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Characterization of Galeal Sugar and Glucosinolate-Sensitive Cells in *Entomoscelis americana* Adults

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Summary. A restrained, whole animal preparation was used to study chemical sensitivity and specificity of three cells in the medial galeal chemosensillum of adult red turnip beetles. One cell is sensitive to sugars, particularly sucrose and maltose, two cells respond to glucosinolates and two to the glucoside arbutin. Responses to mixtures of these compounds revealed that arbutin stimulates the sugar-sensitive cell and one of the glucosinolate-sensitive cells. The second glucosinolate-sensitive cell was stimulated only by glucosinolates. Dose-response curves were determined for most of the compounds tested.

The adult gustatory system differs in several respects from that of the larva. Sugar sensitivity is similar in both, though only in the larva is this cell also sensitive to amino acids. One of the glucosinolate-sensitive cells is similar in both stages, while the second cell is much more dose-dependent in adults. A fourth cell has been morphologically identified in both sensilla, but we were unable to determine its specificity.

Introduction

Studies on physiology of gustation in insects have included species in the orders Lepidoptera and Diptera (see review by Hansen 1978) providing a sound basis for future investigations. However, representatives from other orders must be studied before a truly comparative view of insect gustatory mechanisms can be developed. We have begun a series of studies on leaf beetles (Fam. Chrysomelidae), beginning with *Entomoscelis americana* Brown, the red turnip beetle. In addition to comparisons among orders (see Mitchell and Gregory 1979, 1981), we wanted to compare sensory systems of larvae and adults of the same species, especially when the trophic relationships of the two stages are similar, as in *E. americana* (Stewart 1972). In this paper we describe electrophysiological responses of three cells in the adult galea to sugars, amino acids, glucosinolates and the glucoside arbutin. Comparisons are made with similar cells in red turnip beetle larvae studied by Mitchell and Gregory (1979, 1981), and implications of the differences and similarities for feeding behaviour in the two stages are discussed.

Materials and Methods

Preparation of Animal. We used lab-reared, adult *Entomoscelis americana* (Brown) in this study (see Mitchell 1978 for rearing details). Except where noted, beetles were used within 24 h of eclosion, and, until prepared, were held at 4 °C and high R.H. None were allowed to feed as this led to excessive regurgitation during manipulation. A beetle was anaesthetized with carbon dioxide and a sharpened silver reference electrode was inserted through the ventral posterior wall of the metathorax and pushed in until the point penetrated to the neck region. The electrode was anchored in place with a small drop of molten beeswax-rosin mixture (Fig. 1). The beetle was then placed posterior end first, into a tapering glass tube (cut off end of a Pasteur pipette) such that only its head and prothorax projected, and further restrained by fusing the dorsum of the prothorax to the glass tube with beeswax-rosin. The head was waxed to the prothorax in a prognathous position.

To expose the galea, a fine human head hair was tied into a single knot, slipped around the maxillary palp and pulled snugly around its base, being careful not to sever the palp (Fig. 2). Because the basal palpal segments flare distally, the hair did not slip. The palp was then pulled laterally and slightly downwards and backwards, rotating the galea and lacinia out from under the mandible. To minimize movement, the maxilla was fully extended and the free end of the hair was waxed to the edge of the glass tube. The prepared insect was mounted on a micromanipulator and given one half hour to recover from the carbon dioxide. A small amount of 500 mM NaCl in the end of the glass tube served as a conducter between the silver reference and holder electrodes. A Leitz Laborlux compound microscope with long working distance objectives was used at 480× during stimulus applications.

Even with these measures, the galea usually had some freedom of movement and mandibular movements also perturbed it. This activity was usually greatest just after recovery from carbon dioxide and lessened with time. Exposing the insect to light from the micro-

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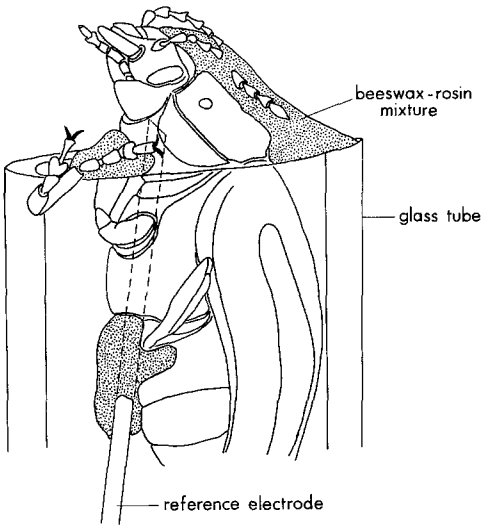

Fig. 1. Lateral view of adult beetle mounted for recording

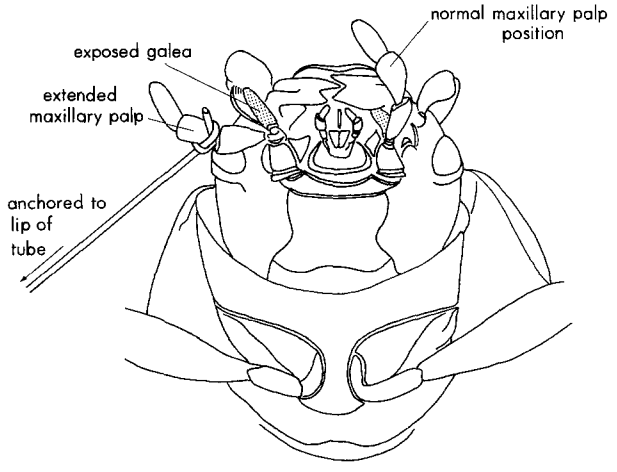

Fig. 2. Ventral view of adult beetle showing method for orientating and restraining maxilla

scope condenser also elicited activity so this was done only at the time of stimulus application.

Recording Method. All recordings were from the medial sensillum (Mitchell and Sutcliffe 1980) and the tip recording method first described by Hodgson et al. (1955) was used. Glass micropipettes of 6–8 µm tip diameter were filled, just before use, with stimulus compounds dissolved in 25 mM NaCl. A disadaptation time of 3 min was allowed between applications. Potentials were amplified using a non-blocking preamplifier and recorded with a Philips Mini-Log 4 tape recorder. Hard copy records were made with a Honeywell 1858 Visicorder on Linagraph paper. Impulse counts and all other measurements were done manually from the visicorder records. Unless otherwise stated discharge frequencies are impulses/s derived by counting the first half s of a record, and multiplying the result by two. Impulse trains were prepared for illustration by tracing original visicorder records.

Results and Discussion

Response to Sugars

Response to sucrose, and to other sugars tested, was from a single cell (Fig. 3a) and was dose-dependent over a sucrose concentration range of 1–200 mM with a threshold of approximately 0.2 mM (Fig. 4a, open circles). Response frequency rose sharply between 1 and 20 mM giving a K_b (concentration eliciting half-maximal response) of 10 mM, as estimated by eye. Disadaptation was rapid at low and mid-range concentrations, even after multiple successive stimulations (Fig. 5). Clearly, a disadaptation period of 1–2 min is sufficient for dose-response studies with this system.

Three other sugars were tested (Fig. 6), their choice based on a previous study of larvae (Mitchell and Gregory 1979). Sucrose and maltose were equally stimulatory, while monosaccharides comprising them were ineffective (glucose) or weakly stimulatory (fruc-

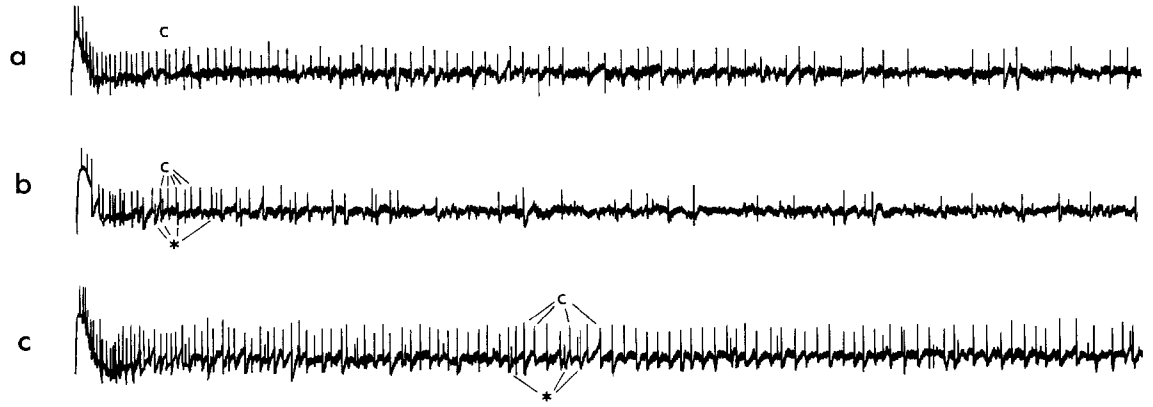

Fig. 3. Responses of adult galeal sensillum to **a** 10 mM sucrose, **b** 10 mM arbutin, **c** 10 mM mixture of sucrose and arbutin. All records from the same preparation. *c* impulses from sucrose-sensitive cell; * impulses from the second arbutin-sensitive cell (perhaps cell B or cell D)

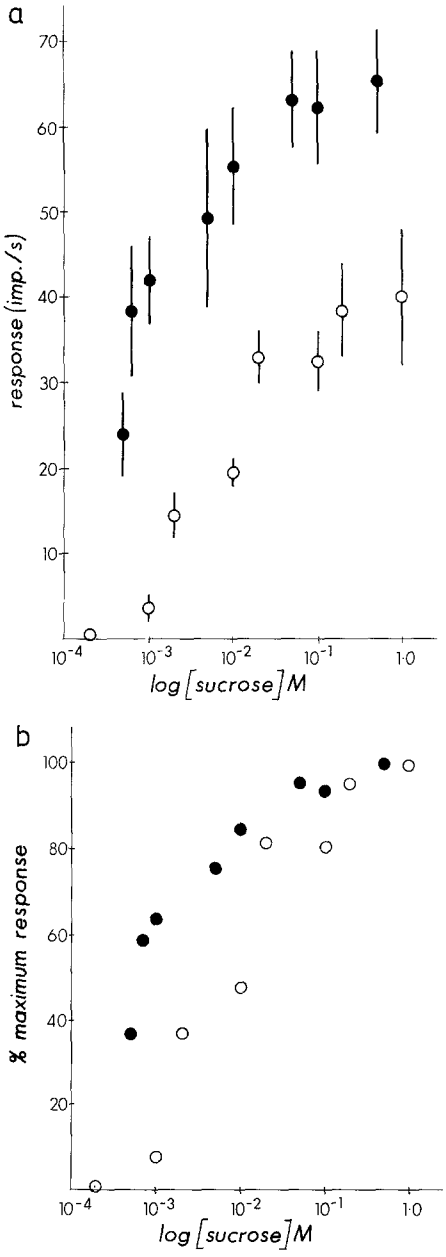

Fig. 4. **a** Dose-response curves for sucrose on galeal sensillum; *open circles*: beetles of mixed ages, $n=6$; *closed circles*: beetles less than 24 h old, $n=5$. *Error bars*: S.E. **b** Same data as in **a** with responses expressed relative to maximum for each group

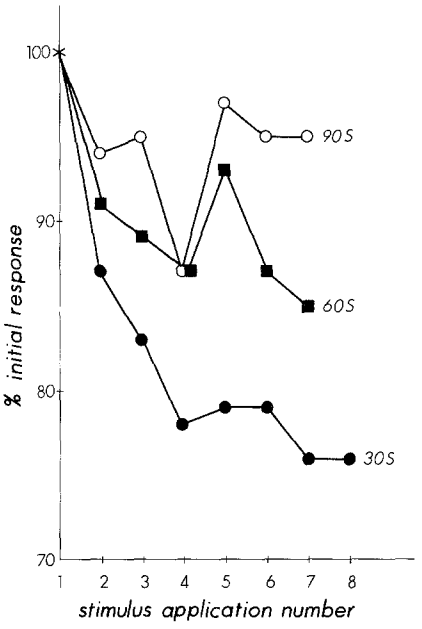

Fig. 5. Response of adult galeal sensillum to 10 mM sucrose after successive disadaptation periods of 20 s, 60 s and 90 s

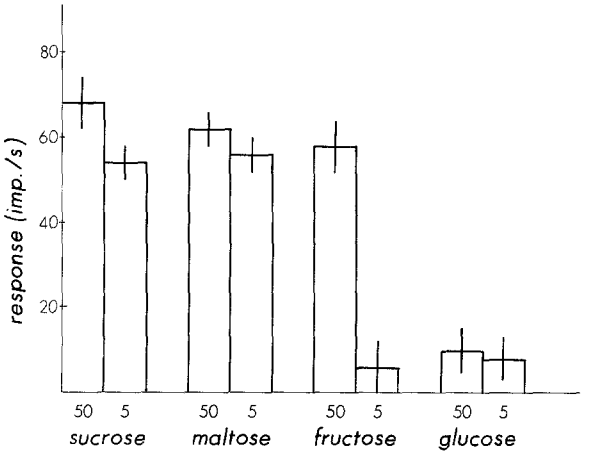

Fig. 6. Response of adult galeal sensilla to four sugars at 5 and 50 mM. *Error bars*: S.E. $n=6$

tose). This is consistent with the well known observation that glucosidic linkages are important in insect sugar-sensitive cells (Schoonhoven 1974; Hansen 1978).

Beetles used in dose-response experiments mentioned above varied in age from twelve hours to four days. Though exact age records were not kept, it seemed that sensilla of younger insects were more sensitive to sucrose. To investigate this, a second series of beetles, all less than 24 h old, was tested (Fig. 4a, closed circles). Response threshold was similar in both groups but overall impulse frequency was significantly greater in the younger group, as was maximum response (65 imp./s compared with 40 imp./s). This heightened sensitivity was also reflected in the concentration range over which the greatest change in response occurred 0.5–10 mM (young group), 1–20 mM (mixed group). Plots of rela-

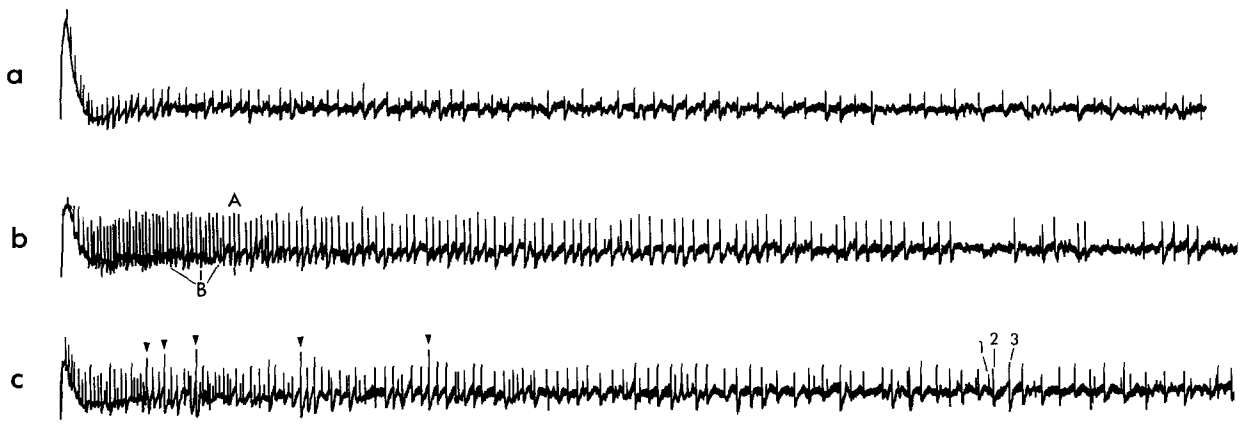

Fig. 7. Response of adult galeal sensillum to **a** 10 mM sucrose, **b** 10 mM glucosinalbin, **c** 10 mM mixture of sucrose and glucosinalbin. All recordings from the same preparation. *A* and *B*: impulses from cells A and B; *arrowheads*: electrically added impulses; *1, 2, 3*: evidence for activity from three cells in this record

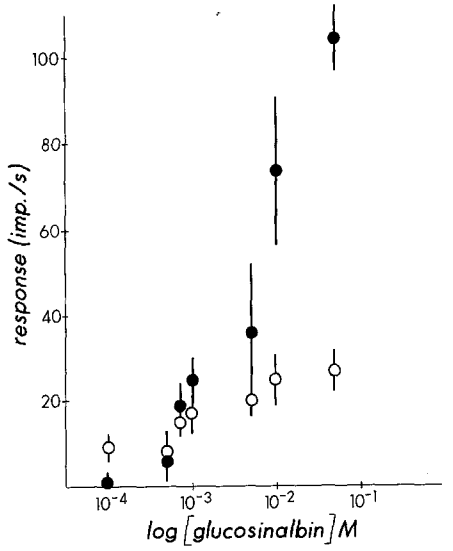

Fig. 8. Dose-response curves for glucosinalbin on adult galeal sensilla. *Closed circles*: response of cell A; *open circles*: response of cell B. *Error bars*: S.E. $n=6$

Table 1. Comparison of cells A and B in adult and larva response to glucosinalbin

Parameter	Cell A		Cell B	
	Larva	Adult	Larva	Adult
K_b (mM)	1.0	2.0	?	0.4
R_{max}	36	100	15	30
Most effective dose range (mM)	0.5–10	1–50	?	0.1–10
Threshold (mM)	0.01	0.1	0.001	<0.01

tive response vs sucrose concentration (maximum response defined as 100%) facilitate comparison of these two groups (Fig. 4b). For mid-range concentrations, the curves are approximately parallel with an apparent K_b change from 1.0 mM (young group) to 10 mM (mixed group).

How could this K_b shift with age be interpreted? Relationship between dose and response is complex, involving more than binding of stimulus and receptor molecules. The dissociation constant of the stimulus-receptor complex (K_d) cannot be reliably estimated from the K_b (half-maximum response) derived from the experimental curve, because a chain of processes occurs between stimulus-receptor binding and impulse initiation. We must therefore be careful not to read too much into the shape of dose-response curves. This problem is thoroughly discussed by Waud (1975, 1976).

If we assume K_d is the same in both groups (i.e. that the receptor molecules are the same between experimental groups), and that the curve shift is related to events occurring after stimulus-receptor binding, three possible explanations come to mind. Firstly, absolute number of receptor molecules may differ between groups, with younger cells having the greater number. Response from older beetles would then be analogous to that expected after treating sensilla of younger beetles with an irreversible blocking agent thus reducing maximum response. Secondly, the dendrite, which presumably transmits a generator potential from the cell's apical region to a spike initiating region (Hansen 1978), may do this with a smaller decrement in very young beetles (i.e. dendritic cable properties may differ between very young and older cells). Thirdly, the threshold of the spike initiating region may be lower in younger cells. Any or none of these explanations may be correct. We mention them to emphasize the complexity of the system and to point out the possibility that age-related phenomena may influence results in such investigations.

Response to Amino Acids

Sugar-sensitive cells in flesh flies and larval red-turnip beetles also respond to amino acids (Shimada 1975,

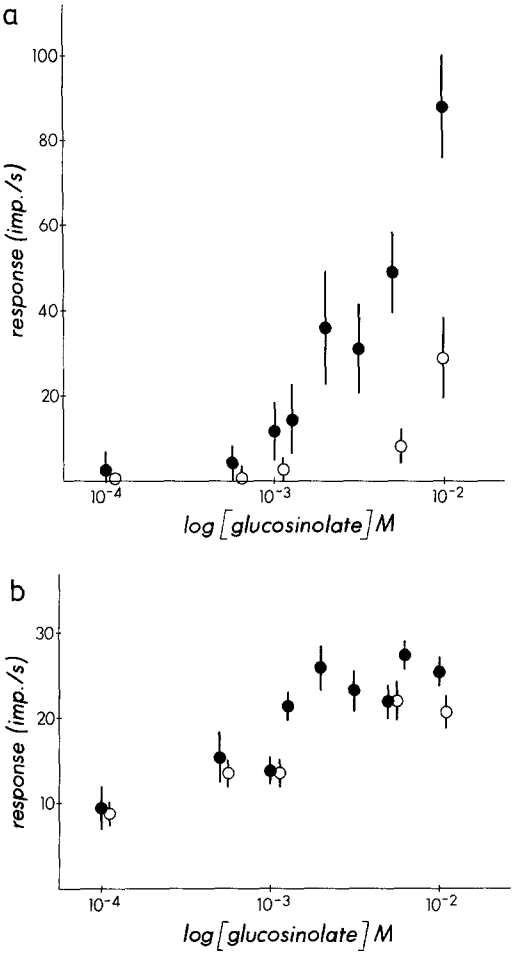

Fig. 9a, b. Dose-response curves for glucosinalbin (*closed circles*) and glucotropaeolin (*open circles*) on adult galeal sensilla. **a** Response of cell A; **b** response of cell B. Data from a different group of beetles than for Fig. 8. *Error bars*: S.E. $n=8$

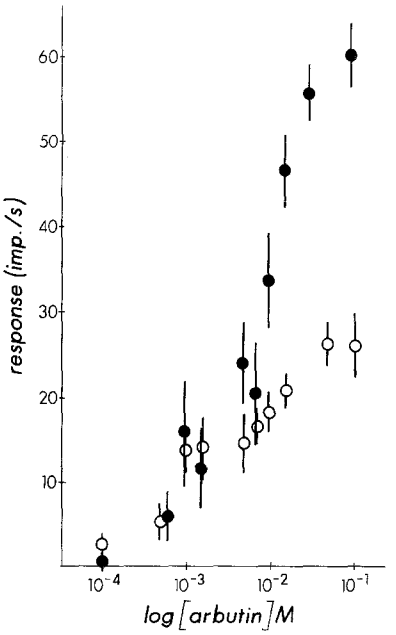

Fig. 10. Dose-response curves for arbutin on adult galeal sensilla. *Closed circles*: response of cell C. *Open circles*: response of cell B (see text for discussion on cell identification). *Error bars*: S.E. $n=7$

1978; Shiraishi and Kuwabara 1970; Mitchell and Gregory 1979). Alanine, glycine, lysine, and glutamine (50 mM) were tested on this sensillum with equivocal results. Responses were multi-celled, had low S/N ratios and low frequency (ave. 8 imp./s), making it impossible to determine if the sugar-sensitive cell was one of those responding. At best, amino acids must be considered only slightly effective stimuli.

Response to Glucosides

Based on experiments with *E. americana* larvae (Mitchell and Gregory 1979, 1981), three glucosides were chosen for our work on adults. These were the glucosinolates glucosinalbin and glucotropaeolin, and the non-glucosinolate glucoside arbutin. Glucosinalbin and arbutin were clearly the most effective; both stimulated two cells (Figs. 3b and 7b). The cells responding to glucosinalbin were both dose-dependent (Fig. 8), and are designated A (large impulse) and B (small impulse) as in the larvae (Mitchell and Gregory 1981). Though cell B had a lower impulse frequency, it was more sensitive than cell A (Table 1).

Evidence of different functions for cells A and B comes from their responses to other glucosinolates, especially glucotropaeolin. Cell A's threshold to glucotropaeolin was higher than to glucosinalbin (1 mM vs. 0.1 mM) and its impulse frequency at all concentrations was lower (Fig. 9a). In contrast, cell B's response was the same for both compounds (Fig. 9b). This suggests that cell B is a more general glucosinolate, or even glucoside, sensor than cell A. Glucotropaeolin differs from glucosinalbin only in lacking an hydroxyl group. Apparently, this is an important feature for the receptor of cell A but not cell B.

Arbutin also stimulated two cells (Figs. 3b and 10) and, as in the experiments with glucosinolates, the cell with larger impulse amplitude had higher activity at most concentrations. Otherwise, the two cells had similar response parameters (Table 2).

Identification of Cells Responding to Various Stimuli

Galeal taste sensilla of red turnip beetle adults have four chemosensitive neurons (Sutcliffe and Mitchell 1980). How are responses to the various compounds tested distributed among these neurons? To facilitate this discussion the four cells are designated A, B, C and D following the scheme Mitchell and Gregory (1981) used for galeal sensilla of red turnip beetle larvae. Cell A was defined as the cell producing the

larger impulses in response to glucosinalbin and cell B as that producing the smaller ones, while cell C was that responding to sucrose. It is important to note that these labels have no morphological significance, i.e., we cannot identify, for instance, cell A in a micrograph.

We compared records of sensillar responses to combined solutions of compounds x and y to records produced by stimulation with solutions of x and y alone. If x and y stimulate different cells, the combined record will show two independent spike sizes. In addition, at high frequencies, the combined record will tend to be complex as a result of numerous electrical additions of impulses. Alternatively, if x and y stimulate the same cell, the combined response will be qualitatively similar to each single response but will have higher impulse frequencies (at least below R_{max}). In addition, no electrical additions will occur.

Combinations of sucrose, glucosinalbin and arbutin were used in practice; interpretation of these records was complicated by the fact that both glucosinalbin and arbutin elicit responses from two cells (Figs. 3 and 7). The first 1.5 s of the combined record for sucrose and glucosinalbin has numerous impulse additions (Fig. 7c, arrowheads). In the later portion of the record there are three independently occurring impulse sizes (Fig. 7c; 1, 2, 3). Because glucosinalbin alone elicits impulses from two cells only, the third impulse in these records must be attributed to sucrose. Therefore, the sucrose-sensitive cell is neither cell A nor cell B and, as in Mitchell and Gregory (1981), is designated as cell C.

In the combined sucrose-arbutin record there is predominantly one regularly occurring impulse size plus a few smaller impulses from the second arbutin cell (Fig. 3c). From this we conclude that cell C, the sucrose-sensitive cell, also responds to arbutin. To confirm this, we counted impulses in records of responses to sucrose alone (Rs), to arbutin alone (Ra), and to sucrose and arbutin in combination (Rc). If sucrose and arbutin both stimulate cell C, then Rs+Ra will be greater than or equal to Rc because both sucrose and arbutin would make a contribution to the combined impulse frequency up to, but not exceeding, the cell's R_{max}. Out of 20 such experiments, Rs+Ra was equal to Rc eight times, exceeded it 10 times and was less only twice, supporting the conclusion that cell C is stimulated by both sucrose and arbutin.

The smaller impulse in the arbutin response may be from cell A, B or D. It is unlikely to be cell A, because cell A typically produces impulses with much larger amplitude. Although cell D cannot be ruled out, the characteristics of the smaller arbutin-sensitive cell's response (impulse amplitude, dose-response parameters) are similar to those of cell B. It is therefore possible that cell B is a general glucoside sensor in adult red turnip beetles. Combined stimulations with arbutin and glucosinalbin which may have helped establish the source of the smaller arbutin impulses were not done.

Comparison of Larval and Adult Sensilla

We hypothesised previously that, since adults and larvae of this species have similar food plant requirements, features of their mouthpart sensilla would also be similar (Sutcliffe and Mitchell 1980). There are morphological similarities between larval and adult chemosensilla (Mitchell et al. 1979; Sutcliffe and Mitchell 1980) but these are of the order of similarity exhibited by insect contact chemosensilla in general. Here we compare the two systems physiologically using data presented above and in Mitchell and Gregory (1979, 1981).

Response to sucrose was from a single cell with a K_b of 1.0 mM in larvae and young adults and 10 mM in older adults. In both stages sucrose and maltose were equally effective while fructose and glucose were much less so. Arbutin also stimulated the sugar-sensitive cell (C) in both stages. There was some difference in disadaptation rates following stimulation with sucrose; larval cells recovered to between 80 to 90% of original response after only 4 s disadaptation at all but the highest concentration tested (100 mM). Equivalent recovery in adults required 60 s.

The two stages differ markedly in their response to amino acids. Larval cells responded to 9 of 20 amino acids tested, and for five of these (alanine, serine, proline, asparagine and glycine) the response was from a single cell. Experiments with sucrose-alanine mixtures showed that the amino acid stimulated the sugar-sensitive cell. Adult sensilla, on the other hand, were barely stimulated by amino acids. It is possible that amino acids and sugars react with different receptor molecules on cell C. Shimada et al. (1974) and Shimada (1975) have demonstrated that different receptors for amino acids and sugars can occur on the same cell in insects.

Two cells responded to glucosinalbin in both stages (Table 1) but, in larvae, the activity of cell B showed little correlation with stimulus concentration. Sensitivity was high in both stages (threshold approaching 0.001 mM) but in larvae it reached R_{max} (15 imp./s) at low concentrations and stayed in the background at that rate throughout the remainder of the stimulus concentration range. In adults, the activity of cell B was strongly correlated with stimulus concentration over a range similar to that of Cell A.

The possible role of cell B in detecting glucosides like arbutin only became apparent in studies with

Table 2. Response to arbutin

Parameter	Cell C[a]	Cell B[a]
K_b (mM)	10	5
R_{max}	60	27
Most effective dose range (mM)	1–20	0.5–20
Threshold (mM)	0.1	0.1

[a] See below for discussion on cell identification

adults. Larval sensilla responded well to arbutin, but often with a single cell which proved to be identical with the sugar-sensitive cell. Activity in a second cell was sometimes evident, especially at high concentrations, but this was never predictable enough to study. In adults, on the other hand, cell B responded to arbutin over essentially the same concentration range as did cell A (Table 2). This cell presumably detects plant glucosides and it is possible that these play a more important role in adult feeding than they do in larval feeding.

In summary, sugar sensitivity remains relatively unchanged from larva to adult, though the sugar-sensitive cell in larvae is also sensitive to amino acids. Sugar-sensitive cells of both larvae and adults respond to arbutin. Of the glucosinolate-sensitive cells, cell A does not appear to differ between adults and larvae but cell B is more dose-dependent in adults than in larvae. Arbutin also stimulates cell B in adults but does so poorly, if at all, in larvae.

To our knowledge, this is the first demonstration of such specificity and sensitivity differences between larval and adult forms where there are presumably limited differences in trophic behaviour. The more complex nature of the adult gustatory system may result from greater demands in terms of food plant selection. The insensitivity of the adult to amino acids may only be apparent since all sensilla were not studied, but the difference between the sugar-sensitive cells in the two stages deserves further study. From the results one might also predict that adult feeding would be more influenced by combinations of arbutin and glucosinalbin. Behavioural experiments would be useful for clarification of some of these points. Unfortunately, both larvae and adults of this species are refractory to many kinds of artifical feeding substrates (Mitchell 1978). The main value of the comparative aspect of this work lies in the demonstration that measurable differences between adult and larval systems do exist. From this we expect similar differences in other chrysomelid species more amenable to behavioural studies.

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