0 diet (Table 3A).

The proportion of time spent in contact with each diet

The proportion of time that larvae spent in contact with the choice diets, during the 9 h choice period, was influenced by the nature of the conditioning diet (Table 1) and the effect was greater with the C choice diet than with the P choice diet (Table 3B). The proportion of time spent in contact with the C choice diet was greater when the larvae had been conditioned on diets lacking digestible carbohydrate (P and 0), whereas larvae conditioned on the C diet spent a greater proportion of their time on the P choice diet (Table 3B).

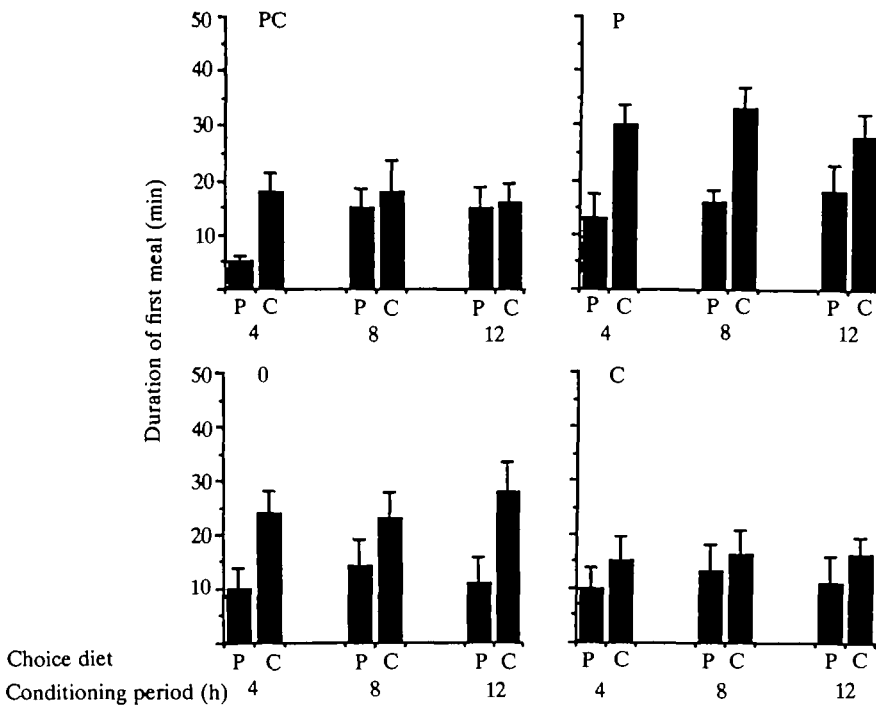


Fig. 1. Effect of conditioning diet (PC, P, C, 0) and conditioning period (h) on the duration of the first meal taken by *Spodoptera littoralis* on the P choice diet or the C choice diet. Values are mean \pm S.E.M.; N varies from 9 to 21.

The proportion of time spent moving and resting

The proportion of time that larvae spent moving and resting during the 9 h choice period was influenced by the nature of the conditioning diet (Tables 1, 3B). When not in contact with one of the choice diets, larvae conditioned on the nutrient-complete diet (PC) spent significantly more time resting than moving (Mann-Whitney U -test, $P < 0.05$), whereas larvae conditioned on diets lacking protein (C and 0) spent significantly more time moving than resting during the choice period (Mann-Whitney U -test, $P < 0.05$) (Table 3C).

Relationship between amount eaten and time spent in contact with choice diets

During the first hour of the choice period it was possible to identify the occasions when the larvae were eating, but during the succeeding 8 h period we could only identify, with certainty, when larvae were in contact with the food. It would be useful to know whether these periods of contact actually involved feeding. To investigate this we have compared the duration of such contacts with the amount of food eaten by these larvae during the entire choice period. Fig. 2 shows a clear relationship between the amount of time spent in contact with the choice diets

Table 3. *The effect of the nature of the conditioning diet on the behaviour of larvae during the 9 h choice period*A. *Frequency with which larvae contact the choice diets*

Conditioning diet	Number of contacts with choice diet (mean \pm S.E.M.)	
	P	C
PC	4.8 \pm 1.7	5.3 \pm 2.8
P	6.0 \pm 2.8	8.0 \pm 3.2
C	11.1 \pm 2.5	6.2 \pm 1.9*
0	10.1 \pm 2.8	9.1 \pm 2.9

B. *Percentage of time spent contacting the choice diets and resting and moving*

Conditioning diet	Time spent contacting the choice diets (mean \pm S.E.M.)		Time spent (mean \pm S.E.M.)	
	P	C	Resting	Moving
PC	11 \pm 1.8	13 \pm 2.4	56 \pm 2.8*	20 \pm 3.9
P	19 \pm 5.8	36 \pm 6.7*	24 \pm 2.9	21 \pm 4.8
C	30 \pm 1.9	17 \pm 5.3*	14 \pm 3.9	39 \pm 5.4*
0	23 \pm 1.9	37 \pm 2.4*	13 \pm 2.9	27 \pm 3.8*

Data have been pooled for the three conditioning periods.

* Significant difference between number of contacts or duration of time larvae are in contact with each choice diet (Mann-Whitney *U*-test, $P < 0.05$).

during the 9 h choice period and the amount eaten of the respective choice diets. We can be reasonably confident, therefore, that the amount of time spent contacting a choice diet is a good indication of the amount of eating activity on that diet.

Duration of intervals between contacts

The duration of intervals between contacts with the choice diets was significantly influenced by the conditioning diet (Table 1). The intervals between contacts with the P choice diet, especially the interval between the first and second contacts, were significantly longer for larvae conditioned on diets containing protein (PC and P) than for larvae conditioned on diets lacking protein (C and 0) (Fig. 3; Mann-Whitney *U*-test, $P < 0.01$). The inverse relationship occurred with the C choice diet; larvae conditioned on diets containing digestible carbohydrate (PC and C) had longer intervals than larvae conditioned on diets lacking digestible carbohydrate (P and 0) (Fig. 3; Mann-Whitney *U*-test, $P < 0.01$).

Neural response

Overall, the responsiveness to sucrose (Fig. 4A) was greater in the lateral

sensilla than in the medial sensilla and the responsiveness to the amino acid mixture (Fig. 5A) was greater in the medial sensilla than in the lateral sensilla. Sucrose stimulated neurones in both sensilla, but only one neurone in the lateral sensillum gave a dose-dependent response (Fig. 4; neurone C, see Simmonds and

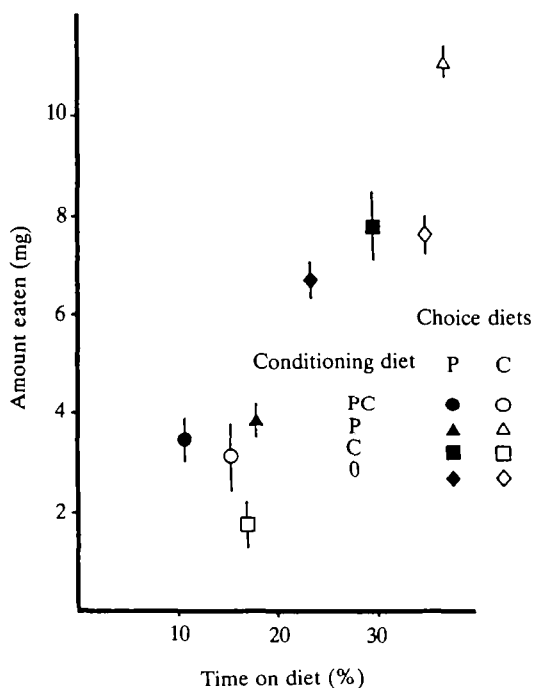


Fig. 2. Effect of conditioning diet on the relationship between the percentage of time spent on each choice diet during the 9 h choice period and the amount eaten (mg).

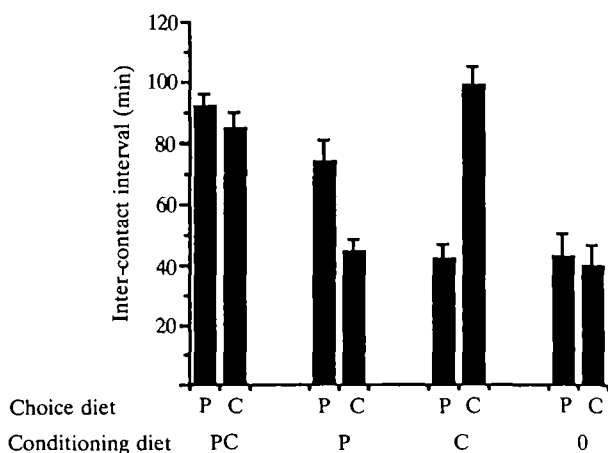


Fig. 3. Effect of conditioning diet (three conditioning periods pooled) on the duration of the intervals between first and second contacts with the P choice diet and the C choice diet during the 9 h choice period. Values are mean \pm s.e.m.; $N=30-55$.

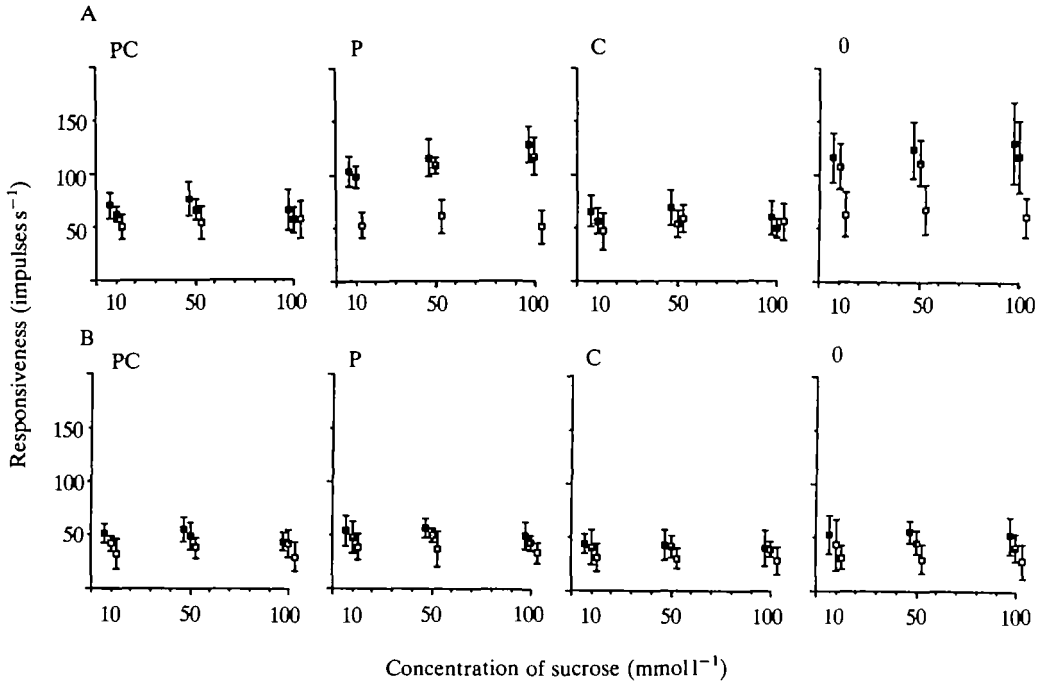


Fig. 4. Effect of the conditioning diet (PC, P, C, 0) on the responsiveness (mean \pm s.e.m., impulses s^{-1}) of the lateral (■) and medial (□) maxillary styloconica and neurone C in the lateral sensillum (□) to stimulation with three concentrations of sucrose, 10, 50 and 100 $mmol\ l^{-1}$. (As the duration of the conditioning period did not significantly affect the neural activity, the data from the three conditioning periods have been pooled.) (A) Neural response to sucrose after larvae had been conditioned on one of the four conditioning diets. (B) Neural response to sucrose after larvae had been conditioned on one of the four conditioning diets and then exposed to the choice diets for 9 h.

Blaney, 1991). In contrast, one neurone (Fig. 5; neurone B, see Simmonds and Blaney, 1991) in the medial sensillum gave dose-dependent responses to the amino acid mixture.

Neural response after the conditioning period: group A

The neural responses to sucrose (Fig. 4A) and the amino acid mixture (Fig. 5A) were affected by the nature of the conditioning diet. The neural responses from the lateral sensilla to sucrose from larvae conditioned on the diets lacking digestible carbohydrate (P and 0) were significantly greater than the responses obtained from larvae conditioned on diets containing carbohydrate (PC and C) (Kruskal–Wallis test, $P < 0.01$; Fig. 4A). Similarly, the neural responses to the amino acid mixture from the medial sensilla, especially neurone B, of larvae conditioned on the diets lacking protein (C and 0) were significantly greater than the responses from larvae conditioned on diets containing protein (PC and P) (Kruskal–Wallis test, $P < 0.01$; Fig. 5A). Thus, in both cases when there has been a deficiency in a dietary constituent, the neural response to that constituent is

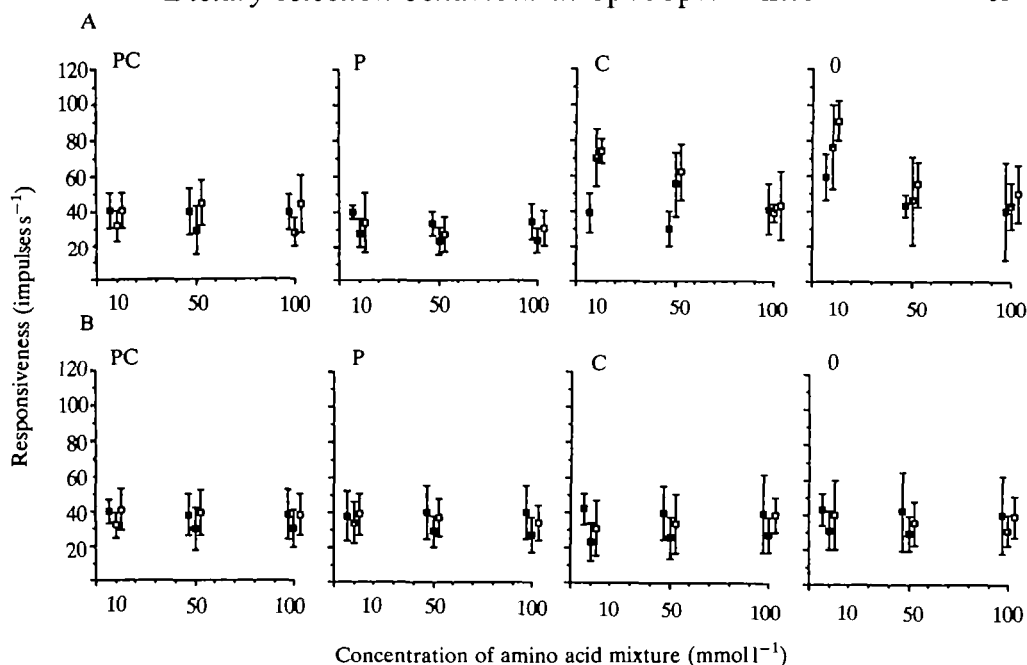


Fig. 5. Effect of the conditioning diet (PC, P, C, 0) on the responsiveness (mean \pm S.E.M., impulses s^{-1}) of the lateral (■) and medial (□) maxillary styloconica and neurone B in the medial sensillum (⊠) to stimulation with three concentrations of the amino acid mixture, 10, 50 and 100 $mmol\ l^{-1}$. (As the duration of the conditioning period did not significantly affect the neural activity, the data from the three conditioning periods have been pooled.) (A) Neural response to the amino acid mixture after larvae had been conditioned on one of the four conditioning diets. (B) Neural response to the amino acid mixture after larvae had been conditioned on one of the four conditioning diets and then exposed to the choice diets for 9 h.

increased. However, the dose-related nature of the response differs between the two situations, that to amino acids being negative (decreased firing with increased concentration), while that to sucrose is positive. Positive dose-response curves for sugars and amino acids have been reported for many species of insects (Blom, 1978; Wiczorek, 1981; van Loon and van Eeuwijk, 1989). At high concentrations, the response commonly plateaus and may even decline (Wiczorek and Koppl, 1982; Blaney and Simmonds, 1983; Blaney *et al.* 1991). Thus, it is possible that the differences in dose-dependency reported here may simply reflect the position on the dose-response curve of the concentrations of sucrose and the amino acid mixture used.

Neural response after the choice period: group B

The neural responses to sucrose and the amino acid mixture obtained from larvae after the 9 h choice period were no longer significantly influenced by the diet that the insects had been conditioned on (Kruskal-Wallis test, $P > 0.05$; Figs 4B, 5B).

Relationship between neural responses and the duration of contacts with the choice diets

The relationship between behaviour on the choice diets and the neural responsiveness of group A larvae to sucrose and the amino acid mixture was affected by the nature of the conditioning diets (Fig. 6Ai,ii, Bi,ii). For example, the duration of the first meal on the C choice diet, taken by larvae conditioned on the diets lacking digestible carbohydrate (P and 0), was positively correlated with a greater neural response to sucrose (0.01 mol l^{-1}) than that obtained from larvae conditioned on diets containing digestible carbohydrate (PC and C). Overall, the response of neurone C (the 'sugar' neurone) in the lateral sensillum ($r=0.777$,

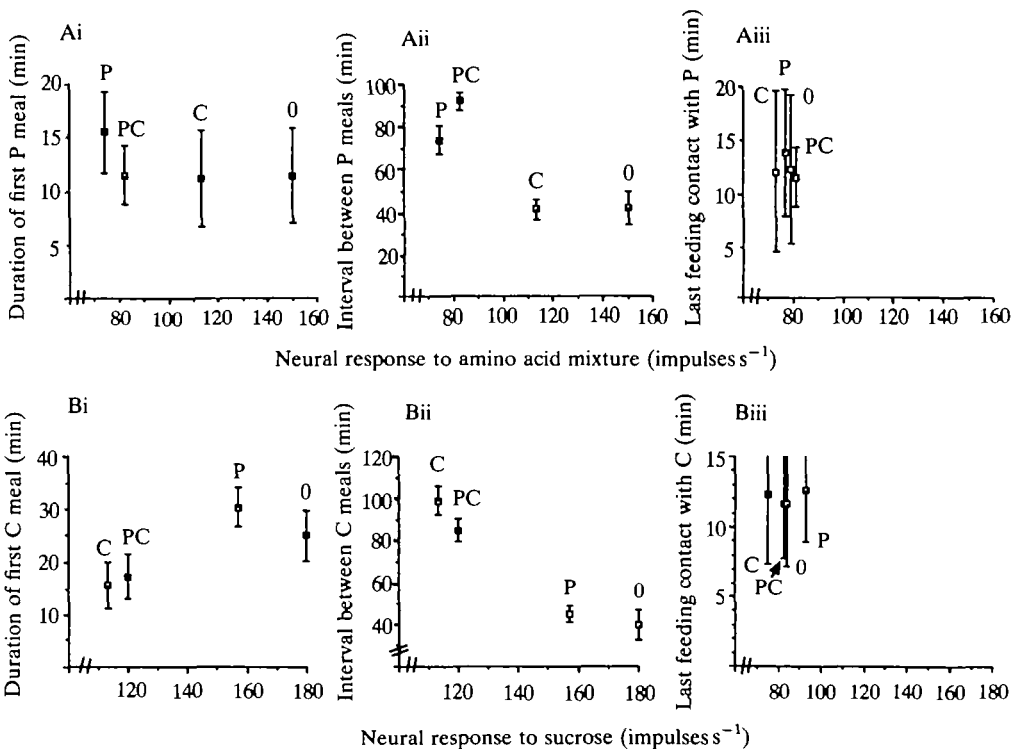


Fig. 6. Effect of the conditioning diet (PC, P, C, 0) on the relationship between the total combined neural response (mean \pm s.e.m., impulses s^{-1}) from the medial and lateral sensilla to the amino acid mixture or sucrose and various aspects of feeding behaviour. (A) The neural response to the amino acid mixture (0.01 mol l^{-1}); (i) the duration of the first meal on the P choice diet (group A larvae), (ii) the interval between the first and second feeding contacts with the P choice diet (group A larvae); and (iii) the duration of the last observed feeding contact on the P choice diet (group B larvae). (B) The neural response to sucrose (0.01 mol l^{-1}); (i) the duration of the first meal on the C choice diet (group A larvae), (ii) the interval between the first and second feeding contacts with the C choice diet (group A larvae); and (iii) the duration of the last observed feeding contact on the C choice diet (group B larvae).

$P < 0.003$) and the combined response from the lateral and medial sensilla (Fig. 6Bi, $r = 0.746$, $P < 0.005$) were correlated with the duration of the first meal on the C choice diet, although the neural response was lower and the duration of the meal taken by the larvae conditioned on the P diet was longer than those of larvae conditioned on the 0 diet. There was no correlation between the duration of the first meal on the P choice diet and the neural responsiveness to the amino acid mixture (Fig. 6Bi, $r = 0.21$, $P = 0.8$). However, the duration of the interval between the first and second feeding contacts on the P choice diet was negatively correlated with the response of neurone B in the medial sensillum ($r = -0.815$, $P = 0.002$) and the total combined response from the lateral and medial sensilla (Fig. 6Aii; $r = -0.764$, $P = 0.004$) to the amino acid mixture. Thus, larvae conditioned on the diets lacking protein (C and 0) were more responsive to the amino acid mixture and took shorter intervals between feeding contacts than those conditioned on diets containing protein (PC and P). A similar relationship occurred between the neural response to sucrose and the duration between the first and second feeding contact with the C choice diet (Fig. 6Bii; $r = -0.819$, $P = 0.005$).

After the 9 h choice period (group B), the conditioning diet did not differentially influence either the duration of the last observed contact with the choice diet or the neural responsiveness to the amino acid mixture or sucrose (Fig. 6Aiii, Biii).

Discussion

We have demonstrated that *S. littoralis* compensates for feeding on a nutrient-deficient conditioning diet by spending more time in contact with and eating more of a diet containing the deficient nutrient in a subsequent choice test.

Compensatory selection could be effected at a number of stages during feeding behaviour: the probability of contacting a food might be influenced, as could the probability of initiating, maintaining or terminating feeding. It is well documented that phagostimulation is critical for the initiation and maintenance of feeding in herbivorous insects, with both sugars and amino acids acting as important phagostimulants (Dethier, 1973; Bernays and Simpson, 1982; Simpson and Abisgold, 1985; Abisgold and Simpson, 1987; van Loon and van Eeuwijk, 1989). The present results indicate that the phagostimulatory power of a compound can vary with the type of food previously eaten by the insect. In this experiment, *S. littoralis* larvae consumed some of the first diet contacted, but the diet they selected for that first contact had some significance in relation to the nutrients that the insects had been deprived of in the immediately preceding conditioning period. Thus, insects conditioned on the P diet, and therefore deficient in carbohydrate, made that first contact predominantly with the C choice diet. By contrast, although about half of the insects conditioned on the C diet chose the P diet, the remainder selected the C diet, even though it would not have alleviated their dietary deficiency. However, the meals taken by these insects on the C choice diet were of shorter duration than those taken on the P choice diet.

It would appear that the larvae are capable of making a selection between the P

and C choice diets before making physical contact with them. The means by which this is done have not been critically assessed but presumably the olfactory system is involved. Both in the present results and in those of C. L. Simpson *et al.* (1990) the observed selection of diets made apparently before contact could result from modulation of the responsiveness of the olfactory sensilla. This, in turn, might result from the previous exposure to the aroma of the conditioning diet or from an internal negative feedback related to the amount of the conditioning diet eaten. Although we have no evidence of this occurring, such changes are recorded in olfactory (Davis, 1984; Simpson and White, 1990) and gustatory (Bernays and Chapman, 1972; Bernays *et al.* 1972; Bernays and Mordue, 1973; Blaney *et al.* 1986; Abisgold and Simpson, 1988) chemoreceptor systems of other insects.

In a comparative study of the dietary selection behaviour of *Locusta migratoria* and *S. littoralis* (S. J. Simpson *et al.* 1988, 1990), it is notable that *S. littoralis* larvae invariably consume the first diet they contact, whereas the locusts frequently rejected a diet on first contact. The observation that *S. littoralis* larvae consumed some of the choice diet first contacted could be a characteristic of the constraints associated with these experiments, or it could be a normal feature of the food-selection behaviour of these larvae. Being less mobile than locusts, they are only able to contact a few of the potential food materials within a habitat. This being so, it may be an advantage to take in at least some food when the opportunity arises.

Over the duration of our experiments the larvae compensated for carbohydrate deficiency by taking meals of a longer duration on the C choice diet and for an amino acid deficiency by taking meals more frequently of the P choice diet. This effect could be associated with the choice diet, the C choice diet promoting long meals and the P choice diet promoting short intercontact intervals, or it could be associated with the effect of preconditioning, or both. Inspection of the data suggests that the preconditioning has the greater influence. Thus, overall, meals on the C choice diet tend to be of a longer duration than those on the P choice diet, but the difference is much greater with insects deprived of carbohydrate. Similarly, intervals between contacts with the P choice diet are shorter than those with the C choice diet, but the greatest discrepancy occurs with insects conditioned on the C diet and, therefore, deficient in protein. This effect is not shown with insects conditioned on the 0 diet, which are deficient in both carbohydrate and protein and which have equally short intervals between contacts with both choice diets.

The neural responses of insects to taste stimuli are rarely simple but, by careful consideration, it may be possible to determine those aspects of the response that are likely to be significant to the insect in the decision-making process. In general, if a reliably identifiable response shows dose-related characteristics, it is likely that it carries usable information (Blom, 1978; Blaney, 1980; Blaney and Simmonds, 1990; Simmonds *et al.* 1990). In this study, the responses to sucrose and amino acids are modulated independently. The response of neurone B in the medial sensillum to amino acids is modulated by protein deficiency, whereas the response of neurone C in the lateral sensillum to stimulation with sucrose is modulated by

carbohydrate deficiency. By these criteria, the responses identified here in the lateral sensilla to sucrose and in the medial sensilla to the amino acid mixture might play a part in determining the behaviour of the insects. Notable in these responses is the observation that, after the conditioning period, larvae likely to be deficient in carbohydrate show higher neural responsiveness to carbohydrate and, similarly, those likely to be deficient in protein show higher neural responsiveness to the amino acid mixture. These differences in neural responsiveness can no longer be detected after the 9 h choice period when, arguably, the insects have restored their nutritional balance. If these arguments are correct, it seems likely that the compensatory feeding behaviour is driven, at least in part, by changes in peripheral neural responsiveness. This leaves the question of how the neural responsiveness itself is mediated.

Abisgold and Simpson (1988) have shown that levels of free amino acids in the blood of locust nymphs influence the neural responses to stimulation with amino acids. It is likely that the same mechanism is involved in the response of *S. littoralis* larvae to amino acids found in the present study, with larvae conditioned on the C diet presumably having lower blood levels of free amino acids than larvae conditioned on the PC or P diets. Preliminary studies (M. S. J. Simmonds and W. M. Blaney, unpublished results) have indicated the possibility that a similar mechanism exists for sugars: as blood sugar level increases, so the firing rate of neurones responding to sugar stimulation decreases.

Reports of dietary quality affecting the responsiveness of chemoreceptors in other insects are scarce, but there are some suggestive data for other caterpillars. The responsiveness of neurones in the maxillary sensilla of *Heliothis virescens* and *Spodoptera exempta*, as well as *S. littoralis*, are influenced by the nature of the rearing diet (Blaney and Simmonds, 1983). In particular, specific changes in activity have been reported for neurones responding to allelochemicals, with a reduction in firing activity occurring when conditioning diets contain the stimulating compound (Schoonhoven, 1969, 1976; Stadler and Hanson, 1976; Blaney and Simmonds, 1987; Schoonhoven *et al.* 1987).

Blaney *et al.* (1986) have reviewed the early reports of changes in gustatory responsiveness with developmental or reproductive state in insects. Recent studies have shown that the relative behavioural and neural responses of *S. littoralis* larvae to sucrose change during a stadium (Simmonds *et al.* 1991) and that these changes can vary with the diet on which the larvae are reared (Schoonhoven *et al.* 1991). C. L. Simpson *et al.* (1990) showed that in adult *Locusta migratoria* the neural response of sensilla on the maxillary palps to amino acids and sucrose changes during somatic growth. Such variations in neural activity are consistent with changes in the pattern of protein and carbohydrate ingestion that occurred when locust adults were able to select their intake of these nutrients (Chyb and Simpson, 1990). In these two cases, the behaviour of the insects, as mediated by the sensory system, varies in accordance with either the diet imposed on the insect or the diet that the insect selects for itself. The effect on the sensory system of the dietary constituents could be mediated, as suggested above, by haemolymph titres of

these constituents. The haemolymph levels, in turn, could be determined by two or more possible mechanisms: either they could reflect the dietary constituents directly or they might be modulated by differential absorption and/or changes in rates of uptake and synthesis by the fat body or other metabolic systems.

Thus, we have good evidence to suggest that changes in the level of nutrients in the haemolymph are associated with the changes in neural responsiveness reported here. However, we do not yet know whether the effect of the dietary constituents on the peripheral neurones is direct or whether some other factor, such as a second messenger or hormone, is involved. Experiments are in progress to investigate this question further.

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