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Chemosensory tuning to a host recognition cue in the facultative specialist larvae of the moth *Manduca sexta*

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Summary

Larvae of *Manduca sexta* are facultative specialists on plants in the family Solanaceae. Larvae reared on solanaceous foliage develop a strong preference for their host; otherwise, they remain polyphagous. The host-specific recognition cue in potato foliage for *Manduca* larvae is the steroidal glycoside, indioside D. Two pairs of galeal taste sensilla, the lateral and medial sensilla styloconica, are both necessary and sufficient for the feeding preferences of host-restricted larvae. We conducted electrophysiological tip recordings from sensilla of solanaceous or wheat germ diet-reared larvae. For each animal, recordings of the responses to indioside D, glucose, tomatine and KCl were compared. All responses included both phasic and tonic portions. The sensilla styloconica of solanaceous-reared larvae were tuned to indioside D, defined as maintaining a high sensitivity to indioside D, while showing lower sensitivity to other plant compounds. Half of the sensillar neurons of solanaceous-reared larvae were 'tuned' to indioside D, whereas those of wheat germ diet-reared larvae were not. The different responses between the two types of animals were a result of changes of individual receptor cells' responses in the sensilla. Feeding on solanaceous foliage therefore appears to result in a modification of the physiological responses of individual taste receptor cells that causes them to be tuned to the host-recognition cue indioside D. We propose that this tuning is the basis for the host-restricted larvae's strong behavioral preferences for solanaceous foliage.

Key words: indioside D, *Manduca sexta*, larva, sensilla, taste receptor, host recognition.

Introduction

In many animals, food choices are modified by dietary experience. Some of the most extensive studies of the neural bases for the effect of experience on food selection have been carried out in phytophagous insects. Insects are good models for these studies because of their clear food preferences and their relatively simple nervous systems (Bernays and Chapman, 1987; Bernays and Weiss, 1996; Szentesi and Jermy, 1990). One approach in the search for chemosensory mechanisms involved in food choices has been to add chemicals to artificial diets, test the animals' food choices after this experience, and record the neural responses in these animals' chemosensory organs (Glendinning and Gonzalez; 1995; Schoonhoven, 1987; van Loon, 1990). Many of these studies have focused on the behavioral and neuronal responses to deterrent chemicals. Such studies have demonstrated that deterrent compounds added to artificial diets modify taste sensillar responses by habituation, resulting in a decreased sensitivity to that stimulus (Bernays and Chapman, 1987, 1994; Bernays and Weiss, 1996; Shepherd, 1988; Szentesi and Jermy, 1990). While these findings have helped explain why phytophagous insects might become insensitive to feeding deterrents, they did not address the fundamental question of why, in the first place, phytophagous insects initiate feeding.

The larvae of *Manduca sexta* (Sphingidae) are facultative specialists on plants in the family Solanaceae. When they feed on solanaceous foliage, the larvae develop a strong preference for these plants, rejecting any other potential food. In contrast, when they feed on non-solanaceous plants or diets based on non-solanaceous foliage, they remain polyphagous (del Campo, 1999; del Campo and Renwick, 1999, 2000; del Campo et al., 2001; Jermy et al., 1968; Rothschild et al., 1979; Schoonhoven, 1967; Yamamoto, 1974; Yamamoto and Fraenkel, 1960). In previous studies, we found that at least one of the reasons why *Manduca* larvae become host-restricted when they feed on solanaceous plants is that they develop a preference for one plant compound, indioside D, a steroidal glycoside so far only found in Solanaceae (del Campo, 1999; del Campo and Renwick, 2000; del Campo et al., 2001; Yahara et al., 1996). Host-restricted *Manduca* larvae would eat non-solanaceous food when it was treated with indioside D or mixtures of plant compounds containing indioside D (del Campo, 1999; del Campo and Renwick, 1999, 2000; del

Campo et al., 2001). Indioside D was isolated from potato foliage by bioassay guided fractionation (del Campo, 1999; del Campo and Renwick, 2000; del Campo et al., 2001). Of all the potato foliage extracts and their chemical fractions containing thousands of plant compounds, only indioside D caused host-restricted larvae to feed on a non-host (del Campo, 1999; del Campo and Renwick, 1999, 2000; del Campo et al., 2001). It was therefore concluded that for potato, the recognition cue used by host-restricted *Manduca* larvae was indioside D.

Host-restricted larvae choose their food based on input from taste receptor cells located within chemosensory sensilla on their mouthparts. There are four sets of external chemosensory sensilla. Antennal, maxillary palp and epipharyngeal sensilla respond to chemical cues in *Manduca* larvae but they do not appear to play a significant role in host-restricted feeding behavior (de Boer, 1991a,b, 1993; de Boer and Hanson, 1987; Glendinning et al., 1998). This behavior is mediated entirely by the sensilla styloconica located on the galea, as removal of these sensilla completely eliminates food preference by host-restricted larvae (del Campo, 1999; del Campo et al., 2001; Flowers and Yamamoto, 1992; Waldbauer and Fraenkel, 1961). Thus, the sensory input from these sensilla is both necessary and sufficient for host recognition by host-restricted larvae. The bilaterally paired lateral and medial sensilla styloconica each contain four chemoreceptor cells, which project to the subesophageal ganglion, where the circuitry for chewing is located (Griss, 1990; Griss et al., 1991; Kent and Hildebrand, 1987; Rohrbacher, 1994a,b). The responses of the sensilla styloconica to a variety of chemical compounds have been examined (Bernays et al., 1998; Glendinning et al., 2001; Glendinning and Hills, 1997; Glendinning et al., 1998, 1999a,b, 2000, 2002; Dethier and Crnjar, 1982; Peterson et al., 1993; Schoonhoven, 1969a,b, 1977; Schoonhoven and Dethier, 1966; Schoonhoven and van Loon, 2002; Städler and Hanson, 1976). In a few cases, comparisons have been made of the responses of these sensilla to plant or other compounds in plant-reared and wheat germ diet-reared larvae (del Campo, 1999; del Campo et al., 2001; Schoonhoven, 1969a; Städler and Hanson, 1976; van Loon, 1990). Larvae of the cabbage butterfly, *Pieris*, reared on a wheat germ-based diet, showed reduced sensitivities of the sensilla styloconica to deterrent compounds (van Loon, 1990). For *Manduca* larvae, it has been difficult to explain the differences in food preferences of diet-reared and solanaceous-reared larvae by differences in the responses of the sensilla styloconica to chemical compounds. One missing factor in these studies has been the relevant natural cue(s) for *Manduca* to recognize suitable food (Bernays, 1996; del Campo and Renwick, 2000; del Campo et al., 2001). Our recent discovery of indioside D allows us to study the effects of dietary experience on the responses of the sensilla to natural cues that the larvae use to identify their normal host plants.

In a previous study we found that in plant-reared larvae, the lateral sensilla styloconica become ‘tuned’ to indioside D by reducing their responses to at least three other compounds that can be found in foliage, while maintaining their sensitivity to indioside D. We called this change in response chemosensory tuning (del Campo et al., 2001). Here, we present additional evidence for chemosensory tuning in both the lateral and medial sensilla styloconica. We selected representative compounds from four major plant chemical classes that might be relevant to a feeding larvae: sucrose representing a generalized nutrient, KCl representing a generalized salt, tomatine representing a solanaceous compound that is not used as a specific recognition cue, and indioside D representing a solanaceous compound that is a specific recognition cue for *Manduca* larvae. From these data, we develop a model to describe how the host-restricted behavior of solanaceous-reared larvae might be accomplished by sensory tuning and its integration in the CNS.

Materials and methods

Animals

To study the effect of dietary experience on the responses of the lateral and medial sensilla in *Manduca sexta* Johan larvae, eggs were collected from a laboratory colony reared on a wheat germ-based diet (Bell and Joachim, 1976) at Binghamton University. Hatchlings that were to be reared on wheat germ diet were transferred to individual transparent plastic cups containing the diet. Hatchlings that were to be reared on solanaceous plants were transferred to potato foliage. The potato plants used for rearing these larvae were at least 3 weeks old and, before flowering, maintained in a greenhouse under a 16 h:8 h L:D photoperiod. Larvae reared on either diet were allowed to feed *ad libitum*, at ~25°C under a 16 h:8 h L:D photoperiod. Larvae continued their development on either of these diets until their fifth instar, when they were collected for experimental procedures.

Electrophysiological recordings

Satiated larvae were anesthetized by submerging them in water. They were prepared for recording by placing them in vials of tapwater so that all spiracles remained submerged with the head exposed for recording, using a modification of the techniques described by Gothilf and Hanson (1994). The head was fixed into position with soft wax so that the medial and lateral sensilla styloconica were accessible to recording electrodes. A ground electrode was inserted into the mandibular musculature through a small hole in the head capsule. Tip recordings were performed on the lateral and medial sensilla of one side of the mouthparts of each animal using glass microelectrodes filled with the test solutions described below. The order in which the test solutions were applied to each sensillum was randomized. The sensilla’s response was recorded for at least 1 min per test solution. Between recordings, mouthparts were rinsed with distilled water and dried with a tissue (Kimwipe®, Kimberly-Clark, Roswell, WI, USA). The signals were amplified with a high impedance amplifier (Getting Instruments, Iowa City, IA, USA) and digitally recorded (Axon Instruments, Union City, CA, USA). Immediately after the electrode contacted the

sensilla a brief stimulus artifact obscured the recording. This artifact ('blocking artifact') had an average duration of 13.4±0.23 ms (mean ± 95% CI; *N*=228 recordings). Even for the highest firing frequencies of approx. 250 Hz, the data lost would produce an error of only about 3.3 spikes, or 1.3% of the total.

Test solutions

All compounds tested were carried in a 50 mmol l^{-1} KCl conducting solution (KCl control solution). This control solution included 0.16% polyvinylpyrrolidone-80 (PVP-80; Sigma Chemical, St Louis, MO, USA) to increase viscosity and reduce evaporation from the electrode tip. It also included 0.1% ethanol and 1% methanol to account for the solubility requirements of tomatine and indioside D, respectively, as described below. Tomatine (Sigma Chemical) was first solubilized in 10% ethanol to a concentration of 10^{-2} mol l^{-1}, and then diluted 100× in 50 mmol l^{-1} KCl with 0.16% PVP-80 and 1% methanol, giving a final tomatine concentration of 100 μmol l^{-1}. Glucose (Sigma Chemical) was dissolved directly in the KCl conducting solution to a concentration of 100 mmol l^{-1}. Indioside D was obtained from fresh potato foliage, using the methods described in del Campo and Renwick (2000), and stored in methanol at a concentration of 1.2×10^{-4} mol l^{-1}. For neurophysiological recordings, it was diluted 100× in 50 mmol l^{-1} KCl with 0.16% PVP-80 and 0.1% ethanol, to a final concentration of 1.2 μmol l^{-1}. All solutions were refrigerated between recording sessions and used within 1 week of preparation.

Data and statistical analyses

For each recording, digital traces were sampled for spike frequency at the onset of the recording (time=0), 1, 5, 15 and 30 s. Spikes were counted for periods of 200 ms at times 0 and 1 s, and 500 ms at all other times. Analyses were conducted independently on lateral and medial sensilla. At analysis, the responses of the sensilla to glucose, tomatine and indioside D were always compared to their responses to the KCl solution. Responses were considered 'sensitive' to a solution when they were significantly higher than the response to KCl alone. For the phasic portion of the response of each sensillum, the first 200 ms after contact were analyzed using two-way analysis of variance (ANOVA) tests. The factors in these fully factorial models were diet, solution and their interaction. When a significant effect was found, a Dunnett *post-hoc* test was conducted to contrast responses to different solutions against the KCl control solution. For the tonic portion of the responses, repeated-measure analyses were conducted on the sampled values for each sensillum. Independent variables in the model were diet, solution, and their interaction. Animal was included in the model as a covariant factor to control for variability between the animals. The effect of these variables was studied over time and independently from time. To contrast further the responses to each solution, repeated measure analyses were conducted for each type of dietary experience (solanaceous or wheat germ diet) to detect different sensillar responses to the solutions. In addition, the digitized responses of the sensilla styloconica were analyzed by individual cell spike counts between 1 and 2 s after contact. At this time, spike shapes are quite constant. Each sensillum contains four sensory cells. These have been classified in a number of studies as sugar-sensitive, salt-sensitive, inositol-sensitive and deterrent cells (for a review, see Schoonhoven and van Loon, 2002). We did not attempt to categorize the cells by their sensitivities to specific substances, but classified them based on their relative amplitudes, their rise times and interspike intervals, using commercial software (Synaptosoft, Decatur, GA, USA), and confirmed the results by visual inspection. Four categories of cells were distinguished: a small amplitude (S) cell, two medium amplitude units (M1 and M2), distinguished by their rise times, and a large amplitude unit (L). For the lateral sensillum, S cells had about half the amplitude of the M cells; L cells had approximately twice the amplitude of the M cells. M cells were discriminated by their rise times, which were approximately 0.3 ms for M1 and 0.8 ms for M2. For the medial sensillum, the S cells also had about half the amplitude of the M cells. The L cells were approximately 50% larger in amplitude than the M cells. M cells were discriminated by their rise times of 0.4 ms for M1 and 0.9 ms for M2. Two-way ANOVA statistical tests were conducted for each individual cell type data set. The factors in these fully factorial models were diet, solution, and their interaction. All statistical analyses were conducted using SPSS 10.0 (Chicago, IL, USA).

Results

In the lateral and in the medial sensilla styloconica, 3–4 units were active. For solanaceous-reared and diet-reared larvae,

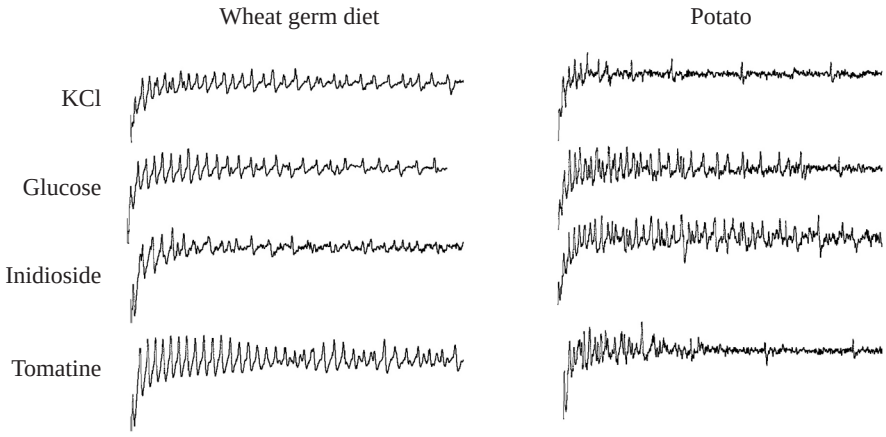

Fig. 1. Typical taste receptor responses to chemostimulation of the lateral sensillum at initial contact. Traces are for a 200 ms period, and for each diet all traces are from the same larva.

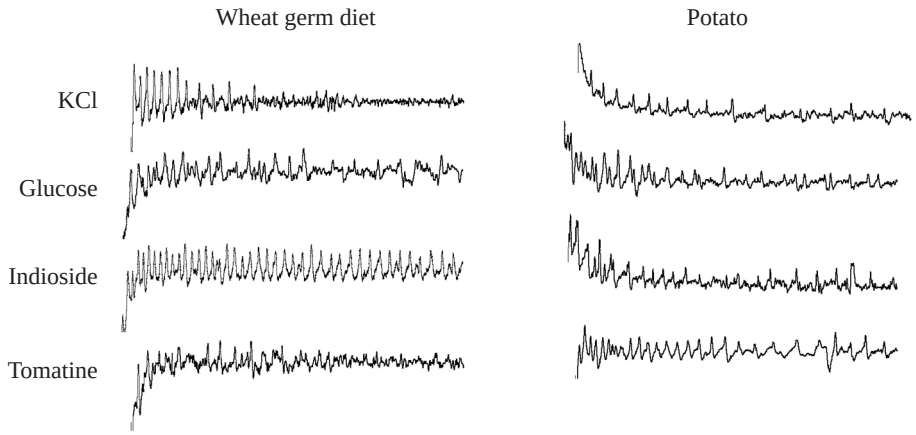

Fig. 2. Typical taste receptor responses to chemostimulation of the medial sensillum at initial contact. Traces are for a 200 ms period, and for each diet all traces are from the same larva.

both sensilla responded to all tested solutions (KCl, tomatine, glucose and indioside) in a phasic–tonic fashion. The phasic portion of the responses typically lasted less than 5 s, showing a rapid decline in spike frequency (>60%). The subsequent tonic portion of the response continued with little decline in spike frequency for the rest of the recording period, which lasted at least 1 min. All recordings showed at least a low level of firing throughout the recording period.

Phasic responses of the lateral and medial sensilla

During the first 200 ms after contact (Figs 1, 2), the responses of the lateral sensillum (Fig. 3A) to the tested solutions were strongly affected by the dietary experience of the larvae (P<0.001, F=24.28, d.f.=1), and the type of solution applied (P<0.001, F=11.74, d.f.=3). The responses of the lateral sensilla to the different solutions were not affected by dietary experience in the same fashion (P<0.001, F=6.64, d.f.=3). For larvae reared on wheat germ diet, the firing frequency of the lateral sensillum did not differ significantly from the response to KCl control solution for glucose, indioside or tomatine (Dunnett *post-hoc* tests, P>0.05, for all solution contrasts against KCl). In contrast, for solanaceous-reared larvae, the average firing frequency of the lateral sensillum was over twofold higher for indioside than for KCl (Dunnett *post-hoc* test, contrasts against KCl: P<0.001). However, as in wheat germ diet-reared larvae, responses to tomatine and glucose did not differ significantly from the responses to KCl alone (Dunnett *post-hoc* test, contrasts against KCl: P>0.05 for tomatine and glucose). Moreover, in solanaceous-reared larvae, responses to KCl, tomatine and glucose were significantly lower than the responses to these solutions shown by diet-reared larvae. The firing frequency in response to indioside was similar in both sets of larvae. The substantial sensitivity to indioside shown by the lateral sensilla of the solanaceous-reared larvae was thus primarily due to their much lower responses to the KCl control solution.

In contrast, the responses of the medial sensilla (Fig. 3B) to the tested solutions were weakly affected by the dietary experience of the larvae (P=0.04, F=4.58, d.f.=1), but strongly affected by the type of solution applied (P<0.01, F=4.93, d.f.=3). For wheat germ diet-reared larvae, the medial sensillum showed a significantly higher firing frequency in response to glucose or indioside when compared to its response to the KCl control solution (Dunnett *post-hoc* test, contrasts against KCl: P<0.001 for indioside and glucose). The response to tomatine, however, was not significantly different from the

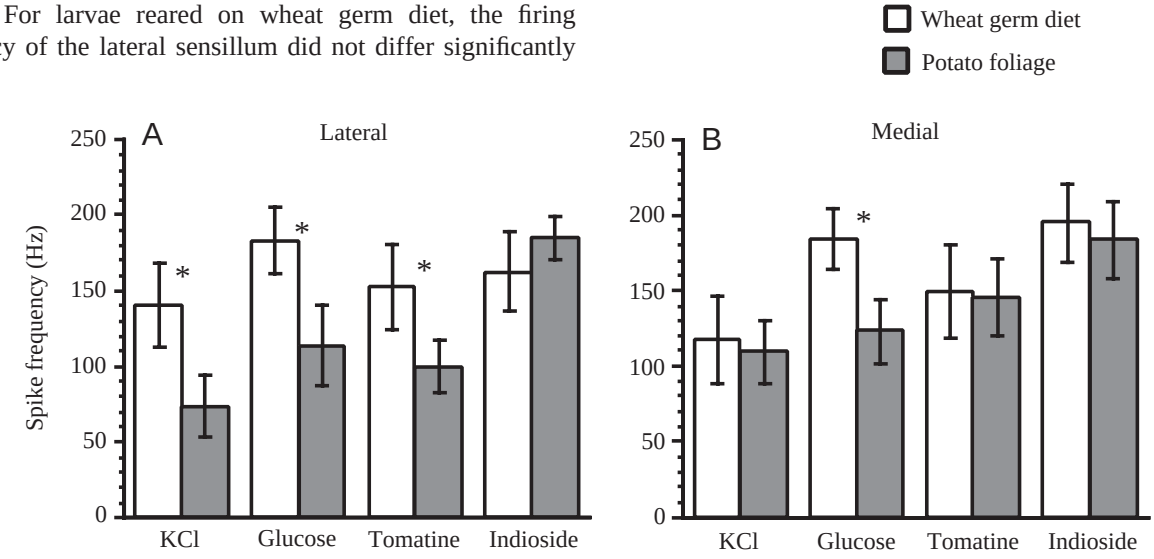

Fig. 3. Phasic portion (first 200 ms after contact) of the chemosensory responses of sensilla styloconica. (A) Lateral sensilla responses of solanaceous-reared larvae (N=16) and wheat germ diet-reared larvae (N=16). (B) Medial sensilla responses of solanaceous-reared larvae (N=10) and wheat germ diet-reared larvae (N=10). Values are means ± S.E.M. *P<0.05. For details, see text.

response to KCl (Dunnett *post-hoc* test, contrast against KCl: $P>0.05$, for tomatine). In contrast, while the medial sensillum of solanaceous-reared larvae showed significantly higher responses to indioside compared to KCl (Dunnett *post-hoc* test, contrast against KCl: $P<0.001$ for indioside), its response to glucose was not significantly different from its response to KCl (Dunnett *post-hoc* test, contrast against KCl: $P>0.05$, for glucose). As for the diet-reared larvae, the response to tomatine was not significantly different from KCl (Dunnett *post-hoc* test, contrast against KCl: $P>0.05$, for tomatine).

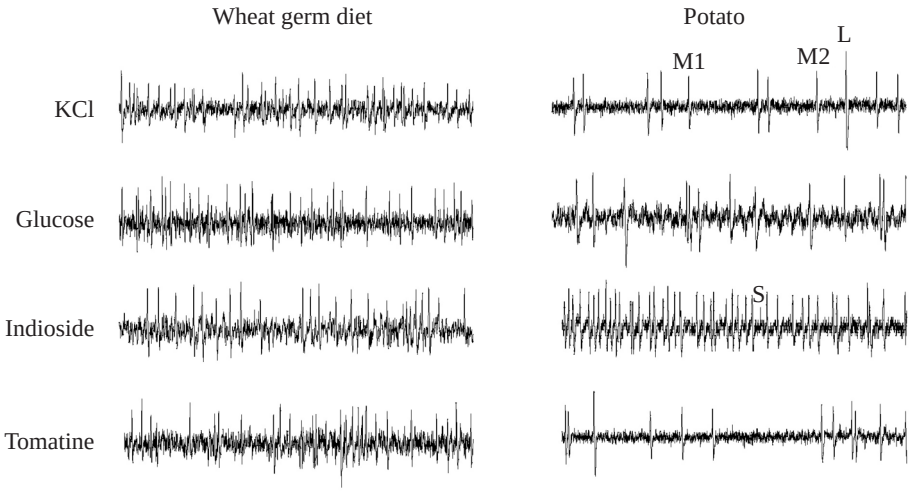

Fig. 4. Typical taste receptor responses to chemostimulation of the lateral sensillum at 1 s after initial contact. Traces are for a 500 ms period, and for each diet all traces are from the same larva. S, M1, M2 and L indicate the different types of cells we identified based on the characteristics described in Materials and methods.

Tonic portion of the responses of the lateral and medial sensilla

The tonic portions (1–30 s after contact) of the responses of lateral sensilla styloconica (Fig. 4) were modified by dietary experience (d.f.=1, $F=28.34$, $P<0.001$) and type of solution (d.f.=3, $F=16.84$, $P<0.001$). Moreover, there was a strong interaction between dietary experience and type of solution tested, which suggested that not all responses to the solutions were modified by dietary experience in the same fashion (d.f.=3, $F=11.14$, $P<0.001$). In wheat germ diet-reared larvae, the tonic portion of the responses of the lateral sensillum to glucose, indioside and tomatine did not differ from the KCl control solution (Fig. 5A, repeated-measures analysis; $P>0.05$). In contrast, the tonic portion of the response of solanaceous-reared larvae to indioside was significantly higher than the responses to the KCl control solution (Fig. 5B). This response was sustained for at least 30 s (over time effect, $P<0.001$), while there was no significant response to glucose ($P>0.05$) or tomatine ($P>0.05$) in comparison to the responses to KCl.

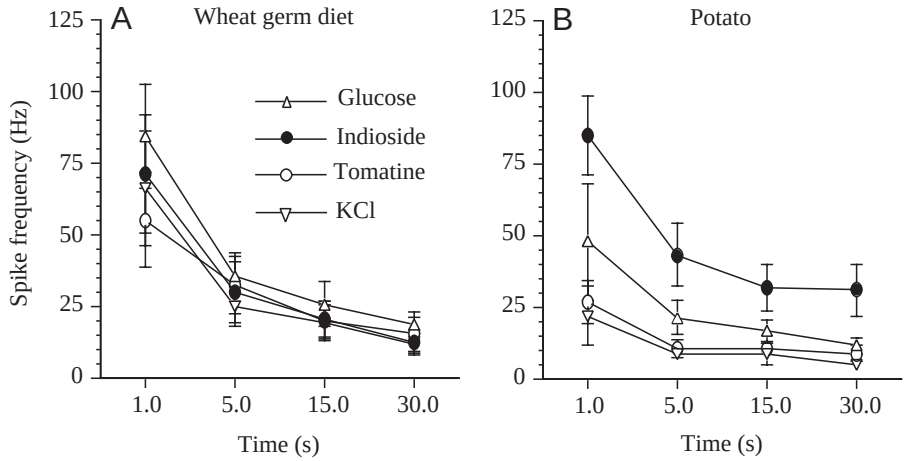

Fig. 5. Tonic portion (1–30 s after contact) of chemosensory responses of the lateral sensilla styloconica. (A) Responses of wheat germ diet-reared larvae ($N=16$). (B) Responses of solanaceous-reared larvae ($N=16$). Values are means ± S.E.M.

The tonic portions of the responses of the medial sensilla styloconica (Fig. 6) were also modified by dietary experience of the larvae (d.f.=1, $F=5.79$, $P<0.02$) and type of solution (d.f.=3, $F=8.98$, $P<0.001$). In this case, however, there was no interaction between dietary experience and type of solution (d.f.=3, $F=0.50$, $P>0.05$). In wheat germ diet-reared larvae (Fig. 7A), the tonic portions of the medial sensilla responses to indioside were higher than KCl control solution for at least 5 s ($P=0.001$), while there were no significant responses to tomatine ($P>0.05$) or glucose ($P>0.05$) in comparison to KCl. This indicates that the sensitivity to glucose that was found in the phasic portion of the medial sensillum's response was lost during the tonic portion. For solanaceous-reared larvae (Fig. 7B) the tonic portions of the responses to indioside were significantly higher than the responses to KCl ($P=0.001$), while there were no significant responses to glucose ($P>0.05$) or tomatine ($P>0.05$). The response to indioside was sustained for at least 30 s after contact (over time effect, $P<0.001$).

Responses of individual cells of the lateral sensillum

Responses of some individual cells in the lateral sensilla styloconica were modified by dietary experience and type of solution at 1 s after contact (Fig. 8). The S cell responses (Fig. 8A) were not significantly modified by the dietary experience of the larvae (d.f.=1, $F=1.67$, $P>0.05$), or type of solution applied (d.f.=3, $F=2.51$, $P>0.05$). There was no

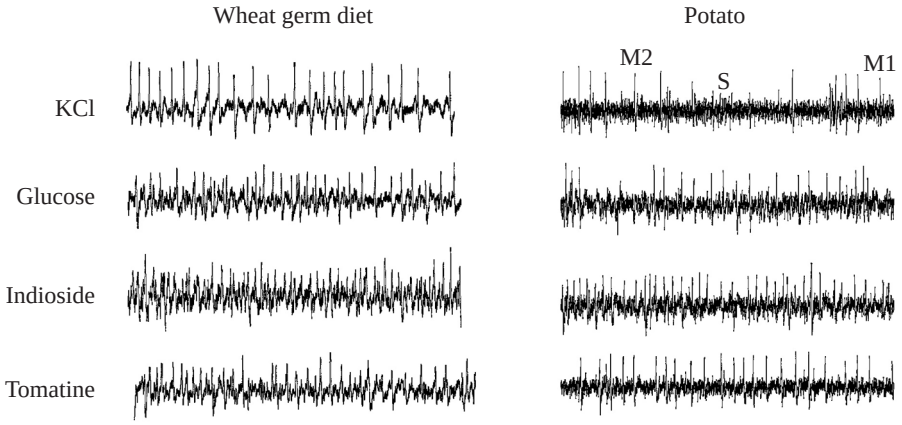

Fig. 6. Typical taste receptor responses to chemostimulation of the medial sensillum at 1 s after initial contact. Traces are for a 500 ms period, and for each diet all traces are from the same larva. The letters S, M1 and M2 indicate the different types of cells we identified based on the characteristics described in the methods. None of the recordings in this data set included an example of an L cell.

interaction of dietary experience and solution applied (d.f.=3, F=0.29, P>0.05). The M1 cell responses of solanaceous-reared larvae were significantly lower than M1 cell responses (Fig. 8B) in wheat germ diet-reared larvae for all solutions except indioside (dietary effect: d.f.=1, F=8.07, P=0.005; solution effect: d.f.=3, F=0.21, P>0.05; interaction of dietary effect and solution applied: d.f.=3, F=2.85, P<0.05). This cell was sensitive to indioside in solanaceous-reared larvae (Dunnett *post-hoc* contrast: P=0.001), but insensitive to all solutions in wheat germ diet-reared larvae. The M2 cell responses (Fig. 8C) of solanaceous-reared larvae were lower than the responses of wheat germ diet-reared larvae for KCl and tomatine; however, the responses to indioside and glucose were not significantly modified by larval diet (dietary effect: d.f.=1, F=12.80, P<0.001; solution effect: d.f.=3, F=2.67, P=0.05; interaction of dietary effect and solution applied: d.f.=3, F=0.44, P>0.05). For wheat germ diet-reared larvae, no M2 responses to any of the solutions applied differed from the KCl control solution, while in solanaceous-reared larvae this cell was sensitive to glucose and indioside (Dunnett *post-hoc* contrasts for indioside and glucose: P<0.05). The L cell responses (Fig. 8D) of solanaceous-reared larvae were significantly lower than responses of wheat germ diet-reared larvae only for KCl control solution (dietary effect: d.f.=1, F=5.02, P<0.01; solution effect: d.f.=3, F=5.02, P<0.01; interaction of dietary effect and solution applied: d.f.=3, F=2.26, P>0.05). This cell was sensitive only to indioside in solanaceous-reared larvae (Dunnett *post-hoc* contrast: P<0.05). For wheat germ diet-reared larvae, none of the applied solutions resulted in a firing frequency in the L cell that was significantly higher than its response to the KCl control solution.

Responses of individual cells of the medial sensillum

In the medial sensillum, only the S cell responses (Fig. 9A) were modified by dietary experience of the larvae and type of solution at 1 s after contact. The S cell responses of solanaceous-reared larvae were significantly lower to glucose, while responses to KCl, indioside and tomatine were not significantly different from the responses of wheat germ diet-reared larvae (dietary effect: d.f.=1, F=4.01, P<0.01; solution effect: d.f.=3, F=3.12, P<0.05; interaction of dietary effect and solution applied: d.f.=3, F=1.12, P>0.05). In solanaceous-reared larvae, this cell was sensitive to indioside when compared to the KCl control solution (Dunnett *post-hoc* contrast: P<0.01). The responses of the M1, M2, and L cells (Fig. 9B–D) were not significantly modified by dietary experience, or type of solution applied (each analysis showed no significant dietary effect or solution effect at the P=0.05 level). No interaction of dietary experience and type of solution was detected in both wheat germ diet- and solanaceous-reared larvae.

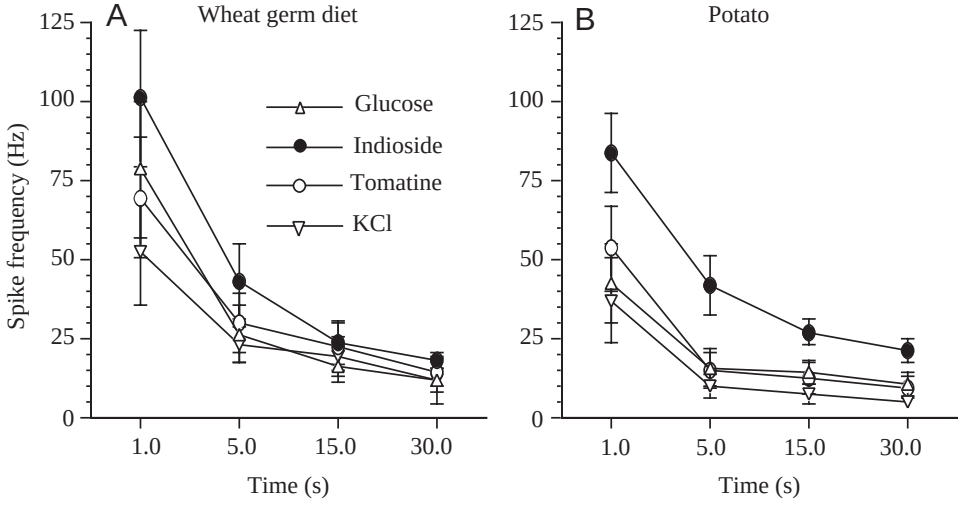

Fig. 7. Tonic portion (1–30 s after contact) of the responses of chemosensory responses of the medial sensilla styloconica. (A) Responses of wheat germ diet-reared larvae (N=11). (B) Responses of solanaceous-reared larvae (N=11). Values are means ± S.E.M.

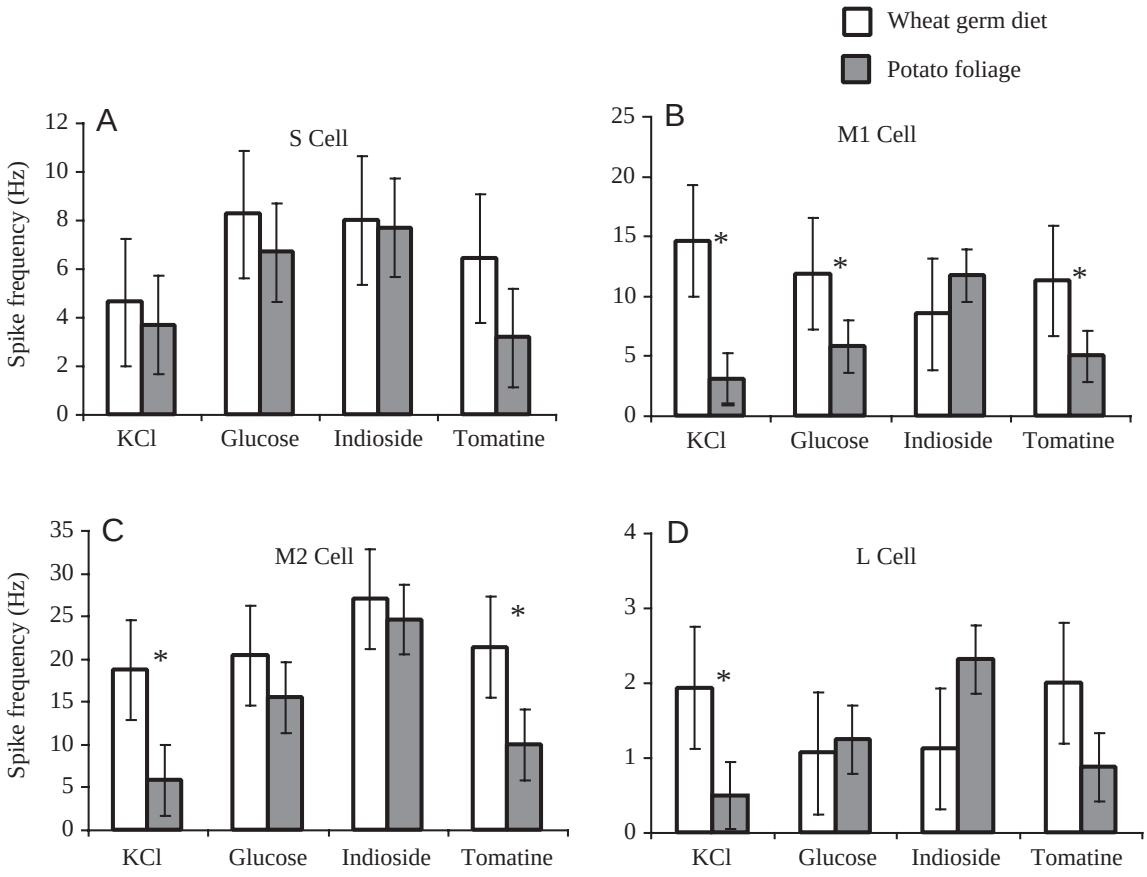

Fig. 8. Responses of individual cells in the lateral sensilla styloconica at 1 s after contact. (A) S cell, (B) M1 cell, (C) M2 cell and (D) L cell. Values are means ± S.E.M. *P<0.05.

Discussion

Our results show that dietary experience determines the responses of chemosensory neurons in the sensilla styloconica of *Manduca sexta* larvae to a variety of plant compounds. Sensilla styloconica of larvae that are reared on their natural host plants have significantly higher firing frequencies to the host recognition cue indioside D than to several other plant compounds. This difference is maintained for both phasic and tonic portions of the sensory response. However, if larvae are reared on a non-solanaceous wheat germ diet, responses of the sensilla styloconica are equally strong to indioside D and the other plant compounds tested. We propose that these differences in the responses of the chemosensory neurons in the sensilla styloconica form the basis for the dramatically different food choices of host-restricted and polyphagous *Manduca* larvae. The sensilla styloconica of polyphagous larvae respond robustly to many different plant compounds and the larvae readily feed on a wide variety of plants. In contrast, the sensilla styloconica of host-restricted larvae are tuned to produce their strongest responses to the host plant cue, indioside D, and these larvae will only feed on plants that contain it. It is certainly possible that other solanaceous plants contain compounds that cause the same behavioral and neurophysiological response. If these compounds exist, it would be interesting to identify them and test their properties in contrast to the responses to indioside D at the behavioral and neurophysiological level.

In our model, tuning the sensilla styloconica to have their strongest responses to a host-specific recognition cue is a central component for the induction of host specificity. There are thousands of chemical compounds found throughout the plant kingdom, and many plant compounds have been tested on the sensilla styloconica of *Manduca* larvae. Many of these plant metabolites produce excitatory responses in the chemosensory neurons of the sensilla styloconica (Bernays et al., 1998; del Campo, 1999; del Campo et al., 2001; Frazier and Hanson, 1986; Glendinning and Hills, 1997; Glendinning et al., 1999a,b; Glendinning et al., 2000, 2001; Peterson et al., 1993; Schoonhoven, 1969a,b, 1972; Schoonhoven and Dethier, 1966). Specific firing patterns for different compounds have been proposed to play a key role in food selection by *Manduca* larvae (Glendinning and Hills, 1997; Peterson et al., 1993). However, these studies did not address the role of dietary experience and host recognition by means of the specific recognition cue for *Manduca* larvae, indioside D (del Campo and Renwick, 2000; del Campo et al., 2001). In our study, we tested indioside as well as three other plant compounds: KCl and glucose, which are ubiquitous among plants, and tomatine, which is restricted to the solanaceae (Boll, 1966; Schreiber et al., 1961).

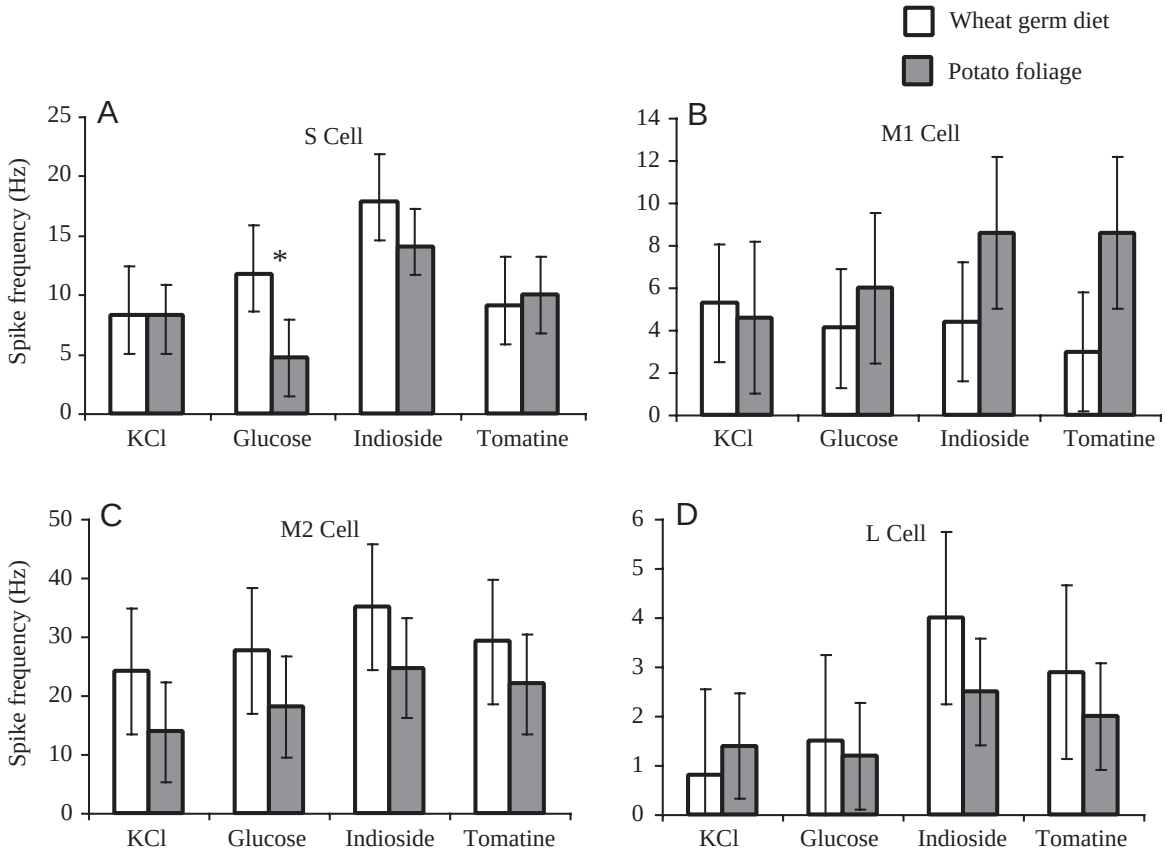

Fig. 9. Responses of individual cells in the medial sensilla styloconica at 1 s after contact. (A) S cell, (B) M1 cell, (C) M2 cell and (D) L cell. Values are means ± S.E.M. *P<0.05.

Response to KCl

We used 50 mmol l^{-1} KCl as our conducting solution for recording the responses of the sensilla styloconica to different plant compounds. This concentration was approximately 50% of the average KCl concentration in potato foliage (Duchateau et al., 1953; Duke and Atchley, 1986) and wheat germ diet (Bell and Joachim, 1976). For the lateral sensilla styloconica, responses to this concentration of KCl were dramatically lower in solanaceous-reared larvae in comparison to larvae reared on wheat germ diet, for both the phasic and tonic potions of the response. Because the concentrations of KCl in wheat germ diet and potato foliage are essentially the same, this difference cannot be attributed to exposure to different KCl concentrations in the respective diets. In contrast, responses of the medial sensilla styloconica to KCl in both types of larvae were not significantly different, being robust for both types of animals. These data suggest that some difference in the two types of diets besides KCl concentration leads to the different sensory responses of the lateral sensilla to KCl in the two sets of larvae. Furthermore, of the eight sensory neurons in the sensilla styloconica of potato-reared larvae, only three of them showed significant lower responses to KCl when compared to wheat germ diet-reared larvae. This indicates that the dietary effect on neural response may be specific for only certain sensory neurons, in this case the L, M1 and M2 neurons of the lateral sensillum.

Response to glucose

Both medial and lateral sensilla styloconica of larvae reared on solanaceous foliage showed lower responses to the glucose solution than sensilla from larvae reared on wheat germ diet. However, this result was significant only for the phasic portion of the response, and was not maintained after 200 ms. In the lateral sensillum of both types of animals, responses to glucose solution were not significantly different from the response to KCl, which was a component of the glucose recording solution. This suggests that during the phasic portion of the response, the lateral sensilla styloconica do not show any specific sensitivity to glucose in either group of animals, and the lower response to glucose solution shown by the solanaceous-reared larvae is due to their lower KCl sensitivity. During the tonic portion of the response, there is a suggestion of sensitivity to glucose at the 1 s and 5 s time points. This may be due to responses of the M2 cell, which shows a significant response to glucose at 1 s. The medial sensilla of wheat germ diet-reared larvae showed significant responses to glucose during the first 200 ms after contact, in comparison to the KCl control solution. As was the case for the lateral sensilla, medial sensilla of solanaceous-reared animals had lower responses to glucose than wheat germ diet-reared animals. This lower response to glucose was not different from the response to the KCl control solution shown by solanaceous-reared animals. Thus, the

response to glucose in the medial sensillum was dependent upon dietary experience. One of the four taste cells in the medial sensillum of wheat germ diet-reared larvae showed a significant response to glucose after 1 s, but we did not determine whether the total sensillar response to glucose during the first 200 ms was due entirely to this cell. Our findings are consistent with the results of Glendinning et al. (2000), who also described a taste receptor cell that responded to glucose in a distinct phasic–tonic manner in wheat germ diet-reared larvae.

It should be noted that our study, like many earlier studies of the responses of taste sensilla to glucose, used a much higher concentration of glucose than is found in foliage, which is typically around 10 mmol l^{-1} (Schoonhoven, 1969b). The concentration of glucose of 100 mmol l^{-1} was selected in this study because it was closest to the concentration used by other researchers, and was done with the intention of comparing our findings with those of previous studies. Concentrations of 100 mmol l^{-1}, 150 mmol l^{-1}, 250 mmol l^{-1} and 300 mmol l^{-1} have all been used experimentally (e.g. Frazier and Hanson, 1986; Glendinning et al., 2000; Peterson et al., 1993; Schoonhoven, 1969b). In feeding assays, even wheat germ diet-reared *Manduca* larvae show only weak or no responses to glucose (Bowdan, 1995; Glendinning et al., 2000). Thus, while a glucose response may be useful for experimental studies of the individual properties of the taste receptor neurons, it appears unlikely to play a direct role in food selection by the animal (Glendinning et al., 2000).

Response to tomatine

We did not find a significant response to the solanaceous alkaloid tomatine, in either the medial or lateral sensilla, of solanaceous or wheat germ diet-reared larvae. We used a concentration of tomatine that is within the normal range for solanaceous foliage (Boll, 1966; Schreiber et al., 1961), and similar to the concentration used in earlier experiments. The earlier studies described a tomatine-sensitive sensory neuron in the sensilla styloconica, which responded to this alkaloid with a delayed bursting firing pattern starting about 30 s after contact (Peterson et al., 1993; Schoonhoven, 1969b). Although we display data for only the first 30 s after the start of a recording, our recordings typically lasted at least 1 min, and we expected to have recorded the bursting response well before the recording period ended. We observed bursting activity in the sensilla styloconica in some recordings, but this did not appear to be a unique response to tomatine as it was found in only 50% of all tomatine recordings, as well as in a number of animals in response to glucose. One difference between the previous studies and our own, is the conducting solution (Peterson et al., 1993; Schoonhoven, 1969b). We used KCl in our experiments, while Peterson et al. (1993) used 100 mmol l^{-1} NaCl. Potato foliage has a very low (<5 mmol l^{-1}) sodium content (Duke and Atchley, 1986), and it is possible that a concentration of 100 mmol l^{-1} in the recording pipette would influence the sensory neurons' responses to other plant compounds, as discussed by Schoonhoven (1969b). Because *Manduca* larvae are unlikely to ever encounter these levels of NaCl in their natural diets, the ecological significance of this response for host selection by *Manduca* larvae in a natural situation is debatable.

Response to indioside D

The clearest responses to a compound we tested on the sensilla styloconica were to indioside D. Both the lateral and medial sensilla styloconica of solanaceous-reared larvae had significantly higher responses to indioside D than to any other compound we tested. This sensitivity was apparent throughout the phasic and tonic portions of the recording.

For wheat germ diet-reared larvae, the lateral sensillum did not have a significant indioside D response. A significant phasic response to this compound was observed for the medial sensillum. This was eliminated by 5 s after contact with indioside D.

The lateral and medial sensilla styloconica of larvae reared on their natural host plants were clearly sensitive to the host recognition cue, indioside D. If the mean spike frequencies of indioside D at each time point are compared with the corresponding indioside D responses in wheat germ diet-reared larvae, they are not significantly different. The sensitivity to indioside D that was shown by solanaceous-reared larvae was thus not due to a higher responsiveness to this compound, but was a function of the lower responses shown by the sensilla of these larvae to other compounds. The sensilla had become 'tuned' to indioside D. Indioside D was the only compound of the three we tested that elicited a significantly higher response than the KCl recording solutions and, therefore, was the only compound to which the sensilla specifically responded. Three of the four sensory cells in the lateral sensillum responded to indioside D, as did one of the four cells in the medial sensillum. Thus, half of the total number of taste receptor neurons in the sensilla styloconica responded to the chemical cue that is both necessary and sufficient for host-restricted feeding behavior. The cellular mechanism(s) by which sensilla exposed to potato foliage are induced to become tuned to indioside D is not known. This could be by direct contact of indioside D and/or other compounds in the foliage with the sensilla, or by way of a post-ingestive feedback mechanism, as has been described for sensillar sensitivity to amino acids in the locust (Abisgold and Simpson, 1988).

We expect that indioside D or a structurally related compound(s) is present in the foliage of most Solanaceae, although this remains to be determined. A number of earlier studies have tested responses of the sensilla styloconica to solanaceous and other types of plant saps. These recordings are comparable to the phasic portion of our longer lasting recordings, and for saps from tomato or Jerusalem cherry (both Solanaceae) the approximately 200 Hz spike frequencies reported by Dethier and Crnjar (1982) are consistent with our values for the phasic portion of the response to indioside D. For the phasic–tonic portion of the response (0.2–1.2 s after contact), Schoonhoven (1969a) reported that sensilla

styloconica of tomato-reared larvae were sensitive to solanaceous plant saps, which resulted in spike frequencies comparable to the responses we found for indioside D alone for this time period. Interestingly, plant saps from non-host plants, which we expect do not contain indioside D, produced initial spike frequencies that were substantially lower than the responses we observed for indioside (Peterson et al., 1993; Schoonhoven, 1969a).

Individual cells vs. total sensillar response

The four sensory cells in each sensillum styloconicum of *Manduca* larvae have been classified by their sensitivities and patterns of response to particular compounds or classes of compounds (Schoonhoven, 1969a; Peterson et al., 1993; Glendinning and Hills, 1997). Early studies recognized that chemosensory neurons in the sensilla styloconica responded to multiple plant compounds, and that it was difficult to determine whether the individual cell responses and/or the sum of cell responses was the relevant information for the CNS to initiate feeding (Dethier, 1973; Frazier and Hanson, 1986; Schoonhoven, 1969a,b, 1977; Schoonhoven and Dethier, 1966). More recently, Bernays et al. (2002) also found that relevant chemical cues for the larvae of the Arctiidae moth *Estigmene acraea*, the pyrrolizidine alkaloids, stimulated three cells in the lateral sensilla at their natural concentration. In addition, they found that only one cell responded in a dose-dependent manner to pyrrolizidine alkaloids, and its sensitivity was very high, although the lowest concentrations that could stimulate this cell were probably not behaviorally significant (Bernays et al., 2002). Our results showed that multiple cells respond to indioside at approximately a fourth of its normal concentration in potato foliage. We did not conduct a dose-dependent study with indioside D in *Manduca*. It is possible that different neurons within the sensilla styloconica of *Manduca* have different sensitivities to indioside, and that we may be able to identify a cell that is particularly sensitive to it by lowering its concentration in our recording solutions. However, the focus of the present study was to understand how the responses of the sensilla styloconica to approximately natural concentrations of host and non-host plant compounds might be interpreted by the CNS to determine whether or not a feeding bout is initiated and sustained. Thus, while an individual neuron's responses to specific compounds may be critical for understanding different mechanisms of how taste sensilla function and are modified by experience in *Manduca*, the role of the total input from the sensilla in triggering a feeding bout cannot be ignored.

Phasic versus *tonic responses*

Both phasic and tonic responses of the sensilla styloconica are likely to be important for feeding. Prior to the initiation of a feeding bout, larvae 'taste' the surface of a leaf by repeatedly touching it with their mouthparts without biting it, producing repeated brief contacts between the taste sensilla and the leaf surface (Devitt and Smith, 1985; Miles and Booker, 2000). More interestingly, Devitt and Smith (1985), using high speed video recordings of feeding *Euxoa messoria* caterpillars, found that only one set of chemosensilla, the lateral and medial sensilla styloconica located on the galeae, remains in contact with foliage throughout a feeding sweep. Each sweep is a set of bites on a leaf with no interruption, and several sweeps comprise a full feeding bout. Only a few milliseconds before the caterpillar's mandibles close on the foliage to complete the first bite, the galeal chemosensilla (lateral and medial sensilla styloconica) contact the leaf surface, remaining in contact with the foliage until just a few milliseconds prior to the end of the feeding sweep. This would first produce a phasic input to the CNS, probably essential for the initiation of the first bite in a feeding bout. Once feeding has been initiated, the tonic responses of the sensilla styloconica, still in contact with the foliage, would provide a continuous source of excitatory inputs to the feeding circuitry. The importance of such input for maintaining a feeding bout has been described in a number of insects (Abisgold and Simpson, 1988; Barton-Browne, 1975; Bernays, 1985; Bernays and Simpson, 1982). Moreover, another piece of evidence for the requirement of continous sensillar contact with food to sustain a feeding bout was provided by Dethier and Crnjar (1982). They reported that *Manduca* larvae do not immediately stop feeding if host foliage is quickly replaced by non-host foliage during a feeding bout; they only began to react about 15 s after the exchange. This phenomenon may be at least partly due to the continued excitation of the chemosensory neurons by plant sap remaining on the mouthparts: an almost constant stimulation to the sensilla styloconica.

Role of sensilla inputs on feeding circuitry; a model for initiating feeding

A central pattern generator (CPG) for chewing has been localized to the subesophageal ganglion (Griss, 1990; Rohrbacher, 1994a,b; Bowden and Wyse, 2000). Elimination of thoracic input to the subesophageal ganglion causes continuous chewing movements of the mandibles, leading to the conclusion that there is an inhibitory input to this circuit of thoracic origin (Griss et al., 1991; Rowell and Simpson, 1992). Excitatory sensory input resulting from the application of solanaceous plant sap to the mouthparts increases the frequency of the chewing rhythm in larvae with intact or cut connectives (Griss et al., 1991; Rowell and Simpson, 1992). These observations led Rowell and Simpson (1992) to propose that feeding was triggered when the level of excitation from chemo- and mechano-sensilla on the mouthparts surpassed a threshold of inhibition to the chewing CPG that was set in the thoracic ganglia. The source of this inhibition is not known, but it was hypothesized to be related to the hunger status of the larva. An additional source of inhibition to the chewing CPG could be from taste receptor cells that respond to deterrent compounds in plant saps (Frazier and Hanson, 1986; Glendinning and Hills, 1997; Glendinning et al., 1999a,b, 2000, 2001; Peterson et al., 1993; Schoonhoven, 1969a,b, 1972). Because host-restricted feeding behavior in *Manduca* is dependent only on the sensilla styloconica (Waldbauer and

Fraenkel, 1961; del Campo et al., 2001), the dramatically different food choices shown by wheat germ diet-reared and solanaceous foliage-reared larvae must be based on differences in the contributions of these sensilla to the total excitatory and inhibitory input to the feeding circuitry. We propose that in order to activate the chewing circuit and initiate feeding, the total excitatory input from all taste sensilla on the mouthparts must be sufficient to surpass a threshold level of inhibition to this circuitry that is determined by thoracic inhibition and any inputs from deterrent sensory cells. For wheat germ diet-reared larvae, the excitatory inputs from the sensilla styloconica help exceed this threshold by responding robustly to many different plant compounds, and should therefore initiate feeding bouts on many different plants, as has been described (de Boer, 1992; Schoonhoven, 1967; Yamamoto, 1974; del Campo and Renwick, 1999). In contrast, the sensilla styloconica of larvae reared on solanaceous foliage are tuned to indioside D, with relatively low responses to other plant compounds, and thus would not contribute sufficient excitatory input to initiate a feeding bout unless the host-specific indioside D is part of the stimulus. Our study tested representatives of four types of potentially relevant chemical stimuli; a salt, a sugar, a solanaceous specific compound that is not used as a recognition cue and the specific host recognition cue for these larvae. Expanding the variety of tested compounds to include more types of salts, sugars and deterrents at different concentrations would be a useful way to further test our model.

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