Contact-chemoreception, or gustation, is a fundamentally important sense to many animals, including insects. Chemical stimuli sampled at close quarters may be implicated in the successful execution of many of the major behavioural tasks an insect must accomplish. These include finding and selecting appropriate foods (Dethier, 1976), regulating dietary intake (Simpson and Raubenheimer, 1993a), finding a mate and suitable oviposition sites (Roessingh et al., 1991; Blaney and Simmonds, 1990) and avoiding potentially poisonous or otherwise harmful chemicals in the environment (White and Chapman, 1990). Despite its importance, the principals of gustatory processing in the central nervous system are much less well understood than those for olfaction, the other chemical sense.

In insects, contact-chemosensory neurones are contained within cuticular structures, commonly taking the form of small hairs (basiconic sensilla), each pierced by a single (usually) apical pore (Blaney et al., 1971; Zacharuk, 1985). In locusts, these sensilla are 25–45 μm long and each contains 2–10 chemosensory neurones, depending on their location on the body (Chapman, 1982). Commonly, they also contain a single mechanosensory neurone (Haskell and Schoonhoven, 1969; Blaney, 1975; Newland and Burrows, 1994). In addition to well-defined aggregations on the mouthparts, orthopteroid insects possess a large number of these sensilla scattered over the entire body surface (Chapman, 1982). Basiconic sensilla on the legs of locusts are thought to possess four chemosensory neurones and a single mechanosensory afferent (Chapman, 1982).

The role of gustatory sensilla on the mouthparts in assessing potential foods is clear and, in general terms, chemicals that may stimulate or inhibit feeding have been extensively analysed, as have the responses of chemosensory neurones to stimulation by these chemicals (Haskell and Schoonhoven, 1969; Blaney and Chapman, 1970; Blaney, 1975; Simpson et al., 1991). In contrast, the functions of chemoreceptors on the...
legs and other body regions are rather less well known. There is some evidence that receptors on the tarsi have a role in the initial stages of food selection and may cue further sampling behaviour by the mouthparts (Dethier, 1976; Bernays and Simpson, 1982). White and Chapman (1990) described a prolonged leg-waving behaviour in response to droplets of nicotine hydrogen tartrate and sodium chloride applied to the tarsus. Blaney and Winstanley (1982) correlated the spiking rates of chemosensory neurones in basiconic sensilla on the fore legs of locusts to various chemicals with the likelihood of feeding on a glass-fibre disc impregnated with those chemicals. Newland (1998) demonstrated a local leg withdrawal response to acidic vapours mediated by chemosensory afferents in the basiconic sensilla on the leg and analysed the patterns of motor neurone activation that accomplish this. However, behavioural responses at a local level arising from stimulation of the legs with different chemical solutions have received little systematic investigation. It is important to define the responses of the leg to a variety of different chemicals before proceeding to investigate the underlying neural processing because different chemical stimuli are used to cue many varied and contrasting behavioural outcomes, e.g. feeding or avoidance. This, in turn, may reflect on how chemosensory stimuli are processed in the central nervous system.

The chemicals used in the present study, water, sodium chloride (NaCl), sucrose, nicotine hydrogen tartrate (NHT) and lysine glutamate, were chosen because they represent a variety of qualities to locusts. They were presented over a wide range of concentrations and in various combinations. Sucrose and lysine glutamate are representative of the two classes of macronutrient, carbohydrate and protein respectively, and actively promote feeding (Simpson and Raubenheimer, 1993a). Salts are required in small quantities in the diet, but become feeding deterrents at higher concentrations (Simpson, 1994), whereas NHT is a potent feeding deterrent, even at low concentrations, and may provoke active avoidance responses (White and Chapman, 1990).

Materials and methods

Locusts

Desert locusts, Schistocerca gregaria (Forskál), of either sex were taken from a colony maintained at the University of Southampton, reared under crowded conditions and fed on seedling wheat and oats. Adult locusts were also occasionally obtained from Blades Biological Supplies. Adult locusts were used in all experiments, aged from 4 to approximately 9 days post-moult, before the onset of breeding. All locusts were examined prior to use to ensure that all the limbs were intact and undamaged. They were kept isolated without food overnight prior to the experiments.

Experimental protocol

Experiments were performed on groups of 12 locusts. Five aqueous solutions of a test chemical (NaCl, sucrose, NHT or lysine glutamate), prepared over a range of concentrations, and a control of distilled water were used in each experiment. All the locusts in each group were tested with all six solutions, with the presentation order altered systematically. The distilled water control was performed primarily to measure the effectiveness of mechanical stimulation alone in eliciting a response. As shown in the Results section, only 11±2.6% of tests with water droplets evoked a response. There was an interval of at least 20 min between subsequent presentations of solutions, and the animals were placed in individual containers between tests. During each test, the locust was removed from its container, and an opaque hood fashioned from heat-shrink insulation was placed over its head, covering the eyes and chemosensory receptors on the mouthparts and antennae (Newland, 1998). The locust was placed on a test arena consisting of a rigid 1 mm mesh nylon sheet raised 25 mm above the work surface. The solutions were applied as droplets using a Pasteur pipette held 10–15 mm above the right hind tarsus. The nylon mesh allowed applied droplets to run easily around the entire surface of the tarsus but prevented them from falling straight through. More importantly, it allowed the locust a firm purchase on the substratum, ensuring that any movement was a positive reaction, not merely the result of the animal losing its grip and sliding away from the point of contact. The droplets had a mean volume of 0.04±0.008 ml (mean ± S.E.M., N=40), and there was no difference in droplet volume between water and the most concentrated solutions used of each of the test chemicals (analysis of variance, F4,35=0.25, P=0.903, each compared using eight pipettes). The mechanical component of the stimulus presented was, therefore, always similar. Droplets were only applied when the locust had come completely to rest on the mesh and the hind leg was at an angle of approximately ±30° from vertical. All experiments were performed at 23–25°C.

All tests were filmed using a video camera (Panasonic WV-BP500) mounted on a tripod with a 50 mm lens at 25 frames s⁻¹ and recorded on a Panasonic NV-HD680 video recorder for 10 s following the application of the droplets. A date/time marker (For.A video timer) was mixed with the video signal, allowing easier analyses of the responses of the animals. After each test, the tarsus was rinsed with distilled water, and the locust was returned to its container. Each experiment was repeated five times with new locusts for each replicate, so that there were 60 tests with any given solution.

Test chemicals

The concentrations used varied for each chemical, and the likely effective ranges were estimated by preliminary experiments. All solutions were made up in distilled water. Concentrations used were as follows: for NHT (Sigma Chemical Co.), 0.05 mmol l⁻¹, 0.5 mmol l⁻¹, 2.5 mmol l⁻¹ and 50 mmol l⁻¹; for NaCl (Fisher), 10 mmol l⁻¹, 25 mmol l⁻¹, 50 mmol l⁻¹, 75 mmol l⁻¹ and 100 mmol l⁻¹; for sucrose (BDH) and lysine glutamate (Sigma Chemical Co.), 10 mmol l⁻¹, 100 mmol l⁻¹, 250 mmol l⁻¹, 500 mmol l⁻¹ and 1000 mmol l⁻¹.
Recordings of tests were played back and analysed frame-by-frame to calculate the latency between the first contact of a drop with the hind tarsus and the start of any subsequent movements by the hind leg. The types of movement were described, and the duration of the first response was measured. In the measurements of latency to first response and response duration, there is an inherent timing resolution limitation of 40 ms and a further half-frame timing error of 20 ms.

All the tests were recorded for 10 s following application of the droplet. As the locusts were free to move at any time, there is potentially some difficulty in separating movements due to the application of the stimulus from spontaneous movements. In the following results, only movements that occurred within 1 s of the droplet being applied are included. Using this upper latency limit of 1 s excludes all movements of the hind leg that were preceded by movements of other limbs and were therefore clearly part of a non-local sequence of motion. The latency to first response followed an approximately exponential function, with 62.8% of all the recorded movements of the hind leg (in response to all the test chemicals) occurring within a latency of 1 s. The proportion of locusts responding at longer latencies declined rapidly over the remaining 9 s recording period.

The natural logarithm of concentration was used in all statistical analyses to render a more linear dose–response relationship. The latencies to first response were not normally distributed and were analysed using Spearman’s correlations when compared against \( \log_{e}(\text{concentration}) \) as a continuous variable and Kruskal–Wallis tests where comparing distinct stimulus groups. The other data were more normally distributed, although the duration of behaviour data were \( \log_{e} \)-transformed to reduce the skew in the distributions. The frequencies and durations of response were analysed using analysis of covariance (ANCOVA) or analysis of variance (ANOVA) using \( \log_{e}(\text{concentration}) \) as covariate and factors such as replicate group or behavioural response category as main effects.

### Results

#### Categories of response

All responses occurring within 1 s of the application of a droplet consisted of the locust moving its hind leg away from the stimulus site on the tarsus. Lifting of the leg occurred in isolation and was not observed to be part of a larger motor pattern involving the other limbs, although the locust may subsequently have walked away from the stimulation site. These behaviour patterns can be categorised into two major groups. The first of these, replacement behaviour, started with the lifting of the leg out of the applied droplet followed by the repositioning of the tarsus in a new location on the substratum in a continuous motion (Fig. 1A). Although the sequence of movement was similar in all cases, the extent of the movement and the final destination of the tarsus relative to its starting position varied considerably. The second major category of response, withdrawal behaviour, was more stereotyped in execution and consisted of a sequence of movements starting with levation of the tarsus. This was followed by levation of the femur, flexion of the tibia and, frequently, adduction of the femur to the side of the abdomen (Fig. 1B), after which the tarsus was held clear of the substratum for a period of not less than 400 ms and frequently for much longer. In the case of responses to sodium chloride solutions only, a third class of movement was occasionally observed in which both hind legs were folded and symmetrically repositioned under the abdomen, followed by the animal jumping out of the arena.

#### Frequencies of response

The proportion of locusts within each group of 12
responding to the applied chemical solutions was strongly correlated with the concentration of the chemical in the droplet for all the tested substances (Table 1; Fig. 2). However, there was considerable variation in effective concentrations between the different chemicals, as shown in Fig. 2. The concentration of chemical in a droplet sufficient to evoke a response (within 1 s) in 50 % of the locusts in each group ranged from approximately 2.5 mmol l\(^{-1}\) for NHT to over 500 mmol l\(^{-1}\) for sucrose, with NaCl (50 mmol l\(^{-1}\)) and lysine glutamate (between 250–500 mmol l\(^{-1}\)) having intermediate values.

Fig. 2. The frequencies of response depend on both chemical identity and concentration. Mean ± S.E.M. frequencies of locusts moving their hind leg out of droplets of water and solutions of increasing concentration of NaCl, sucrose, nicotine hydrogen tartrate (NHT) and lysine glutamate. Each frequency was calculated from the number of locusts in a group of 12 that responded within 1 s to each of the solutions, and each point is the mean of five replicates.

Table 1. Results of an analysis of covariance examining the effects of chemical concentration and test group on the frequencies of locusts responding to different chemical solutions

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Concentration (mmol l(^{-1}))</th>
<th>F-ratio, d.f.=1</th>
<th>F-ratio, d.f.=4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaCl</td>
<td>109.1***</td>
<td>0.87</td>
<td>5.29**</td>
</tr>
<tr>
<td>Sucrose</td>
<td>14.3**</td>
<td>0.60</td>
<td>3.63*</td>
</tr>
<tr>
<td>NHT</td>
<td>61.6***</td>
<td>0.77</td>
<td>0.54</td>
</tr>
<tr>
<td>Lysine glutamate</td>
<td>61.25***</td>
<td>0.77</td>
<td>0.71</td>
</tr>
</tbody>
</table>

Where replicate as a factor was significant (for NaCl and sucrose), this indicates that there was some consistent variation in the frequency of response between different groups of locusts over the entire concentration range.

Concentration was log\(_e\)-transformed for the analysis to allow for a linear regression fit.

Significant results are marked as follows: *P<0.05; **P<0.01; ***P<0.001.

NHT, nicotine hydrogen tartrate.

Locusts were tested in groups of 12 animals, and the numbers of animals in each group responding to various concentrations of a particular chemical were counted. There were five replicate test groups for each chemical.

Where replicate as a factor was significant (for NaCl and sucrose), this indicates that there was some consistent variation in the frequency of response between different groups of locusts over the entire concentration range.

Although overall the frequency of behavioural response increased with greater concentration, there was some variation in the type of response, whether replacement or withdrawal, that the locust was likely to perform (Fig. 3). The incidence of withdrawal responses increased significantly with concentration for all four chemicals (ANCOVA; NaCl, \(F_{1,19}=41.23, P<0.001\); sucrose, \(F_{1,19}=10.38, P=0.004\); NHT, \(F_{1,19}=94.43, P<0.001\) and lysine glutamate, \(F_{1,19}=16.12, P=0.004\); test group was used as a factor in all tests but was non-significant in all cases), but the distribution of replacement movements exhibited more variation. There was no significant concentration-dependent correlation with the incidence of replacement movements for sucrose (ANCOVA; \(F_{1,19}=2.51, P=0.13\); Fig. 3B) or NHT (\(F_{1,19}=0.27, P=0.609\); Fig. 3C), which occurred at similar, low frequencies for all the concentrations used (9±1.9 % of cases for sucrose and 8±1.4 % for NHT; means ± S.E.M., \(N=25\) each). The pattern of response to NaCl (Fig. 3A) was more complex. When tested over the entire concentration range, there was again no significant variation in the frequencies of replacement movements with concentration (\(F_{1,19}=1.2, P=0.288\)). However, the incidence of replacements increased steadily over the three lower concentrations used, before levelling off at the higher concentrations. When the three lower concentrations (10–50 mmol l\(^{-1}\)) only were analysed, this increase in frequency was significant (\(F_{1,9}=5.99, P=0.037\)). For these three chemicals, NaCl, sucrose and NHT, behavioural responses occurred infrequently at the lower end of the concentration ranges used and were equally likely to be withdrawals or replacements. At the most effective concentrations, responses were between 2.4 (sucrose) and
Local movements evoked by chemical stimulation

7.8 (NHT) times as likely to be withdrawals as replacements. Lysine glutamate (Fig. 3D) was the only chemical for which the frequencies of replacement movements increased significantly over the whole concentration range (ANCOVA; \( F_{1,19} = 10.67, P = 0.004 \)) and the only chemical in response to which locusts were as likely to perform replacement as withdrawal movements to all the test solutions.

As locusts within an experiment were repeatedly stimulated with chemical solutions (albeit of different concentrations and in a randomised order), the potential effect of repeated presentation on overall responsiveness and on the type of response they exhibited was also analysed (Table 2). There was no overall increase or decrease in responsiveness with repeated presentations of any of the chemical stimuli. For sucrose and NHT, the incidence of withdrawal behaviours was always significantly greater than of replacement behaviours, whilst the frequency of both types of behaviour was similar for lysine glutamate. For each of these chemicals, the relative frequencies of both behaviours were similar throughout the experiments. NaCl, however, does display a small but significant change in the frequency of the two types of behaviour over the duration of the experiments, with a slight increase in the frequency of withdrawal responses at the expense of replacement responses on repeated presentations. Whether this represents some form of sensitisation, with the frequency of the longer-lasting and more rapidly initiated (see below) behaviour being more likely to occur when stimulated with certain chemical stimuli, must await a more critical investigation.

The latency to response

Movements elicited by sucrose (Fig. 4B) and NHT (Fig. 4C) both had similar median latencies from the first contact of a droplet with the tarsus to the start of response, with values of 350 ms (\( N = 92 \)) and 320 ms (\( N = 137 \)) respectively. The median latencies to response when stimulated with lysine glutamate

### Table 2 Results of an analysis of covariance examining the effects of repeated stimulation with a chemical on the overall incidence and type of response

<table>
<thead>
<tr>
<th>Chemical, Repeat order</th>
<th>Movement type</th>
<th>Interaction, repeat order × movement type</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>0.076</td>
<td>0.25</td>
</tr>
<tr>
<td>Sucrose</td>
<td>2.112</td>
<td>3.985*</td>
</tr>
<tr>
<td>NHT</td>
<td>1.549</td>
<td>13.049**</td>
</tr>
<tr>
<td>Lysine glutamate</td>
<td>0.062</td>
<td>3.514</td>
</tr>
</tbody>
</table>

The repeat order category tests for any overall difference in response frequency with repeated stimulation of the chemicals. The movement type category tests whether there is any consistent difference in behavioural response type over the entire experiment, and the interaction (repeat order × movement type) tests whether the relative frequencies of withdrawal and replacement behaviour change with repeated stimulation.

Significant results are marked as follows: * \( P < 0.05 \); ** \( P < 0.01 \).

NHT, nicotine hydrogen tartrate.
(Fig. 4D) or concentrations of NaCl (Fig. 4A) less than 50 mmol l\(^{-1}\) were greater, with median values of 480 ms for lysine glutamate (\(N=119\)) and 560 ms (\(N=28\)) for NaCl. Although responses to water were very infrequent (27 out of 240 tests), the median latency to the start of the response was rapid, only 200 ms. The most rapid reaction times were seen in response to NaCl droplets with concentrations of 50 mmol l\(^{-1}\) and greater, with a median response time of 160 ms (\(N=112\)). As these results suggest, the latencies to NaCl-stimulated responses were negatively correlated with concentration, (\(N=140\), Spearman’s coefficient \(-0.2148\), \(P=0.011\)). However, there was no correlation between concentration and latency except for responses stimulated by NaCl. Values of \(N\) are shown in parentheses.

The duration of movement

The mean duration of the first behavioural response following stimulation was similar, regardless of whether it was a replacement or a withdrawal, for all the chemicals except NaCl (Table 3; Fig. 5A), where replacement responses took on average 44 ms longer than withdrawal movements for the same concentration. The duration of the avoidance behaviour was strongly affected by the concentration of NaCl [Table 3; Fig. 5A, withdrawals decreasing from 260±10 ms (\(N=6\)) at 10 mmol l\(^{-1}\) to 170±20 ms (\(N=32\)) at 100 mmol l\(^{-1}\)]. The concentration of NHT also significantly affected subsequent movement duration (Table 3), but the scale of the effect was less than for NaCl (Fig. 5C), decreasing from 360±50 ms (\(N=10\)) at 0.05 mmol l\(^{-1}\) to 270±30 ms (\(N=44\)) at 50 mmol l\(^{-1}\). There was no concentration-dependent effect on movement duration for sucrose (Fig. 5B) or lysine glutamate (Fig. 5D), which had similar mean durations of 360±10 ms (\(N=338\)) and 350±20 ms (\(N=119\)), respectively. Responses to water droplets were on average 290±40 ms in duration. Taken together, response durations when stimulated with sucrose, lysine glutamate or lower concentrations of NHT were similar, but all responses to NaCl, even at the lowest concentrations, were

<table>
<thead>
<tr>
<th>Chemical</th>
<th>d.f. residual</th>
<th>Concentration F-ratio, d.f.=1</th>
<th>Movement type F-ratio, d.f.=1</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>122</td>
<td>10.10**</td>
<td>3.96*</td>
</tr>
<tr>
<td>Sucrose</td>
<td>86</td>
<td>0.1</td>
<td>2.31</td>
</tr>
<tr>
<td>NHT</td>
<td>134</td>
<td>5.26*</td>
<td>0.16</td>
</tr>
<tr>
<td>Lysine glutamate</td>
<td>116</td>
<td>0.07</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Both concentration and movement duration were log\(_e\)-transformed for the analysis to produce a more linear covariate and to reduce skew in the data, respectively.

Only replacement and withdrawal behaviour types were included in the analysis, the small number of jump responses being excluded. Significant results are marked as follows: *\(P<0.05\); **\(P<0.01\).

NHT, nicotine hydrogen tartrate.
briefer and declined to values approximately half those of the movements elicited by the other chemicals.

The duration of the first behaviour following contact with a stimulus was the only measure of response magnitude taken, because the orientation of the locusts towards the camera varied considerably and, consequently, the extent of movement could not be accurately measured. Response duration as a measure confounds the rapidity of the movement per se with the magnitude of the movement, since larger movements would be expected to take longer to perform for a given velocity of movement. However, a behavioural response was marked as being small if the final position of the tarsus was less than one tarsus length from its starting position. The proportion of such small movements out of the total number of responses was not significantly correlated with concentration for either NaCl (Spearman’s coefficient 0.169, N=25, P=0.419) or NHT (Spearman’s coefficient 0.369, N=25, P=0.07). Differences in the extent of the movement cannot therefore be used to explain the differences in duration with increasing concentration observed in response to these chemicals.

Responses to NaCl and sucrose mixtures

The frequencies of locusts responding to 50 mmol l⁻¹ NaCl, 250 mmol l⁻¹ and 500 mmol l⁻¹ sucrose alone and to solutions containing them in combination were tested in a separate experiment. A concentration of 50 mmol l⁻¹ NaCl was chosen because it elicited responses in approximately 50% of cases in the previous experiment; 250 mmol l⁻¹ sucrose was chosen because it had a very weak behavioural effect on its own, and 500 mmol l⁻¹ sucrose was chosen to provide a contrast, because it had a greater behavioural effect in isolation (Fig. 2). The incidence of responses to 50 mmol l⁻¹ NaCl combined with 250 mmol l⁻¹ sucrose was significantly less than to 50 mmol l⁻¹ NaCl alone (paired t-test of frequencies within each replicate, t=4.81 with 4 d.f., P=0.009, Fig. 6A). However, pairing 50 mmol l⁻¹ NaCl with 500 mmol l⁻¹ sucrose, a concentration of sucrose that in itself was sufficient to induce a higher frequency of response than to water controls (paired t-test, 10 mmol l⁻¹ NaCl alone than to the sucrose solutions (250 mmol l⁻¹ sucrose, median 460 ms, 500 mmol l⁻¹ sucrose, median 360 ms, N=19). Both NaCl/sucrose mixtures had latencies to response more like that of 50 mmol l⁻¹ NaCl alone than to the sucrose solutions (Fig. 6B; Kruskal–Wallis test comparing latencies to sucrose solutions alone and in combination with 50 mmol l⁻¹ NaCl, \( \chi^2=9.46, P=0.0238 \)). Comparing the duration of responses to mixtures and their individual components (Fig. 6C) reveals a similar pattern to that found with the latencies. Movements in response to stimulation with 50 mmol l⁻¹ NaCl and to the NaCl-containing mixtures were more rapid than to sucrose alone (ANOVA of log-transformed data, F₄,₁₃₄=3.103, P=0.018). Thus, even though
Fig. 6. Responses to mixtures of chemicals cannot be predicted simply from the responses to their individual constituents. (A–C) Results of an experiment applying droplets of water (black), 50 mmol l\(^{-1}\) NaCl (diagonal hatching), 250 mmol l\(^{-1}\) sucrose (green), 500 mmol l\(^{-1}\) sucrose (blue) and combinations of these chemicals to the hind tarsus. (A) Frequency of locusts, out of groups of 12, responding to each chemical (N=5). Values are means ± S.E.M. (B) Box plots depicting the median, interquartile and 95% ranges of the latencies between first contact of the droplet with the tarsus and the start of the ensuing response. (C) Mean ± S.E.M. duration of the withdrawal response. (D–F) Results of an experiment applying droplets of water (black), 50 mmol l\(^{-1}\) NaCl (diagonal hatching), 0.05 mmol l\(^{-1}\) nicotine hydrogen tartrate (NHT) (yellow), 0.5 mmol l\(^{-1}\) NHT (red) and combinations of these chemicals to the hind tarsus. (D) Frequency of locusts, out of groups of 12, responding to each chemical (N=6). Values are means ± S.E.M. (E) Box plots depicting the latencies between first contact of the droplet with the tarsus and the start of the ensuing response. (F) Mean ± S.E.M. duration of the withdrawal response.
the presence of low concentrations of sucrose can depress the likelihood of the locust responding to behaviourally stimulating concentrations of NaCl, the duration of a response, when one does occur, follows a similar dynamic to that of NaCl alone.

Responses to NaCl and NHT mixtures

The second mixture experiment compared 50 mmol l\(^{-1}\) NaCl, 0.05 mmol l\(^{-1}\) NHT, 0.5 mmol l\(^{-1}\) NHT and solutions containing both 50 mmol l\(^{-1}\) NaCl and either of the two concentrations of NHT. As in the sucrose/NaCl experiments, 0.05 mmol l\(^{-1}\) NHT was chosen because it elicited responses in very few cases when applied alone, and 0.5 mmol l\(^{-1}\) NHT was chosen because by itself it elicited responses in approximately 50% of tests. The proportion of locusts (out of 12) responding to each of the test solutions within 1 s is shown in Fig. 6D (N=6 trials). The frequency of locusts responding to the mixtures was slightly greater than to 50 mmol l\(^{-1}\) NaCl on its own (mean frequency 38.9±4.8%), but was not significantly greater for either 0.05 mmol l\(^{-1}\) NHT+50 mmol l\(^{-1}\) NaCl (mean frequency 44±4%; paired t-test, t=0.88, 5 d.f., P=0.421) or for 0.5 mmol l\(^{-1}\) NHT+50 mmol l\(^{-1}\) NaCl (mean frequency 52.8±2.8%; paired t-test, t=1.63, 5 d.f., P=0.164). If the frequencies of response to different chemical solutions were simply additive, the expected frequency of animals responsive to the 50 mmol l\(^{-1}\) NaCl+0.5 mmol l\(^{-1}\) NHT mixture would be 62%. This is calculated as 38.9% (the mean frequency of locusts responsive to 50 mmol l\(^{-1}\) NaCl) plus 37.5% (the frequency of response to 0.5 mmol l\(^{-1}\) NHT) of the remaining 61.1% of locusts. This gives a value of 0.389+(1–0.389)0.375=0.62. A one-sample t-test of this value against the observed frequency of response to the mixture of 0.52±0.028 (t=3.32, 5 d.f., P=0.021) suggests that frequencies of response were not simply additive.

The latencies to response within 1 s broadly followed the same pattern seen in the previous two experiments (Fig. 6E). The time to the start of the response when stimulated by 50 mmol l\(^{-1}\) NaCl or 50 mmol l\(^{-1}\) NaCl+0.05 mmol l\(^{-1}\) NHT was more rapid than to solutions only containing NHT or 50 mmol l\(^{-1}\) NaCl combined with the higher concentration of NHT. Similarly, as shown in Fig. 6F, movements elicited by NaCl-containing solutions were more rapid than those in response to the NHT solutions (ANOVA, F\(_{5,135}=2.74, P=0.017\)), but there was a trend for the movements elicited by the NaCl solution containing the higher concentration of NHT to be of longer duration than that in response to NaCl alone (Fig. 6F).

Discussion

Responses to stimulation with chemical solutions

All the observed behavioural responses occurring within 1 s of stimulation by chemical solutions were avoidance responses, and the likelihood of evoking a response was strongly linked to both chemical identity and concentration. Moreover, the frequency of response appeared to be a function of the combination of chemicals present in the stimulus and could not be simply predicted from the responses to its individual constituents. The responses were broadly similar regardless of the chemical used in the droplet. The withdrawal type of movement is similar to responses evoked by tactile stimuli (Pflüger, 1980; Siegler and Burrows, 1986) or by acidic vapour (Newland, 1998). The replacement class of movement has not been explicitly described in these other studies. The initial sequence of movements involved in lifting the leg is similar for both the withdrawal and replacement types of behaviour and also resembles the ‘tarsus on tarsus’ repositioning reflex seen in walking insects (Graham, 1985). As no data are available describing the period during which the leg was held in the lifted position in previous studies, it is possible that both categories of response may have been treated together. It is unlikely that replacement movements are specific to contact-chemosensory stimuli because responses to water droplets were equally likely to be replacements as withdrawals and, with the exception of lysine glutamate, were a minor fraction of the responses at higher chemical concentrations.

The major effect of adding a chemical stimulus to a water droplet was to increase the likelihood of eliciting a response, and this probability was strongly correlated with chemical concentration. For three of the four test chemicals, increasing concentration had little observable effect on the dynamics of the ensuing movement, only on the probability of the response happening. NaCl differed from the other test chemicals in that higher concentrations led to a large decrease in both the latency to response and the duration of the movement. The duration of the avoidance response to acetic acid odour found by Newland (1998) was 193±11.6 ms, which compares closely with the mean duration of responses to 100 mmol l\(^{-1}\) NaCl of 170±20 ms. The mean movement duration in response to the other chemicals was over 100 ms longer. It remains to be seen whether these apparent differences in response dynamics, between rapid responses to NaCl (and possibly acetic acid) and the longer time course of responses to the other chemicals, reflect differences in the central processing of these chemical stimuli.

The frequency with which locusts responded to any particular solution may be derived from one of two possible factors. It may represent a genuine behavioural decision and be related to the probability of whether an individual animal will respond to a given stimulus. If so, individual locusts would be expected to vary considerably in response to concentrations of intermediate effectiveness. The second possibility is that the frequency of response represents absolute differences in concentration threshold between individuals, in which case individual locusts would be expected to behave more consistently. This may be a difficult question to resolve because chemoreceptor sensitivity varies with factors such as the time since the last meal (Bernays et al., 1972; Simpson and Raubenheimer, 1993b) and over shorter time scales is subject to considerable adaptation (White and Chapman, 1990).

The applied droplets unavoidably combined both mechanical and chemical stimuli and may be expected to
have activated exteroceptive and possibly proprioceptive mechanosensory neurones. Stimulation of individual tactile hairs is a fairly weak stimulus and only infrequently evokes withdrawal movements (Pflüger, 1980). It seems likely that the response frequency to water and to the lowest concentrations of the test chemicals (approximately 10–20 % of cases) represents a baseline level of response to the mechanical component of the stimulus. It is possible, however, that there is some hygrosensory or other sensory input onto the neural pathways controlling the reflex, but this does not appear to have a strong influence on withdrawal behaviour.

**Significance of response patterns to chemosensory processing**

The movements evoked by chemical stimulation are very similar to the responses to tactile stimulation. A great deal is already known about the neural organisation of tactile withdrawal reflexes within the thoracic ganglia, and many of the principles underlying the integration of local sensory stimuli have been extensively analysed (Burrows, 1996). The signals from the mechanosensory afferents in the basiconic sensilla are already known to be integrated in the metathoracic ganglion to form part of an extensive exteroceptive detector system sensitive to the displacement and velocity of contact (Newland and Burrows, 1994). Furthermore, it has recently been shown that a midline population of spiking local interneurones, which are important components in the organisation of tactile reflexes, may be involved in the integration of both mechanosensory and chemosensory stimuli (Newland, 1999; Rogers and Newland, 1998).

An investigation of chemosensory processing at the level of the thoracic ganglia may present practical advantages over studying chemoreception by the mouthparts and may provide a useful general model in understanding the coding and integration of gustatory signals. The neuronal networks in the suboesophageal ganglion and brain responsible for integrating sensory information and controlling feeding behaviour are largely unknown. At the behavioural level, analysing local leg responses to chemical stimulation is simpler than unravelling the complex interlocking rhythms of activity by multiple body parts that constitute feeding behaviour.

The chemical stimuli used in this study have previously been demonstrated to signify a variety of qualities to locusts, particularly in terms of feeding. The site of stimulation used here, the hind leg, would appear at first to have little direct connection with the process of feeding. Nevertheless, it might be expected that chemosensation over the entire body surface would be directed towards similar overarching behavioural outcomes and that different parts of the body should not produce strongly conflicting responses to a particular chemical stimulus. The apparent paradox that all the chemicals used in this study produced aversive withdrawal responses can be resolved when the concentration ranges at which the chemicals became effective stimuli are examined.

The two classes of nutrient chemical only became effective stimuli at concentrations more than two orders of magnitude greater than those sufficient to stimulate responses to NHT in 50 % of cases, while NaCl was of intermediate effectiveness. Recently, studies on food selection and feeding by locusts have strongly suggested that gustatory stimuli need not be intrinsically phago-stimulatory or deterrent but that their appetitive quality may depend greatly on the blend and concentration of all the constituent chemicals in the potential food (Simpson and Raubenheimer, 1993a, 1996; Simpson, 1994). Even nutrient chemicals may not promote feeding if their concentration is too great and they are present in unbalanced amounts relative to other essential compounds. The concentrations and blends of chemicals sufficient to elicit a leg withdrawal reflex may provide a corollary to food choice in locusts. This is not to suggest that the strong leg withdrawal reflexes of the kind described here need be a common feature of normal locust exploratory behaviour. Indeed, the animals were disturbed, hooded and presented with the stimulus in an unfamiliar context. However, these data may reflect on how chemosensory information is used to make local adjustments to posture and movement during normal walking behaviour in a manner similar to that suggested for tactile local reflexes (Burrows, 1996). The chemosensory inputs may, for example, dissuade a locust from remaining on an unsuitable substratum before other food sampling behaviour even begins.

Although it is clear that chemosensory inputs may promote local responses, this does not in any way exclude the presence of other pathways in the central nervous system in which chemosensory qualities may be coded in a different manner. In particular, if appropriate chemosensory stimulation of the tarsi cues food sampling behaviour by the mouthparts, then chemical concentrations lower than those that elicit reflex withdrawal movements of the legs would be expected to excite neurones conveying this information from the thorax to the head. Such intersegmental coordination is of necessity highly complex and may be expected to be highly context-dependent. Food sampling behaviour by the mouthparts may only follow on from sampling by the legs when the locust is in an appropriate motivational state, undisturbed by experimental manipulation and/or hungry, for example. Local reflex movements and chemosensory processing in the neuronal circuits organising them will probably be more easily evoked than feeding behaviour in physiological preparations, and the data from this study will give a vital context to our investigations of chemosensory processing within the metathoracic ganglion. In particular, they give some indication of the behavioural effects of different chemical types and the ends towards which chemosensory processing may be directed at the local level.

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References


