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SENSORY CODING FOR FEEDING DETERRENCE IN THE GRASSHOPPER *SCHISTOCERCA AMERICANA*

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Summary

The electrophysiological responses of sensilla on the tibia of *Schistocerca americana* (Drury) to six compounds were examined. All the compounds were shown to cause feeding deterrence at high concentrations. Nicotine hydrogen tartrate, quinine, hordenine (all alkaloids) and salicin (a phenolic glycoside) all stimulated one cell in each sensillum. This was shown by differential adaptation experiments to be the same cell. In some sensilla this cell also responded to linamarin (a cyanogenic glycoside). Earlier work had shown that the activity of this cell was correlated with feeding deterrence. However, canavanine (a non-protein amino acid) did not stimulate this cell, although it caused feeding deterrence. All the compounds, except salicin, produced a marked depression in the activity of cells responding to sucrose, and at higher concentrations of the compounds this inhibition was almost complete.

The activity of the deterrent cell and inhibition of the activity of sucrose-sensitive cells appear to act together to produce the behavioural effects of most chemicals, but canavanine appears to act only by suppressing the activity of other cells and salicin primarily through activity of the deterrent cell. In addition, quinine disrupts the activity of all the cells and in its presence the deterrent cell adapts very slowly so that the message signalling deterrence is sustained.

At low concentrations, salicin, and probably hordenine, increased the duration of feeding. In the case of hordenine this was due to an increase in the firing rate of sucrose-sensitive neurones; with salicin the increase was associated with a high threshold of response and a rapid rate of adaptation of the deterrent cell.

Thus, similar behavioural effects are produced by a variety of sensory phenomena with each compound acting in a slightly different manner from the others.

Introduction

The sensory codes by which insects distinguish palatable from unpalatable foods have remained elusive. In the larva of *Pieris brassicae* a relatively simple,

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numerical balance between the inputs of receptors responding to phagostimulatory and deterrent compounds may be adequate to account for food acceptance or rejection (Blom, 1978; Schoonhoven, 1987; Schoonhoven and Blom, 1988). Recently, Mitchell *et al.* (1990) have suggested that the fly *Sarcophaga bullata* uses the variance of the signal to distinguish between food types.

The chemosensory systems of these insects, however, are quantitatively, and perhaps qualitatively, different from those of grasshoppers, which possess an extensive array of apparently largely non-specific receptors. In an attempt to take this into account, Blaney (1980; Blaney and Winstanley, 1980; Winstanley and Blaney, 1978) has computed an overall relative sensory input from a number of receptors, which, he suggests, correlates with behaviour. However, this approach gives rise to certain anomalies (Chapman, 1988) and in this paper we attempt a different approach to the understanding of sensory coding in a polyphagous grasshopper.

Previous studies have shown that contact chemosensilla on the tibia and tarsus of the grasshopper *Schistocerca americana* are stimulated by nicotine hydrogen tartrate (NHT), which is a feeding deterrent (White and Chapman, 1990). This compound was found to stimulate a single cell in each sensillum, raising the question of whether this cell was also stimulated by other deterrent compounds and whether it might be considered a general 'deterrent' cell which dominated food selection. In this paper we describe the effects on meal duration of a small number of plant secondary compounds, both chemically related and unrelated to NHT, and describe the neurophysiological responses of contact chemosensilla on the tibia to these same compounds. We investigate the possibility that a general deterrent cell exists and also attempt to elucidate the role of sensory input in governing the effects of the different chemicals on meal duration. We believe that the outcome goes some way towards an understanding of the sensory codes underlying food selection and meal size regulation in a grasshopper.

Materials and methods

Experiments were carried out on nymphs of the grasshopper *Schistocerca americana* on the third to fifth days of the final instar, which lasted 10 days. The insects were fed on seedling wheat and during the final instar were maintained in a controlled environment room on a 12 h:12 h light:dark cycle at a temperature of 32.5 °C during the photophase and 27.5 °C during the scotophase.

The chemicals were selected to include compounds that were both chemically related and unrelated to NHT and that were known to be deterrent to the closely related species *Schistocerca gregaria*. Quinine and hordenine are, like NHT, alkaloids, although in chemically different classes. Salicin is a phenolic glycoside, canavanine is a non-protein amino acid and linamarin is a cyanogenic glycoside.

For the behavioural assays, the chemicals were presented on 4.25 cm diameter glass-fibre discs (Whatman GF/A) with 5 % dry weight of sucrose. The sucrose was necessary in order to detect the reduction in feeding induced by the

deterrents. Test chemicals were added at 0.01, 0.1, 1.0 and 10.0% dry weight of the disc, but in the results these are presented as μmol/disc because the sensory response probably depends on the number of molecules present. The discs were presented to the insects singly, i.e. no choice, in small enclosures 11 cm×11 cm×4 cm high, and to make the disc easily accessible to the insect it was attached to a cork so that it was close to the glass lid in one corner of the enclosure.

Immediately prior to testing, the insects were kept without food for 3 h at 30°C, then their behaviour in the experimental enclosures was videotaped for 3 h. The behaviour of 12 insects, in separate enclosures, was taped simultaneously so that in any one experimental period insects were tested across the whole concentration range. Eight to 16 insects were tested at each concentration of each chemical. Subsequently the tapes were analysed. The duration of the first meal on a disc in the test period was recorded, a 'meal' being defined as any bite or feeding period separated from any other by an interval of at least 4 min. In most cases the meals were clearly defined in this respect. This method did not allow rejection on palpation to be recognised with certainty, but rejection following biting was clear.

Electrophysiological recordings were made using the tip recording technique (Hodgson *et al.* 1955). The records were amplified using a World Precision Instruments DAM 50 amplifier in differential mode (with a 100 MΩ impedance in parallel with the preparation to reduce the stimulus artefact) or with a Johnson clamping preamplifier. Data were recorded on magnetic tape using a Vetter model B instrumentation recorder. All recordings were made from contact chemosensilla on the ventral surface of the tibia of an excised mesothoracic leg. Preliminary electron microscopical investigations show that each of the sensilla has four chemosensitive cells. Cell identification was based on spike size and shape as determined by the 'SAPID Tools' software package (Smith *et al.* 1990), as well as by the pattern of firing.

The basic electrophysiological response to each chemical was investigated using 10^{-2} mol l^{-1} solutions in 5×10^{-2} mol l^{-1} NaCl. The same concentration was used in differential adaptation experiments to obtain data on which cells were responding. Adaptation was carried out with 5×10^{-1} mol l^{-1} NaCl or 5×10^{-2} mol l^{-1} NHT in 5×10^{-2} mol l^{-1} NaCl. In these experiments the sequence of stimulations was as follows: (a) test compound for approximately 2 s; (b) 5 min to disadapt followed by 30 s stimulation with NaCl or NHT to produce adaptation; (c) immediate reapplication of NaCl or NHT to confirm complete adaptation, approximately 2 s; (d) immediate reapplication of test compound, approximately 2 s.

The responses to the test compound at a and d were then compared. Elimination of the response at d was taken to indicate that the test chemical normally stimulated the same cells as the adapting compound; similar responses at a and d indicated that the test chemical affected a different cell from the adapting compound.

In studying the effects of the chemicals over a range of concentrations each was dissolved in 5×10^{-2} mol l^{-1} NaCl with 10^{-2} mol l^{-1} sucrose. The sucrose was

added because it was present in the behavioural assays, but also because evidence from other insects had indicated that deterrents may function by inhibiting the activity of other cells (e.g. Dethier and Bowdan, 1989; Mitchell and Sutcliffe, 1984). Concentrations were tested from 10^{-5} to 5×10^{-2} mol l^{-1} for all the compounds and higher levels were tested with salicin and canavanine. Each chemical was tested at each concentration on two sensilla on each of five insects. So that direct comparisons of the responses of single sensilla were possible, NHT, hordenine and canavanine were tested in one experiment and salicin, quinine and linamarin in another; it was not practicable to test all six compounds on one preparation. The sequence of chemicals and concentrations was randomised and 6 min elapsed between successive stimulations of any one sensillum.

Results

Behavioural responses

All six of the compounds were deterrent at high concentrations, but salicin was strongly phagostimulatory at low concentrations (Fig. 1). Hordenine also produced a slight increase in the feeding time at low concentrations. This increase was not statistically significant, but a similar result was obtained in two separate experiments (results from only one experiment are shown). Quinine and NHT were the most effective deterrents, causing a 90 % reduction in the duration of the first meal at concentrations of about 1 μmol/disc.

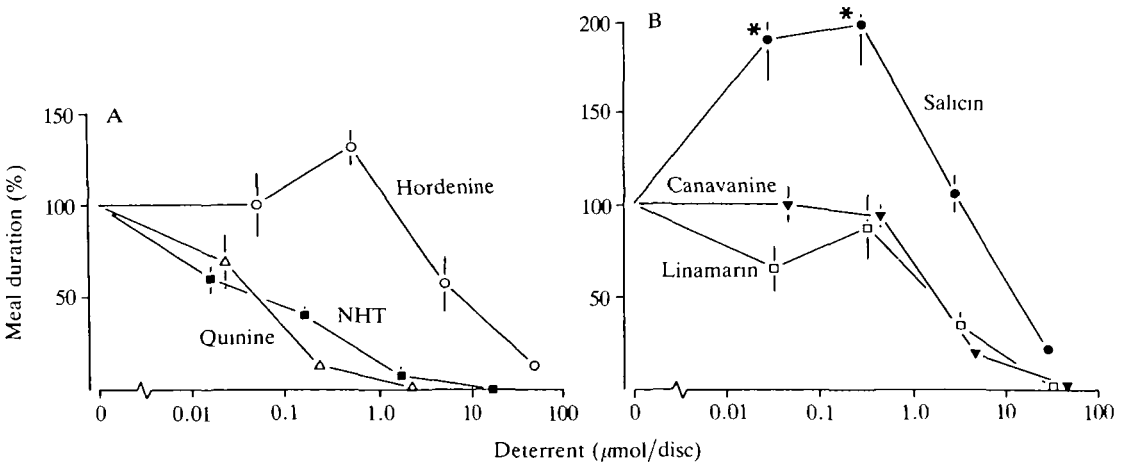

Fig. 1. Duration of the first meal on sucrose-impregnated glass-fibre discs with different concentrations of deterrents, expressed as a percentage of the meal duration on sucrose alone. (A) Responses to three alkaloids; (B) responses to three other deterrent compounds. Vertical bar denotes standard error, N=8–16 for each point. *denotes significantly longer than on sucrose alone (P<0.01)., NHT, nicotine hydrogen tartrate.

Neurophysiological responses

Spike identification

Great difficulty was experienced with spike identification and the shape of action potentials from different cells is apparently much less characteristic than has been described for other insects (e.g. Mitchell *et al.* 1990; Schnuch and Hansen, 1990, in the fly; Wieczorek, 1976, in a caterpillar). Only the NHT-sensitive cell was identified with reasonable confidence, using spike shape combined with its regular firing pattern. Previous work had established that the NHT-sensitive cell was distinct from the cells responding to NaCl and sucrose (White and Chapman, 1990). Cross-adaptation experiments further demonstrated that the same cell was active in response to hordenine, salicin and quinine since following adaptation by NHT the response to these compounds was greatly reduced or completely eliminated (Table 1). The response of the cells in one sensillum to quinine was, however, unaffected by previous treatment with NHT. With linamarin, adaptation with NHT had no effect in those sensilla in which only a single cell responded, but where two or three cells responded to linamarin, adaptation with NHT reduced the number to one or two and in most cases the overall firing rate was significantly decreased. This suggests that in some sensilla the NHT-sensitive cell also responded to linamarin. Differential adaptation indicated that the cells that were active in the presence of canavanine did not include the NHT-sensitive cell but were probably the same as those responding to NaCl.

In our dose–response studies we frequently recorded a cell responding to sucrose alone which we could not differentiate from the NHT-sensitive cell. This cell continued to fire at all concentrations of NHT, hordenine, salicin and quinine, but the firing rate only exhibited a sustained increase in firing at 10^{-4} mol l^{-1} with NHT and quinine, at 5×10^{-4} mol l^{-1} with hordenine and at 5×10^{-3} mol l^{-1} with salicin. These may represent threshold values for the sensitivity of the cell to these compounds. At lower concentrations it is possible that the NHT-sensitive cell was firing spontaneously or was sensitive to the other compounds. However, in these cases we should have expected the response to sucrose to be reduced, but not eliminated, following adaptation to NHT, but this was not the case (White and Chapman, 1990). We conclude that in our dose–response curves we are probably confounding the activity of two cells with similar-shaped action potentials and that the true NHT-sensitive cell really only becomes active at around 10^{-4} mol l^{-1} while the activity of the other cell is suppressed. In the following account, the true NHT-sensitive cell is considered to become active at the low point in the curve of firing rate which marks the probable threshold level.

Responses of sensilla to NHT

Forty seven sensilla on seven insects were tested with 10^{-2} mol l^{-1} NHT in 5×10^{-2} mol l^{-1} NaCl. In nearly every instance the response was dominated by a single, regularly firing cell and in about one-third of the traces this was the only cell firing. In a majority of sensilla, this cell produced more than 10 spikes in the first

Table 1. *Differential adaptation with NaCl and nicotine hydrogen tartrate (NHT)*

	Adaptation with NaCl					Adaptation with NHT				
		Before adaptation		After adaptation			Before adaptation		After adaptation	
Chemical	Number of sensilla	No.*	Rate†	No.	Rate	Number of sensilla	No.	Rate	No.	Rate
Hordenine	4	1	17.7±8.6	1	11.7±9.1	4	1	27.5±14.0	0–1	1.5±3.0
	2	2	27.0±14.1	1–2	40.6±22.6					
Salicin	5	1	7.4±8.8	1	9.6±6.9	1	1	8.0	0	0
						7	2	18.3±10.0	0–1	0.6±0.8
Quinine	3	1	11.0±14.0	1	11.0±2.6	2	1	6.5±0.7	0–1	3.0±4.2
	1	3	18.0	2	23.0	2	2	31.5±14.8	0	0
Canavanine	4	1	6.0±5.5	0–1	0.25±0.5	4	1	4.2±4.4	1	4.5±2.1
	7	2	11.1±8.3	0–1	0.6±1.1	3	2	4.7±3.1	1–2	12.3±2.1
Linamarin	1	1	11.0	1	12.0	2	1	7.0±1.4	1	7.5±6.4
	5	2	17.0±10.4	1–2	15.8±7.9	5	2	15.4±3.6	1	6.2±1.3
						1	3	8.0	2	9.0

* Number of cells firing.

† Firing rate refers to the number of spikes in the first 500 ms of stimulation; values are mean±S.D.

Table 2. *Response to different compounds at 10^{-2} mol l^{-1} in 5×10^{-2} NaCl*

Compound	Number of insects	Number of sensilla	Number with one spike class	Firing rate of dominant cell	Number with more than 10 spikes	Firing rate of secondary cell
NHT	7	47	16	5–47	45	0–8
Hordenine	3	25	15	6–48	23	0–14
Salicin	5	18	7	3–36	13	0–17
Quinine	5	26	12	9–83	25	0–50

Except for quinine, firing rate refers to the number of spikes in the first 500 ms of stimulation, for quinine the period is 1 s.
NHT, nicotine hydrogen tartrate.

500 ms of stimulation (Table 2) and, in some, its firing rate was over 40 per 500 ms. The rate of firing of the second cell, when it was present, was always very low. Nevertheless, when the second cell did fire, it often briefly inhibited the dominant cell, eliminating a single spike from the otherwise regular pattern of firing (see White *et al.* 1990).

At low concentrations of NHT in the mixture with sucrose and NaCl, it was usual for two, or sometimes three, cells to be active (Fig. 2). The combined rate of firing of the two or three cells in the first 250 ms of stimulation at 10^{-5} and 5×10^{-5} mol l^{-1} NHT was not significantly different from the firing rate of cells in the same sensilla in response to sucrose alone (mean±S.D. sucrose 7.2±3.1 spikes 250 ms^{-1}, NHT 9.1±4.7 spikes 250 ms^{-1}, t=1.24, 28 d.f., P>0.1). The decline in the rate of firing of all cells combined over the first second of stimulation was also similar to that with sucrose alone (Fig. 3).

Above 5×10^{-5} mol l^{-1} NHT, the NHT-sensitive cell exhibited an increase in firing rate which commonly reached a maximum at 10^{-3} mol l^{-1} and 5×10^{-3} mol l^{-1} and then declined. In four sensilla (out of 15) the firing rate continued to increase up to the maximum concentration tested, 10^{-2} or 5×10^{-2} mol l^{-1}. The higher the concentration at which the peak firing rate

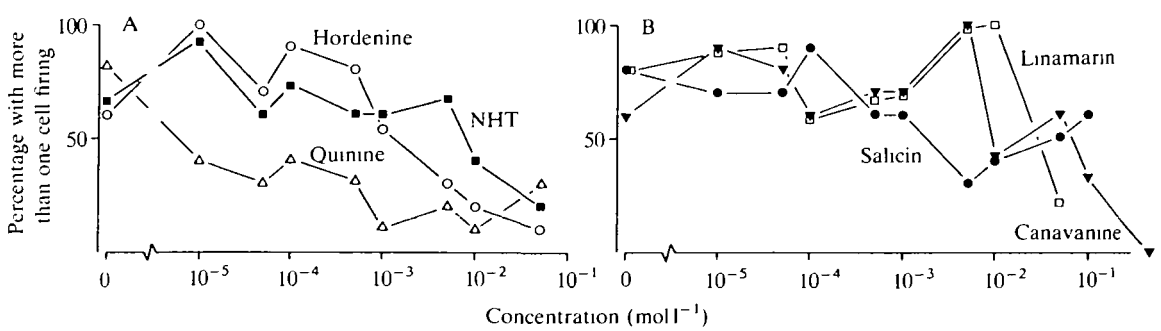

Fig. 2. Percentage of sensilla in which more than one cell was firing in response to different concentrations of deterrents. The line for each chemical is based on 10 sensilla, except NHT, which is based on 15. (A) Responses to three alkaloids; (B) responses to three other deterrent compounds.

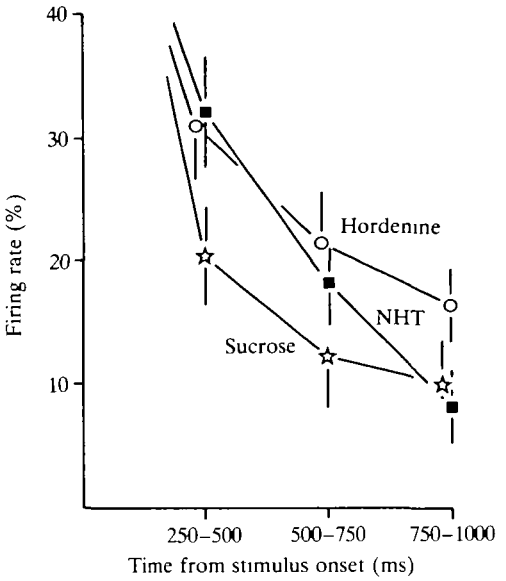

Fig. 3. Combined firing rates of all cells in a sensillum in response to sucrose and sucrose plus NHT or hordenine at 10^{-5} and 5×10^{-5} mol l^{-1} (data for two concentrations pooled). Rates are expressed as percentages of the firing rate in the first 250 ms of stimulation. Vertical bar denotes standard error. The line for each chemical is based on the responses of 10 sensilla.

occurred, the higher the rate of firing at the peak (r=0.7015, P<0.01, N=15). When the peak occurred at 10^{-2} mol l^{-1} or above the rate of firing was twice as high (about 20 spikes 250 ms^{-1}) as that of a cell with a peak at 5×10^{-4} mol l^{-1}. Over this part of the concentration range the number of cells firing declined and often only the cell responding to NHT was active (Fig. 2). Adaptation rates of the NHT-sensitive cell did not differ from 10^{-3} mol l^{-1} upwards, irrespective of the rate of firing, and Fig. 4 shows the data for all these concentrations and all sensilla combined. After 250 ms the rate of firing was reduced to about 30 % of the original value and by 750 ms this was further reduced to less than 20 %.

The average response of 10 sensilla from five insects is shown in Fig. 5A. This shows that, despite the variability between sensilla, the average input from the NHT-sensitive cells of all the sensilla combined increases with the concentration of NHT above 5×10^{-5} mol l^{-1} and, at the highest concentrations, the input from other cells is almost totally suppressed.

Responses of sensilla to other compounds

Both hordenine and salicin produced similar responses to NHT at 10^{-2} mol l^{-1} in 5×10^{-2} mol l^{-1} NaCl; in nearly every case one cell responded with a relatively high and regular rate of firing and in most cases produced more than 10 spikes i

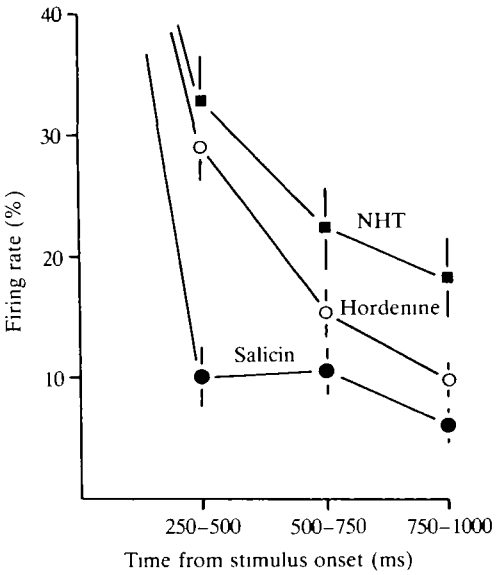

Fig. 4. Adaptation rates of the NHT-sensitive cell during stimulation by NHT, hordenine or salicin at concentrations of 10^{-3} to 5×10^{-2} mol l^{-1} (data for all concentrations pooled). Rates are expressed as percentages of the firing rate in the first 250 ms of stimulation. Vertical bar denotes standard error. The line for each chemical is based on the responses of 10 sensilla.

the 500 ms following application of the stimulus (Table 2). In some cases a second cell also fired and this was more common with salicin.

In the dose–response experiment, threshold values at which the firing rate of the NHT-sensitive cell started to increase were 10^{-4} mol l^{-1} for hordenine and 10^{-3} mol l^{-1} for salicin (Fig. 5). With hordenine the firing rate of all the cells together at 10^{-5} and 5×10^{-5} mol l^{-1} was significantly higher than that with sucrose alone (sucrose 7.2±3.1 spikes 250 ms^{-1}, hordenine 11.1±4.9 spikes ms^{-1}, t=2.29, 28 d.f., P<0.05). With salicin the mean rate of firing was almost exactly the same as with sucrose and this level of firing was maintained up to 10^{-3} mol l^{-1} (sucrose 8.7±2.8 spikes 250 ms^{-1}, salicin 8.5±4.7 spikes 250 ms^{-1}). At these low concentrations it was usual for two, or occasionally three, cells to be active and the decline in firing rate with stimulus duration followed the same pattern in all cases (Fig. 3).

At higher concentrations the rate of firing of the NHT-sensitive cell increased to a maximum, which for salicin was usually about 10^{-2} mol l^{-1} and for hordenine was usually at 5×10^{-3} mol l^{-1}. The adaptation rate to hordenine was very similar to that with NHT, but with salicin the adaptation rate was significantly faster than with either of the other compounds. In the second 250 ms interval the firing rate with salicin was only 10 % of the original rate (Fig. 4; NHT *versus* salicin t=5.13, 72 d.f., P<0.01; hordenine *versus* salicin t=5.05, 72 d.f., P<0.01).

Hordenine, like NHT, produced almost total suppression of other cells at higher

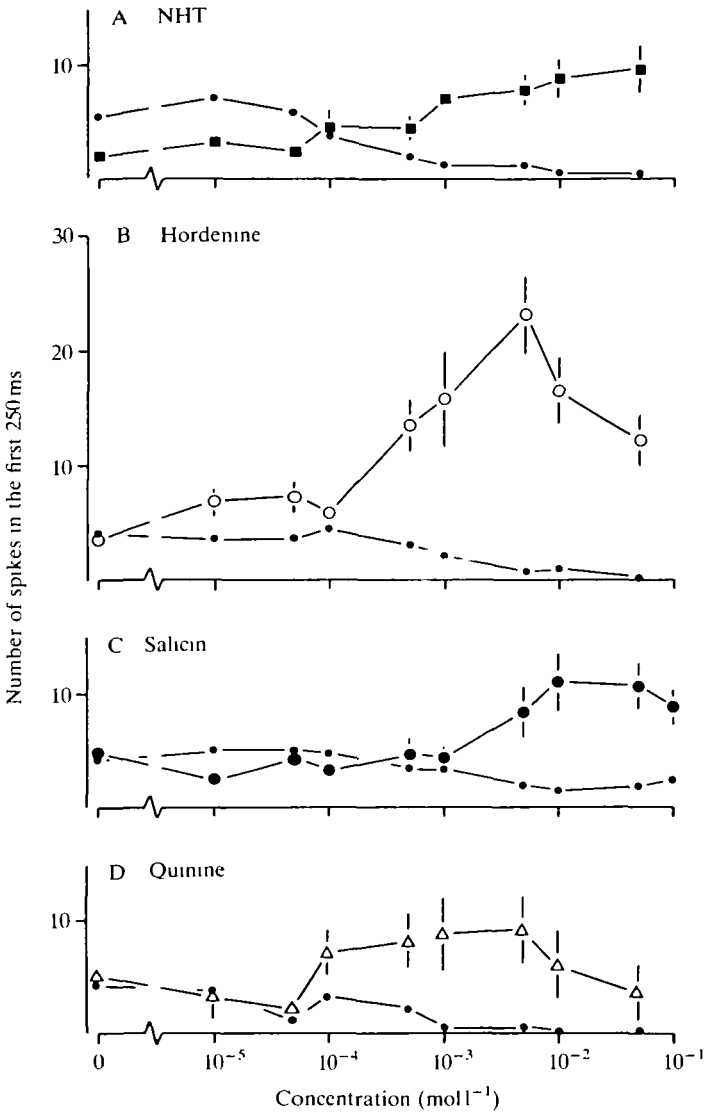

Fig. 5. Average firing rates of the NHT-sensitive cell and other cells in response to (A) NHT, (B) hordenine, (C) salicin and (D) quinine over a range of concentrations all in the presence of 10^{-2} mol l^{-1} sucrose. The activity of the NHT-sensitive cell is indicated by large symbols with standard errors. Activities of all other cells combined are shown by small filled circles. The line for each chemical is based on the responses of 10 sensilla.

concentrations, but with salicin one other cell often continued to fire at a low rate at all concentrations (Fig. 2). The average spike frequencies from 10 sensilla are shown in Fig. 5B,C.

The response to quinine, although it usually involved the NHT-sensitive cell, was much more variable than that to the other compounds. Twenty six sensilla on

five insects were stimulated with a concentration of 10^{-2} mol l^{-1} quinine. In seven instances there was a delay in the response varying from 100 ms to over 1 s (one example). In addition, the NHT-sensitive cell did not always fire regularly, but sometimes responded with a series of irregular bursts. For these reasons, the general account of the response to quinine is based on the number of spikes in the first second of the response, rather than the first 500 ms as with the previous chemicals.

In 25 of the 26 sensilla tested with quinine, the NHT-sensitive cell fired at a rate greater than 10 spikes s^{-1}, the highest rate being 83 spikes s^{-1} (Table 2). In 12 of these instances this was the only cell firing; in others it was accompanied by one other cell firing at low frequency, less than 5 spikes s^{-1}; in 10 sensilla the NHT-sensitive cell was accompanied by one, two or three other cells firing at higher rates and occasionally exceeding the firing rate of the NHT-sensitive cell.

The threshold of activity of the NHT-sensitive cell for quinine appeared to be at 5×10^{-5} mol l^{-1}, but even at 10^{-5} mol l^{-1} quinine affected the activity of other cells within a sensillum, usually suppressing the activity of all but two of the cells (Fig. 2), so that the overall rate of firing was significantly reduced compared with the rate with sucrose alone (sucrose 8.7 ± 2.8 spikes 250 ms^{-1}, quinine 4.85 ± 5.5 spikes 250 ms^{-1}, $t=2.54$, 18 d.f., $P<0.02$). The activity of the cells tended to be suppressed over the whole concentration range and this sometimes included the NHT-sensitive cell itself. The activity of the NHT-sensitive cell in different sensilla peaked at concentrations in the range 10^{-4} to 5×10^{-2} mol l^{-1}, with four cells (out of 10) firing at maximum rate at 10^{-3} mol l^{-1}.

The adaptation rate of the NHT-sensitive cell was examined for those cells with a firing rate greater than 10 s^{-1} and, because the initiation of firing was sometimes delayed, the rate of firing over the first 500 ms is compared with the rate over the second 500 ms. The average response in the second 500 ms period, for 26 cells, was 84 % of the initial response. In five instances the rate of firing increased (these were not cells with a long delay before the onset of firing) and in only three was the firing rate reduced to less than 50 %.

The response to canavanine at 10^{-2} mol l^{-1} in NaCl, but without sucrose, usually involved two cells, but in a few of the records only one cell was active (tested on three insects, 20 sensilla). The firing rate was generally lower than with the previous compounds and the overall spike frequency was usually less than 10 in the first 500 ms of stimulation. Where it was possible to compare the rate of firing with the activity of the same sensillum in response to 5×10^{-2} mol l^{-1} NaCl without canavanine, there was no significant difference (paired t-test, $t=0.69$, 3 d.f., $P>0.1$). Thus, these data offer no evidence that canavanine at 10^{-2} mol l^{-1} produced a sensory response that differed from the response to the NaCl solution in which it was dissolved.

The dose–response curve for canavanine mixed with sucrose was examined on 10 sensilla with a single application of each concentration. The average firing rate at the two lowest concentrations was not significantly different from the response to sucrose alone (mean±S.D., sucrose, 7.2 ± 3.1 spikes 250 ms^{-1}; canavanine,

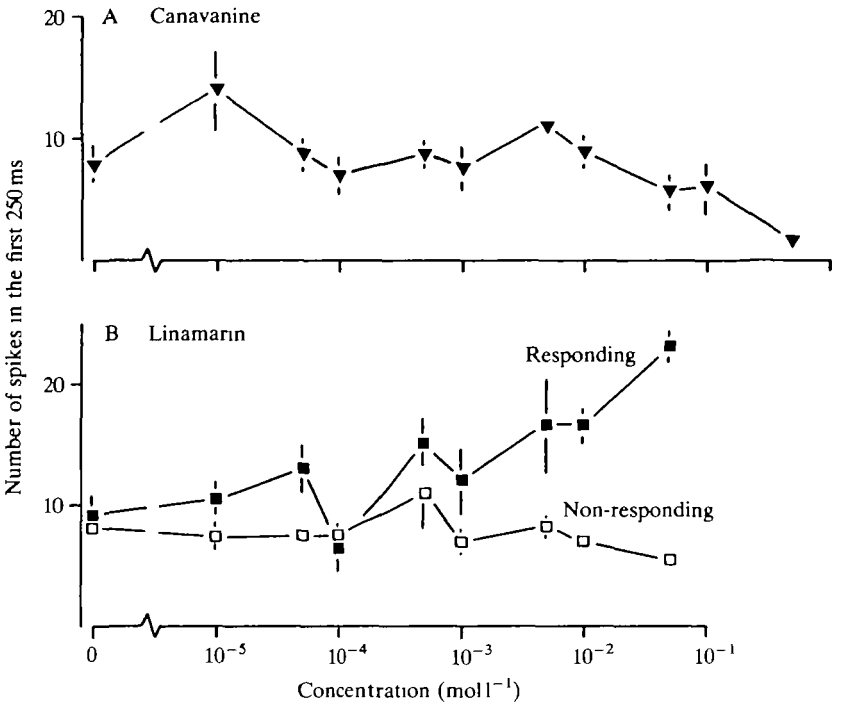

Fig. 6. Average firing rates of all cells in a sensillum in response to a range of concentrations of (A) canavanine (N=10) and (B) linamarin. Data for linamarin are divided into those sensilla that did not respond to the chemical (N=6) and those that did (N=4). Vertical bar denotes standard error.

11.45±8.4 spikes 250 ms^{-1}; t=1.752, 28 d.f., P>0.05), nor was the firing rate at 5×10^{-3} and 10^{-2} mol l^{-1} (10.05±4.20 spikes 250 ms^{-1}; t=1.77, 28 d.f., P>0.05), and over the whole range of concentrations the dose–response curve was more or less flat, declining above 10^{-2} mol l^{-1} (Fig. 6). (The high rate at 10^{-5} mol l^{-1} is not significantly different from adjacent values. It was due to two sensilla, from the same insect, firing at an exceptionally high rate.) There was thus no evidence of any response to stimulation by canavanine. Over most of the range two cells were active, but above 5×10^{-2} mol l^{-1} the number declined to one (Fig. 2; only four sensilla were tested at 5×10^{-1} mol l^{-1}). The rate of firing of the cell remaining active also declined.

Adaptation rates were not calculated for the cells separately because of the variability of response, but the decline in firing rates of the cells together indicates that adaptation rates were very similar to those of the NHT-sensitive cell with NHT and hordenine (Fig. 7).

Linamarin at 10^{-2} mol l^{-1} in 5×10^{-2} mol l^{-1} NaCl was tested on 27 sensilla on four insects. Nearly all the sensilla responded with two cells firing, in five only one cell fired. Where direct comparison with the response of the same sensilla to NaCl alone was possible there was generally no difference in the overall spike frequency

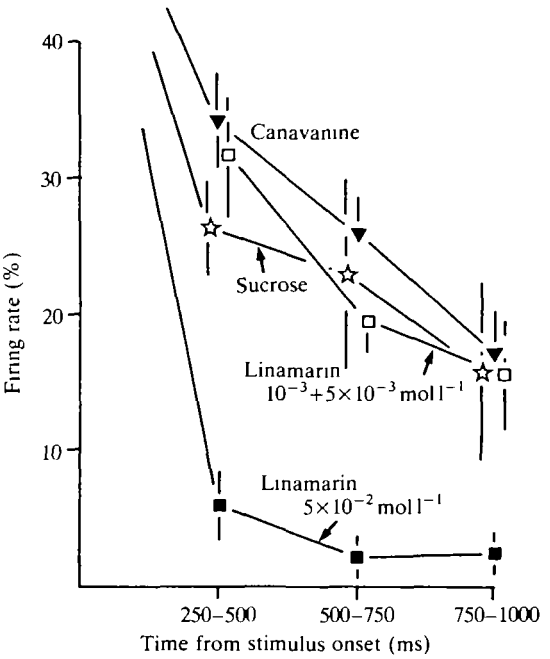

Fig. 7. Firing rates of all cells in a sensillum in response to stimulation with sucrose plus canavanine at 10^{-3} to 5×10^{-2} mol l^{-1}, and to sucrose plus linamarin at different concentrations. Rates are expressed as percentages of the firing rate in the first 250 ms of stimulation. Vertical bar denotes standard error. The line for each chemical is based on the responses of 10 sensilla.

from that observed with 5×10^{-2} mol l^{-1} NaCl. In other cases the spike number was greatly reduced by linamarin and in some cases the signal was completely eliminated for up to 1 s. Only one sensillum appeared to respond positively to linamarin with an increase in firing rate, but this possibly reflected an unusually low rate of firing in response to NaCl alone, i.e. sampling error. These data offer no evidence that the cells respond to linamarin, although some inhibition does seem to have occurred.

The dose–response curves in which linamarin was presented together with sucrose fall into two classes. Six sensilla on four insects appeared not to respond to linamarin. The average rate of firing did not change over the whole range of concentrations tested, except for a possible (not statistically significant) reduction at 5×10^{-2} mol l^{-1}, the highest concentration tested (Fig. 6). At this concentration, too, usually only one cell was active (Fig. 2). Four other sensilla, on three insects, exhibited a positive response to linamarin above 10^{-3} mol l^{-1} (Fig. 6). Although in three of these sensilla two cells were active in the first 250 ms of stimulation at the highest concentration, nearly all this activity came from a single cell, believed to be the NHT-sensitive cell, and other cells were effectively silent.

The decreases in firing rates during the course of a single stimulation of both

non-responding and responding sensilla were similar up to 5×10^{-3} mol l^{-1} and were comparable to the response to sucrose alone. At the highest concentration, however, when firing was dominated by the NHT-sensitive cell, the firing rate decreased markedly by the second 500 ms of stimulation (Fig. 7).

Discussion

The tibial contact chemosensilla usually play little or no part in the process of food selection. However, other studies (White and Chapman, 1990; P. R. White and R. F. Chapman, unpublished results) show that the tibial receptors respond to different chemicals in an essentially similar manner to other sensilla on the tarsi and arolium that are more directly involved in food selection. More subjectively, the patterns of responses that we observed are generally similar to those documented by Blaney (1974, 1975) on the maxillary palps of *Locusta migratoria*. That is to say, the receptor cells appear to have a broad spectrum of response and there is little evidence of enhanced sensitivity to particular chemicals. As a result, we feel justified in using our results to attempt to account for the observed behavioural responses of the insects.

A feature of the contact chemosensory system of grasshoppers is the very large number of sensilla (Chapman, 1982*a*). This is associated with the relative lack of tuning of the receptor cells and it appears obvious that decision-making by a grasshopper is based on assessing the value of inputs from a number of receptors (across-fibre patterning) (Chapman, 1988). This is clearly recognised in the work of Blaney (Blaney, 1975, 1980; Blaney and Winstanley, 1980; Winstanley and Blaney, 1978). However, because we do not know how information from the peripheral receptors is integrated in the central nervous system, we have not attempted any general quantified explanation of the feeding behaviour, but base our arguments more subjectively on the responses of cells in individual sensilla and in populations of sensilla. Such an approach is to some extent inevitable because it is not possible to relate directly the concentration of dry material on a glass-fibre disc to a concentration in aqueous solution.

In considering populations of sensilla we have treated sensilla from different insects as if they were part of the same population of receptors. We believe this is justified because it has been demonstrated that sensilla in one insect do differ significantly in their responses to stimulation and differences between insects appear to be no greater than differences within an insect.

Chemosensory input from contact chemosensilla affects feeding by grasshoppers in various ways. A phagostimulatory input is necessary to initiate and to maintain feeding (Chapman, 1982*b*). At the opposite extreme, a feeding deterrent applied to the surface of an otherwise acceptable food may lead to immediate rejection without feeding (e.g. White and Chapman, 1990). In these situations the insect is often seen to make its decision to feed or to reject the food within a fraction of a second and, for this reason, much of this discussion focuses on the physiological response in the first 250 ms of contact.

However, Bernays and Chapman (1974) showed that the meal size of *Locusta migratoria* was increased if the insect was stimulated for a prolonged period with food sap before it started to feed. Blaney and Duckett (1975), also working with *L. migratoria*, showed that a meal was lengthened or shortened depending on whether they ensheathed the palp-tip sensilla in a solution of sucrose or a deterrent compound. In their experiments the meal ended long after the receptors would have been fully adapted. These results led Chapman (1982*b*) to suggest that the chemical inputs, probably acting over a period of time, were affecting the level of a central excitatory state that modulated meal size. For this reason it is important to consider the results of stimulation over at least the first second, in addition to the immediate effects.

Ultimately, however, meal size is governed by stretch receptor inputs from the fore and hind guts (Simpson *et al.* 1988) and, if the insect is eating maximally sized meals, further enhancement of sensory input may have no effect on meal size. This may have affected some of the results in the behavioural study because the sucrose concentration to which potential deterrents were added was already highly stimulating. Thus, phagostimulatory effects may sometimes have been obscured. It is also known that meal size may be affected by nutritional and, perhaps, other chemical feedbacks from the haemolymph (Lee and Bernays, 1988; Abisgold and Simpson, 1987). These have been shown to operate only after an initial meal on a substrate containing the chemical and it was to reduce the possibility of such confounding effects on the sensory input that the behavioural observations in this study were made on the duration of the first meal on any given substrate.

The clear association of the activity of the true NHT-sensitive cell with a deterrent response demonstrated by White and Chapman (1990) seems to indicate that this cell signals unpalatability. The activity of this cell at high concentrations of NHT, hordenine, quinine, salicin and linamarin, at which deterrence occurs, supports the concept that this is a general deterrent cell since, although the first three compounds are alkaloids, they differ markedly in structure, while the structures of salicin and linamarin are quite different. It has previously been suggested that the deterrent effects of NHT and azadirachtin on *S. gregaria* may result from labelled line responses (Blaney, 1980; Winstanley and Blaney, 1978). It seems likely that these responses were due to a cell with characteristics similar to the deterrent cell in *S. americana*.

Only four out of ten sensilla responded positively to linamarin and the experiments with 10^{-2} mol l^{-1} linamarin in NaCl suggest that the proportion may be lower than this. When a sensillum does respond it appears to be the NHT-sensitive cell that is activated, but not all NHT-sensitive cells exhibit this response since they were found in all, or nearly all, sensilla.

The effect of quinine on the deterrent cell is often abnormal, with spikes occurring in irregular bursts. This effect of quinine on contact chemoreceptors has frequently been remarked upon (Dethier, 1976).

However, activation of the deterrent cell is not the only correlate of rejection behaviour. High concentrations of all the compounds tested, except salicin,

suppress the activity of all cells except the cell responding to these compounds. The suppression of activity of sensory cells by alkaloids has been documented on a number of occasions in different insects (Mitchell and Sutcliffe, 1984; Dethier and Bowdan, 1989) and other classes of compound have been shown to have the same effect (Schoonhoven, 1982; Blaney and Simmonds, 1990). Amongst the Acrididae, Winstanley and Blaney (1978) found that the addition of nicotine suppressed the response of contact chemoreceptors on the maxillary palps of *Locusta migratoria* to sucrose and sodium tartrate and they obtained the same effect in some sensilla on the palps of *Schistocerca gregaria*. These results, together with our data, suggest that this is a widespread phenomenon.

We believe that the cells that are active at low concentrations of all the compounds tested, including the cell that resembles the NHT-sensitive cell, are the cells that respond to sucrose (plus NaCl) since they also respond when sucrose alone is present (but see below for linamarin). These cells presumably signal palatability and suppression of their activity inhibits feeding.

In the case of linamarin, the position is not clear cut. There was no enhancement of firing activity, above that in response to sucrose alone, at low concentrations, and from the dose–response data it appears that activity is due to the sucrose/salt cells. However, adaptation with NaCl produces no depression of activity and NHT produces only partial suppression. This implies that at least one of the cells responding to linamarin is different from both the sucrose/NaCl-sensitive cells and the NHT-sensitive cell. We could not distinguish such a cell in our traces. If it exists, it apparently did not influence behaviour in our experiments because at low concentrations linamarin neither enhanced nor decreased the duration of meals.

In most cases, the chemicals tested, when at low concentration, had no effect on the firing rates of the sucrose-sensitive cells, but low concentrations of hordenine produced an enhancement of their activity. Whether this results from a direct response of the cells to hordenine or from an increase in their sensitivity to sucrose in the presence of hordenine, we do not know. It would be expected that this increase in firing rate might lead to an increase in consumption. There was a slight, although not statistically significant, effect, perhaps because the insects were already feeding almost maximally. However, since this occurred in two independent experiments (only one is documented) it is likely that it is a real effect.

Mitchell and Sutcliffe (1984) and Schoonhoven (1982) have suggested that the suppression of activity of cells responding to phagostimulants may contribute to feeding deterrence. This certainly appears to be the case in *S. americana*, where suppression of the cells responding to sugar complements the activity of the deterrent cell with nearly every chemical. In the case of canavanine, this suppression of activity is apparently the only factor resulting in a failure to feed at higher concentrations. With quinine, such suppression occurred even at low concentrations and this probably contributed to the powerful deterrent effect of this compound. In addition, quinine sometimes produced a delay in the response of the receptors so that the insect has no information about whether the substrate is palatable or not.

Insofar as sensory input regulates the duration of a meal, as opposed to switching on or totally inhibiting feeding, it may be that rates of adaptation are important. The response to quinine, a most effective deterrent, adapts very slowly and it may be supposed that the sustained input depresses the central excitatory state.

Salicin had a phagostimulatory effect at lower concentrations, but there is no evidence that it enhanced the input from the sugar-sensitive cells in the way that hordenine did. However, the apparent threshold level of response of the NHT-sensitive cell to salicin was considerably higher than that for other compounds tested. In addition, this cell adapts very quickly when stimulated with salicin, while adaptation of the sugar-sensitive cells occurs more slowly. If meal duration is regulated *via* the central excitatory state we should expect salicin to be less effective as a deterrent than hordenine or NHT. Since the sugar-sensitive cells are not strongly inhibited by salicin, it appears that the activity of the deterrent cells at higher concentrations of salicin is sufficient to override the phagostimulatory effects of the sucrose, despite the high rate of adaptation.

The response to linamarin at high concentrations also adapts very quickly, although this is not true up to 5×10^{-3} mol l^{-1}. This was true both in those sensilla in which the firing rate increased, apparently through the activity of the deterrent cell, and in those in which it did not. Perhaps this suggests that even in the latter the deterrent cell was active, though firing at a low rate and unrecognised.

It was often true that the activity of some deterrent cells peaked at concentrations below the maximum tested, but, despite this, discs with high concentrations of the chemicals were not eaten. That is, the decline in the sensory input from the deterrent cells was not associated with an increase in meal durations. However, even when the average input declined at high concentrations, some cells reached their maximum firing rate at these levels. In the case of NHT it was also true that these cells had the highest firing rate, so that the average input from 10 sensilla increased progressively up to the maximum concentration tested. Whether the insect responds to total spike input, to the number of cells firing, or to the input from particular cells, we do not know, but the population of receptors with different sensitivities appears to provide the means by which the insect can make graded behavioural responses.

These results demonstrate that, in the polyphagous grasshopper *Schistocerca americana*, rejection of potential food owing to the presence of a deterrent compound may involve two different sensory mechanisms. Most of the chemicals tested activate a deterrent cell, the input from which, presumably, is interpreted in some negative way. But deterrence can occur also simply by suppression of cells that are normally active in response to phagostimulants and in the case of canavanine this appears to be the only mechanism. This suppression of activity is a common phenomenon and usually complements the activation of the deterrent cell. Quinine acts in both ways, but also appears to disrupt the normal activity of the deterrent cell. Each of the different compounds tested seems to produce its effects in a slightly different way from each of the others.

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