Sex-specific non-pheromonal taste receptors in *Drosophila*

Nicolas Meunier*, Jean-François Ferveur† and Frédéric Marion-Poll*

Taste receptors have recently been reported in *Drosophila* [1,2], but little is known of the relation between receptor and response. Morphological studies of the distribution of chemosensory sensilla indicate that the fruit fly has two major sites of gustation: the proboscis and the legs [3]. The taste sensilla on both these sites are similar in structure and each sensillum generally houses four gustatory neurons [4]. Early anatomical observations have demonstrated a sexual dimorphism in the number of tarsal sensilla [5] and in their central projections [6]. We measured the electrophysiological responses of the prothoracic taste sensilla to non-pheromonal substances – salts, sugars and water – and found a clear sexual dimorphism. From the response profile of individual sensilla, we were able to distinguish three types of tarsal sensilla in females as against only two types in males. The female-specific type, which responded specifically to sugar, was absent in males except when male gustatory neurons were genetically feminised. The fact that tarsal gustatory hairs exhibit a sexual dimorphism that affects the perception of non-pheromonal compounds suggests that sexual identity is more complex than has previously been thought [7,8].

Addresses: *I.N.R.A., Centre de Versailles, F-78026 Versailles Cedex, France. †UMR-CNRS 5548, Faculté des Sciences, Université de Bourgogne, 6 Boulevard Gabriel, 21000 Dijon, France.

Correspondence: Nicolas Meunier
E-mail: nmeunier@versailles.inra.fr

Received: 18 August 2000
Revised: 23 September 2000
Accepted: 24 September 2000

Published: 8 December 2000

Current Biology 2000, 10:1583–1586

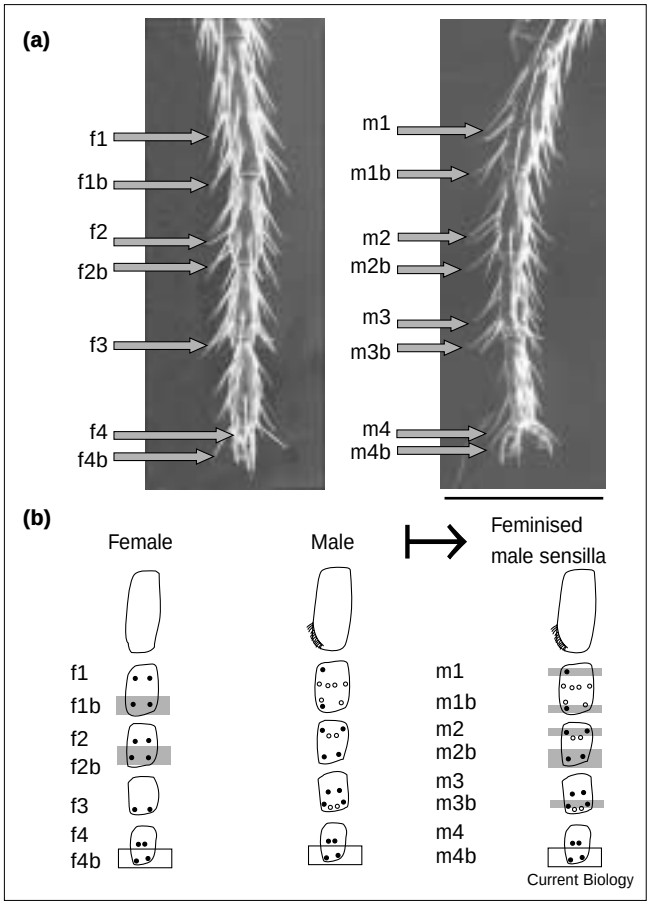
0960-9822/00/$ – see front matter

Results and discussion

Electrophysiological characterisation of tarsal taste sensilla

We found 14 functional sensilla in females and 24 in males on the last four segments of the prothoracic leg (Figure 1b). On the same segments, using ethanolic silver staining, Nayak and Singh [5] found 19 taste sensilla in females and 33 in males, whereas, using crystal violet and scanning electron microscopy techniques, Venard *et al.* [7] reported only 12 and 22 taste bristles in females and males, respectively. Although we cannot be certain that we probed all taste sensilla, our sampling seems to be consistent with previous work.

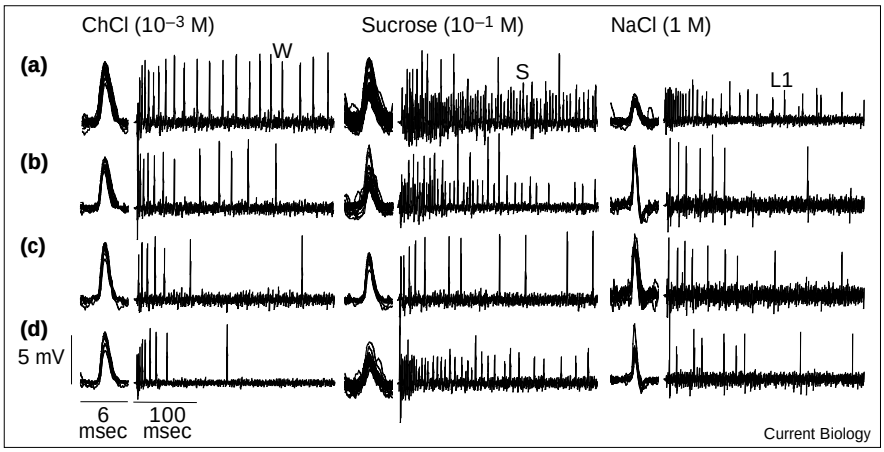
Figure 1



Tarsal segments of female and male *Drosophila melanogaster* prothoracic legs. (a) The taste bristles tested in this study are labelled with arrows. They are coded according to the sex (f, female; m, male), tarsal segment number (1–4 from proximal to distal) and approximate location within a segment (b, distal). The scale bar represents 100 μm. (b) Distribution of the three types of tarsal sensilla on the last four tarsal segments of the prothoracic leg of female, male and feminised male *Drosophila* according to their sensitivity to sugars, salts and water. The male sensilla tested in feminisation experiments (black circles) were selected on the basis that they were located at an equivalent tarsal position as in female flies. Type A sensilla (framed) responded to sugar, salt and water. Female-specific type B sensilla (grey rectangles) responded only to sugar. Type C sensilla (black circles) did not respond to any of the stimuli tested. Sensilla designated by unshaded circles represent male taste sensilla of type C that were characterised during preliminary experiments (with $n = 5$ for each bristle).

Behavioural experiments, such as the proboscis-extension reflex in response to stimulation of tarsal sensilla, indicate that tarsal taste sensilla can encode differences between sugars, salts and water, with the same specificity as the taste sensilla borne by the proboscis [9]. This indicates that

Figure 2



Typical responses of sensilla to stimulation with 10^{-3} M choline chloride (ChCl, left), 10^{-1} M sucrose (centre) and 1 M NaCl (right). (**a**) Type A sensilla in a Canton S (Cs) female; (**b**) type B sensilla in a Cs female; (**c**) type C sensilla in a Cs male; (**d**) type C sensilla in a feminised *Voila1* × *UAS–tra* male. Three spike classes were consistently found: S spikes (sucrose) had an amplitude of 2–3 mV and a duration of 3 msec; L1 spikes (salt) had an amplitude of 2–3 mV and a duration of 2 msec; and W spikes (water) reached a much larger amplitude of 5–7 mV. They are believed to originate from three different taste neurons. The S cell behaved differently according to the type of sensillum. In type A sensilla, it responded to sugar with a sustained discharge whereas, in type B sensilla, it responded with an initial burst. The W cell also exhibited different properties according to the type of sensillum. In type A sensilla, it was inhibited by increasing concentrations of choline chloride. In type B and type C sensilla, it maintained the same discharge rate. The L1 cell was only active in type A sensilla.

tarsal taste sensilla house neurons that are sensitive to salts, sugars and water. We stimulated the tarsal taste sensilla with different concentrations of salts (NaCl, KCl), sugars (sucrose, fructose, trehalose, glucose, inositol), amino acids (leucine, proline) and molecules derived from amino acids (γ-aminobutyric acid, choline chloride). Three types of cells, differing by their spike amplitudes and shapes, were found to be active in response to these stimuli. These cells were labelled following the typology proposed in earlier studies of the taste sensilla of the proboscis [10]. One cell type responded to water (cell W; Figure 2, left). A second cell type responded to sugar (cell S; Figure 2, middle). The third cell type responded to salt (cell L1; Figure 2, right).

The responses to sucrose, NaCl and water allowed us to divide tarsal sensilla into three groups. In type A, the three cells L1, S and W were active. The best stimulus that elicited action potentials from the L1 cell was NaCl. The best stimulus for the S cell was sucrose. The W cell was inhibited by increasing osmolarity (Figure 2a). In type B sensilla, one cell responded to sugar. This cell showed different responses to the equivalent cell in type A sensilla: instead of a sustained response, it mainly fired at the onset of stimulation (Figure 2b, compare with Figure 2a). Sucrose was also the best stimulus for this cell. A W-like cell was active in most recordings, but failed to be inhibited by increasing concentrations of solutes (that is, increasing osmolarity; Figure 2b). In type C sensilla, no cell responded to either stimuli. A W-like cell was active in most recordings, but failed to be inhibited as in type B sensilla, even at 1 M concentration of solutes (Figure 2c).

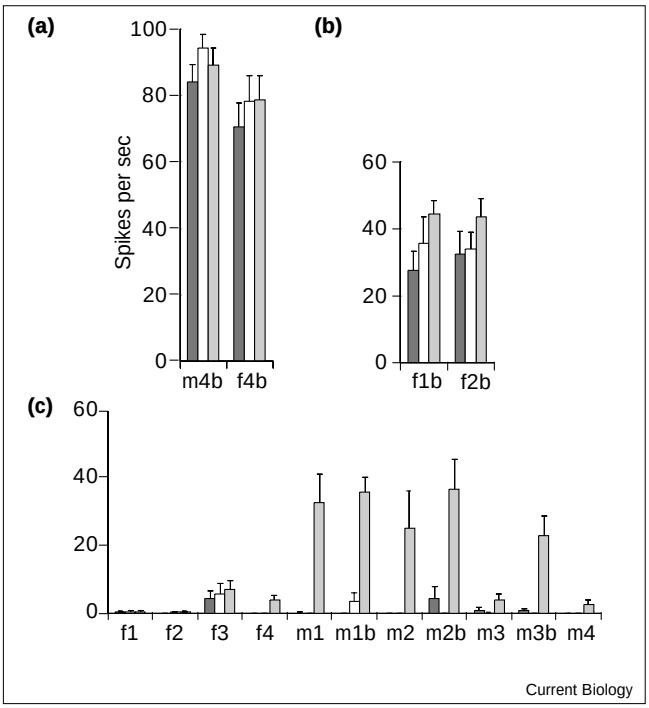
Sensilla were consistently found at specific locations on the legs. Type A sensilla were found as a distal pair on the last tarsal segment only. The terminal position of type A sensilla (Figure 1) is compatible with their ability to respond to a variety of molecules, all of which can represent food cues. Type B sensilla were found in pairs on the distal part of tarsal segments II and III and exclusively in females (Figure 1b; grey rectangles). All other (non-responsive) sensilla were classified as type C.

Feminisation of tarsal taste sensilla

To determine whether the sex-specific response of type B sensilla was due to the sexual identity of these neurons, we feminised male gustatory neurons following ectopic expression of the transgene *UAS–transformer* (*UAS–tra*) in adult gustatory organs. Genetic feminisation was done using *PGal4–Voila1* [11], an enhancer-trap strain that drives the expression of *UAS–tra* mainly in the peripheral gustatory nervous system of the fly. Untransformed *Voila1* males showed identical electrophysiological responses to control flies (Figure 3a–c), and transformed flies showed a typical male-specific distribution of sensilla on the tarsi [12] (data not shown). Although the morphology and location of the taste sensilla were unchanged following feminisation, the behaviour of the receptor neurons of *Voila1* × *UAS–tra* males was profoundly altered. Five out of seven feminised sensilla showed type B female-specific responses (Figure 2d, compare with Figure 2b; Figure 3c), despite being in the same position as type C sensilla in control males. The absence of any modification in the distribution of taste sensilla together with the clear response to sucrose indicates that genetic feminisation has specifically switched the sex-specificity of the S neuron in type B sensilla.

Sex differences in contact chemodetection were expected on the basis of behavioural responses to sex pheromones

Figure 3



Spike frequency of the responses of S cells in (a) type A, (b) type B and (c) type C sensilla after stimulation with 10^{-1} M sucrose. The genotypes tested were *Voila1*/TM3 (dark grey bars), Cs (white bars) and *Voila1* × *tra* feminised strain (light grey bars). Types A and C were present in both sexes whereas type B was female specific. Only type C was affected by feminisation and yielded a response resembling that of type B sensilla. Results were similar for Cs and *Voila1*/TM3 flies. For each histogram, the bar indicates the mean (± SEM) of at least ten observations.

[8,13], but these data constitute the first example of a sex-specific peripheral insect chemoreceptor that is not related to pheromone detection. This demonstrates that sexual identity may encompass unexpected aspects of the individual's interaction with the environment. No sex differences have been described in behavioural responses of fruit flies to gustatory stimulation *per se* [12], but our data suggest that such a dimorphism does exist, perhaps in relation to the choice of the oviposition site.

Materials and methods

D. melanogaster stocks were maintained at 25°C on a standard cornmeal agar food. We used the cDNA of the sex-determination gene *transformer*, downstream of four UAS_{Gal4} enhancer elements (*P[UAS–tra]* [14]).The expression of the enhancer-trap line *Voila1* has been precisely characterised [11]. In this strain, Gal4 is strongly expressed in the peripheral gustatory system during larval and adult development. Because Gal4-driven *transformer* expression was tested in *Voila1–P[Gal4]; P[UAS–tra]* flies, heterozygous *Voila1–P[Gal4]*/Cs and *P[UAS–tra]*/Cs flies were chosen as controls.

Electrophysiological recordings were performed on single tarsal sensilla of a decapitated fly over 1.5 sec intervals. The tip of each tested sensillum was covered with a recording and stimulating electrode [15]. The insect was grounded using an electrically conductive gel (Spectra 360 electrode gel, Parker). The electrode used for simultaneous stimulation and recording was a glass capillary with a tip of about 20 μm in diameter. It was connected to a TastePROBE amplifier [16] (Syntech) and further amplified and filtered (CyberAmp 320, Axon Instrument; gain: 1000; eighth order Bessel pass-band filter: 1Hz–2800 Hz). Each stimulus trial was digitised (sampling rate 10 kHz, 12 bits; DT2821 Data Translation) and stored on a computer. These data were then analysed with Awave custom software [17]. Spikes were counted during the first second of the stimulation. They were detected from a visually adjusted threshold set across the digitally filtered signal. Different classes of spikes were sorted with the help of interactive software procedures on the basis of their amplitudes and shapes [18].

All chemicals were purchased from Sigma. Salt solutions (KCl, NaCl) were prepared in advance and stored at 4°C. Choline chloride was used to determine the response to water because it does not elicit a response by itself but changes the osmotic pressure [10]. Sugars (sucrose, trehalose, fructose, glucose, inositol), amino acids (leucine, proline) and γ-aminobutyric acid were prepared as dilutions in 10^{-3} M KCl less than 15 days before the experiment and stored at 4°C. Each stimulus was presented only once to the tested sensillum, by bringing a capillary electrode into contact with its tip.

Acknowledgements

We thank M. Cobb and two anonymous reviewers for critical reading of the manuscript, and D. Tauban for her work in microscopy. N.M. was supported by the French Ministry of Education and research. J.-F.F. was supported in part by the Burgundy Region and by the French Ministry of Education and Research.

References

1. Ishimoto H, Matsumoto A, Tanimura T: Molecular identification of a taste receptor gene for trehalose in ***Drosophila***. *Science* 2000, 289:116-119.
2. Clyne PJ, Warr CG, Carlson JR: Candidate taste receptors in ***Drosophila***. *Science* 2000, 287:1830-1834.
3. Stocker RF: The organization of the chemosensory system in ***Drosophila melanogaster***: a review. *Cell Tissue Res* 1994, 275:3-26.
4. Pollack GS, Balakrisnan R: Taste sensilla of flies: function, central neuronal projections, and development. *Microsc Res Tech* 1997, 39:532-546.
5. Nayak SV, Singh RN: Sensilla on the tarsal segments and mouthparts of adult ***Drosophila melanogaster*** Meigen (Diptera: Drosophilidae). *Int J Insect Morphol Embryol* 1983, 12:273-291.
6. Possidente DR, Murphey RK: Genetic control of sexually dimorphic axon morphology in ***Drosophila*** sensory neurons. *Dev Biol* 1988, 132:448-457.
7. Venard R, Antony C, Jallon JM: ***Drosophila*** chemoreceptors. In *Neurobiology of Sensory Systems*. Edited by Naresh SIngh R, Strausfeld NJ. Plenum: New York; 1989:377-385.
8. Ferveur JF, Savarit F, O'Kane CJ, Sureau G, Greenspan RJ, Jallon JM: Genetic feminization of pheromones and its behavioral consequences in ***Drosophila*** males. *Science* 1997, 276:1555-1558.
9. Rodrigues V, Siddiqi O: A gustatory mutant of ***Drosophila*** defective in pyranose receptors. *Mol Gen Genet* 1981, 181:406-408.
10. Fujishiro N, Kijima H, Morita H: Impulse frequency and action potential amplitude in the labellar chemosensory neurones of ***Drosophila melanogaster***. *J Insect Physiol* 1984, 30:317-325.
11. Balakireva M, Stocker RF, Gendre N, Ferveur JF: ***Voila***, a new ***Drosophila*** courtship variant that affects the nervous system: behavioral, neural and genetic characterization. *J Neurosci* 1998, 18:4335-4343.
12. Singh RN: Neurobiology of the gustatory system of ***Drosophila*** and some terrestrial insects. *Microsc Res Tech* 1997, 39:547-563.
13. Savarit F, Sureau G, Cobb M, Ferveur JF: Genetic elimination of known pheromones reveals novel chemical bases of mating and isolation in ***Drosophila***. *Proc Natl Acad Sci USA* 1999, 96:9015-9020.
14. Ferveur JF, Störkuhl KF, Stocker RF, Greenspan RJ: Genetic feminization of brain structures and changed sexual orientation in male ***Drosophila***. *Science* 1995, 267:902-905.

15. Hodgson ES, Lettvin JY, Roeder KD: Physiology of a primary chemoreceptor unit. *Science* 1955, 22:417-418.
16. Marion-Poll F, Van der Pers J: Un-filtered recordings from insect taste sensilla. *Entomol Exp Appl* 1996, 80:116-119.
17. Marion-Poll F: Object-oriented approach to fast display of electrophysiological data under MS-Windows. *J Neurosci Methods* 1995, 63:197-204.
18. Marion-Poll F: Display and analysis of electrophysiological data under MS-Windows. *Entomol Exp Appl* 1996, 80:113-115.