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# VARIANCE: A POSSIBLE CODING MECHANISM FOR GUSTATORY SENSILLA ON THE LABELLUM OF THE FLESHFLY *SARCOPHAGA BULLATA*

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## Summary

In behavioural tests, 2-day-old female *Sarcophaga bullata* consumed more liver or fish powder in solution than 100 mmol $l^{-1}$ sucrose. We investigated the chemosensory basis of this discrimination by recording electrophysiological responses of 177 medium-length labellar taste sensilla from 10 different flies to two applications of each of these three solutions. Responses from three chemosensory cells were evident in most records. Cell 1 produced a mean response of 37.6 impulses $s^{-1}$, and similar responses to all three stimuli. It was the most active of the three cells. Cell 2 produced a significantly greater response to fish than to liver or sucrose in one of the two stimulus applications. Cell 3, the least active, responded with twice the firing rate to fish than to liver or sucrose. However, the mean firing rates did not provide information that could account for the observed behavioural discrimination. The only difference in the electrophysiological responses to the three stimuli which correlated with the behavioural discrimination was the variance of the response of cell 1, which was much higher to sucrose than to either fish or liver. We propose that variance itself could provide the necessary information to allow the fly's nervous system to distinguish between a 'simple' stimulus such as sucrose and a 'complex' stimulus such as fish or liver.

Key words: *Sarcophaga*, proboscis, gustatory chemosensilla, variance, electrophysiology, computer analysis.

## Introduction

The electrical responses of insect gustatory sensilla have been the subject of many investigations since a suitable method for recording from them was developed (Hodgson *et al.* 1955). Traditionally, the labellar chemosensory sensilla of the blowfly *Phormia regina* were thought to contain one sensory cell responsive to cations, one to anions, one to sugars and one to water, in addition to a cell responding to mechanical displacements of the sensillum. The concept that these cells were narrowly specific was challenged by Dethier (1974).

Van der Starre (1972) showed in *Calliphora* tarsal sensilla that as many as three cells could be active when sucrose was used as the stimulating solution. He also concluded that water and sucrose actually stimulated the same cell. Impulse frequencies of the different sensory cells showed a very high degree of variability not only among different sensilla but also between successive stimulations of the same sensillum. Van der Molen *et al.* (1985) analyzed the sources of variability in *C. vicina* tarsal sensilla from 10 flies, using a large data set of 1600 spike trains obtained from stimulations with an equimolar glucose:fructose mixture. Inter-fly variability accounted for almost 50% of the variation in responses. The authors thus argued that similar experiments should be done on individual flies rather than averaging the responses from different flies. Residual variation (about 40% of the total variability) not explained by inter-individual variability (50%), sensillar topology (6%) and decreasing response values with time (<5%), was attributed either to random fluctuations in membrane potential or, more likely, to changes in the accessory structures at the tip of a sensillum.

Den Otter (1971) also found a high degree of variation in the responses of tarsal sensilla of *C. vicina* to stimulations with sodium chloride solutions. He found both intra-sensillum variation in response to repeated presentations of the same stimulus, as well as inter-sensillum variation in response to the same stimulus. Three cell types were proposed, based on their response patterns to sodium chloride. Maes and Den Otter (1976) identified two cell types responding to solutions of $1\,\mathrm{mol\,l^{-1}}$ potassium chloride in the labellar sensilla of *C. vicina*. They were able to localize 'type A' responses mostly in large aboral setae, and 'type B' responses in short aboral setae and in adoral setae. More recently, Maes and Harms (1986) and Maes and Ruifrok (1986) examined the responses of labellar sensilla of *C. vicina* to various salts, and discussed possible coding mechanisms: a temporal code, which they concluded was not very probable, and an ensemble code (across-fibre pattern), which they favoured.

These studies show that a high degree of intra- and inter-animal variability exists in the responses of gustatory chemosensilla in flies. However, since flies have been shown behaviourally to distinguish between different solutions, a sensory code must exist which provides unambiguous information concerning such solutions. One would assume that such a code would be the same for different flies of the same species, and that it must operate in any given animal despite the high degree of variability. Moreover, flies typically are exposed to, and presumably feed on, complex mixtures rather than simple solutions. How, then, does the individual fly

make a correct choice to feed or not to feed when presented with a specific solution?

Preliminary experiments showed that adults of the fleshfly *Sarcophaga bullata* were able to discriminate between 100 mmol $l^{-1}$ sucrose, and a solution of either powdered fish extract or powdered liver extract. In this paper we report the results of a first series of experiments on the numerous medium-size sensilla on the labellar lobes of *S. bullata*, to investigate: (a) whether the variability in single-cell response seen in *C. vicina* and *P. regina* is more general in flies; (b) whether the response is more or less variable when the stimuli are complex and more 'natural'; (c) what the sensory code or codes are that the fly might use to distinguish such stimuli and that would work in the face of high variability; (d) what the mechanism and function of the variability might be.

## Materials and methods

### *Insects*

Ten 2-day-old adult *Sarcophaga bullata* females were used. They were obtained from a laboratory colony kept at an ambient temperature of 22°C and a 16 h:8 h L:D cycle. Larvae were reared on either beef or pork liver. The diet for adults was a 2:1 mixture of sucrose and instant dried skim milk, sugar cubes and water.

### *Electrophysiology*

Labellae were excised and mounted on glass micropipettes containing 100 mmol $l^{-1}$ NaCl. All recordings were from medium-size sensilla on either the left or the right labellar lobe using the tip recording technique (Hodgson *et al.* 1955). 12–22 sensilla per animal was sampled. Stimuli were: (1) 100 mmol $l^{-1}$ sucrose in 100 mmol $l^{-1}$ NaCl (Sucrose); (2) liver powder (Sigma Chemical Co., stock no. 202-3) as a 10 % solution in distilled water (Liver); (3) fish meal (Sigma Chemical Co., stock no. F-6381) as a centrifuged, 40 % solution in distilled water (Fish). To achieve equivalent conductances for all three solutions, it was necessary to add a small amount of 100 mmol $l^{-1}$ NaCl to the fish meal solution described above. Stimuli were presented in random order; thus a typical protocol would be: stimulate eight different sensilla with Fish (FA) for 3 s each; stimulate the same sensilla with Sucrose (SA); then stimulate the same sensilla with Liver (LA); repeat, again with a random order of presentation (e.g. LB, FB, SB). This would result in six stimuli for each sensillum: FA, FB, LA, LB, SA, SB. Stimuli were thus presented to each sensillum twice, with the two presentations anywhere from 10–30 min apart. 177 sensilla were sampled from 10 flies, resulting in 1062 spike trains. Records were digitized at 10 000 points $s^{-1}$ for a 1-s period starting 0.3 s after contact with the sensillum. The first 0.3 s of the response was not used since the high initial firing frequency of some cells produced too many superimposed spikes for accurate classification. Spikes were classified on a sensillum by sensillum basis, and assigned to one of four spike classes using the MS-DOS compatible software package 'SAPID Tools' developed by the authors (Smith *et al.* 1990).

Entire blocks of data for each fly were examined to ensure that we had achieved correct assignments of spike classes. Spike firing frequencies were then analyzed using DataDesk Professional (Odesta Corp., 4084 Commercial Avenue, Northbrook, IL, USA), Systat (Systat Inc., 1800 Sherman Avenue, Evanston, IL, USA) and Number Cruncher Statistical System (J. L. Hintze, 865 East North, Kaysville, UT, USA) programs on a Macintosh computer.

### *Feeding experiments*

2- to 3-day-old flies were starved for 18–24 h prior to their use in experiments with the one-choice apparatus described by Blades and Mitchell (1986). An individual fly was placed in a 25 ml clear glass vial fitted with a plastic cap, through which protruded the tip of a 100 $\mu$l capacity pipette. Small holes in the plastic cap provided aeration. The pipette was filled with one of the three solutions described in Materials and methods, or with 100 mmol $l^{-1}$ NaCl. Readings were taken of the volume of solution ingested by each fly in a 6-h period.

## Results

### *Feeding behaviour*

The questions about neural coding which form the major portion of this paper are predicated on behavioural responses of these flies to the mixtures of Fish, Liver and Sucrose. Fig. 1 shows the amount of feeding by 34 2- to 3-day-old female *S. bullata* on each of the above stimuli and on 100 mmol $l^{-1}$ NaCl during a 6-h period. One group of 48 flies was observed at hourly intervals to obtain an estimate of drink size. On average, the single largest consumption in 1 h amounted to 70 % of total consumption in 6 h. Time to the first drink varied considerably, from a few minutes to several hours. On the basis of these results, we decided that 6 h was a

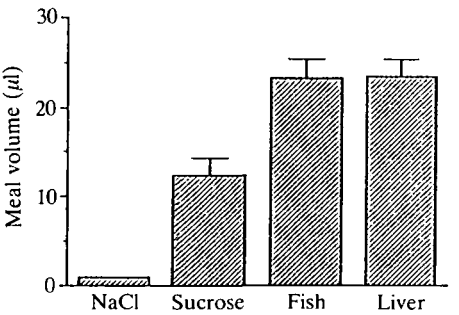


Fig. 1. Feeding ($\mu$l) in 6 h by 2- and 3-day-old female *Sarcophaga bullata* on Fish, Liver, Sucrose and NaCl (see text for details). Values for Sucrose, Fish and Liver are means±S.E. The results for NaCl were highly skewed, with 5/34 flies taking meals comparable to those on Fish and Liver, and 27/34 taking meals of 3 $\mu$l or less; consequently the median response is shown for NaCl, with no S.E. bar. Feeding on Sucrose is significantly lower than on Fish or Liver ($P$=<0.001 Kruskall–Wallis multiple comparisons).

reasonable time over which to conduct short-term feeding tests. Since most of the consumption was from feeding in a single hour, it is likely that any differences observed among stimuli reflected differential sensory input rather than metabolic phenomena and associated internal feedback. As is clearly shown in Fig. 1, Liver and Fish were equally stimulatory while Sucrose stimulated only about half as much consumption ($P<0.001$ Kruskal–Wallis analysis of variance). 100 mmol $l^{-1}$ NaCl was included as a control, even though it was excluded from electrophysiological experiments for logistical reasons (see below). Feeding was minimal on NaCl, with only a few flies consuming more than 1 or 2 $\mu$l of solution.

### *Preliminary electrophysiological results*

In attempting to establish the responses to various stimuli, the number of recordings per fly is limited by the useful lifetime of the preparation. The total number of recordings that can reasonably be managed is limited by the digitization and analysis procedures which are still labour-intensive, despite extensive use of computers. Thus, it was necessary to optimize the numbers of different stimuli, repetitions of the same stimulus, different sensilla tested, and different flies. Initially, we had chosen to use two complex stimuli (Fish and Liver extracts) and two simple stimuli (Sucrose plus NaCl, and NaCl alone in solution). Preliminary results from 11 sensilla comparing Sucrose and 100 mmol $l^{-1}$ NaCl as stimuli produced the following mean activity for NaCl (cell 1, 9.3±5.3; cell 2, 1.6±3.4; cell 3, 1.0±1.8 impulses $s^{-1}$). Results for Sucrose were substantially higher and were similar to those reported in the main data set. Because of its low activity, NaCl was not included in the main experiment.

To test the possibility that another part of the response might be more important for neural coding than the 0.3–1.3 s segment we used, the complete set of recordings for one fly was digitized both for the 0.3–1.3 s period and the 1.3–2.3 s period. Overall, there was less activity in the second period, owing to adaptation. However, the comparisons of means, variance and reproducibility across stimuli had the same trends in both periods. For the purposes of this study, we concluded that the 0.3–1.3 s period would be the more appropriate.

### *Nature of the chemosensory responses*

Fig. 2 depicts the types of responses obtained from medium-length labellar sensilla of *S. bullata* to the three stimuli tested. Note that because of variation in total activity and number of cells firing, single records say little about differences that emerge upon analysis of a large number of records. These records merely illustrate the general similarity and complexity of responses to the three stimuli.

Fig. 3. illustrates one of the final stages in the classification of cellular activity from the same sensillum whose response is shown in Fig. 2. The first three results relate directly to the traces shown in Fig. 2, and are from the first application of each of the three solutions to this sensillum. The last three results in Fig. 3 summarize activity recorded in the second application series using the same stimuli. Note that the relative shapes and amplitudes of the waveforms from the

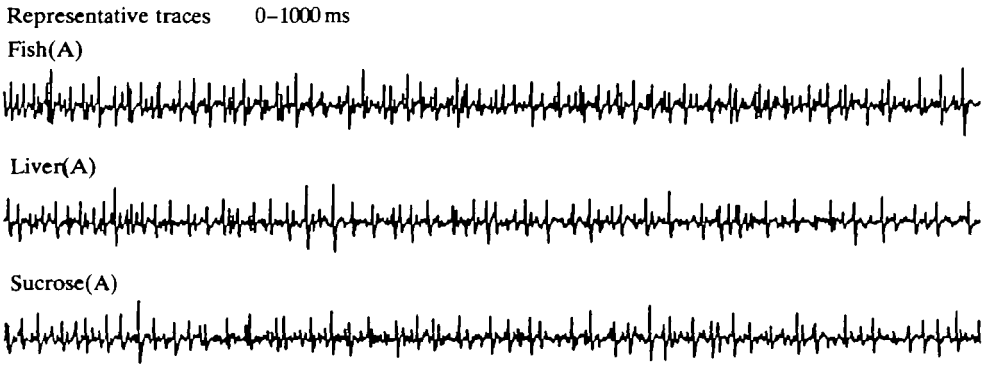


Fig. 2. Electrophysiological records showing representative responses of a medium-length labellar sensillum to the stimuli used in this study. The response from 0.3 to 1.3 s is shown in each case. Stimuli were: (1) Fish extract, (2) Liver extract (see Materials and methods) and (3) 100 mmol $l^{-1}$ Sucrose in 100 mmol $l^{-1}$ NaCl. (A) relates to Fig. 3.

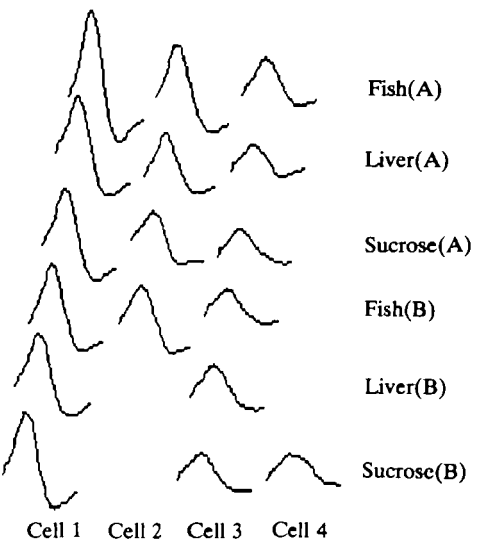


Fig. 3. Printout of part of one of the computer screens from the data-analysis package showing results of a classification for the six stimuli applied to one sensillum [same sensillum as shown in Fig. 2; stimuli are, in order, Fish(A), Liver(A), Sucrose(A), Fish(B), Liver(B) and Sucrose(B)]. Note that, although the amplitudes of waveforms from any one cell differ between records, the relative amplitudes and shapes remain consistent across cells. Use of such displays provides the basis for assigning waveforms to specific cells. Results for each of the 177 sensilla were viewed in this manner to determine if the assignment of waveforms to specific cell classes was appropriate. The frequency of firing of each cell type in each sensillum was only saved after we had viewed the classification results in this manner (on a sensillum by sensillum basis) and on a fly by fly basis.

different cells in each record (stimulus) are similar. The programs allow this type of comparison on a sensillum by sensillum basis (as shown here) and on an animal by animal basis. Both were used in this analysis to check the results of the template-matching program. In the case of this sensillum, there was no activity from cell 2 during the second applications of Liver and of Sucrose. This is unusual, but it illustrates the type of control that the user must have over the classification software. In this case, since the smallest waveform in the Fish(B) record was very similar to those from cell 3 in other recordings from this sensillum, it was placed in that category. A similar process led to the decision that there was no activity from cell 2 in the Sucrose(B) application. The fourth cell, seen during the last application in Fig. 3, was very sporadic in its response. Analyses of records from sensilla that were deliberately moved during recording showed high activity from a cell with a waveform similar to that of cell 4. The waveform's characteristically long time course and low amplitude make it easy to recognize, and similar waveforms from any record were considered to be from a mechanosensitive cell. Therefore, only activity from the first three cells was used in subsequent analyses.

### *Overall results*

Tables 1, 2 and 3 along with Fig. 4 summarize the results for 177 sensilla from 10

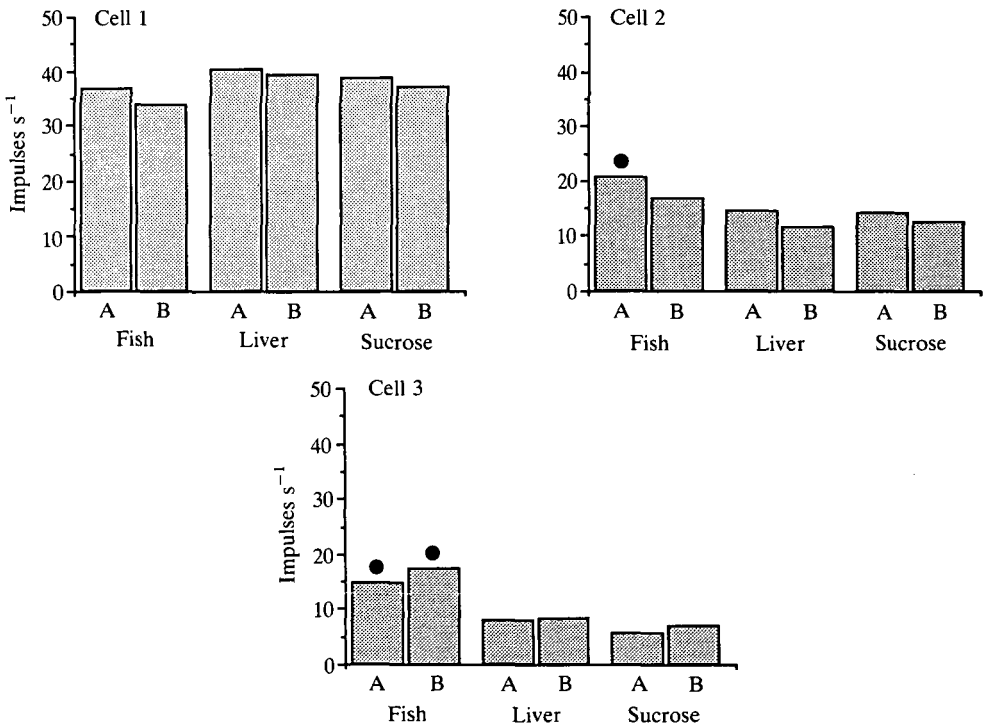


Fig. 4. Mean activity from three cells to duplicate applications of Fish, Liver and Sucrose. Pooled data from all 10 flies, total of 177 sensilla. The dots indicate a significant stimulus effect within an application series.

Table 1. *Mean activity from cell 1 to both applications of each of the three stimuli shown on a per fly basis*

| Fly | *N* | Fish(A) | Fish(B) | Liver(A) | Liver(B) | Sucrose(A) | Sucrose(B) |
|---|---|---|---|---|---|---|---|
| 1 | 22 | 42.7±13.6 | 44.2±12.5 | 48.1±15.2 | 50.1±14.3 | 48.9±22.6 | 47.4±22.2 |
| 2 | 14 | 15.2±7.1 | 15.8±8.5 | 19.8±8.4 | 20.6±9.4 | 44.4±26.0 | 43.7±29.1 |
| 3 | 16 | 48.3±21.2 | 40.3±13.5 | 55.3±18.5 | 52.4±24.5 | 48.8±17.9 | 32.8±19.1 |
| 4 | 19 | 48.2±15.1 | 43.5±12.6 | 48.3±14.2 | 44.7±13.1 | 41.4±15.5 | 51.6±16.3 |
| 5 | 20 | 29.9±7.6 | 30.4±6.1 | 34.8±9.9 | 36.1±11.0 | 28.0±6.3 | 15.5±9.7 |
| 6 | 19 | 39.9±11.2 | 37.9±15.6 | 52.1±15.1 | 46.6±13.5 | 31.9±19.9 | 52.2±19.0 |
| 7 | 19 | 27.8±13.8 | 21.1±10.0 | 28.2±10.0 | 25.1±10.5 | 38.6±23.9 | 29.5±16.4 |
| 8 | 12 | 32.7±8.0 | 29.9±9.2 | 34.1±11.6 | 28.8±11.9 | 28.6±8.8 | 21.3±12.3 |
| 9 | 18 | 42.1±16.5 | 36.3±14.3 | 41.9±11.2 | 40.9±11.6 | 27.3±23.3 | 25.2±20.4 |
| 10 | 18 | 34.5±11.2 | 32.0±13.0 | 32.7±15.6 | 37.8±15.9 | 45.8±17.6 | 45.9±17.0 |
| Grand mean | | 36.7±16.0 | 33.8±14.6 | 40.2±16.8 | 39.1±16.9 | 38.5±20.5 | 37.0±22.3 |
| CV* | | 43.6 | 43.2 | 41.8 | 43.2 | 53.2 | 60.3 |
| Grand mean A and B | | 35.3 | | 39.7 | | 37.8 | |
| Correlation(%) mean A and B | | 95.7 | | 95.0 | | 58.9 | |

* Coefficient of variation (%) (comparable across all columns since *N* values are equal at 177).
Means are expressed ±S.D.
Correlation is Pearson product-moment.
The grand mean shows responses across all flies, as does the coefficient of variation.
The grand mean of A and B combines the two applications for any one stimulus on any one sensillum [e.g. Fish(A), Fish(B)].
The correlation of means A and B shows the correlation between the means of the two applications of any one stimulus across flies.

female *S. bullata*. Fig. 4 illustrates the mean activity from all three cells to both applications of the three stimuli. It is the most condensed presentation of the data offered. Note that, on this basis, there is no difference in cell 1 activity related to type of stimulus or time of application. Cell 2 was generally less active than cell 1, and only in one trial (first application of Fish) did its activity differ significantly from that in other trials ($P<0.001$ Kruskal–Wallis analysis of variance). Cell 3 was the least active of all cells, but it fired significantly more often in both applications of Fish compared with Liver and Sucrose ($P<0.001$ Kruskal–Wallis analysis of variance). The means used in these plots, together with their standard deviations are shown in Tables 1, 2 and 3 under Grand mean.

The data are separated by fly in Tables 1, 2 and 3 to provide a better idea of the structure of the data set. For any particular fly, it may be possible to show significant differences among trials, both for stimulus effect and for time of application. However, there was no consistent pattern in this variance, as shown by the results of Kruskal–Wallis analyses of variance for each fly in Table 4.

Table 2. *Mean activity from cell 2 to both applications of each of the three stimuli shown on a per fly basis*

| Fly | *N* | Fish(A) | Fish(B) | Liver(A) | Liver(B) | Sucrose(A) | Sucrose(B) |
|---|---|---|---|---|---|---|---|
| 1 | 22 | 13.6±10.5 | 9.7±5.9 | 11.2±10.2 | 9.5±9.2 | 10.7±12.2 | 8.9±7.1 |
| 2 | 14 | 10.7±16.1 | 8.1±12.2 | 8.9±11.0 | 7.4±11.5 | 7.4±7.7 | 4.0±5.6 |
| 3 | 16 | 15.3±10.1 | 17.3±18.2 | 8.9±5.4 | 9.8±13.2 | 8.7±5.0 | 11.9±6.1 |
| 4 | 19 | 13.5±8.3 | 13.4±12.1 | 10.3±7.9 | 5.6±7.0 | 20.1±12.3 | 14.2±12.5 |
| 5 | 20 | 18.0±13.4 | 15.9±10.9 | 13.8±8.0 | 12.2±7.2 | 17.9±9.4 | 18.5±10.5 |
| 6 | 19 | 30.8±11.5 | 18.5±15.6 | 14.3±8.6 | 9.0±6.8 | 28.4±20.1 | 22.5±18.5 |
| 7 | 19 | 32.9±18.5 | 30.9±18.5 | 20.5±14.9 | 18.1±15.6 | 15.1±13.3 | 11.4±10.2 |
| 8 | 12 | 15.7±15.4 | 17.4±20.4 | 17.3±16.0 | 9.8±9.0 | 14.3±9.9 | 11.8±13.6 |
| 9 | 18 | 21.2±16.9 | 17.9±17.3 | 17.3±11.8 | 14.5±7.9 | 7.6±8.6 | 4.5±6.9 |
| 10 | 18 | 32.0±18.1 | 21.0±19.2 | 23.2±16.8 | 15.9±14.9 | 7.9±9.5 | 12.9±16.0 |
| Grand mean | | 20.7±16.0 | 16.8±16.2 | 14.5±12.1 | 11.3±11.0 | 14.1±13.2 | 12.3±12.5 |
| CV* | | 77.3 | 96.4 | 83.4 | 97.3 | 93.6 | 101.6 |
| Grand mean A and B | | 18.8 | | 12.9 | | 13.2 | |
| Correlation(%) mean A and B | | 83.0 | | 84.2 | | 84.9 | |

* Coefficient of variation (%) (comparable across all columns since *N* values are equal at 177).
Means are expressed as ±S.D.
Correlation is Pearson product-moment.
The grand mean shows responses across all flies, as does the coefficient of variation.
The grand mean of A and B combines the two applications for any one stimulus on any one sensillum [e.g. Fish(A), Fish(B)].
The correlation of means A and B shows the correlation between the means of the two applications of any one stimulus across flies.

## *Detailed summary*

### *Cell 1*

Cell 1 was by far the most consistent responder and the most active cell in over 98% of the records. An analysis of its mean activity as stimuli were varied showed no effect of stimulus on this cell at the level of the population (Table 1 and Fig. 4). With individual flies, there were no significant differences across stimuli in three flies. Sucrose caused significantly less activity than Liver or Fish in three flies and significantly more activity that these two stimuli in two other flies. Liver elicited the highest response in one fly, and Fish the lowest response in another fly (Table 4).

Consistent differences among stimuli did emerge for cell 1 when variance and reproducibility were considered. We use variance to describe the variability across sensilla either within flies or for all 10 flies combined, thus summarizing spatial variance. Reproducibility describes variability in the response of single cells over time. In this study, applications were repeated only once, so reproducibility refers

Table 3. *Mean activity from cell 3 to both applications of each of the three stimuli shown on a per fly basis*

| Fly | *N* | Fish(A) | Fish(B) | Liver(A) | Liver(B) | Sucrose(A) | Sucrose(B) |
|---|---|---|---|---|---|---|---|
| 1 | 22 | 21.0±19.0 | 22.6±20.3 | 15.0±13.7 | 16.1±14.0 | 5.5±10.4 | 4.5±6.2 |
| 2 | 14 | 32.1±18.0 | 25.7±19.7 | 9.4±10.1 | 13.7±12.2 | 1.8±3.1 | 4.4±8.6 |
| 3 | 16 | 7.4±8.4 | 12.8±12.1 | 8.6±7.0 | 6.3±6.6 | 9.9±11.5 | 11.8±15.5 |
| 4 | 19 | 10.3±14.0 | 10.2±13.9 | 5.7±9.1 | 6.4±12.2 | 10.4±13.4 | 6.6±8.9 |
| 5 | 20 | 13.7±10.1 | 15.3±13.5 | 4.2±6.5 | 2.0±3.8 | 3.5±6.3 | 6.1±8.1 |
| 6 | 19 | 10.6±14.7 | 13.7±13.3 | 1.2±3.8 | 2.7±5.2 | 8.8±11.8 | 12.1±9.5 |
| 7 | 19 | 2.3±7.4 | 5.9±11.8 | 3.1±8.0 | 5.1±11.4 | 2.9±5.1 | 0.9±2.5 |
| 8 | 12 | 19.3±14.5 | 14.5±9.9 | 6.5±6.7 | 8.8±12.1 | 13.3±10.6 | 16.0±11.4 |
| 9 | 18 | 12.5±11.4 | 20.7±17.5 | 6.2±8.2 | 3.1±5.6 | 0.6±2.4 | 3.3±6.1 |
| 10 | 18 | 22.4±19.8 | 32.3±21.9 | 17.1±14.1 | 17.1±15.0 | 2.9±5.5 | 6.5±16.7 |
| Grand mean | | 14.7±16.1 | 17.3±17.4 | 7.7±10.4 | 8.1±11.6 | 5.7±9.5 | 6.9±10.6 |
| CV* | | 109.5 | 100.6 | 135.1 | 143.2 | 166.7 | 153.6 |
| Grand mean A and B | | 16.0 | | 7.9 | | 6.3 | |
| Correlation(%) mean A and B | | 82.1 | | 89.2 | | 87.5 | |

* Coefficient of variation (%) (comparable across all columns since *N* values are equal at 177).
Means are expressed as ±S.D.
Correlation is Pearson product-moment.
The grand mean shows responses across all flies, as does the coefficient of variation.
The grand mean of A and B combines the two applications for any one stimulus on any one sensillum [e.g. Fish(A), Fish(B)].
The correlation of means A and B shows the correlation between the means of the two applications of any one stimulus across flies.

to differences in a cell's response between the two applications, and is a rough measure of temporal variance.

Table 1 lists the coefficient of variation in cell 1 responses across all sensilla for each application of each stimulus. The response to Sucrose was significantly more variable than the responses to Liver or Fish [coefficients of variation 53.2 % and 60.3 % *vs* 41.8 %, 43.2 %, 43.6 % and 43.2 %; *t*-test for paired samples taking the coefficient of variation for each fly/stimulus as a variable, *P* values: Fish(A) *vs* Sucrose(A) $P<=0.0338$; Liver(A) *vs* Sucrose(A) $P<=0.0589$; Fish(B) *vs* Sucrose(B) $P<=0.012$; Liver(B) *vs* Sucrose(B) $P<=0.009$; $N=10$ in each case]. This pattern largely held for individual flies (Fig. 5). In all but fly number 10, the response to Sucrose had the greatest variance.

Table 1 also summarizes reproducibility for cell 1 under Correlations(%) A and B. This was determined by correlating the means of the first and second applications for each stimulus (A and B) on a fly by fly basis. Overall, the correlation was high for Liver and Fish but much lower for Sucrose. Both this temporal variation and the variation between sensilla are clearly shown in scatter

Table 4. *Significance of stimulus effect on activity of each cell from each fly*

| Fly | Cell 1 | Source | Cell 2 | Source | Cell 3 | Source |
|---|---|---|---|---|---|---|
| 1 | NS | | NS | | 0.001 | S (−) |
| 2 | 0.001 | S (+) | NS | | 0.001 | F (+) |
| 3 | 0.037 | L (+) | 0.028 | F (+) | NS | |
| 4 | NS | | 0.001 | L (−) | NS | |
| 5 | 0.001 | S (−) | NS | | 0.001 | F (+) |
| 6 | 0.008 | F (−) | 0.001 | L (−) | 0.001 | L (−) |
| 7 | NS | | 0.001 | S (−) | NS | |
| 8 | 0.038 | S (−) | NS | | 0.006 | L (−) |
| 9 | 0.001 | S (−) | 0.001 | S (−) | 0.001 | S (−) |
| 10 | 0.004 | S (+) | 0.002 | S (−) | 0.001 | S (−) |

*P* values determined by Kruskal–Wallis analysis of variance.

Sources of significant differences indicated are largest single source for each case (usually the only source). F, fish; L, liver; S, sucrose. (+) indicates that the activity elicited by this stimulus was significantly higher than the others, (−) indicates that it was lower.

*P* values reported as 0.001 are that value or lower; NS, not significant.

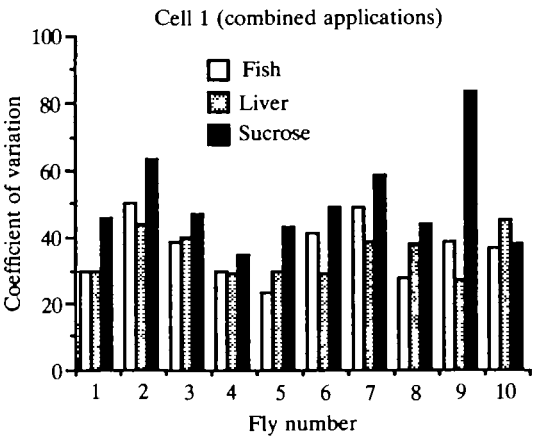


Fig. 5. Coefficients of variation for each stimulus for each fly showing that, even in a single fly, sucrose usually elicited the most variable response from cell 1.

plots of the two applications for each stimulus. Plots for Fish and Sucrose are shown in Fig. 6. In a perfect correlation, all points would fall on the diagonal.

*Cell 2*

Table 2 summarizes data for cell 2. On a population basis, there was a tendency for Fish to stimulate more activity than Liver and Sucrose (Grand mean), and this was significant for Fish(A) (see Fig. 4). The variability in the responses to all stimuli was high, but roughly similar. Table 4 shows that the significant differences in means were not consistently related to Fish. Correlations between applications

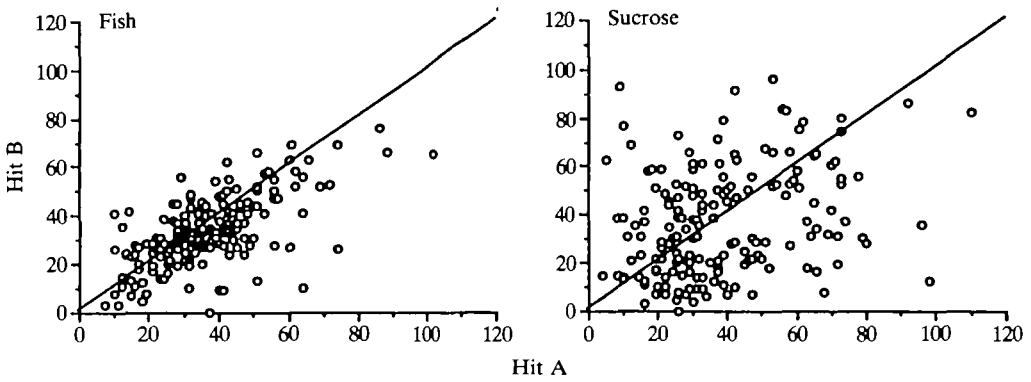


Fig. 6. Reproducibility of responses (impulses $s^{-1}$) of cell 1 to Fish and Sucrose, as seen by viewing the entire data set. Responses from the same cells are included in both graphs. The two applications of any one stimulus were separated by 10–30 min. Note the poor reproducibility of the Sucrose response relative to Fish. The plot for Liver is similar to that for Fish. See Table 1 for the actual variances.

A and B showed no difference (Table 2). All three stimuli showed weaker correlations (Fish 83 %, Liver 84 % and Sucrose 85 %) than was the case for Liver and Fish on cell 1 (95 % and 96 %, respectively).

*Cell 3*

Table 3 summarizes data for cell 3. On a population basis, Fish stimulated significantly more activity than Liver or Sucrose. From the perspective of a single fly, however, this relationship did not hold (Table 4). Of the seven flies where there was a significant difference among stimuli, Fish stimulated significantly greater activity in two while Liver or Sucrose was significantly less potent in the other five. In these five cases, it was not a question of Fish stimulating greater activity, but rather one of Liver or Sucrose stimulating less activity than did the two other mixtures. Correlations between applications for cell 3 were similar across all three stimuli: Fish 82 %, Liver 89 % and Sucrose 88 % (Table 3). The coefficient of variation for all stimuli was very high. Although it appears to be lower for Fish stimuli, this is likely to be attributable to the larger numbers of spikes produced in response to these stimuli.

*Three-axis plots of combined data*

While cell 1 was highly active in the vast majority of the 1062 recordings, this was not the case for cell 2 and cell 3. There were many instances of zero activity from these two cells. The three-axis scatter plots of Fig. 7 reveal that there was some structure in the data set when the above differences were highlighted. In these plots, the position of a point for a particular cell indicates its relative response to the three stimuli. Cells which responded to only one of the three stimuli are plotted at the apex representing the stimulus to which a response occurred, while cells which responded to only two of the three stimuli are plotted along the axis

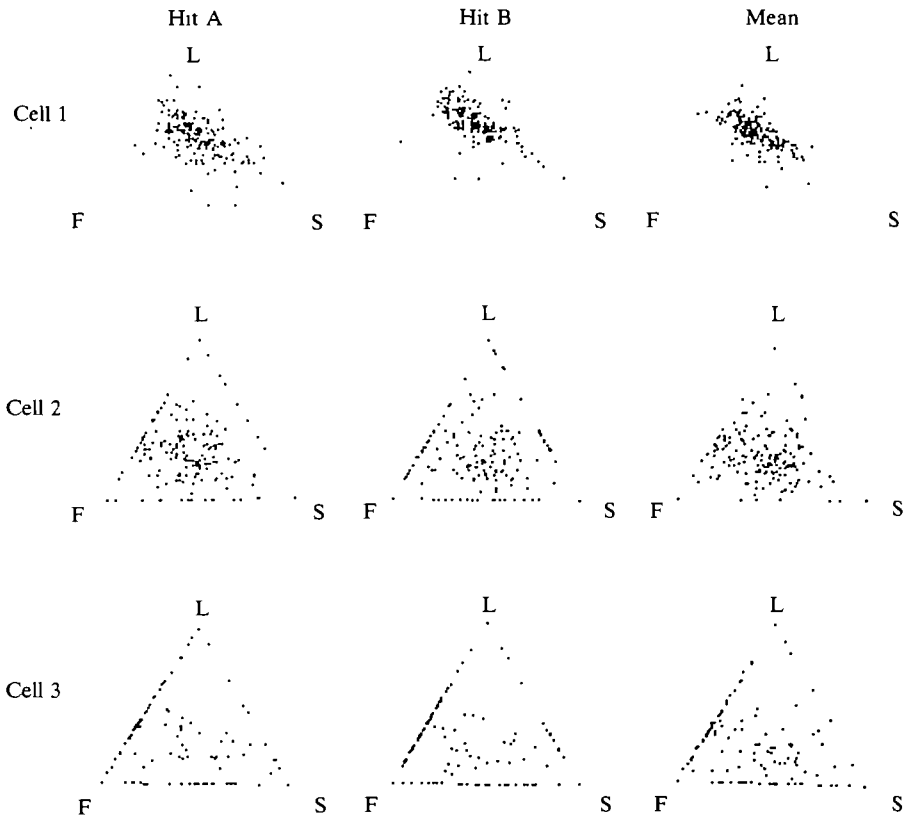


Fig. 7. Summary of responses from cells 1, 2 and 3 to all three stimuli. Cells which responded with less than 10 spikes per application to all six applications (Fish, Liver, and Sucrose each repeated once) were excluded. The data on each cell were transformed as follows: (response to Fish)/(response to Fish+Liver+Sucrose), or briefly F/(F+L+S) for responses to Fish; L/(F+L+S) for responses to Liver; and S/(F+L+S) for responses to Sucrose. This normalized the data and made it possible to display the three-dimensional relationship in two-dimensional space. Data from the first and second applications are plotted in the left-hand and centre columns. Data derived from averaging the first and second applications for each cell/stimulus combination are plotted in the right-hand column.

joining the two effective stimuli. The relative response of cells responding to all three stimuli occurs in the inner portion of the triangle outlined by the three axes. There were no examples where cells 1 responded to only one, and few where they responded to only two of the three stimuli. With cells 2, many instances of responses to only a pair of stimuli occurred with each application (A and B). However, when responses were expressed as means of applications A and B most of the points fell into the central region, indicating that most cells 2 responded, at least some of the time, to all three stimuli.

The most interesting result of the three-axis plots relates to cell 3. Even when responses were expressed as means of applications A and B (bottom right in

Fig. 7), many points remained on the periphery, particularly on the Liver/Fish and the Fish/Sucrose axes. Of the cells which responded to all three stimuli, most responded poorly to Liver (note the empty space under the Liver apex). The significantly greater activity for cell 3 in response to Fish referred to earlier can be seen graphically in Fig. 7. However, the clustering of units on the two major axes suggests that the cell 3 of our classification may include more than one functional type of cell.

## Discussion

Which sensilla are involved in discrimination? Behavioural results reported in Fig. 1 show that adult females of *S. bullata* could clearly distinguish between 100 mmol $l^{-1}$ sucrose in NaCl and either of the more complex solutions offered (Liver or Fish). The apparatus used for the feeding experiments required flies to drink solutions through a small opening such that, at least while drinking, only labellar sensilla and interpseudotracheal sensilla could be in contact with the food. The short time over which feeding was measured (6 h), and the observation in preliminary experiments that flies imbibed about 70 % of their 6-h total intake in 1 h or less, strongly suggests that the differences in intake were due to differences in chemosensory stimuli received during feeding, as opposed to any kind of internal feedback. Labellar sensilla are the most likely candidates for providing the necessary input. Our experimental design did not test for the importance of olfactory input to the observed behavioural result. While this could be done, it is not the main point of the study. Instead, we assume that labellar sensilla are likely to be involved in the discrimination, even if olfaction also plays a role, and that differences in gustatory activity stimulated by the four behavioural stimuli should be measurable. That olfaction plays only a limited role, if any, in modulation of food intake by *P. regina* is suggested by the work of Evans (1961). Since there are no morphological data regarding labellar sensilla of *S. bullata* we used the literature on the black blowfly *P. regina* as a guide in selecting sensilla to study. Preliminary observations with the scanning electron microscope showed *S. bullata* to be similar in sensillar distribution. In the female blowfly there are about 130 trichoid sensilla on each labellar lobe, and these can be divided into four groups on the basis of length (Wilczek, 1967). There are also interpseudotracheal papillae distributed over the surface of the labellum. All these sensilla could be variously important in mediating the behavioural results. Differences in physiology across a field of morphologically similar sensilla have been documented for tarsal sensilla of *Calliphora* sp. (Van der Molen *et al.* 1985). Also, our preliminary recordings from *S. bullata* indicated that sensitivity decreased with increasing sensillar length, using a rough classification of long, medium and short sensilla. Since recording from all types of sensilla which might possibly be involved was beyond the scope of this study, we chose to work on the intermediate-length sensilla, which correspond to group II and 'different' sensilla described by Wilczek (1967) for *P. regina*. In *P. regina* females there are about 47 sensilla of these types on each labellar lobe

(derived from Table 2 of Wilczek, 1967) or 36% of the total number of labellar sensilla. Our average sample of 17.7 sensilla per fly thus represents approximately 38% of the intermediate-size sensilla on each lobe, and 14% of all sensilla on a lobe.

### *Cell types and neural coding*

Generally, 3–4 cells in each intermediate sensillum responded to the stimuli used. We determined that cell 4, whose responses had a very low amplitude and long duration, was mechanosensitive, based on its increase in firing upon bending of the sensillar shaft. Precautions were taken to move sensilla as little as possible during stimulus applications, which kept activity from this cell to a minimum. When present, these small, wide waveforms were easily separated from the others by the spike-classification software. Subsequent analyses were only done on waveforms representing cell 1, cell 2 and cell 3.

We deliberately avoided using the traditional physiological classification of these three cells into salt, sugar and water cells, partly because detailed analyses using these stimuli are not available for *S. bullata*. In addition, we assumed that many compounds in the mixtures would potentially stimulate each cell, and it was really the pattern across sensilla of responses to the three test stimuli which was of interest. We did take particular care to ensure that the cell we termed cell 1 had the same waveform relative to other cells across sensilla, and this was true for each of the three chemosensitive cells. For example, though spike amplitude can change with application, relative amplitude of the spike for the three cells is more constant, and was used as a guide in classification. In addition, the programs display separately the responses of each apparent class, which allowed us to determine if the predominantly responding cell (usually cell 1) was firing in a regular pattern. The software allowed extensive cross-referencing to confirm waveform classification, as discussed by Smith *et al.* (1990).

A comparison of means in our data yielded only one consistent difference: the activity of cell 3 in response to Fish was significantly higher than to the other two stimuli. Activity of cell 2 in response to Fish was also higher than to other stimuli, but only in the first of the two applications. In any case, the flies consumed equal quantities of Fish and Liver, so these differences could not bear on the question of how flies distinguish between Sucrose and the other two stimuli. Even if the variances were much reduced, the means in general are so similar that significant differences would remain elusive.

The across-fibre patterning hypothesis of neural coding as applied to insect gustatory sensilla (Dethier, 1971, 1976) places a strong emphasis on the mean activity from a group of related cells (called receptors by Dethier, 1971) in that the relative number of impulses from different types of chemosensory cells must differ between stimuli if those stimuli are to be distinguished. Assuming that, in large sensory fields such as fly labella, each sensillum houses a few cell types comparable across the sensillar field, across-fibre-pattern coding requires the mean activity from cells of each type to differ across stimuli. In this view, across-fibre patterning

is similar to the labelled-line hypothesis, except that there are several types of cells involved to achieve the pattern. Labelled lines would be special cases of this type of across-fibre pattern, in which responses of a single cell carry the important information. In either case, if many comparable cells are involved (i.e. if there are cell types) mean activities across cell types must differ. This reliance on means makes across-fibre patterning very sensitive to variability in responses among individual cells. Usually this variability is substantial and it has been the topic of several investigations on fly sensilla (Den Otter, 1971; Van der Starre, 1972; Maes and Den Otter, 1976; Van der Molen *et al.* 1985). It should be noted, however, that not all across-fibre patterns require cell types (cf. Maes and Erickson, 1984). Each one of a large number of unrelated cells could have its own response to a given stimulus, resulting in a response pattern across the population of cells. A different stimulus would probably generate a different pattern. The resolution of this type of across-fibre pattern would depend on a low temporal variance (high reproducibility) of the system: any pattern must be consistent.

*Can variance itself be informative?*

The most striking result of this experiment was the large coefficient of variation in the cell 1 responses to Sucrose compared with those to Fish and Liver. Indeed, both spatial variability (across cells) and temporal variability (reproducibility of two applications) showed this difference (Table 1, Figs 5 and 6). It was also the only substantial difference in the responses of any of the cells which correlates with the results of the feeding experiment. It is possible, therefore, that the fly was using the variation in cell 1 responses as a signal conveying information about the potential food source. The greater homogeneity of responses of cell 1 to Liver and Fish may have indicated a more appropriate food than did the more variable responses to Sucrose. For such a code to be possible, the central nervous system would have to compare activity across some group of related cells and respond 'best' when the differences across cells were least. In this way, variability, an established phenomenon in many chemosensitive systems, could be put to use by the organism. Instead of corrupting the information in a code based on means, variability would be a source of information. This does not imply that mean activity is unimportant, for there are many studies which show greater behavioural responses as stimulus concentration is increased, and this can often be correlated with increased mean receptor cell activity. However, there could be an interplay between mean and variability such that the greatest behavioural responses are elicited when the mean response is optimal (high?) and the variance is low.

We suggest that variation between the responses of related cells (which we shall refer to as 'variance') should be added to the list of mechanisms by which sensory cells are thought to convey information. This list presently includes such parameters as spike frequency integrated over time; the temporal pattern of spike frequency (e.g. phasic and tonic components, 'bursting', etc.); the pattern of discharges compared across cells ('across-fibre patterning'); the identity of the cell(s) ('labelled lines'). It should be noted that variance is not the same as across-

fibre patterning: two patterns could be different but have the same variance. The hypothesis that variance itself conveys information could imply that there are situations where the across-fibre pattern may not, in itself, be important; the central nervous system may only be distinguishing between stimuli producing low variance and those producing high variance. For instance, in the present experiments, Fish and Liver both resulted in cell 1 responses with similarly low variance (and similar means). Both are complex, protein-rich foods, appropriate diets for a female fly commencing oogenesis. It could be important to distinguish between such diets and a simple diet of sugar.

Indeed, the lower variability in responses seen with such complex diets may be related to their complexity. There is good evidence that the 'sugar' cell of fleshflies, cell 1 in our data, has at least four different receptor types on the dendritic membrane [pyranose (P site), furanose (F site), aryl (AR site) and alkyl (R site)] interacting, respectively, with glucose, fructose, valine and phenylalanine (Shimada *et al.* 1985). Other stimuli for the 'sugar' cell of fleshflies and blowflies, some of which interact with one or more of these sites, include a wide array of sugars (Jakinovich *et al.* 1971), amino acids (Shiraishi and Kuwabara, 1970), fatty acids (Shimada, 1978), dipeptides (Shimada and Tanimura, 1981), NaCl (Morita *et al.* 1966) and ATP (Liscia *et al.* 1987). If we regard cell 1 as synonymous with the 'sugar' cell, it must be considered a broadly tuned cell. Such broadly tuned cells would have evolved in a context where naturally occurring stimuli are complex: the real world of insects involves rotting flesh, leaf sap, blood and pheromone blends, not analytical grade sucrose and NaCl. It is reasonable, therefore, that the best (most consistent) chemosensory responses would be to such mixtures (J. Atema, personal communication). Even if variance, *per se*, is not used in chemosensory coding, any code based on pattern or mean responses will have increased resolution with lower variance. We might expect that chemosensory cells would be tuned in this way to ecologically appropriate natural stimulus mixtures.

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