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THE EFFECT OF DIETARY PROTEIN LEVELS AND HAEMOLYMPH COMPOSITION ON THE SENSITIVITY OF THE MAXILLARY PALP CHEMORECEPTORS OF LOCUSTS

BY J. D. ABISGOLD* AND S. J. SIMPSON

Department of Zoology, University of Oxford, South Parks Road, Oxford, OX1 3PS

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SUMMARY

1. Previous work has shown that fifth-instar nymphs of *Locusta migratoria* (L.) compensate for a dilution of their dietary protein by reducing intermeal interval (Simpson & Abisgold, 1985).

2. The effect of dietary protein on intermeal interval is regulated, either directly or indirectly, by the osmolality and free amino acid content of the haemolymph (Abisgold & Simpson, 1987).

3. The possibility that levels of dietary protein and haemolymph composition affect the response of the maxillary palp gustatory receptors is investigated.

4. Insects fed a low-protein diet had a significantly greater receptor response (measured as the total number of spikes in the first second of stimulation of a sensillum) to stimulation with 0·0125 mol l^{-1} leucine in 0·05 mol l^{-1} NaCl, 0·05 mol l^{-1} NaCl alone or 0·025 mol l^{-1} sucrose in 0·05 mol l^{-1} NaCl than did insects fed a high-protein diet, although for both diets the response to sucrose was significantly lower than the response to the other two solutions.

5. Increasing the free amino acid profile of the haemolymph of a low-protein-fed locust up to that of a high-protein-fed locust by injection markedly reduced the response of the receptors to subsequent stimulation with a 0·0125 mol l^{-1} mix of eight of the 10 amino acids injected, but did not reduce the response to stimulation with 0·025 mol l^{-1} sucrose in 0·05 mol l^{-1} NaCl. This reduction was independent of the effect of injection on blood osmolality and was sustained for 50 min after the injection.

6. The response to 0·05 mol l^{-1} NaCl alone was influenced both by increases in blood amino acid levels and by osmolality, but the effect was less marked than the specific reduction in response to amino acid stimulation.

7. The possible significance of a reduction in receptor sensitivity on feeding behaviour and the relative roles of blood osmolality and free amino acid content are discussed.

* Present address: Overseas Development Natural Resources Institute, College House, Wrights Lane, London W8 5SJ.

Key words: maxillary palp, gustatory receptors, protein, blood amino acids, osmolality, compensatory feeding behaviour.

INTRODUCTION

It has been shown that fifth-instar nymphs of *Locusta migratoria* are able to compensate for a 50% dilution of their dietary protein, from 28% to 14%, by reducing the intermeal interval while keeping meal size constant (Simpson & Abisgold, 1985). The effect of dietary protein on intermeal interval is related to the levels of 10 free amino acids in the haemolymph as well as to haemolymph osmolality. Increasing the blood free amino acids levels or osmolality by injection results in an increase in intermeal interval (Abisgold & Simpson, 1987). The question remains as to how blood composition mediates its effect on feeding behaviour.

The sensilla concentrated at the tips of the maxillary palps of locusts are known to play an important role in food selection [Blaney & Chapman, 1970; Blaney & Duckett, 1975; Mordue (Luntz), 1979]. During the testing of potential food, the palps are rapidly vibrated bringing the tips of the sensilla into intermittent contact with the leaf surface (Blaney & Duckett, 1975). Several studies have shown that the sensitivity of the receptors in the sensilla of other insect species varies depending on reproductive state, developmental stage, time of day and feeding history (see Blaney, Schoonhoven & Simmonds, 1986; Schoonhoven, Blaney & Simmonds, 1987). It is possible, therefore, that changes in receptor sensitivity are involved in regulating intermeal interval for locusts fed on artificial diets containing different amounts of protein. The present study addresses two questions. First, does the receptor sensitivity differ between locusts fed a high- and low-protein diet? Second, if there is such a difference, is it caused, either directly or indirectly, by the differences in haemolymph composition associated with feeding?

MATERIALS AND METHODS

Artificial diets

Variations on the artificial diets of Dadd (1961) were used. The high-protein diet (P) contained 28% protein and the low-protein diet (p) 14% protein. The bulk of the two diets was equal and maintained with cellulose which is virtually indigestible to locusts (Morgan, 1976; Martin, 1983). In addition to protein and cellulose the diets contained equal amounts of digestible carbohydrate (28%), salts, fat and vitamins. For the exact content of the diets see Abisgold & Simpson (1987).

Insects

Locusts were reared at the Department of Zoology, Oxford, in the standard manner (Hunter-Jones, 1961), using seedling wheat as the green food source. Males were removed from stock cages during the first day of the fifth instar (termed day 0), and placed individually in 17×12×6 cm clear plastic boxes. Each box contained a strip of expanded aluminium running around three of its sides which acted as a perch. Locusts were kept at 30 ± 1°C under a L:D 12 h:12 h light regime up until electrophysiological recordings were made, when the locusts were maintained at room temperature for the duration of the recordings (90 min). From day 0 until the

beginning of day 3 locusts were fed ample seedling wheat and a dry food in the form of bran given in a 5-cm diameter Petri dish.

Recording technique

Recordings were obtained using a modified version of the tip recording technique of Hodgson, Lettvin & Roeder (1955) (see also Blaney, 1974). Silver/silver chloride electrodes were used to detect, extracellularly, electrical changes within the sensilla. Such signals were preamplified with an Isleworth A103 preamplifier (passing a bandwidth of 200–5000 Hz), displayed on a Tektronix 5103N CRO, and stored for later analysis on tape using a Racal Store 4DS recorder. The preamplifier enabled recording of the early portion of the response without blocking. Hard copies of recordings were taken from the tapes using a Medelec FOR 1004 u.v. recorder.

Electrodes and stimulating solutions

Glass capillaries (1·5 mm outside diameter) were drawn out to form recording electrodes. The tip of each electrode was then fashioned by inserting the capillaries into a bead of low melting point glass on a heated element. By cooling the glass bead the electrode tip could be snapped off to a diameter of approximately 5 μm. Three types of stimulating solutions were used in both experiments 1 and 2. All solutions were made using Analar grade compounds in glass-distilled deionized water. The solutions were stored at −5 °C when not in use. The concentrations of the solutions used were chosen, where possible, to be the concentrations that produced a behavioural response when presented, impregnated on a pith disc, to locusts (Cook, 1977) and that were known to be neither subthreshold nor damagingly high when applied to the palps (Blaney, 1974). Solutions 1 and 2 were the same in both experiments. Solution 1 contained 0·05 mol l^{-1} NaCl in distilled water and solution 2 contained 0·025 mol l^{-1} sucrose in 0·05 mol l^{-1} NaCl. In experiment 1, solution 3 consisted of 0·0125 mol l^{-1} leucine in 0·05 mol l^{-1} NaCl. Leucine was the amino acid whose concentration differed in the blood of P- and p-fed locusts by the greatest amount (Abisgold & Simpson, 1987). In experiment 2, a mixture of amino acids in 0·05 mol l^{-1} NaCl was used as the stimulating solution. The mixture consisted of a 41:46:33:47:52:46:37:28 ratio of leucine:glutamine:serine:methionine:phenylalanine:lysine:valine:alanine (a total of 0·0125 mol l^{-1} amino acids). This represented the ratio, present in the artificial diet, of those amino acids implicated in compensatory feeding (Abisgold & Simpson, 1987; J. D. Abisgold, unpublished observations). In both experiments only the L-isomer of each amino acid was used.

To reduce the tendency for water to evaporate from the tip of electrodes and so increase the concentration of the stimulating solution, the experimental area was maintained at a high humidity using tissue soaked in distilled water. To further ensure that the solution at the tip of the electrode was representative of the concentration of the rest of the solution, fluid was drawn from the tip using a cotton bud immediately prior to each stimulation.

Method of preparation

Experiment 1

At the beginning of the light phase on day 3 insects were placed in clean containers and given a Petri dish filled with 1·5–2 g of either the P- or p-diet ($N = 10$ per diet). Insects were then left for a minimum of 3 h and a maximum of 7 h before any recordings were made. Previous work has shown that interfeed intervals are consistently longer on the P- than the p-diet during this period (Simpson & Abisgold, 1985). Insects were observed to feed *ad libitum* until they had completed a meal of a minimum of 3 min duration followed by a period of 4 min without feeding. The time, to the nearest minute, at which the meal ended was noted and the locust removed to another room for electrophysiological recordings. Insects were restrained by wrapping the thorax and part of the abdomen with double-sided tape, leaving the lower abdominal segments exposed but immobilizing all pairs of legs. The double-sided tape was then used to stick the insect on its right side onto a glass slide. The left maxillary palp was waxed to the mandible which was similarily waxed to the labrum using Paramat low melting point wax. Thus the left palp tip was immovably fixed, pointing outwards from the insect's face. The locust was then placed onto the stage of a microscope set at a magnification of 100× under Nomarski optics, and the indifferent electrode inserted into the haemolymph in the area surrounding the left antennal socket. Three adjacent and accessible chemosensilla were selected for the recordings from the array of approximately 350 chemosensilla on the palp dome, the same hairs being used in successive recordings (see Blaney, Chapman & Cook, 1971 for a description of the appearance of chemosensilla and other sensilla located on the locust maxillary palp). Previous studies have shown a correlation between the input from the sensilla and the feeding behaviour of the insect, suggesting that it is the 'across-fibre' response from all the sensilla (as opposed to the response from the individual neurones within the sensilla) that provides the message to the central nervous system (Blaney, 1974; Blaney & Winstanley, 1979). The exact position of the hairs differed among preparations, it not being possible to identify individual sensilla among locusts. However, in most cases the plane in which the hairs lay and the approximate position of the hairs was the same among insects. Fifteen minutes after the end of the previous meal each of the three stimulating solutions were tested on each of the three hairs in turn, the order in which the solutions were presented to each hair being randomized. Care was taken not to displace the sensillum, and hence stimulate the basal mechanoreceptor which is found in many of the gustatory sensilla (Blaney, 1974), while placing the electrode over the end of the hair. Once the three solutions had been tested on each of the hairs, the palp was gently washed with a cotton bud soaked in distilled water. The recordings were repeated 30, 45, 60, 75 and 90 min after the end of the last meal.

Experiment 2

Preparation for experiment 2 was as for experiment 1 except that from the onset of day 3 all insects were given the p-diet. A recording was made 30 min after the end of

the meal as previously described. Forty minutes after the meal insects were injected, while on the microscope stage, with 10 μl of one of four solutions between tergites four and five using a Hamilton syringe ($N = 9$ per injection). The four solutions were the same as those injected in previous studies (Abisgold & Simpson, 1987). Amino acid injections contained 134 nmol of threonine, 212 nmol of glutamine, 30 nmol of serine, 267 nmol of methionine, 217 nmol of leucine, 110 nmol of phenylalanine, 133 nmol of isoleucine, 251 nmol of lysine, 111 nmol of valine and 283 nmol of alanine. Such injections were designed to raise the free amino acid content of the blood to that found in locusts 40 min after taking a high-protein (P) meal (Abisgold & Simpson, 1987). When the amino acids were mixed in saline isotonic with blood the injection also raised the blood osmolality by 12 mosmol kg^{-1}, approximately a 3 % increase. The amino acid injections were unlikely, however, to alter the pH of the blood owing to the buffering capacity of the haemolymph. When the same amounts of amino acids were mixed in distilled water the injection decreased osmolality slightly (by approximately 4 mosmol kg^{-1}). An injection of xylose in saline raised the blood osmolality by 12 mosmol kg^{-1} and was used to show whether osmolality *per se* affected sensilla responsiveness. Xylose, a non-utilizable sugar for locusts, had previously been shown to have a time course of removal from the blood most similar to an injection of amino acids and therefore acted as an osmotic control for the amino acid injection (Abisgold & Simpson, 1987). An injection of isotonic saline served as a control for the effect of injection alone. Recordings were then taken 45, 60, 75 and 90 min after the end of the last meal.

Analysis of recordings

It is normally not possible to distinguish between spikes from the 6–10 different gustatory neurones within a single locust sensillum (Blaney, 1974, 1975). In addition, the chemosensory cells in a sensillum do not appear to be highly tuned but respond to a range of stimulants (Blaney, 1974, 1975, 1981). Therefore the total number of spikes of all sizes occurring in the first second of stimulation (SPS) was used as the measure of responsiveness. Blaney & Winstanley (1979) found a strong correlation between the response of the sensilla in the first second of stimulation and the behavioural response in the first encounter. The first second of stimulation contains the initial phasic response of the receptors, of approximately 300 ms duration (Blaney & Duckett, 1975), and approximately 700 ms of the following tonic response.

Tape recordings were played through a Tektronix 5223 digitizing oscilloscope which stored and displayed the first second of the response immediately after the stimulus artefact. Thus the total number of spikes in the first second could be counted directly from the oscilloscope screen. The recording technique almost invariably produced a stimulus artefact, and any instances where no stimulus artefact was produced upon placing the electrode over the sensillum were not used in the analysis. Such recordings were rare, however (2 %).

RESULTS

Experiment 1: P- versus p-fed insects

Fig. 1 shows the response of the sensilla to the stimulating solutions for insects fed the P- or p-diet. The data are presented as the mean number of spikes elicited from the three hairs for each solution and at each recording. For all three solutions tested,

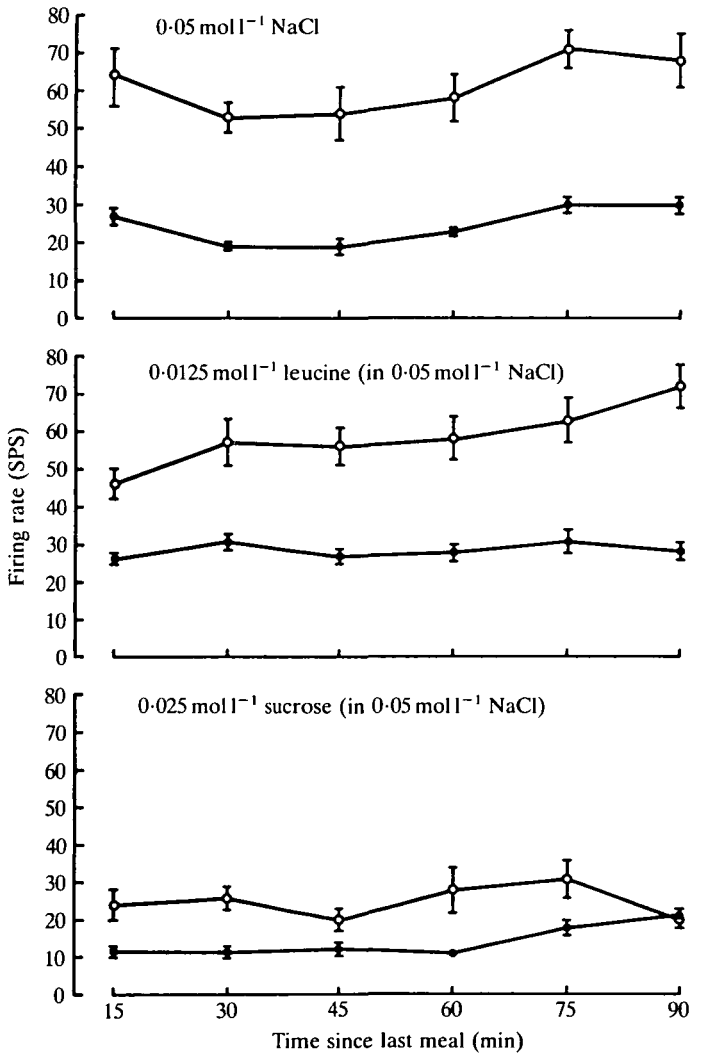

Fig. 1. The mean response (total number of spikes during the first second of stimulation, SPS) of the same three sensilla to different stimulating solutions at various times since the last meal, for insects fed either the P- (●) or p- (○) diet. The mean response of the three sensilla per insect was used to produce an overall mean for all the insects tested. Each point represents the mean of the responses of 10 insects. The same insect was used for successive time recordings. Each of the sensilla was stimulated with the three solutions at each time recording. Bars represent ±S.E.M.

the response of the sensilla of those locusts fed the p-diet was significantly higher than that of locusts fed the P-diet (see Table 1 for the analysis). This difference in sensilla response was most marked when stimulating with either 0·0125 mol l^{-1} leucine in 0·05 mol l^{-1} NaCl or 0·05 mol l^{-1} NaCl solution alone ($P < 0·001$ ANOVA for both solutions). The difference in sensilla response between P- and p-fed insects when stimulating with 0·025 mol l^{-1} sucrose in 0·05 mol l^{-1} NaCl was also significant ($P < 0·01$), although for both diets the response to the sucrose solution was significantly lower than the response to the amino acid or salt solution ($P < 0·001$ in all cases). There was no significant effect of time since the last meal on sensilla activity so that, with the exception of the sucrose stimulation 90 min after the meal, at all times measured the responsiveness of the terminal sensilla of the p-fed insects was higher than that of the P-fed insects.

Experiment 2: injecting p-fed locusts

Fig. 2 shows the response of the sensilla to the three stimulating solutions after one of four injections. The data are presented as the difference between the mean number of spikes elicited from the three hairs at each recording after injection and that elicited from the same hairs at the recording prior to injection (30 min after the meal ended). The firing rate at the pre-injection recording was 46 ± 2 SPS ($\bar{x} \pm$ S.E.) for stimulation with amino acids in 0·05 mol l^{-1} NaCl, 45 ± 2 SPS for 0·05 mol l^{-1} NaCl alone and, as in experiment 1, considerably lower for 0·025 mol l^{-1} sucrose in 0·05 mol l^{-1} NaCl (21 ± 2 SPS). A summary of the analysis of the data is presented in Table 2.

There was a marked and rapid effect on the total number of spikes recorded from hairs stimulated with the 0·0125 mol l^{-1} amino acid mix in 0·05 mol l^{-1} NaCl after

Table 1. *Summary of* F-*ratios from the ANOVA for sensilla response of locusts fed either the P- or p-diet, when stimulated with different solutions at various times after a meal*

		F-ratios Sensilla stimulated with		
Source	*df.*	0·0125 mol l^{-1} leucine (in 0·05 mol l^{-1} NaCl)	0·025 mol l^{-1} sucrose (in 0·05 mol l^{-1} NaCl)	0·05 mol l^{-1} NaCl
Diet	1	60·8***	13·3**	66·9***
Residual 1	54			
Time	5	0·9	0·4	1·3
T × D	5	0·8	0·8	0·1
Residual	54			
Total	119			

The levels of significance are based on the difference between insects fed the two diets, with stimulating solution and time since the meal as other factors.
** $P < 0·01$; *** $P < 0·001$.

injections which increased the free amino acid concentration of the haemolymph to that which would have been experienced had the locust eaten the P- and not the p-diet ($P < 0{\cdot}001$). Five minutes after the injection, sensilla responsiveness had decreased by approximately 70% and remained at this level over the next 50 min. This decrease, however, was independent of the effect of the injection on blood osmolality (injecting with amino acids in saline or amino acids in water has the same

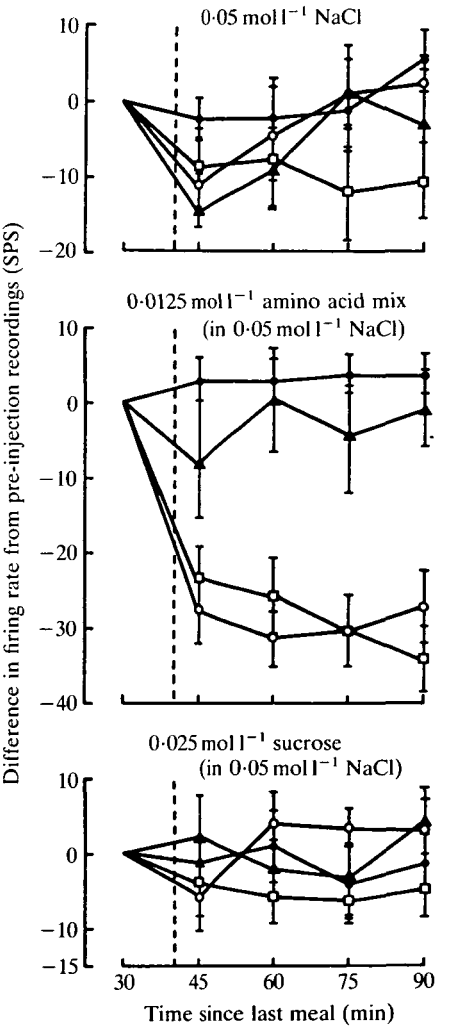

Fig. 2. The difference in response between sensilla stimulated 30 min after the last meal and the same sensilla stimulated at successive times after an injection given 40 min after feeding. The mean for the difference of three sensilla per insect was used to calculate an overall mean for all the insects tested. Insects were injected with one of four solutions, saline (●), xylose in saline (▲), amino acids in water (○) or amino acids in saline (□) (see text). Each of the sensilla was stimulated once with the three solutions in turn at each time after the meal. $N = 9$ for each injection. Bars represent ±S.E.M.

Table 2. *Summary of* F*-ratios from the ANOVA for sensilla response to several stimulating solutions and at several times since the last meal, when injected with one of four solutions*

Source	*df.*	*F*-ratios Sensilla stimulated with 0·0125 mol l^{-1} amino acids (in 0·05 mol l^{-1} NaCl)	0·025 mol l^{-1} sucrose (in 0·05 mol l^{-1} NaCl)	0·05 mol l^{-1} NaCl
Osmolality	1	1·6	0·6	1·0
Amino acids	1	41·6***	0·2	1·4
O × A	1	0·1	1·1	0·0
Residual 1	32			
Time	3	0·5	1·7	4·9**
T × O	3	0·6	2·7	2·3
T × A	3	2·7	1·8	0·0
T × O × A	3	2·0	0·9	4·1**
Residual	96			
Total	143			

The analysis has two levels. Main effects in the first level include changes in blood osmolality (O) and amino acid composition (A), whereas time since injection (T) is the main effect in the second level.

** $P < 0·01$; *** $P < 0·001$.

effect). The locusts injected with xylose in saline or saline only, showed no change in responsiveness.

There was no significant difference among any of the injections in their effect on the total firing rate when the same sensilla were stimulated with 0·025 mol l^{-1} sucrose in 0·05 mol l^{-1} NaCl. There was, however, a significant difference in the response to stimulation with 0·05 mol l^{-1} NaCl alone ($P < 0·01$), although this was not nearly so clear an effect as that demonstrated with amino acid stimulation. A change in either blood osmolality or amino acid concentration caused an initial depression in firing rate of approximately 20 %. Only with the injection of amino acids in saline, where both blood amino acid concentration and osmolality were increased, did the depression last beyond 35 min after the injection.

DISCUSSION

Results from the present experiments demonstrate how specific nutrient feedbacks alter the electrophysiological responses of taste receptors and thus contribute to compensatory feeding. Increasing the amino acid concentration of the haemolymph of locusts by injection has a large and rapid effect on the sensitivity of the palp-tip sensilla. Such an effect is specific; if the sensilla of the injected insect are stimulated with amino acids the response of the receptors is greatly reduced (by approximately

70%), whereas if the sensilla are stimulated with a salt solution the response is reduced to a much lesser extent (by approximately 20%), and if stimulated with sucrose there is no significant reduction. It is therefore likely that the central nervous system (CNS) of a locust well-fed on protein will receive different sensory information regarding the amino acids in a food which the insect contacts than the CNS of a locust which has previously fed on a less protein-rich food.

The mechanisms of coding in gustatory receptors of the locust are only poorly understood and are apparently more complex than in species such as blowflies and lepidopterous larvae, where receptors are more tuned in their responses and more readily identified from spike traces (Blaney *et al.* 1986). Results from the present study, however, agree with those of Blaney (1974) in that 0·025 mol l^{-1} sucrose in 0·05 mol l^{-1} NaCl caused a depression in firing rate as compared with 0·05 mol l^{-1} NaCl alone (Figs 1, 2). Blaney (1980) suggested that low sensory input to the CNS in *Locusta migratoria* signifies high palatibility for sucrose whereas the reverse is true for other compounds such as amino acids. The addition of the 0·0125 mol l^{-1} amino acid mix to 0·05 mol l^{-1} NaCl did not evoke a stronger response (in terms of total number of spikes produced from a sensillum in the first second) than did 0·05 mol l^{-1} NaCl alone (46 ± 2 *vs* 45 ± 2 SPS), although the observation that the response to amino acids in 0·05 mol l^{-1} NaCl declined more than the response to 0·05 mol l^{-1} NaCl after injection indicates that amino acids are distinguished from NaCl.

The changes in sensory information passing to the CNS, which accompany an increase in free amino acid levels in the blood after feeding on a protein-rich food, might have one of two behavioural consequences when the nymph subsequently contacts food. First, the insect might take a meal but reduce the size of that meal. This does not occur, however, since locusts fed on high- or low-protein diets take the same sized meals and ingest at the same rate during meals (Simpson & Abisgold, 1985). The second possible effect is that the insect might be more likely to reject the food before ingestion. Sugars and amino acids are phagostimulants for locusts (Cook, 1977), and a decline in firing rate in response to amino acids might reduce the acceptability of a food when it is contacted. Maxillary palp receptors are known to mediate acceptance or rejection [Blaney & Chapman, 1970; Blaney & Duckett, 1975; Mordue (Luntz), 1979]. As we do not know whether the maxillary sensilla are the only ones influenced by changes in blood amino acids, and gustatory receptors on the tarsi and other mouthpart structures (Chapman, 1982) also mediate acceptance (see Simpson & Bernays, 1983), it is possible that the reduction in the sensory response to amino acids could lead to the locust rejecting food not only upon palpation but also at biting or even tarsal contact, resulting in longer interfeed intervals. Results from behavioural studies with locusts pre-fed a low-protein diet and injected with the same solution as used here to raise the blood amino acid level to that of high-protein-fed insects, support increased rejection (Abisgold & Simpson, 1987; J. D. Abisgold, unpublished observations). Such insects rejected food on first contact more often (33% of insects) than those injected with either xylose, which raises the blood osmolality only (20%), or a saline control (20%). Rejection is defined as leaving the food after feeding for 0–30 s.

In addition to the probability of rejection on contact being affected, latency to first contact also varied. 66 % of insects injected with amino acids in saline did not contact the food within 40 min after injection. 53 % did not contact the food within 40 min after an injection of amino acids in water, which increased the amino acid content of the blood but did not increase the osmolality. 40 % did not contact the food within 40 min after a xylose injection, whereas after an injection of saline, only 13 % failed to contact the food.

Hence, the extended interfeed intervals exhibited by P-fed insects were due both to spending longer quiescent after feeding and to rejecting food more often once it was contacted. The former effect cannot be related to changes in gustatory sensitivity since insects rested some distance from the food, unless there was a change in the rate of 'spontaneous' firing of gustatory receptors as the insect rested. The injection study shows that, under the conditions used, there is little effect of blood osmolality on sensilla responsiveness. It is possible that blood osmolality is more important in regulating the activity of the insect *before* it contacts the food, whereas blood amino acid levels are more important in determining whether the locust feeds once contact with food has been made. A similar mechanism may also underlie dietary selection in locusts (Simpson, Simmonds & Blaney, 1988). Such a system seems reasonable since changes in blood osmolality are a general measure of the quantity and quality of the previous meal, whereas changes in blood amino acids are a more specific measure of the protein requirements of the insect. The effect of blood osmolality and amino acid content on locust activity and olfactory sensitivity is currently being investigated.

Whereas the effects of increasing the amino acid composition of the blood by injection on gustatory responsiveness were clear and specific, the results from the experiment in which insects had fed on the P-diet were somewhat different. The reduction in response to stimulation with 0·0125 mol l^{-1} leucine in 0·05 mol l^{-1} NaCl in the P- *versus* p- experiment was not significantly different from the reduction in the response to 0·05 mol l^{-1} NaCl alone. The degree of reduction in response to 0·05 mol l^{-1} NaCl was greater in the diet experiment than in the injection experiment ($P<p$ by 54 % in the diet experiment as compared with 20 % in the injection experiment). From the injection experiment there is evidence that osmolality as well as amino acid concentration of the blood affect the response to 0·05 mol l^{-1} NaCl. The greatest reduction in firing rate occurred after an injection which increased both blood amino acid levels and osmolality. In the injection experiments osmolality increases were designed to equal that due to the increase in amino acid concentration which occurs in the blood of P- *versus* p-fed locusts. In fact free amino acids only contribute 40 % (12 mosmol kg^{-1} *vs* 40 mosmol kg^{-1}) of the actual difference in blood osmolality between p- and P-fed insects (Abisgold & Simpson, 1987). The extra increase in osmolality has not been characterized but is likely to be due to general increases in the ionic composition of the blood, probably because of a net loss of water from the blood as a result of salivation (Bernays & Chapman, 1974). This extra increase in osmolality could account for the greater decline in response to 0·05 mol l^{-1} NaCl seen in experiment 1. In addition, the amino acid stimulant differed between experiments 1 and 2; whereas a mix of amino acids was used in

experiment 2, leucine alone was used in experiment 1. Leucine was chosen as it had been found to contribute the greatest amount to the difference in blood amino acid concentration between insects fed the P- rather than the p-diet (Abisgold & Simpson, 1987). However, Cook (1977) found that leucine by itself did not elicit a behavioural response when presented impregnated on a pith disc to locusts. It may be, therefore, that leucine in $0{\cdot}05\,\mathrm{mol\,l^{-1}}$ NaCl is not sufficiently stimulating to produce a difference in response from $0{\cdot}05\,\mathrm{mol\,l^{-1}}$ NaCl alone. Such a difference in experimental design is potentially important as we do not know which components of the stimulating amino acid mix used in experiment 2 are the most effective. There exists the possibility that deficiencies in individual amino acids in the blood could evoke specific changes in receptors; it may be that leucine is not one of these amino acids. Further work investigating this question is currently under way.

There was a further difference between experiments 1 and 2. In experiment 1, the sugar response of P-fed insects was less than the response of p-fed insects (by 45 %), while the response was not significantly different after injections which increased either blood amino acids or osmolality (by $12\,\mathrm{mosmol\,kg^{-1}}$). Why this should be is unclear but if, as suggested by Blaney (1980), decreased firing in response to sugar indicates increased acceptability, the effect would be to reduce, not increase, the probability of rejection of food by P-fed insects. This clearly did not happen. Thus, while the injection experiment shows unequivocal and specific changes in receptor responsiveness, these are masked in the diet-fed insects from experiment 1.

An effect of feeding on receptor sensitivity has previously been demonstrated in several instances. In blowflies, the excitability of receptor cells located in mouthpart sensory hairs gradually increases when the insects are deprived of food. The sugar receptor is particularly affected, whereas the water receptor is less affected (Omand, 1971; Omand & Zabara, 1981). In this case it is the amount of food ingested rather than its nutritional quality that apparently determines the receptor response. Rachman (1979), however, did not find an effect of feeding history on receptor sensitivity in blowflies. It is likely that the difference in experimental conditions between the two studies can account for at least some of the differences in results. Rachman used flies which were physically restrained and deprived of food over 24 h periods only, whereas Omand used flies which were allowed to move freely, and followed the effects of food deprivation on their sensory responses over 2 days or longer. Bernays & Chapman (1972) also found a relationship between food quantity and receptor response. They showed that the electrical resistance across the tips of the palps of *Locusta migratoria* increased as a result of filling the foregut with non-nutritive agar.

There are, however, several examples where food quality rather than quantity appears to affect receptor sensitivity. The response of the maxillary taste sensilla of the tobacco hornworm to leaf saps of various plants is different if the insects are reared on an artificial diet as compared with a host plant (Schoonhoven, 1967). It was therefore suggested that the acceptance of foreign plants by larvae grown on an artificial diet occurs because of an increased sensitivity to some acceptable chemicals in these plants, rather than a reduced sensitivity to their deterrent substances. In

addition, when the insects are reared on two different host plant species, sensory responses to the saps of those host plants differ (Stadler & Hanson, 1976). Stoffolano (1973) demonstrated that diapausing female blowflies that had been fed sugar and liver exhibited a reduced sensitivity of their salt and sugar receptors compared with flies which had been fed sugar only, showing a relationship between the nutrient content of the diet and the type of receptors affected. Busse & Barth (1985) also demonstrated a relationship between nutrition and receptor sensitivity. Female blowflies previously fed sucrose only, and so in a state of yeast preference, ingested a solution of sodium and potassium salts mixed in $0{\cdot}2\,\mathrm{mol\,l^{-1}}$ sucrose in amounts similar to their ingestion of a 10% yeast suspension. The salts are thought to stimulate the salt-sensitive receptors that are usually stimulated by alcohols, fatty acids and certain amino acids present in yeast. Busse & Barth (1985) suggested that the activity in the salt-sensitive chemosensory neurones is involved in protein preference.

Behavioural gustatory responses to individual amino acids have been demonstrated in the cockroach (Sugarman & Jakinovich, 1986). Shimada, Maki & Sugiyama (1983) showed dipeptide reception in the fleshfly, and Mitchell (1985) demonstrated the existence of a cell highly sensitive to amino acids contained within a single sensillum of the galea of adult Colorado beetles.

The mechanism whereby blood amino acid levels influence receptor responsiveness is a matter for conjecture. There are several possibilities. First, haemolymph composition may directly affect the composition of the dendritic liquor *via* the secretory function of the tormogen and trichogen cells (Phillips & Vande Berg, 1976). The composition of the dendritic liquor may then affect the sensitivity of receptor cells, as has been suggested for blowfly taste receptors, where there is a significant correlation between the ionic composition of the haemolymph and the response of taste cells (Jachmann, Zweypfenning & van der Molen, 1982). A second possible mechanism is that amino acid levels affect the CNS, either directly or *via* internal receptors, which in turn modulates gustatory receptor sensitivity. The role of internal receptors in mediating 'protein hunger' has been investigated in mammals (Jeanningross, 1982; Mei, 1985) but not in insects. Jeanningross (1982) has demonstrated that vagal nerves in the cat respond to amino acids infused into the duodenum and that the same infusion also alters the firing rate of single neurones in the lateral hypothalamus. Centrifugal modulation of palp receptors by the CNS could be *via* the release of hormones or by efferent neural control. The latter has not, as yet, been demonstrated in the chemosensory system of any insect. Hormones or neurohormones could affect the ionic composition of the dendritic liquor (Kuppers & Thurm, 1975). An effect of a hormone released from the storage lobes of the corpora cardiaca on sensilla response has been demonstrated in *Locusta migratoria* (Bernays, Blaney & Chapman, 1972). However, it is not known whether a mechanism involving hormones would act rapidly enough to produce the rate of changes demonstrated in the present experiments.

Bernays *et al.* (1972) found that resistance across the palp tips, probably signifying pore closure, is high immediately after feeding but falls to a steady level after

approximately 2 h. In the present study, however, there appears to be no effect of the time since feeding or injection on sensilla response. Insects fed the low-protein diet show a slight but insignificant increase in response with time when stimulated with either 0·0125 mol l^{-1} leucine in 0·05 mol l^{-1} NaCl or 0·05 mol l^{-1} NaCl alone and, similarly, high-protein-fed insects show a small increase in response with time when stimulated with either 0·025 mol l^{-1} sucrose in 0·05 mol l^{-1} NaCl or 0·05 mol l^{-1} NaCl alone, but neither increase is significant (Fig. 1). The apparent absence of a change with time in the present study may be due to the fact that locusts were transferred from the temperature-controlled room at 30°C to a room at 20°C for recordings. Hence, 90 min after feeding may have been insufficient to note any changes in responsiveness at the lower temperature.

Whatever the mechanism by which blood amino acids influence receptor sensitivity, the involvement of the peripheral nervous system in the integration of sensory information passing to the CNS may result in increased neural economy in the insect.

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