The Journal of Experimental Biology 210, 3054-3067
Published by The Company of Biologists 2007
doi:10.1242/jeb.004671

The hungry caterpillar: an analysis of how carbohydrates stimulate feeding in *Manduca sexta*

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Accepted 26 June 2007

Summary

In most insects, the taste of carbohydrates stimulates an immediate appetitive response. The caterpillar of *Manduca sexta* is an exception to this general pattern. Despite eliciting a strong peripheral gustatory response, high concentrations of carbohydrates (e.g. glucose or inositol) stimulate the same intensity of biting as water during 2-min tests. We suspected that the lack of feeding stimulation reflected the fact that prior studies used single carbohydrates (e.g. sucrose), which *M. sexta* would rarely encounter in its host plants. We hypothesized that the feeding control system of *M. sexta* responds selectively to carbohydrate mixtures. To test this hypothesis, we ran three experiments. First, we stimulated the two taste sensilla that respond to carbohydrates (the lateral and medial styloconic) with a battery of carbohydrates. These sensilla responded exclusively to sucrose, glucose and inositol. Second, we determined the response properties of the carbohydrate-sensitive taste cells within both sensilla. We found that one class of carbohydrate-sensitive taste cell responded to sucrose, and two other classes each responded to glucose and inositol. Third, we examined the initial biting responses of caterpillars to disks treated with solutions containing single carbohydrates (sucrose, glucose or inositol) or binary mixtures of these carbohydrates. The only solutions that stimulated sustained biting were those that activated all three classes of taste cell (i.e. sucrose+inositol or sucrose+glucose). We propose that the brain of *M. sexta* monitors input from the different classes of carbohydrate-sensitive taste cell, and generates protracted feeding responses only when all three classes are activated.

Key words: taste cell, carbohydrates, sugars, sensory coding, feeding, *Manduca sexta*.

Introduction

In 1988, Schoonhoven and Blom developed a model to explain how taste modulates feeding in insects (Schoonhoven and Blom, 1988). They proposed that the size and nature of an insect's feeding response are determined by the algebraic sum of inputs from two functionally distinct classes of taste cell: one responds to nutrients (e.g. carbohydrates or amino acids) and the other responds to unpalatable compounds (i.e. 'bitter' taste stimuli). Greater input from the nutrient-sensitive taste cells was hypothesized to elicit an appetitive response, whereas greater input from the bitter-sensitive taste cells was hypothesized to elicit an aversive response. There is widespread support for this model, spanning at least four insect orders: Lepidoptera (Ma, 1972; Blom, 1978; Schoonhoven and van Loon, 2002; Omura and Honda, 2003; Sasaki and Asaoka, 2006), Diptera (Dethier, 1976; Wang et al., 2004), Orthoptera (Blaney, 1981; Chapman et al., 1991) and Hymenoptera (Scheiner et al., 2001; de Brito Sanchez et al., 2005).

In the present study, we examined an insect (*Manduca sexta* caterpillars; Sphingidae) that appears to contradict the predictions of the Schoonhoven and Blom model (Schoonhoven and Blom, 1988). Despite displaying a robust aversive response to bitter stimuli (Frazier, 1986; Glendinning et al., 1999; Glendinning et al., 2006), *M. sexta* shows a weak-to-nonexistent appetitive response to amino acids and carbohydrates (Bowdan, 1995; Glendinning et al., 2000). The lackluster appetitive response to carbohydrates is perplexing because the peripheral gustatory system of *M. sexta* responds vigorously to carbohydrates. For instance, even though glucose (100 mmol l^{-1}) and inositol (10 mmol l^{-1}) each strongly stimulate several classes of taste cell, the caterpillars take as many bites (over a 2-min test) from disks treated with glucose or inositol as from disks treated with water alone (Glendinning et al., 2000).

Given that most plant tissues contain multiple carbohydrates (Hardinge et al., 1965; Somogyi and Trautner, 1974; Clements and Darnell, 1980; Nelson and Bernays, 1998), it is possible that a herbivorous insect such as *M. sexta* would have evolved a taste system that responds selectively to mixtures of carbohydrates. If so, then this would explain why single carbohydrates (e.g. glucose or inositol) were so ineffective at eliciting an appetitive response in previous studies (Bowdan, 1995; Glendinning et al., 2000). In humans, carbohydrate mixtures often produce synergistic effects, such that the mixture generates a sweetness intensity that is higher than would be predicted based on the sum of the intensities generated by the individual component carbohydrates (Schiffman et al., 1995; Schiffman et al., 2000).

Similarly, in rodents, binary mixtures of sweeteners can stimulate intake much more effectively than individual sweeteners (Valenstein et al., 1967; Capretta, 1970; Smith et al., 1976).

Like many other insects (Schoonhoven and van Loon, 2002; Thorne et al., 2004; Wang et al., 2004), *M. sexta* has a heterogeneous population of carbohydrate-sensitive taste cells. It has three bilateral pairs of carbohydrate-sensitive taste cells, and at least two of these pairs have different response properties (Schoonhoven, 1969). As a result of this peripheral organization, it follows that (1) no single carbohydrate could stimulate all three classes of carbohydrate-sensitive taste cell, and (2) some carbohydrate mixtures could stimulate more carbohydrate-sensitive taste cells than others. We hypothesized that *M. sexta* monitors the pattern of input from the different classes of carbohydrate-sensitive taste cell, and that it activates an appetitive response only when all three classes of taste cell are activated simultaneously. To test this hypothesis, we performed three experiments. In Experiment 1, we determined which carbohydrates activate the peripheral taste system of *M. sexta*. In Experiment 2, we ascertained the response properties of the different carbohydrate-sensitive taste cells. In Experiment 3, we asked whether the magnitude (or intensity) of the initial feeding response increases with the number of activated carbohydrate-sensitive taste cells.

Materials and methods

Subjects and rearing conditions

We maintained a breeding colony of tobacco hornworms (*Manduca sexta*; Sphingidae) in our laboratory. This colony was derived from eggs that were donated by the *Manduca* rearing facility at the ARL Division of Neurobiology, University of Arizona, AZ, USA. The caterpillars were reared on a wheat germ-based artificial diet (Bell and Joachim, 1976), and were maintained in an environmental chamber with a 16 h:8 h L:D cycle at 25°C. All experiments were performed with caterpillars during the first or second day of their fifth larval growth stage (instar). All caterpillars were naive to the taste stimuli prior to testing. To control for any potential differences among caterpillars from different egg batches, individuals from each batch were interspersed randomly across treatment levels, according to a blind procedure. We provide sample sizes in the figure legends.

Experiment 1: which carbohydrates generate excitatory responses in the lateral and medial styloconic sensilla?

We tested seven carbohydrates: six simple and one complex. Four of the simple carbohydrates are relatively abundant in the insect's host plants [myo-inositol, sucrose, fructose and glucose (Nelson and Bernays, 1998)], and the other two are known to stimulate taste cells in other caterpillar species [maltose and trehalose (Schoonhoven and van Loon, 2002)]. The complex carbohydrate, Polycose™, is a mixture of glucose polymers ranging from G1 (glucose) to G30; it is water-soluble, has an average molecular weight of 1000 Da (provided by the manufacturer), and stimulates feeding in many species of mammal (Sclafani, 1987). We considered polycose to be a representative starch, the most abundant carbohydrate in plants.

We tested all carbohydrates, except inositol and Polycose, at a 200 mmol l^{-1} concentration. We selected 200 mmol l^{-1} because preliminary studies indicated that it is the lowest concentration that elicits a maximal neural response in both sensilla. We used a 1 mmol l^{-1} concentration of inositol because preliminary studies indicated that it elicited an excitatory response similar to that of 200 mmol l^{-1} sucrose. We used 100 mmol l^{-1} Polycose because this concentration is highly palatable to several species of rodent (Sclafani, 1987; Glendinning et al., 2005). For the taste cell recordings, we presented the carbohydrates in an electrolyte solution (i.e. 100 mmol l^{-1} KCl in deionized water). We purchased all carbohydrates, except Polycose, from Sigma-Aldrich (St Louis, MO, USA); we obtained the Polycose from Abbott Laboratories (Columbus, OH, USA).

For those carbohydrates that elicited an excitatory response in at least one class of taste sensillum (i.e. a response significantly greater than that to solvent alone), we generated concentration–response curves. To this end, we selected concentrations that spanned the dynamic range of response.

Electrophysiological recordings

Following 30 min of food deprivation, we recorded neural responses of each taste sensillum with a non-invasive extracellular tip-recording technique (Gothilf and Hanson, 1994). In brief, we placed a glass electrode containing a taste stimulus solution over the tip of a lateral or medial styloconic sensillum (Fig. 1A,B). To minimize any potential carry-over between successive recordings, we paused for at least 3 min between stimulations. To minimize the effects of solvent evaporation at the tip of the recording/stimulating electrode, we drew fluid from the tip with a piece of filter paper immediately before stimulation. For each caterpillar, we made recordings from a single lateral and a single medial styloconic sensillum.

We recorded extracellular alternating current signals with the Tasteprobe amplifier system (Syntech, Hilversum, The Netherlands) (Marion-Poll and Van der Pers, 1996). We preamplified each recording 10×, ran it through a band-pass filter set at 100–1200 Hz, fed it into a computer through a 16-bit analog-to-digital converter board, and then analyzed it off-line with Autospike software (Syntech).

Data analysis

In this experiment, we tallied the total excitatory response (i.e. total number of spikes that occurred during the initial 1000 ms of stimulation) separately for each taste sensillum. To determine which of the carbohydrate solutions elicited a detectable excitatory response, we compared the number of spikes elicited by the electrolyte solution alone with that elicited by each carbohydrate solution, using one-way factorial analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test. For those carbohydrates that generated strong excitatory responses, we also obtained concentration–response curves. We analyzed the curve for each carbohydrate and sensillum separately, using one-way repeated-measures (RM) ANOVA. In this and all subsequent experiments, we set the alpha level at 0.05.

Experiment 2: what are the response properties of each class of carbohydrate-sensitive taste cell?

We used the tip-recording technique to record excitatory

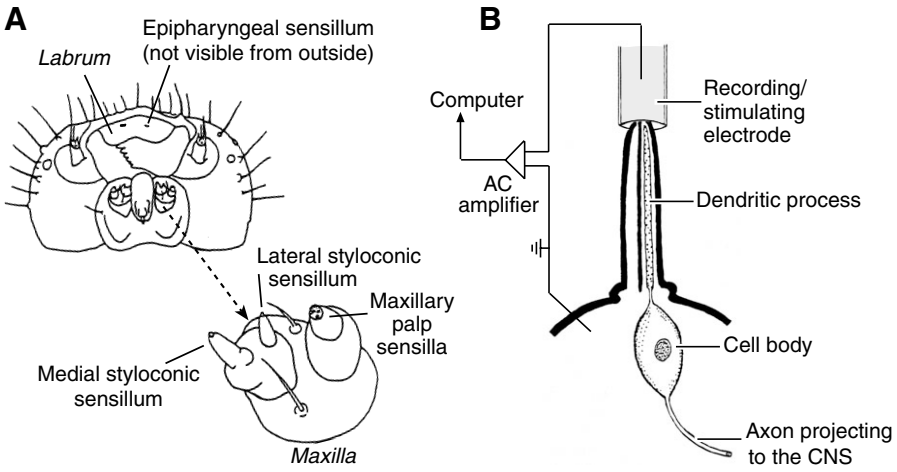

Fig. 1. (A) Cartoon of the head of an *M. sexta* caterpillar, as viewed from below. An enlargement of the maxilla (indicated with an arrow) is provided to clarify the location of the medial and lateral styloconic sensilla. This cartoon was adapted from Bernays and Chapman, fig. 3.4 (Bernays and Chapman, 1994). (B) Illustration of the tip-recording method, which was used to record excitatory responses of individual taste cells located within a taste sensillum. During a tip recording (Hodgson et al., 1955), the tip of a taste sensillum is inserted into the end of a glass recording/stimulating electrode, which is filled with a taste stimulus dissolved in an electrolyte solution (0.1 mol l^{-1} KCl in deionized water). The taste stimulus solution diffuses through a pore in the tip of the sensillum and activates transduction mechanism(s) on the distal end of a taste cell's dendritic process; the electrode detects the ensuing action potentials. For clarity, only one taste cell is indicated. Note that the taste cell's axonal process projects directly to the central nervous system without synapsing.

responses of the lateral and medial styloconic sensilla to the following stimulus triads: (1) glucose, sucrose and the binary mixture of both; (2) glucose, inositol and the binary mixture of both; and (3) sucrose, inositol and the binary mixture of both. We used the same concentrations of each carbohydrate as in the previous experiment, and stimulated one lateral and one medial sensillum from each caterpillar with each stimulus triad. To control for order effects, we randomized the presentation sequence of the triads, and the stimuli within each triad.

Spike assignment procedure

Because the lateral and medial styloconic sensilla each contain four taste cells, any given neural response could contain spikes from several taste cells. Thus, we developed a spike classification procedure based on earlier studies in our laboratory and by others (White et al., 1990; Bernays et al., 2000; Glendinning et al., 2002). We avoided using waveform algorithms to assign spikes to specific taste cells because spike amplitudes often changed with time, and spikes often interacted to produce irregular waveforms. Instead, our spike classification approach was visual, using relative spike height and shape, and the regularity of firing to separate and assign spikes. When a neural record was dominated by spikes of similar size and shape, which occurred in regular temporal sequence, we assumed that a single cell produced the spikes.

During the analysis, we found that the response of the lateral styloconic sensilla to sucrose always contained a population of spikes that followed a tonic pattern of firing (as indicated by a unimodal distribution of inter-spike intervals); the remaining spikes occurred less frequently and out-of-phase with the tonic pattern. In this case, we assigned the spikes that fit the tonic pattern to the 'dominant' category, and the remaining spikes to the 'non-dominant' category. However, the response of the lateral and medial styloconic sensilla to glucose or inositol always contained a population of spikes that followed a phasic-tonic pattern of firing (as indicated by a bimodal distribution of inter-spike intervals); the remaining spikes occurred less frequently and out-of-phase with the phasic-tonic pattern. In this latter case, we assigned the spikes that fit the phasic-tonic pattern to the 'dominant' category, and the remaining spikes to the 'non-dominant' category. Note that we assume that spikes assigned to the dominant category are derived from a single taste cell, and that spikes assigned to the non-dominant category could be derived from up to three taste cells.

We inferred that two carbohydrates (e.g. glucose and inositol) stimulated the same taste cell within a sensillum if the neural responses met two conditions. First, the binary mixture of carbohydrates had to elicit significantly more dominant spikes than each carbohydrate alone. This would indicate that both carbohydrates were acting additively on one taste cell, causing it to fire more rapidly than either carbohydrate alone. Second, the binary mixture of carbohydrates had to elicit the same number of non-dominant spikes as each taste stimulus alone. This would indicate that the non-dominant spikes in each trace reflected the response to the electrolyte solution.

By contrast, we inferred that two carbohydrates (e.g. glucose and sucrose) stimulated different taste cells within a sensillum if the neural responses met a different set of conditions. First, the binary mixture of carbohydrates could not elicit significantly more dominant spikes than either carbohydrate alone. This would indicate (1) that the binary mixture of carbohydrates was not acting additively on a single taste cell, and (2) that the dominant spikes from the binary mixture reflected the response to one of the carbohydrates in the mixture (e.g. glucose). Second, the binary mixture of carbohydrates had to elicit significantly more non-dominant spikes than each carbohydrate alone. This would indicate that the non-dominant spikes from the binary mixture reflected the response to the second carbohydrate in the mixture (e.g. sucrose), whereas the non-dominant spikes from each of the carbohydrates reflected the response to the electrolyte solution.

Data analysis

We sought to determine how many carbohydrate-sensitive taste cells existed in both the lateral and medial styloconic sensilla, and the response properties of each of these taste cells. To this end, we measured the neural response of the dominant

and non-dominant taste cells, separately for each taste stimulus (see above for details). For a given neural response, we tallied the number of spikes that occurred across the initial 1000 ms of stimulation. We used one-way RM-ANOVA to compare the number of dominant (or non-dominant) spikes generated by each triad of taste stimuli (i.e. two carbohydrates alone and in the binary mixture of both). If the ANOVA was significant, then we ran a post-hoc test (Tukey's HSD test, adapted for a within design). We used this information, together with a detailed analysis of the neural traces (e.g. spike shape, amplitude and temporal pattern), to ascertain which of the carbohydrates (i.e. sucrose, glucose and inositol) stimulated the same or different taste cells within each taste sensillum.

Experiment 3: are two carbohydrates more effective than one at stimulating feeding?

We dissolved each carbohydrate in deionized water and then pipetted the test solution onto a glass-fiber disk (Whatman GF/A, 4.25 cm diameter; henceforth, 'disk'). We presented the disk to the caterpillar immediately after adding the test solution so as to reduce evaporative water loss and to make the results comparable to the electrophysiological recordings. We used the following test solutions (at room temperature): water alone, sucrose (200 mmol l^{-1}), glucose (200 mmol l^{-1}), inositol (1 mmol l^{-1}) and binary mixtures of each solution (i.e. 200 mmol l^{-1} sucrose plus 200 mmol l^{-1} glucose; 200 mmol l^{-1} sucrose plus 1 mmol l^{-1} inositol; and 200 mmol l^{-1} glucose plus 1 mmol l^{-1} inositol). We selected the indicated concentration of each carbohydrate because they were used in the previous experiment.

Behavioral test

We recorded the timing of all bites taken over the course of an entire meal. Our biting test consisted of four steps. (1) We placed a caterpillar in the 'food-deprivation arena', which consisted of a clean (inverted) Petri dish covered by a clear plastic cylinder (7.5 cm in diameter, 10 cm tall). We fasted the caterpillar in this arena for 30 min to standardize its 'hunger' state. (2) Then, we transferred the caterpillar to the 'test arena', which was identical to the food-deprivation arena in all respects except that a piece of cork (1 cm in diameter, 3–4 mm high) had been taped to the middle of the inverted Petri dish. Immediately before each test session, we pinned a disk to the piece of cork, and then moistened it with 400 ml of the test solution (see above for details). (3) Next, we placed the caterpillar on the edge of the glass-fiber disk, positioning it so that its legs and prolegs grasped the edge of the glass-fiber disk securely. (4) Once the caterpillar had brought its mouthparts into contact with the surface of the glass-fiber disk and appeared to be tasting it (i.e. drumming the surface of the glass-fiber disk with its chemosensilla), we began the biting test. To be included in the experiment, the caterpillar had to initiate biting on the disk within 8 min of the beginning of the trial. We recorded the timing of all bites until the 'meal' ended. We defined a 'meal' as a period of relatively continuous biting, which ended once the caterpillar ceased biting for 2 min (Bowdan, 1988). To determine the amount of disk area eaten during the meal, we let the disks dry overnight and used SigmaScan (SPSS, Chicago, IL, USA) to quantify the two-dimensional area of disk (in mm^2) that the caterpillar removed during its meal.

We developed three procedures to ensure that the observer could record the timing of each bite easily, accurately and without bias. First, by positioning the test arena on a turntable-like device, we could rotate the caterpillar and keep its mandibles clearly visible as it fed (this rotation did not disrupt the feeding of the caterpillar). Second, we recorded the timing of each bite with a software-based event recorder; this was possible to do accurately because the caterpillars emitted bites at a relatively low frequency (0 to 3 Hz), and because their black mandibles contrasted with the white experimental disks. Third, we kept the observer blind to the identity of the carbohydrate solution in the experimental disk.

Data analysis

Each caterpillar was run through a single meal. We analyzed several features of its biting response: (1) latency to initiate feeding (i.e. the time that elapsed between initially tasting the surface of the disk and initiating biting); (2) total number of bites exhibited during the initial 10 s, 120 s and then across the meal; (3) duration of the meal; (4) disk area consumed (in mm^2) across the meal; and (5) mean bite size (i.e. disk area consumed divided by the total number of bites). For each biting measure, we made comparisons across taste stimuli, using the Kruskal–Wallis test and Dunn's multiple-comparison test. We used nonparametric tests because the data were not normally distributed.

Finally, we determined the molar density of carbohydrates in each type of disk (i.e. moles of carbohydrate per mm^2 of disk). Given that we applied 400 μl of test solution to each disk, and that the area of the disk (at the beginning of each test) was 1418.6 mm^2, it follows that there was 0.282 μl of carbohydrate per mm^2 of disk. Because we knew the molar concentration of carbohydrate in each test solution, we could determine the moles of carbohydrate in each mm^2 of disk.

Results

Experiment 1: which carbohydrates generate excitatory responses in the lateral and medial styloconic sensilla?

We sought to determine which carbohydrates stimulate the peripheral taste system of *M. sexta*. To this end, we focused on the medial and lateral styloconic sensilla (Fig. 1A) because they are the only ones with taste cells that generate excitatory responses to carbohydrates (Schoonhoven and Dethier, 1966; Glendinning et al., 2000). Previous studies demonstrated that carbohydrates do not elicit excitatory responses in any of the other taste sensilla [i.e. the epipharyngeal and maxillary palp sensilla (de Boer et al., 1977; Glendinning et al., 2000) (J.I.G., A.J. and A.T.R, unpublished data)].

The medial styloconic sensillum responded to the carbohydrates with the following relative magnitude of response: inositol>glucose>sucrose=trehalose=fructose=maltose=Polycose=solvent alone (Fig. 2A). However, the lateral styloconic sensillum responded to the carbohydrates with different relative magnitudes of response: inositol=sucrose>glucose>fructose=Polycose=maltose=trehalose=solvent alone (Fig. 2B). Because inositol, sucrose and glucose were the only carbohydrates that elicited responses significantly greater than

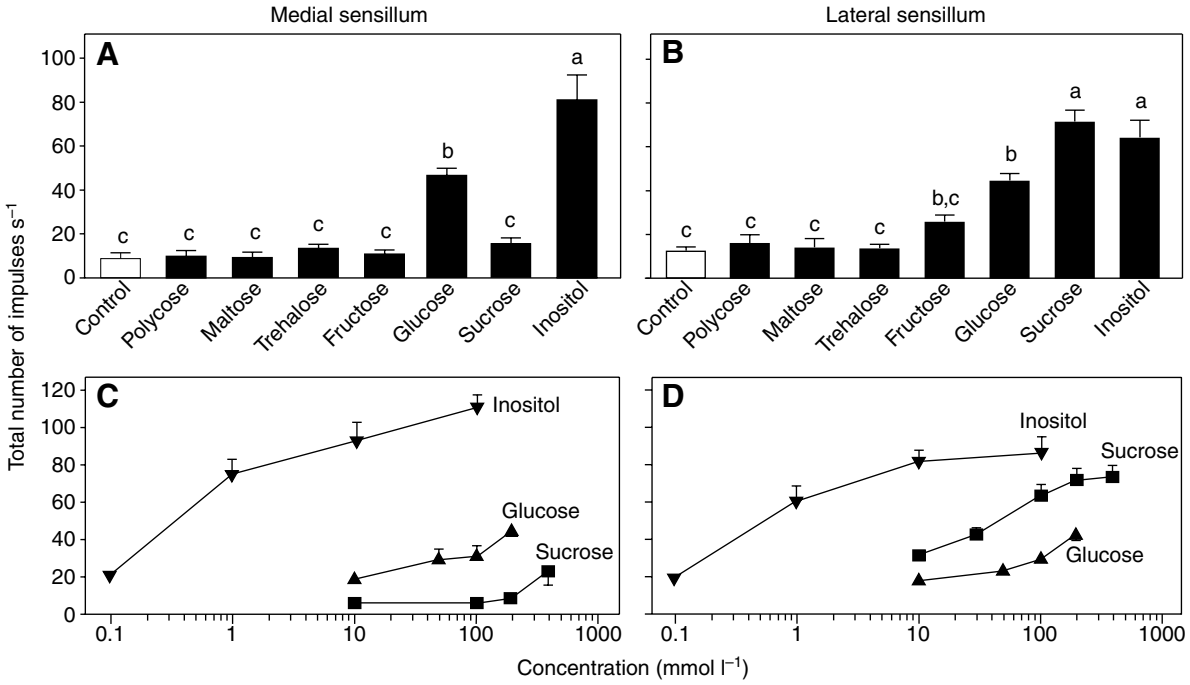

Fig. 2. Total excitatory responses of the medial (A,C) and lateral (B,D) sensilla to stimulation with the control solution (i.e. 100 mmol l^{-1} KCl) or one of the carbohydrates: Polycose (100 mmol l^{-1}), maltose (200 mmol l^{-1}), trehalose (200 mmol l^{-1}), fructose (200 mmol l^{-1}), glucose (200 mmol l^{-1}), sucrose (200 mmol l^{-1}) and myo-inositol (1 mmol l^{-1}). We presented each carbohydrate in the control solution. In panels A and B, we show the total number of spikes elicited in the medial and lateral styloconic sensilla, across the initial 1 s of stimulation. Within each panel, we compare means with a post-hoc test (Tukey's HSD test); different letters (a, b, c or a combination of each) above the bars indicate means that differ significantly from one another ($P \leqslant 0.05$). In panels C and D, we show concentration–response functions for a range of inositol, glucose and sucrose concentrations in both the medial and lateral styloconic sensilla. Each bar or symbol indicates mean ± s.e.m.; N=7–18 sensilla per tastant (each from a different caterpillar).

solvent alone (in at least one class of styloconic sensillum), we limited all subsequent experiments to these three carbohydrates.

We found that the neural responses of each taste sensillum to inositol, sucrose and glucose all increased significantly with concentration (for each concentration–response curve, $P<0.05$; one-way RM-ANOVA), but the magnitude of the response varied considerably across carbohydrates (Fig. 2C,D). The medial styloconic sensilla exhibited robust concentration-dependent increases in response to inositol, but only modest increases to glucose and sucrose. By contrast, the lateral styloconic sensilla exhibited robust concentration-dependent increases in response to inositol and sucrose, but a modest increase to glucose. The most notable difference between the lateral and medial styloconic sensilla concerned the sucrose response – e.g. 200 mmol l^{-1} sucrose elicited an excitatory response of approximately 75 spikes s^{-1} in the lateral, but only 8 spikes s^{-1} in the medial styloconic sensillum.

Experiment 2: what are the response properties of each class of carbohydrate-sensitive taste cell?

According to Schoonhoven (Schoonhoven, 1972), the lateral styloconic sensillum of *M. sexta* contains one taste cell that responds to sucrose and glucose, and another taste cell that responds to inositol. The medial styloconic sensillum, on the other hand, contains one taste cell that responds to glucose and another that responds to inositol. Here, we assessed this peripheral organizational plan.

Response to sucrose and inositol (lateral styloconica)

We examined the neural response of the carbohydrate-sensitive taste cells in the lateral styloconic sensillum to sucrose (200 mmol l^{-1}), inositol (1 mmol l^{-1}) and sucrose+inositol. We found that the binary mixture elicited the same number of dominant spikes as sucrose, but significantly more dominant spikes than glucose (Fig. 3B). By contrast, the binary mixture elicited significantly more non-dominant spikes than sucrose or glucose alone (Fig. 3C). These results, together with analyses of the neural traces (e.g. see Fig. 3A) and temporal patterns of discharge (Fig. 7B,C), indicate (1) that sucrose caused one taste cell to generate a tonic discharge pattern, (2) that inositol caused one taste cell to generate a phasic-tonic discharge pattern, and (3) that the binary mixture of sucrose+inositol caused two taste cells to discharge out-of-phase with one another (one taste cell exhibited a tonic discharge pattern, and the other elicited a phasic-tonic discharge pattern). Based on these findings, we propose that sucrose and inositol each activated different carbohydrate-sensitive taste cells within the lateral styloconic sensillum.

If the two carbohydrate-sensitive taste cells functioned independently of one another, then the binary mixture of sucrose+inositol should have elicited ~70 dominant spikes s^{-1} and ~45 non-dominant spikes s^{-1} (Fig. 3B,C). This was not the case, however. We measured ~70 dominant spikes s^{-1}, but ~25 non-dominant spikes s^{-1}. The low number of non-dominant spikes indicates that inhibitory interactions occurred between the two carbohydrate-sensitive taste cells.

Response to sucrose and glucose (lateral styloconica)

Next, we examined the neural response of the carbohydrate-sensitive taste cells in the lateral styloconic sensillum to sucrose (200 mmol l^{-1}), glucose (1 mmol l^{-1}) and sucrose+glucose. We found that the binary mixture elicited the same number of dominant spikes as sucrose, but significantly more than glucose (Fig. 4B). By contrast, the binary mixture elicited significantly more non-dominant spikes than did either component alone (Fig. 4C). These findings, together with analyses of the neural traces (e.g. see Fig. 4A) and temporal patterns of discharge (Fig. 7B,C), indicate (1) that sucrose caused one taste cell to generate a tonic discharge pattern, (2) that glucose caused one taste cell to generate a phasic-tonic discharge pattern, and (3) that the binary mixture of both carbohydrates caused two taste cells to discharge out-of-phase with one another (one taste cell exhibited a tonic discharge pattern, and another a phasic-tonic discharge pattern). Based on these findings, we propose that sucrose and glucose each activated different carbohydrate-sensitive taste cells in the lateral styloconic sensillum.

If the carbohydrate-sensitive taste cells functioned independently of one another, then the binary mixture of sucrose+glucose should have elicited ~70 dominant and ~40 non-dominant spikes s^{-1} (Fig. 4B,C). Our results roughly match this prediction, indicating that the two carbohydrate-sensitive taste cells functioned independently of one another when stimulated by the mixture of sucrose and glucose.

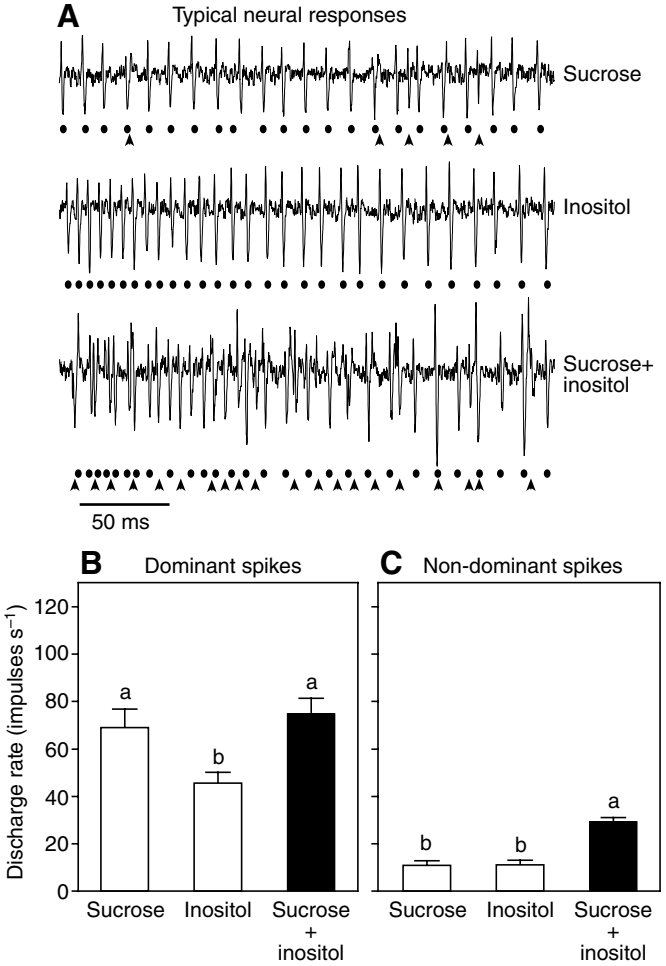

Fig. 3. Demonstration that sucrose and inositol stimulate different taste cells in the lateral styloconic sensillum. In panel A, we show representative neural responses of a lateral styloconic sensillum to sucrose (200 mmol l^{-1}), inositol (1 mmol l^{-1}) and the binary mixture of both. We provide the initial 250 ms of response. Below each trace, we indicate dominant spikes with a filled circle, and non-dominant spikes with an arrowhead; see text for a description of how we distinguished these two classes of spikes. We dissolved the carbohydrates in a 100 $mmol^{-1}$ KCl solution. In panels B and C, we show the number of dominant and non-dominant spikes elicited by each of the taste stimuli. Each bar indicates mean ± s.e.m.; N=13 sensilla (each from a different caterpillar). We compare the means within each panel with a post-hoc test (Tukey's HSD test); different letters (a or b) above the bars within a panel indicate means that differ significantly from one another ($P \leqslant 0.05$).

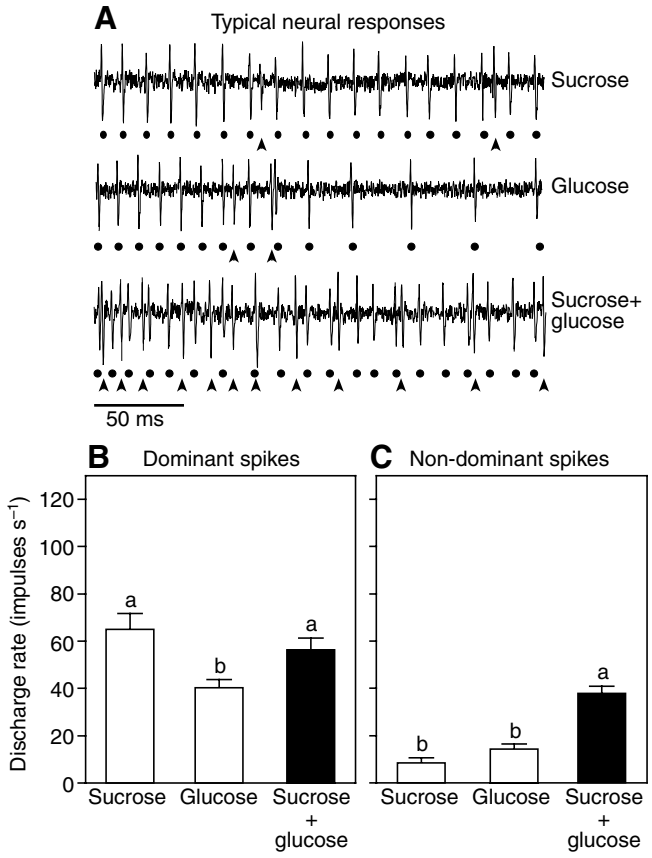

Fig. 4. Demonstration that sucrose and glucose stimulate different taste cells in the lateral styloconic sensillum. In panel A, we show representative neural responses of a lateral styloconic sensillum to sucrose (200 mmol l^{-1}), glucose (200 mmol l^{-1}) and the binary mixture of both. We provide the initial 250 ms of response. Below each trace, we indicate dominant spikes with a filled circle, and non-dominant spikes with an arrowhead; see text for a description of how we distinguished these two classes of spikes. We dissolved the carbohydrates in a 100 $mmol^{-1}$ KCl solution. In panels B and C, we show the number of dominant and non-dominant spikes elicited by each of the taste stimuli. Each bar indicates mean ± s.e.m.; N=12 sensilla (each from a different caterpillar). We compare the means within each panel with a post-hoc test (Tukey's HSD test); different letters (a or b) above the bars within a panel indicate means that differ significantly from one another ($P \leqslant 0.05$).

Response to glucose and inositol (lateral styloconica)

We examined the neural response of the carbohydrate-sensitive taste cells in the lateral styloconic sensillum to glucose (200 mmol l^{-1}), inositol (1 mmol l^{-1}) and glucose+inositol. We found that the binary mixture elicited significantly more dominant spikes than did glucose or inositol alone (Fig. 5B). By contrast, the binary mixture elicited the same number of non-dominant spikes as glucose or inositol alone (Fig. 5C). These results, together with analyses of the neural traces (Fig. 5A) and temporal patterns of discharge (Fig. 7B), indicate that the glucose, inositol and glucose+inositol solutions each caused a single taste cell to generate similar phasic-tonic discharge patterns. The only difference between these discharge patterns was that the binary mixture elicited a higher rate of firing across the entire 1000 ms of response. Based on these findings, we infer that inositol, glucose and the binary mixture each activated the same carbohydrate-sensitive taste cell in the lateral styloconic sensillum.

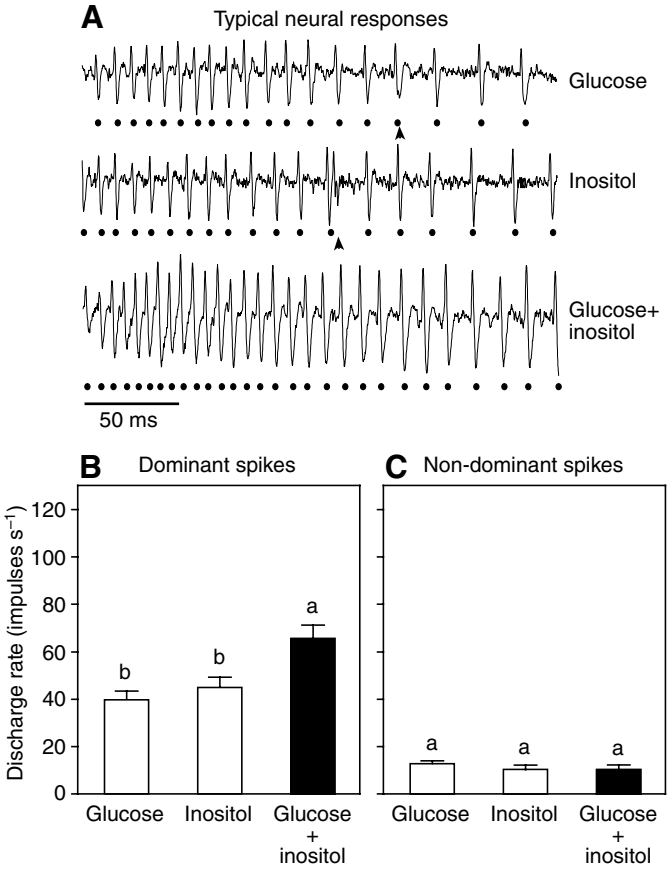

Fig. 5. Demonstration that glucose and inositol stimulate the same taste cell in the lateral styloconic sensillum. In panel A, we show representative neural responses of a lateral styloconic sensillum to glucose (200 mmol l^{-1}), inositol (1 mmol l^{-1}) and the binary mixture of both. We provide the initial 250 ms of response. Below each trace, we indicate dominant spikes with a filled circle, and non-dominant spikes with an arrowhead; see text for a description of how we distinguished these two classes of spikes. We dissolved the carbohydrates in a 100 $mmol^{-1}$ KCl solution. In panels B and C, we show the number of dominant and non-dominant spikes elicited by each of the taste stimuli. Each bar indicates mean ± s.e.m.; N=16 sensilla (each from a different caterpillar). We compare the means within each panel with a post-hoc test (Tukey's HSD test); different letters (a or b) above the bars within a panel indicate means that differ significantly from one another ($P \leqslant 0.05$).

If glucose and inositol each acted independently on the same carbohydrate-sensitive taste cell, then the binary mixture of glucose+inositol should have elicited ~85 dominant spikes s^{-1} (Fig. 5B). Instead, we observed ~65 dominant spikes s^{-1}. This indicates that inhibitory interactions occurred between glucose and inositol.

Response to glucose and inositol (medial styloconica)

We also examined the neural response of the carbohydrate-sensitive taste cells in the medial styloconic sensillum to glucose (200 mmol l^{-1}), inositol (1 mmol l^{-1}) and glucose+inositol. We found that the glucose+inositol solution elicited significantly more dominant spikes than did the glucose or inositol solution (Fig. 6B). Further, the glucose, inositol and glucose+inositol solutions each elicited a low and statistically equivalent number of non-dominant spikes (Fig. 6C). These findings, together with analyses of the neural traces (Fig. 6A) and temporal patterns of discharge (Fig. 7A), indicate that the glucose, inositol and glucose+inositol solutions each caused a single taste cell to generate a phasic-tonic discharge pattern. Based on these findings, we propose that inositol, glucose and the binary mixture each activated the same carbohydrate-sensitive taste cell in the medial styloconic sensillum.

If glucose and inositol each acted independently on the same carbohydrate-sensitive taste cell, then the binary mixture of glucose+inositol should have elicited ~105 dominant spikes s^{-1} (Fig. 6B). This was approximately what we observed, indicating that no inhibitory interactions occurred between glucose and inositol in the medial styloconic sensilla.

Response to sucrose (medial styloconica)

We asked whether sucrose stimulates the same carbohydrate-sensitive taste cell as glucose or inositol in the medial styloconic sensillum. We could not answer this question, however, because the neural response to 200 mmol l^{-1} sucrose was indistinguishable from that to the electrolyte solution.

Taken together, these results show that the carbohydrate solutions (i.e. sucrose, glucose, inositol and the three binary mixtures) activated different populations of carbohydrate-sensitive taste cells. Whereas sucrose activated one class of taste cell in the lateral styloconic sensillum, glucose+inositol both activated another taste cell in both the lateral and medial styloconic sensillum.

Experiment 3: are two carbohydrates more effective than one at stimulating feeding?

In many species of mammal (Spector, 2000) and insect (Ma, 1972; Blaney, 1975; Dethier, 1976; Scheiner et al., 2001; Omura and Honda, 2003; Wang et al., 2004; Sasaki and Asaoka, 2006), the presence of single carbohydrates elicits an immediate concentration-dependent increase in feeding responsiveness. Previous work in *M. sexta* revealed a paradoxical effect of carbohydrates on feeding, however. Although carbohdyrates appear to have a limited (or non-existent) effect on immediate biting responses (Bowdan, 1995; Glendinning et al., 2000), they

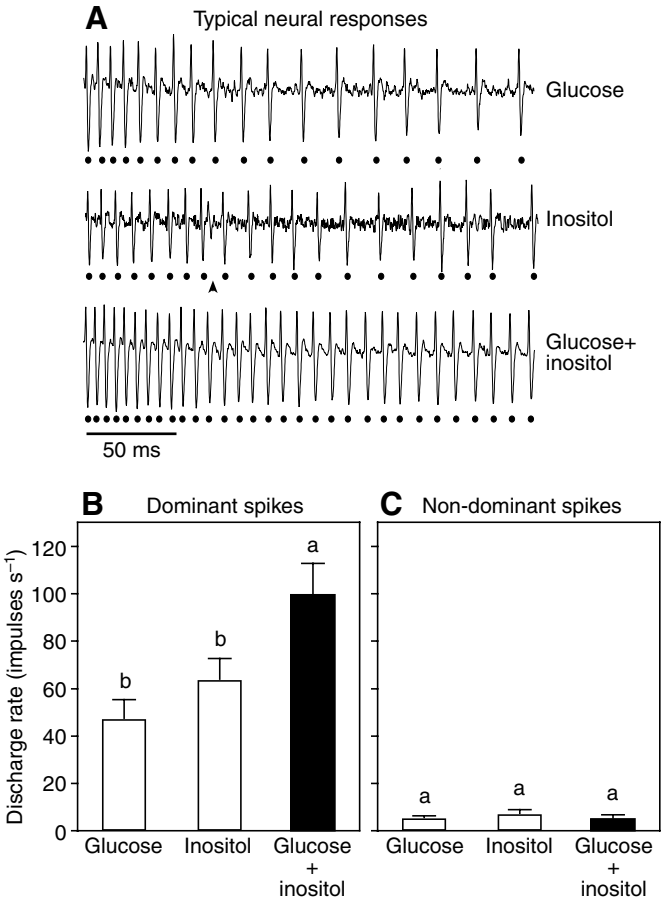

Fig. 6. Demonstration that glucose and inositol stimulate the same taste cell in the medial styloconic sensillum. In panel A, we show representative neural responses of a medial styloconic sensillum to glucose (200 mmol l^{-1}), inositol (1 mmol l^{-1}) and the binary mixture of both. We provide the initial 250 ms of response. Below each trace, we indicate dominant spikes with a filled circle, and non-dominant spikes with an arrowhead; see text for a description of how we distinguished these two classes of spikes. We dissolved the carbohydrates in a 100 $mmol^{-1}$ KCl solution. In panels B and C, we show the number of dominant and non-dominant spikes elicited by each of the taste stimuli. Each bar indicates mean ± s.e.m.; N=12 sensilla (each from a different caterpillar). We compare the means within each panel with a post-hoc test (Tukey's HSD test); different letters (a or b) above the bars within a panel indicate means that differ significantly from one another ($P \leq 0.05$).

nevertheless stimulate intake in long-term feeding studies (Yamamoto and Fraenkel, 1960; Städler and Hanson, 1978; Glendinning et al., 2000).

Here, we addressed three inter-related issues. First, we attempted to explain the discrepancy between the results from short- and long-term feeding tests. To this end, we tested the hypothesis [derived from a study of the terrestrial slug, *Limax maximus* (Reingold and Gelperin, 1980)] that carbohydrates stimulate intake by causing *M. sexta* to bite for more extended periods of time, without increasing biting rate or bite size. We defined feeding stimulation as biting activity greater than that elicited by water alone. Second, we compared the ability of different carbohydrates (alone or in binary mixture) to stimulate feeding. Third, we tested two hypotheses to explain why some carbohydrate solutions stimulated larger meals. If meal size is determined by negative feedback from the midgut or hemolymph [owing to the accumulation of nutrients or osmotically active compounds (Simpson and Raubenheimer, 1993; Timmins and Reynolds, 1992)], then meal size should decrease with increasing molar density of nutrients in the ingested food. Alternatively, if meal size is determined by excitatory input from carbohydrate-sensitive taste cells, then meal size should increase with (a) the total number of spikes elicited across all carbohydrate-sensitive taste cells, or (b) the number of different classes of carbohydrate-sensitive taste cell that are activated.

Behavioral tests

When presented with a water-treated disk, the caterpillars took approximately 12 s to initiate biting, but when presented with any of the carbohydrate-treated disks, they took significantly less time (2–3 s) to initiate biting (K–W statistic=26.0; $P \leq 0.05$; Fig. 8A). All of the carbohydrate solutions, however, were equally effective at reducing the latency to initiate biting.

Once biting commenced, the caterpillars exhibited the same number of bites on the water-treated disk as on the carbohydrate-treated disks during the initial 10 s (K–W statistic=5.2; $P>0.05$) and 120 s (K–W statistic=4.3; $P>0.05$) of the meal (Fig. 8B,C). This shows that carbohydrates did not modulate initial biting rate. To examine more protracted biting responses, we examined three intake parameters across the first meal: mean bite size, total number of bites and meal duration. There were no significant differences in mean bite size (K–W statistic=3.6; $P>0.05$; Fig. 9A) across the carbohydrate solutions, but there were large and significant differences in total number of bites (K–W statistic=27.8; $P \leq 0.05$; Fig. 9B) and meal duration (K–W statistic=26.4; $P \leq 0.05$; Fig. 9C). A post-hoc analysis revealed that despite a trend for all carbohydrate solutions to stimulate feeding, only two carbohydrate solutions, sucrose+glucose and sucrose+inositol, caused the caterpillars to take significantly larger meals than water alone. These findings show that specific mixtures of carbohydrates are required to stimulate feeding, and that these mixtures do so by increasing the amount of time spent biting.

What determined differences in meal size across carbohydrate solutions?

To explain the large variation in meal size in Fig. 9, we tested two hypotheses. The first was that meal size is determined by negative feedback from the gut or hemolymph. Accordingly, meal size should vary inversely with the molar density of nutrients in the ingested food. To test this hypothesis, we regressed meal size (i.e. mean number of bites emitted over meal; Fig. 9B) on the molar density of carbohydrates in the test solutions (Table 1). This regression was not significant (slope=19.0; r^2=0.06; $F_{1,4}$=0.27; $P>0.05$), indicating that meal size varied independently of the molar density of accumulated carbohydrates in the gut and/or hemolymph.

This second hypothesis was that excitatory input from the carbohydrate-sensitive taste cells determined meal size. We examined two possible mechanisms by which this could occur.

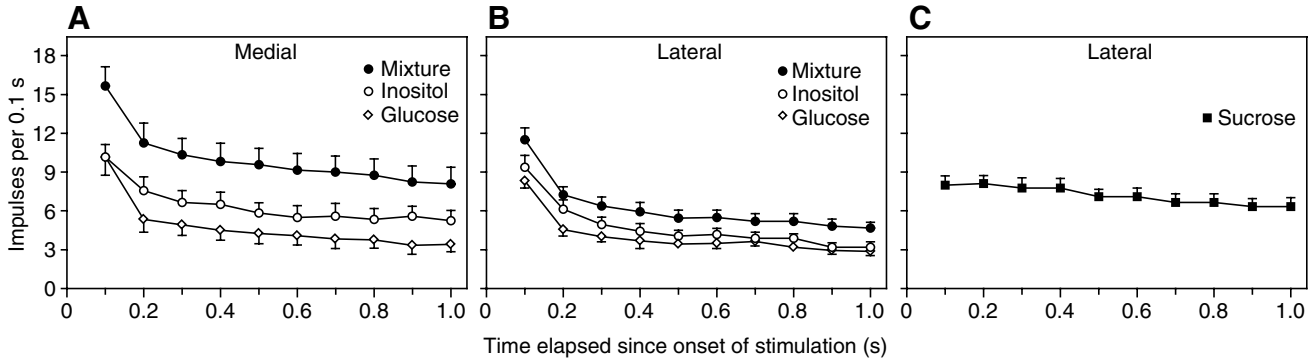

Fig. 7. Instantaneous firing rates of the carbohydrate-sensitive taste cells. (A) Response of the carbohydrate-sensitive taste cell in the medial styloconic sensillum to 1 mmol l^{-1} inositol, 200 mmol l^{-1} glucose and the mixture of both. (B) Response of one carbohydrate-sensitive taste cell in the lateral styloconic sensillum to 1 mmol l^{-1} inositol, 200 mmol l^{-1} glucose and the mixture of both. (C) Response of the second carbohydrate-sensitive taste cell in the lateral styloconic sensillum to 200 mmol l^{-1} sucrose. We show mean ± s.e.m.; N=8–16 taste cells per panel (each from a different caterpillar).

One is that the insect's brain monitors excitatory input from all carbohydrate-sensitive taste cells, and increases meal size in direct proportion to the algebraic sum of this afferent input. To test this possibility, we regressed meal size on the total number of spikes s^{-1} elicited by each carbohydrate solution (Table 1). This regression was not significant (slope=0.3; r^2=0.003; $F_{1,4}$=0.02; P>0.05), indicating that meal size varied independently of total excitatory input. A second mechanism is that the insect's brain monitors the pattern of activity across the three classes of carbohydrate-sensitive taste cell (rather than total excitatory input *per se*), and generates the largest meal when all three classes of carbohydrate-sensitive taste cell are activated strongly. We found clear support for this latter mechanism. Even though the glucose+inositol solution elicited a high total firing rate (166 spikes s^{-1}), it activated only two carbohydrate-sensitive taste cells and failed to generate meals larger than those on water alone (Table 1, Fig. 9B). By contrast, the two other binary mixtures (sucrose+inositol and sucrose+glucose) each elicited an intermediate firing rate (i.e. 126–154 spikes s^{-1}), but nevertheless activated three carbohydrate-sensitive taste cells and caused the caterpillars to take meals significantly larger than those on water alone. In fact, the meals on the sucrose+inositol disks were significantly larger than those on the glucose+inositol disks.

Discussion

The peripheral taste system of *M. sexta* responded vigorously to three carbohydrates (sucrose, glucose and inositol) and weakly to one carbohydrate (fructose); it failed to respond altogether to three others (maltose, trehalose and polycose). Because we used high concentrations of each carbohydrate, it is unlikely that the weak responses to some carbohydrates reflect the use of sub- or peri-threshold concentrations. This finding agrees with prior reports (Schoonhoven, 1972; Glendinning et al., 2000), and confirms that the taste system of *M. sexta* detects a limited number of carbohydrates, as compared with other species of Lepidopteran (Schoonhoven and van Loon, 2002) and Dipteran (Dethier, 1976). It is notable that

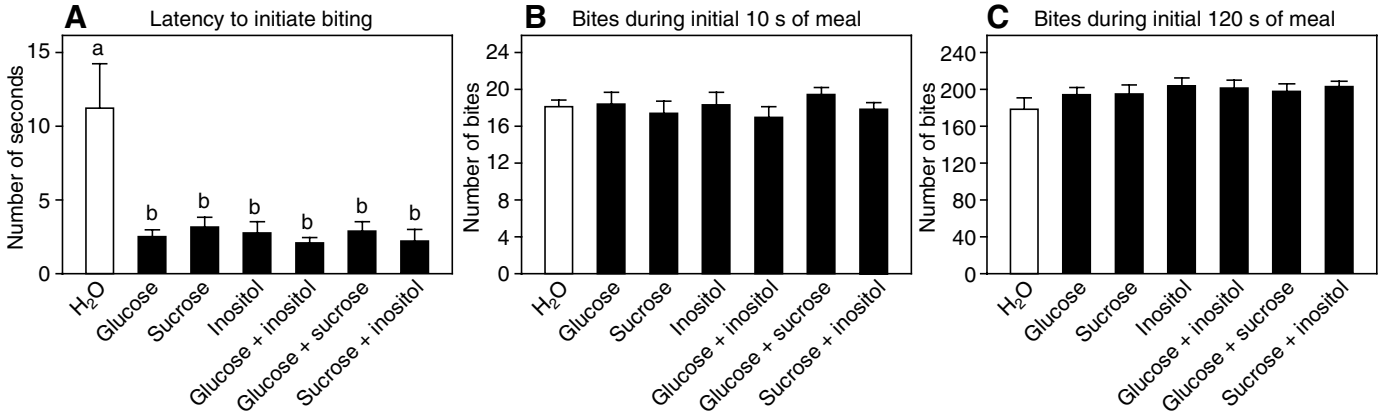

Fig. 8. Analysis of the initial biting responses of caterpillars to disks containing water (H_2O), 200 mmol l^{-1} glucose, 200 mmol l^{-1} sucrose, 1 mmol l^{-1} inositol or binary mixtures of the carbohydrates. We show (A) latency to initiate biting, and number of bites taken across the initial (B) 10 s and (C) 120 s of the meal. Within each panel, we compared medians (±median absolute deviation) with Dunn's multiple comparison test; different letters (a, b, c or combinations of each) above the bars indicate medians that differ significantly from one another (P≤0.05). The absence of letters above the bars in panels B and C reflects a lack of significant difference among the medians (according to a Kruskal–Wallis test, P>0.05). N=19–25 caterpillars per taste stimulus.

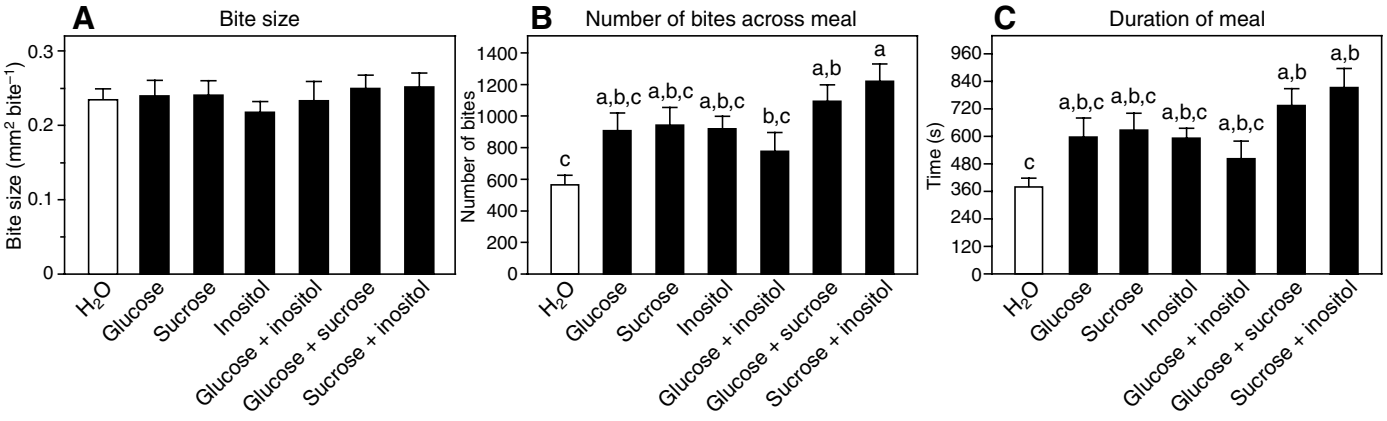

Fig. 9. Analysis of biting activity across the first meal on disks containing water (H_2O), 200 mmol l^{-1} glucose, 200 mmol l^{-1} sucrose, 1 mmol l^{-1} inositol or binary mixtures of the carbohydrates. We show (A) bite size, (B) total number of bites emitted and (C) meal duration. Within each panel, we compare medians (±median absolute deviation) with Dunn's multiple comparison test; different letters (a, b, c or combinations of each) above the bars indicate medians that differ significantly from one another ($P \leqslant 0.05$). The absence of letters above the bars in panel A reflects a lack of significant difference among the medians (according to a Kruskal–Wallis test, $P > 0.05$). N=19–25 caterpillars per taste stimulus.

the taste system of *M. sexta* also responds to a limited number of bitter taste stimuli (Wrubel and Bernays, 1990) (J.I.G., unpublished data), amino acids (Glendinning et al., 2000) and acidic compounds (Bernays et al., 1998). Comparative studies are needed to explain why *M. sexta* evolved such an insensate taste system.

Although several reports have shown that taste cells within the lateral and/or medial sensilla of *M. sexta* exhibit excitatory responses to sucrose, glucose and/or inositol (Frazier, 1986; Lam and Frazier, 1991; Gothilf and Hanson, 1994; Glendinning et al., 2000; Schoonhoven and van Loon, 2002), we are aware of only one systematic attempt to determine the response properties of each carbohydrate-sensitive taste cell – it was performed by Schoonhoven and Dethier (Schoonhoven and Dethier, 1966). These authors examined representative neural responses of the lateral and medial styloconic sensilla to binary mixtures of carbohydrates. For instance, they show a response of the lateral (their fig. 4, trace 12) and medial (their fig. 3, trace 9) styloconic sensilla to a binary mixture of glucose (100 mmol l^{-1}) and inositol (100 mmol l^{-1}); the traces appear to illustrate a single taste cell firing rapidly. In addition, the authors show a response of the lateral styloconic sensilla to a binary mixture of sucrose (100 mmol l^{-1}) and glucose (100 mmol l^{-1}) (their fig. 3, trace 11); the trace appears to illustrate two taste cells firing out-of-phase with one another. These findings corroborate the more extensive results presented herein, and lead us to conclude that (1) each lateral styloconic sensillum contains one carbohydrate-sensitive taste cell that responds to sucrose and another that responds to glucose and inositol; whereas (2) each medial styloconic sensilla contains a single carbohydrate-sensitive taste cell, which responds to glucose and inositol.

In a comprehensive review of caterpillar taste, Schoonhoven and van Loon reported that many species possess taste cells that respond exclusively to sucrose or inositol, but they did not cite any species with taste cells that respond both to glucose and inositol (Schoonhoven and van Loon, 2002). It would appear, therefore, that the presence of a sucrose-sensitive taste cell

Table 1. *Factors hypothesized to influence meal size in the caterpillars*

Test solution*	μmol l^{-1} of carbohydrate per mm^2 of disk	Total spikes s^{-1}†	Number of classes of carbohydrate-sensitive taste cell activated
Glucose	3.6	90 ± 8^{b}	2
Sucrose	3.6	71 ± 10^{b}	1
Inositol	0.02	111 ± 8^{b}	2
Glucose+inositol	3.6	166 ± 14^{a}	2
Sucrose+glucose	7.2	$126\pm9^{a,b}$	3
Sucrose+inositol	3.6	154 ± 8^{a}	3

*See Fig. 8 for the concentration of carbohydrate(s) in each test solution.

†These values (derived from Figs 3–6) show the total number of spikes (mean ± s.e.m.) that were generated by all carbohydrate-sensitive taste cells across the first 1000 ms of response in the lateral and medial styloconic sensilla. In the right-most column, we show the number of taste cells that contributed to the total number of spikes. Even though there are two bilateral pairs of each class of carbohydrate-sensitive taste cell, we included spikes from only one member of each bilateral pair. We compare total spikes across test solutions with an RM-ANOVA; means labeled with distinct superscripts (a or b) differ significantly from one another (Tukey's multiple comparison test, modified for repeated measures; $P<0.05$). The results are derived from one lateral and one medial sensilla from each of 12 caterpillars.

is common among caterpillars, but that the presence of glucose/inositol-sensitive taste cells is unusual.

How do carbohydrates modulate feeding in M. sexta?

Our behavioral studies offer several insights into how gustatory input from the carbohydrate-sensitive taste cells modulate feeding in *M. sexta*. First, the fact that all of the carbohydrate solutions significantly reduced the latency to initiate biting (as compared with water) demonstrates that carbohydrates can incite feeding. It is notable, however, that there were no significant differences in latency to initiate biting across the different carbohydrate solutions. This may reflect the fact that we used relatively high concentrations of most carbohydrates (e.g. 200 mmol l^{-1} glucose). In support of this possibility, we reported previously that a lower concentration of glucose (100 mmol l^{-1}) did not reduce the latency to initiate biting (Glendinning et al., 2000). Given that 200 mmol l^{-1} glucose generates a substantially stronger peripheral gustatory response than 100 mmol l^{-1} glucose (Fig. 2), it would appear that there is a threshold level of input from the carbohydrate-sensitive taste cells required to incite feeding, and that input from 200 mmol l^{-1} glucose (and the other carbohydrate solutions tested herein) surpassed this threshold.

In many species of insect, the taste of sugars stimulates concentration-dependent increases in feeding. For instance, increasing the concentration of sucrose will cause a proportional increase in (1) the strength of the swallowing response in *Bombyx mori* caterpillars (Sasaki and Asaoka, 2006); (2) the rate of biting in *Pieris brassicae* caterpillars (Ma, 1972); and (3) the probability that flies and bees will extend their proboscis (Dethier, 1976; Scheiner et al., 2001) and butterflies will initiate feeding (Omura and Honda, 2003). Based on these findings, it is surprising that *M. sexta* caterpillars emitted the same initial rate of biting on disks treated with water as on disks treated with high concentrations of carbohydrates, both here and elsewhere (Bowdan, 1995; Glendinning et al., 2000). Even though Bowdan reported that *M. sexta* caterpillars tend to bite more avidly from disks treated with 100 mmol l^{-1} than 10 mmol l^{-1} sucrose during the first chewing bout (Bowdan, 1995), the median number of bites on each type of disk did not differ significantly (Mann–Whitney *U*-test, $P>0.05$).

We found that only two of the carbohydrate solutions (sucrose+inositol and sucrose+glucose) significantly increased meal size, relative to water. To explain this observation, we asked whether meal lengths on the different carbohydrate solutions correlated with differences in the (1) amount of postingestive feedback, (2) magnitude of the total peripheral response, or (3) number of activated carbohydrate-sensitive taste cells. Our analysis indicated that meal length correlated with differences in the number of activated carbohydrate-sensitive taste cells, but not with the other two measures. This leads us to propose that meal length on the carbohydrate-treated disks was not modulated by the algebraic sum of inputs from the carbohydrate-sensitive taste cells (Schoonhoven and Blom, 1988) or the accumulation of carbohydrates in the mid-gut or hemolymph (Timmins and Reynolds, 1992; Simpson and Raubenheimer, 1993).

Insects use several coding mechanisms to identify and discriminate taste stimuli, including labeled-line (Marella et al., 2006), temporal (Glendinning et al., 2006) and ensemble (Dethier and Crnjar, 1982; Glendinning et al., 2002) codes. Our findings indicate that *M. sexta* identifies preferred foods based on an ensemble code – i.e. the pattern of activity generated across the entire population of carbohydrate-sensitive taste cells. Accordingly, the ensemble code for a preferred carbohydrate-rich food would be strong and simultaneous activation of all three classes of carbohydrate-sensitive taste cell. A crucial prediction of this coding mechanism is that those carbohydrate solutions that activate only one or two pairs of carbohydrate-sensitive taste should fail to stimulate feeding, irrespective of the magnitude of the peripheral taste response. We found clear support for this prediction. For instance, even though the mixture of glucose+inositol elicited a vigorous peripheral gustatory response (i.e. 166 spikes s^{-1}), the spikes were limited to two classes of carbohydrate-sensitive taste cell and the caterpillars failed to exhibit significant feeding stimulation (Table 1, Fig. 9). However, the mixture of sucrose+inositol elicited a comparable peripheral response (i.e. 154 spikes s^{-1}), but activated all three classes of carbohydrate-sensitive taste cell and stimulated significantly larger meals than water alone.

Caveats

There are three interpretive limitations of our study. The first is that we are relating taste cell responses that span 1 s to biting responses that span several minutes. As we illustrate in Fig. 7, adaptation processes can cause large reductions in the absolute firing rates of taste cells over periods as short as 1 s. Before we can address this caveat, however, we need to determine the temporal pattern of stimulation that taste cells experience over the course of the meal. This is because caterpillars repeatedly retract their styloconic sensilla from the surface of food items as they are feeding (Devitt and Smith, 1985). Whenever a sensillum is retracted, the taste cells contained within it should experience partial or complete disadaptation (Blaney, 1975). If so, then our 1 s neural responses would accurately reflect what caterpillars experienced over the course of a meal.

The second interpretive limitation is that we focused on the first meal, and ignored consumption across successive meals. We did so to maximize our chances of measuring taste-mediated feeding responses. It is notable, however, that both sucrose and inositol alone have been reported to stimulate feeding in *M. sexta* during feeding tests that span several hours (Yamamoto and Fraenkel, 1960; Städler and Hanson, 1978; Glendinning et al., 2000). We can propose two non-mutually exclusive explanations for the discrepancy between the results of these protracted feeding tests and the one presented herein. First, in the present study, there was a trend for sucrose and inositol to stimulate feeding, but the difference was not significant. It is possible that the feeding stimulation would have become significant had we performed the test over a longer period of time. Second, it is possible that post-oral response mechanisms indirectly stimulate feeding during long-term intake tests. This possibility is based on the observation that the positive postingestive actions of carbohydrates alone can stimulate robust intake in mammals (Sclafani, 2001; Sclafani and Glendinning, 2005).

The third interpretive limitation of our study is that the

sucrose+glucose mixture contained both 200 mmol l^{-1} sucrose and 200 mmol l^{-1} glucose. Thus, it is possible that the peripheral gustatory response to this binary mixture differed from that to each carbohydrate alone simply because it contained 400 mmol l^{-1} of total sugar. This possibility is contradicted, however, by the observation that the neural response of the lateral styloconic sensillum to 200 mmol l^{-1} sucrose (or glucose) is indistinguishable from that to 400 mmol l^{-1} sucrose (or glucose) (J.I.G., unpublished data). That is, each carbohydrate strongly activates one dominant taste cell at both the 200 and 400 mmol l^{-1} concentrations.

Interactions among taste cells within the same sensillum

Previous studies have reported that when insect taste sensilla are stimulated with taste stimulus mixtures, the discharge rate is often less than the sum of the discharge rates from the individual component stimuli. These inhibitory interactions involve stimulus mixtures that activate different taste cells within the same sensillum (Ishikawa, 1967; Schoonhoven, 1978; Mitchell, 1987; Blaney and Simmonds, 1990; White et al., 1990; Chapman et al., 1991; Schoonhoven et al., 1992; Shields and Mitchell, 1995; Bernays and Chapman, 2000; Glendinning et al., 2000; Bernays and Chapman, 2001) or different signaling pathways within the same taste cell (Glendinning and Hills, 1997). Here, we report similar findings. For example, despite activating different taste cells within the lateral sensillum, one of the binary mixtures (i.e. inositol+sucrose) elicited fewer non-dominant spikes than would have been predicted based on the firing rate elicited by the individual component stimuli (Fig. 3). This indicates that inhibitory interactions occurred between the two carbohydrate-sensitive taste cells. Likewise, despite activating the same taste cell, the glucose+inositol mixture elicited fewer dominant spikes in the lateral styloconic sensillum than expected based on the response to each carbohydrate alone (Fig. 5). This indicates that the inhibitory interactions also occurred within the same taste cell.

The mechanistic bases for inhibitory mixture interactions in the lateral sensillum are unclear. For these interactions to occur between taste cells, there could be direct electrical communication via gap junctions (Steinbrecht, 1989; Isidoro et al., 1993) or ephaptic interactions among adjacent taste cells (Jefferys, 1995; Bokil et al., 2001). For inhibitory interactions to occur between taste stimuli that activate the same taste cell, there could be antagonistic interactions at the cell-surface receptor. Alternatively, if the two carbohydrates activate different signaling pathways, then activation of one pathway could inhibit the response of the other (Dethier and Bowdan, 1989).

Functional implications

There are at least two reasons why *M. sexta* should benefit from feeding selectively on leaves containing mixtures of sucrose+inositol (or sucrose+glucose). Whereas many plant tissues contain relatively high concentrations of glucose and low concentrations of sucrose (Somogyi and Trautner, 1974), solanacous plants contain roughly equal concentrations of glucose and sucrose, and relatively high concentrations of inositol (Nelson and Bernays, 1998). Thus, *M. sexta* could use the simultaneous activation of all three classes of carbohydrate-sensitive taste cell as a sign stimulus for its solanacous host plants. A second reason is that high carbohydrate consumption increases fat accumulation in both the larval (Thompson et al., 2003) and adult (Ojeda-Avila et al., 2003) stages. This extra fat would help pupae undergo successful metamorphosis and adults fuel flight when nectar reserves are limited (Ziegler and Schultz, 1986). Given that carbohydrate levels vary greatly among leaves within solanacous plants (Nelson and Bernays, 1998), any gustatory mechanism that helps *M. sexta* identify leaves with relatively high concentrations of several carbohydrates (i.e. sucrose, glucose and inositol) should promote fat accumulation, and thus be adaptive.

Future directions

We found that a specific ensemble code from the peripheral taste system (i.e. activation of all three classes of carbohydrate-sensitive taste cell) was necessary to stimulate feeding in *M. sexta*. This ensemble code increased the duration of biting, but had no effect on bite size or the rate at which bites were emitted. Further work is needed to determine the mechanisms by which the ensemble code increases meal length. They could involve raising the insect's central excitatory state at the onset of the meal, or delaying activation of the mechanisms that terminate feeding.

Additional work should attempt to identify other ecologically relevant situations that enhance behavioral responsiveness to carbohydrates. For instance, synergistic interactions could occur between gustatory input from carbohydrates and host plant chemicals. This is based on the observation that rearing *M. sexta* on their host plants (e.g. tomato, potato or tobacco leaves) induces a strong preference for leaf disks treated with host plant extracts (Hanson and Dethier, 1973; de Boer, 1993; del Campo et al., 2001; del Campo and Miles, 2003; Haribal et al., 2006). Another possibility is that taste-mediated responsiveness to carbohydrates increases following starvation or prolonged consumption of a carbohydrate-deficient diet. If so, then this elevated responsiveness would facilitate the re-establishment of normal blood sugar levels. The existence of such a mechanism is supported by the observation that maintaining *M. sexta* on a carbohydrate-deficient diet for 24 h causes them to prefer a carbohydrate-rich diet over a carbohydrate-deficient diet (Thompson and Redak, 2000). This deprivation-induced preference for carbohydrates could be mediated by enhanced peripheral (Simpson et al., 1990; Simmonds et al., 1992) or central taste responsiveness.

We thank James Frazier and two reviewers for valuable editorial comments. This project was supported in part by research grant number 5 R29 DC 02416 from the National Institute on Deafness and Other Communication Disorders, National Institutes of Health (to J.I.G.), and a grant from the Howard Hughes Medical Institute (to Barnard College).

References

Bell, R. A. and Joachim, F. A. (1976). Techniques for rearing laboratory colonies of tobacco hornworms and pink bollworms. *Ann. Entomol. Soc. Am.* **69**, 365-373.

Bernays, E. A. and Chapman, R. F. (1994). *Host-plant Selection by Phytophagous Insects*. New York: Chapman & Hall.

Bernays, E. A. and Chapman, R. F. (2000). A neurophysiological study of sensitivity to a feeding deterrent in two sister species of *Heliothis* with different diet breadths. *J. Insect Physiol.* **46**, 905-912.

Bernays, E. A. and Chapman, R. F. (2001). Taste cell responses in a polyphagous arctiid: towards a general pattern for caterpillars. *J. Insect Physiol.* **47**, 1029-1043.

Bernays, E. A., Glendinning, J. I. and Chapman, R. F. (1998). Plant acids modulate chemosensory responses in *Manduca sexta* larvae. *Physiol. Entomol.* **23**, 193-201.

Bernays, E. A., Chapman, R. F. and Singer, M. S. (2000). Sensitivity to chemically diverse phagostimulants in a single gustatory neuron of a polyphagous caterpillar. *J. Comp. Physiol. A* **186**, 13-19.

Blaney, W. M. (1975). Behavioural and electrophysiological studies of taste discrimination by the maxillary palps of larvae of *Locusta migratoria* (L.). *J. Exp. Biol.* **62**, 555-569.

Blaney, W. M. (1981). Chemoreception and food selection in locusts. *Trends Neurosci.* **4**, 35-38.

Blaney, W. M. and Simmonds, M. S. J. (1990). A behavioural and electrophysiological study of the role of tarsal chemoreceptors in feeding by adults of *Spodoptera*, *Heliothis virescens* and *Helicoverpa armigera*. *J. Insect Physiol.* **36**, 743-756.

Blom, F. (1978). Sensory activity and food intake: a study of input-output relationships in two phytophagous insects. *Neth. J. Zool.* **28**, 277-340.

Bokil, H., Laaris, N., Blinder, K., Ennis, M. and Keller, A. (2001). Ephaptic interactions in the mammalian olfactory system. *J. Neurosci.* **21**, RC173.

Bowdan, E. (1988). Microstructure of feeding by tobacco hornworm caterpillars, *Manduca sexta*. *Entomol. Exp. Appl.* **47**, 127-136.

Bowdan, E. (1995). The effects of a phagostimulant and a deterrent on the microstructure of feeding by *Manduca sexta* caterpillars. *Entomol. Exp. Appl.* **77**, 297-306.

Capretta, P. J. (1970). Saccharin and saccharin-glucose ingestion in two inbred strains of *Mus musculus*. *Psychon. Sci.* **21**, 133-135.

Chapman, R. F., Ascoli-Christensen, A. and White, P. R. (1991). Sensory coding for feeding deterrence in the grasshopper *Schistocerca americana*. *J. Exp. Biol.* **158**, 241-259.

Clements, R. S. and Darnell, B. (1980). Myo-inositol content of common foods: development of a high-myo-inositol diet. *Am. J. Clin. Nutr.* **33**, 1954-1967.

de Boer, G. (1993). Plasticity in food preference and diet-induced differential weighting of chemosensory information in larval *Manduca sexta*. *J. Insect Physiol.* **39**, 17-24.

de Boer, G., Dethier, V. G. and Schoonhoven, L. M. (1977). Chemoreceptors in the preoral cavity of the tobacco hornworm, *Manduca sexta*, and their possible function in feeding behavior. *Entomol. Exp. Appl.* **21**, 287-298.

de Brito Sanchez, G. M., Guirfa, M., de Paula Mota, R. T. and Guauthier, M. (2005). Electrophysiological and behavioural characterization of gustatory responses to antennal "bitter" taste in honeybees. *Eur. J. Neurosci.* **22**, 3161-3170.

del Campo, M. L. and Miles, C. I. (2003). Chemosensory tuning to a host recognition cue in the facultative specialist larvae of the moth *Manduca sexta*. *J. Exp. Biol.* **206**, 3979-3990.

del Campo, M. L., Miles, C. I., Schroeder, F. C., Muellerk, C., Booker, R. and Renwick, J. A. (2001). Host recognition by the tobacco hornworm is mediated by a host plant compound. *Nature* **411**, 186-189.

Dethier, V. G. (1976). *The Hungry Fly: A Physiological Study of the Behavior Associated with Feeding*. Cambridge, MA: Harvard University Press.

Dethier, V. G. and Bowdan, E. (1989). The effect of alkaloids on the sugar receptors of the blowfly. *Physiol. Entomol.* **14**, 127-136.

Dethier, V. G. and Crnjar, R. M. (1982). Candidate codes in the gustatory system of caterpillars. *J. Gen. Physiol.* **79**, 549-569.

Devitt, B. D. and Smith, J. J. B. (1985). Action of mouthparts during feeding in the dark-sided cutworm, *Euoxa messoria* (Lepidoptera: Noctuidae). *Can. Entomol.* **117**, 343-349.

Frazier, J. L. (1986). The perception of plant allelochemicals that inhibit feeding. In *Molecular Aspects of Insect-plant Interactions* (ed. L. B. Brattsten and S. Ahmad), pp. 1-42. New York: Plenum Press.

Glendinning, J. I. and Hills, T. T. (1997). Electrophysiological evidence for two transduction pathways within a bitter-sensitive taste receptor. *J. Neurophysiol.* **78**, 734-745.

Glendinning, J. I., Tarre, M. and Asaoka, K. (1999). Contribution of different bitter-sensitive taste cells to feeding inhibition in a caterpillar (*Manduca sexta*). *Behav. Neurosci.* **113**, 840-854.

Glendinning, J. I., Nelson, N. and Bernays, E. A. (2000). How do inositol and glucose modulate feeding in *Manduca sexta* caterpillars? *J. Exp. Biol.* **203**, 1299-1315.

Glendinning, J. I., Davis, A. and Ramaswamy, S. (2002). Contribution of different taste cells and signaling pathways to the discrimination of "bitter" taste stimuli by an insect. *J. Neurosci.* **22**, 7281-7287.

Glendinning, J. I., Bloom, L. D., Onishi, M., Zheng, K. H., Damak, S., Margolskee, R. F. and Spector, A. C. (2005). Contribution of alpha-gustducin to taste-guided licking responses of mice. *Chem. Senses* **30**, 299-316.

Glendinning, J. I., Davis, A. and Rai, M. (2006). Temporal coding mediates discrimination of "bitter" taste stimuli by an insect. *J. Neurosci.* **26**, 8900-8908.

Gothilf, S. and Hanson, F. E. (1994). A technique for electrophysiologically recording from chemosensory organs of intact caterpillars. *Entomol. Exp. Appl.* **72**, 304-310.

Hanson, F. E. and Dethier, V. G. (1973). Role of gustation and olfaction in food plant discrimination in the tobacco hornworm, *Manduca sexta*. *J. Insect Physiol.* **19**, 1019-1034.

Hardinge, M. G., Swarner, J. B. and Crooks, H. (1965). Carbohydrates in foods. *J. Am. Diet. Assoc.* **46**, 197-204.

Haribal, M., Renwick, J. A. A., Attygalle, A. B. and Kiemle, D. (2006). A feeding stimulant for *Manduca sexta* from *Solanum surattenses*. *J. Chem. Ecol.* **32**, 2687-2694.

Hodgson, E. S., Lettvin, J. Y. and Roeder, K. D. (1955). Physiology of a primary chemoreceptor unit. *Science* **122**, 417-418.

Ishikawa, S. (1967). Maxillary chemoreceptors in the silkworm. In *Olfaction and Taste 2* (ed. T. Hayashi), pp. 761-777. Oxford: Pergamon Press.

Isidoro, N., Solinar, M., Baur, R., Roessingh, P. and Städler, E. (1993). Functional morphology of a tarsal sensillum of *Delia radicum* L. (Diptera, Anthomyiidae) sensitive to important host-plant compounds. *Int. J. Insect Morphol. Embryol.* **39**, 275-281.

Jefferys, J. G. (1995). Nonsynaptic modulation of neuronal activity in the brain: electric currents and extracellular ions. *Physiol. Rev.* **75**, 689-723.

Lam, P. Y.-S. and Frazier, J. L. (1991). Rational approach to glucose taste chemoreceptor inhibitors as novel insect antifeedants. In *Synthesis and Chemistry of Agrochemicals II* (ed. D. R. Baker, J. G. Fenyes and W. K. Moberg), pp. 400-412. Washington, DC: American Chemical Society.

Ma, W.-C. (1972). Dynamics of feeding responses in *Pieris brassicae* Linn as a function of chemosensory input: a behavioral and electrophysiological study. PhD thesis, Wageningen Universiteit, The Netherlands.

Marella, S., Fischler, W., Kong, P., Asgarian, E. and Scott, K. (2006). Imaging taste responses in the fly brain reveals a functional map of taste category and behavior. *Neuron* **49**, 285-295.

Marion-Poll, F. and Van der Pers, J. (1996). Un-filtered recordings from insect taste sensilla. *Entomol. Exp. Appl.* **80**, 113-115.

Mitchell, B. K. (1987). Interactions of alkaloids with galeal chemosensory cells of colorado potato beetle. *J. Chem. Ecol.* **13**, 2009-2022.

Nelson, N. and Bernays, E. A. (1998). Inositol in two host plants of *Manduca sexta*. *Entomol. Exp. Appl.* **88**, 189-191.

Ojeda-Avila, T., Woods, H. A. and Raguso, R. A. (2003). Effects of dietary variation on growth, composition, and maturation of *Manduca sexta* (Sphingidae; Lepidoptera). *J. Insect Physiol.* **49**, 293-306.

Omura, H. and Honda, K. (2003). Feeding responses of adult butterflies, *Nymphalis xanthomelas*, *Kaniska canace*, and *Vanessa indica*, to components of tree sap and rotting fruits: synergistic effects of ethanol and acetic acid on sugar responsiveness. *J. Insect Physiol.* **49**, 1031-1038.

Reingold, S. C. and Gelperin, A. (1980). Feeding motor programme in *Limax*. II. Modulation by sensory inputs in intact animals and isolated central nervous systems. *J. Exp. Biol.* **85**, 1-19.

Sasaki, K. and Asaoka, K. (2006). Swallowing motor pattern triggered and modified by sucrose stimulation in the larvae of the silkworm, *Bombyx mori*. *J. Insect Physiol.* **52**, 528-537.

Scheiner, R., Page, R. E. and Erber, J. (2001). The effects of genotype, foraging role, and sucrose responsiveness on the tactile learning performance of honey bees (*Apis mellifera* L.). *Neurobiol. Learn. Mem.* **76**, 138-150.

Schiffman, S. S., Booth, B. J., Carr, B. T., Losee, M. L., Sattely-Miller, E. A. and Graham, B. G. (1995). Investigation of synergism in binary mixtures of sweeteners. *Brain Res. Bull.* **38**, 105-120.

Schiffman, S. S., Sattely-Miller, E. A., Graham, B. G., Booth, B. J. and Gibes, K. M. (2000). Synergism among ternary mixtures of fourteen sweeteners. *Chem. Senses* **25**, 131-140.

Schoonhoven, L. M. (1969). Gustation and foodplant selection in some lepidopterous larvae. *Entomol. Exp. Appl.* **12**, 555-564.

Schoonhoven, L. M. (1972). Plant recognition by lepidopterous larvae. In *Insect/Plant Relationships* (ed. H. F. van Emden), pp. 87-99. Oxford: Blackwell Scientific Publications.

Schoonhoven, L. M. (1978). Long-term sensitivity changes in some insect taste receptors. *Drug Res.* **28**, 2367.

Schoonhoven, L. M. and Blom, F. (1988). Chemoreception and feeding

behavior in a caterpillar: towards a model of brain functioning in insects. *Entomol. Exp. Appl.* **49**, 123-129.

Schoonhoven, L. M. and Dethier, V. G. (1966). Sensory aspects of host-plant discrimination by lepidopterous larvae. *Arch. Neerland. Zool.* **16**, 497-530.

Schoonhoven, L. M. and van Loon, J. J. A. (2002). An inventory of taste in caterpillars: each species is its own key. *Acta Zool. Acad. Sci. Hung.* **48** Suppl. 1, 215-263.

Schoonhoven, L. M., Blaney, W. M. and Simmonds, M. S. J. (1992). Sensory coding of feeding deterrents in phytophagous insects. In *Insect-Plant Interactions*. Vol. IV (ed. E. A. Bernays), pp. 59-79. Boca Raton: CRC Press.

Sclafani, A. (1987). Carbohydrate taste, appetite, and obesity: an overview. *Neurosci. Biobehav. Rev.* **11**, 131-153.

Sclafani, A. (2001). Post-ingestive positive controls of ingestive behavior. *Appetite* **36**, 79-83.

Sclafani, A. and Glendinning, J. I. (2005). Sugar and fat conditioned flavor preferences in C57BL/6J and 129 mice: oral and postoral interactions. *Am. J. Physiol.* **289**, R712-R720.

Shields, V. D. C. and Mitchell, B. K. (1995). The effect of phagostimulant mixtures on deterrent receptor(s) in two crucifer-feeding lepidopterous species. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **347**, 459-464.

Simmonds, M. S. J., Simpson, S. J. and Blaney, W. M. (1992). Dietary selection behaviour in *Spodoptera littoralis*: the effects of conditioning diet and conditioning period on neural responsiveness and selection behavior. *J. Exp. Biol.* **162**, 73-90.

Simpson, C. L., Chyb, S. and Simpson, S. J. (1990). Changes in chemoreceptor sensitivity in relation to dietary sensitivity by adult *Locusta migratoria*. *Entomol. Exp. Appl.* **56**, 259-268.

Simpson, S. J. and Raubenheimer, D. (1993). The central role of the haemolymph in the regulation of nutrient intake in insects. *Physiol. Entomol.* **18**, 395-403.

Smith, J. C., Williams, D. P. and Short Jue, S. (1976). Rapid oral mixing of glucose and saccharin by rats. *Science* **191**, 304-305.

Somogyi, J. C. and Trautner, K. (1974). Der Glukose-, Fruktose- und Saccharosegehalt verscheidener Gemüsearten. *Schweiz. Med. Wochenschr.* **104**, 177-182.

Spector, A. C. (2000). Linking gustatory neurobiology to behavior in vertebrates. *Neurosci. Biobehav. Rev.* **24**, 391-416.

Städler, E. and Hanson, F. E. (1978). Food discrimination and induction of preference for artificial diets in the tobacco hornworm, *Manduca sexta*. *Physiol. Entomol.* **3**, 121-133.

Steinbrecht, R. A. (1989). The fine structure of thermohygrosensitive sensilla in the silkmoth *Bombyx mori*: receptor membrane structure and sensory cell contacts. *Cell Tissue Res.* **255**, 49-57.

Thompson, S. N. and Redak, R. A. (2000). Interactions of dietary protein and carbohydrate determine blood sugar level and regulate nutrient selection in the insect *Manduca sexta* L. *Biochim. Biophys. Acta* **1523**, 91-102.

Thompson, S. N., Borchardt, D. B. and Wang, L.-W. (2003). Dietary nutrient levels regulate protein and carbohydrate intake, gluconeogenic/glycolytic flux and blood trehalose level in the insect *Manduca sexta* L. *J. Comp. Physiol. B* **173**, 149-163.

Thorne, N., Chromey, C., Bray, S. and Amrein, H. (2004). Taste perception and coding in *Drosophila*. *Curr. Biol.* **14**, 1065-1079.

Timmins, W. A. and Reynolds, S. E. (1992). Physiological mechanisms underlying the control of meal size in *Manduca sexta* larvae. *Physiol. Entomol.* **17**, 81-89.

Valenstein, E. S., Cox, V. C. and Kakolewski, J. W. (1967). Polydipsia elicited by the synergistic action of a saccharin and glucose solution. *Science* **157**, 552-554.

Wang, Z., Singhvi, A., Kong, P. and Scott, K. (2004). Taste representations in the *Drosophila* brain. *Cell* **117**, 981-991.

White, P. R., Chapman, R. F. and Ascoli-Christensen, A. (1990). Interactions between two neurons in contact chemosensilla of the grasshopper, *Schistocerca americana*. *J. Comp. Physiol. A* **167**, 431-436.

Wrubel, R. P. and Bernays, E. A. (1990). The relative insensitivity of *Manduca sexta* larvae to non-host plant secondary compounds. *Entomol. Exp. Appl.* **54**, 117-124.

Yamamoto, R. T. and Fraenkel, G. (1960). Assay of the principal gustatory stimulant for the tobacco hornworm, *Protoparce sexta*, from solanaceous plants. *Ann. Entomol. Soc. Am.* **53**, 499-503.

Ziegler, R. and Schultz, M. (1986). Regulation of lipid metabolism during flight in *Manduca sexta*. *J. Insect Physiol.* **32**, 997-1001.