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gustJ, a salt-insensitive mutant of *Drosophila melanogaster*

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Abstract. A set of 340 P insert lines on the X chromosome and the autosomes were screened for altered responses of the labellar chemoreceptors to salts and sucrose. A mutant line *gustJ* was isolated in which the electrophysiological response of the salt-sensitive neuron to Na^+ in the sensilla of the proboscis is reduced. The responses to KCl and sucrose are unaffected. In feeding tests, *gustJ* flies have Na^+-specific defects. Heterozygotes of *gustJ* with two other salt mutants *gustE* and *BE1323* are normal. Multiple alleles of *gustJ* have been obtained by excision of the original P element. All mutants have defects in Na^+ sensing specifically, thus defining a new gene that affects Na^+ response of the fly.

Keywords. Gustatory; sensory physiology; chemosensory.

1. Introduction

The mechanism of sensory transduction in the taste pathway has been investigated in vertebrates and invertebrates. In the amphibian *Necturus maculosus* sour and salty tastants are believed to be detected by the direct entry of H^+ and Na^+ ions through specialized channels on the sensory membrane (Kinnamon and Roper 1988). Sweet substances are detected by receptors coupled to G-proteins that activate adenyl cyclase (Bernhardt *et al.* 1996). Bitter taste is also mediated by a G-protein-coupled pathway (Ruiz-Avila *et al.* 1995), and in different systems either a phosphoinositide-phospholipase C or a cyclic nucleotide phosphodiesterase-mediated signalling mechanism is used.

Not much is known about the biochemistry of gustatory transduction in insects. In the blowfly *Phormia regina* it has been shown that membrane-permeable analogues of cGMP evoke a vigorous discharge of spikes from the sugar-sensitive labellar neuron (Amakawa *et al.* 1990, 1992). A number of groups have attempted to analyse the mechanisms of gustatory behaviour in *Drosophila* by studying taste mutants (Isono and Kikuchi 1974; Falk and Atidia 1975; Rodrigues and Siddiqi 1978; Tompkins *et al.* 1979; Tanimura *et al.* 1982; Morea 1985; Siddiqi *et al.* 1989; Inamdar *et al.* 1993; Rodrigues *et al.* 1995). Some of these mutants exhibit altered neurophysiological response in the labellar gustatory neurons. In the mutants *gustA* and *tre*, for instance, responses to pyranose sugars or trehalose are blocked (Rodrigues and Siddiqi 1981; Tanimura *et al.* 1982). Other gustatory mutants show either an increase or a reduction in salt-induced firing (Siddiqi *et al.* 1989; Rodrigues *et al.* 1991).

To gain an understanding of the mechanism of salt detection, we have studied mutants which show reduced electrophysiological responses to NaCl, correlated with

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behavioural deficits. Two such genes are known: *gustJ*, which is described in this paper, and *gustE* (Siddiqi *et al.* 1989). We present a detailed characterization of *gustJ* with a comparative account of electrophysiological responses and gustatory behaviour of this class of genes.

2. Materials and methods

2.1 *Stocks*

The wild-type strain in all experiments was the Canton Special strain (CS(Bz)) of *Drosophila melanogaster,* originally obtained from S. Benzer (Division of Biology, California Institute of Technology, Pasadena, USA). The deficiency Df(1)N19 strain (17A3–6; 17F2–18A3) and the stable source of transposase TM2 *Ubx*[(Δ2-3)ry^+] were obtained from the Bloomington stock centre at Bloomington, USA. The A4IM2 strain carrying the transposon PlArB used for generating the mutant lines was kindly provided by W. Gehring (Biozentrum, Basel, Switzerland). The flies were reared at 25°C with 12-h light and 12-h dark cycle on standard cornmeal–yeast medium. Standard procedures were used in handling *Drosophila* cultures (Roberts 1986).

2.2 *Electrophysiological recordings*

The recordings were done by the tip recording method of Hodgson *et al.* (1955) which has been used to study *Drosophila* by other workers (Rodrigues and Siddiqi 1978; Fujishiro *et al.* 1984; Arora *et al.* 1987). For recording extracellular responses from proboscis hairs, a three-day-old fly was mounted on a micropipette tip and the head and proboscis immobilized with myristic acid. Tarsal recordings were made from amputated limbs inserted in a glass capillary containing *Drosophila* Ringer solution. The sensilla were stimulated with a glass capillary of tip diameter 40 μm, containing the stimulating solution. The same capillary also served as the recording electrode. In the case of sucrose recordings 0.5 mM of NaCl was added to the solution to allow adequate electrical conductance. Impulses were passed through a WPI amplifier and recorded on a storage oscilloscope. Spikes were discriminated visually on the basis of their shape and amplitude. The frequency of spikes was measured over a 450-ms interval in each trace, leaving out 50 ms after the stimulus artifact. The responses were measured from the medial or mediolateral bristles of the labellum (Arora *et al.* 1987). In the tarsi, measurements were made from two medium-sized bristles (MB) on the distal end of the ventral surface of the 4th tarsal segment and two large bristles (LB) on the distal end of the 5th segment (Nayak and Singh 1983).

2.3 *Feeding preference test*

The behavioural responses of the flies were measured by the feeding preference test (Tanimura *et al.* 1982; Arora *et al.* 1987; Joshi *et al.* 1989). Flies were fed on a 60-well microtitre plate where the wells were filled with 1% agar. In this test alternate wells contain a red food dye, Carmoisine Red. When an attractant such as sucrose is to be tested, the sugar in appropriate concentrations is added to the uncoloured wells.

Normal flies eat preferentially from sugar-containing wells and their abdomens remain white. Mutants that cannot sense the attractant eat from both coloured and white wells and develop red abdomens. The proportion of uncoloured flies is thus a measure of attraction. When repellents such as NaCl or KCl have to be tested, the stimulus is added to the coloured wells. Normal flies avoiding salt remain uncoloured. The proportion of uncoloured flies is, in this case, a measure of avoidance. Two-to-four-day-old flies were starved for 18–20 h (but were allowed to drink distilled water) prior to testing. Approximately 100 flies were introduced over each plate and the flies were allowed to feed for 1 h in the dark. They were then killed by freezing and the colour of their abdomens was scored.

2.4 *Excision of the P insert in gustJ*

Males carrying the stable source of transposase *TM2 Ubx*[(Δ2–3)ry^+] (Robertson *et al.* 1988) on the third chromosome and a first-chromosome balancer FM6 were crossed to homozygous *gustJ*[Pry^+] females. Virgin *Ubx* F_1 females were picked and single-pair-mated to FM6;*ry* males. From the progeny non-*Ubx rosy* females, which had lost the P insert, were picked and again crossed to *FM6*; *ry* males to set up independent lines. In subsequent generations these became homozygous if there were no lethal mutations.

2.5 *In situ hybridization to polytene chromosomes*

DNA probes for the ry^+ gene labelled with biotinylated 16-dUTP were used for hybridization to salivary gland polytene chromosomes. The methods used for preparation of the chromosomal spreads and for probing were as described in Ashburner (1989). The chromosomes were counterstained with Giemsa stain and the signals were visualized using horseradish peroxidase (HRP)-labelled antibodies by the DAB colour reaction.

2.6 *Genetic methods*

For carrying out the meiotic mapping of the electrophysiological phenotype in *gustJ* a cross was made between *gustJ* females and *f car ; ry/ry* males. The male progeny in the F_2 were classified as parental or the recombinant genotype. This was possible as the three strains P[ry^+] *car ; ry/ry*, *car ; ry/ry*, and *ry/ry* were distinguishable by differences in their eye colour. All the recombinant progeny were tested for their electrophysiological phenotype; a few flies of the parental genotype were also tested.

3. Results

3.1 *Isolation of gustJ*

A large-scale transposon mutagenesis was carried out in which 120 lines mutated on the X chromosome and 1240 lines mutated on the autosomes carrying single P insertions were set up. These were screened for chemosensory mutations by behavioural tests. Three hundred and forty of these lines were also screened for altered electrophysiological responses to gustatory stimuli.

The taste sensilla of *Drosophila* are located on its tarsi and labellum. Typically the chemoreceptors have five sensory neurons, four of which are chemosensory and one is mechanosensory. The chemosensory neurons have been designated W, S, L1 and L2 (Arora *et al.* 1987), and they respond to stimulation by sugars and salts (Rodrigues and Siddiqi 1978; Fujishiro *et al.* 1984). The extracellular spikes from these neurons can be distinguished by their amplitude and shape. We tested the labellar hairs of four or five flies from each P insertion line against NaCl, KCl and sucrose. In one of the lines the response of the cell L1 to NaCl was 12 spikes in 450 ms against the 52 spikes in 450 ms seen in the wild type. The responses to KCl and sucrose were unaltered. This mutant line has been designated *gustJ* and is described below.

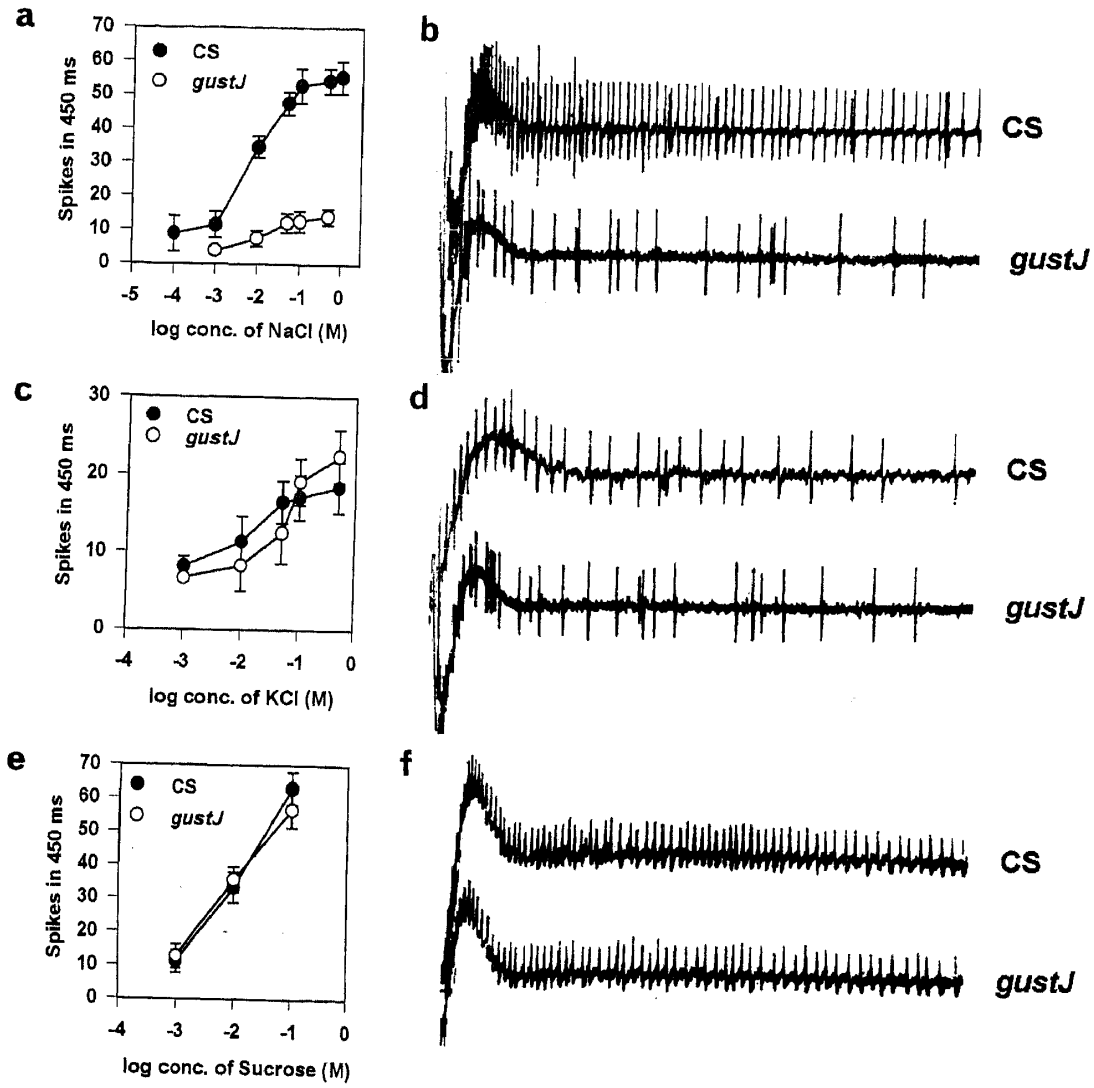

Figure 1. Electrophysiological responses of the labellar chemoreceptors of *gustJ* and CS adults to taste stimuli. The responses were measured by the tip recording method. Spikes arising in a 450-ms interval beginning 50 ms after the initiation of the response were counted. The response of CS and *gustJ* flies to different concentrations of NaCl (*a*), KCl (*c*) and sucrose (*e*), and the sample spike trains for stimulation by 0·1 M NaCl (*b*), 0·1 M KCl (*d*) and 0·1 M sucrose (*f*) are shown. Each point represents the mean for at least 60 chemoreceptors; standard deviations are also shown.

3.2 *Electrophysiology*

The electrophysiological responses of the L1 and S neurons in the labellar hairs of *gustJ* were compared with the responses of the wild type (figure 1). In the mutant the response of L1 to NaCl is greatly reduced while its response to KCl is not significantly altered. The response of the S neuron is normal. Figure 2 shows the recordings from the tarsal sensilla. The salt cell of the tarsal hair is unaffected. The mutation *gustJ* thus affects the L1 neuron in the proboscis but not in the tarsi.

3.3 *Behaviour*

The gustatory responses of *gustJ* were examined in the feeding preference test (Tanimura *et al.* 1982; Arora *et al.* 1987). In this test normal flies are attracted by sugars and low concentrations of NaCl but avoid concentrations of NaCl higher than 0·075 M. KCl is a strong repellent. The responses of *gustJ* are presented in figure 3. When NaCl is added to the white wells, at all concentrations, fewer *gustJ* flies eat out of the white wells (figure 3a). When NaCl is added to the coloured wells, at all concentrations, more *gustJ* flies avoid NaCl (figure 3b). These experiments show that, in the

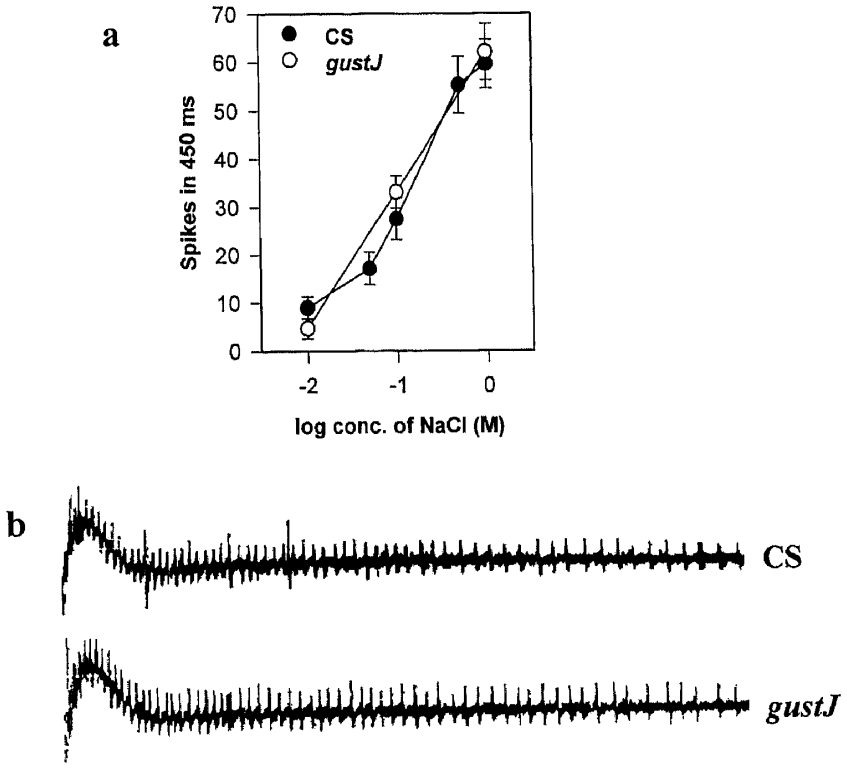

Figure 2. Electrophysiological responses of the tarsal chemoreceptors of *gustJ* and CS adults to NaCl. All responses were measured from the two large bristles on the distal end of the 5th segment and two medium bristles on the distal end of the ventral surface of the 4th segment. Spikes arising in a 450-ms interval beginning 50 ms after the initiation of the response were counted. The response of CS and *gustJ* flies to different concentrations of NaCl (*a*) and sample spike trains for stimulation by 1 M NaCl (*b*) are shown. Each point represents the mean for at least 45 chemoresceptors; standard deviations are also shown.

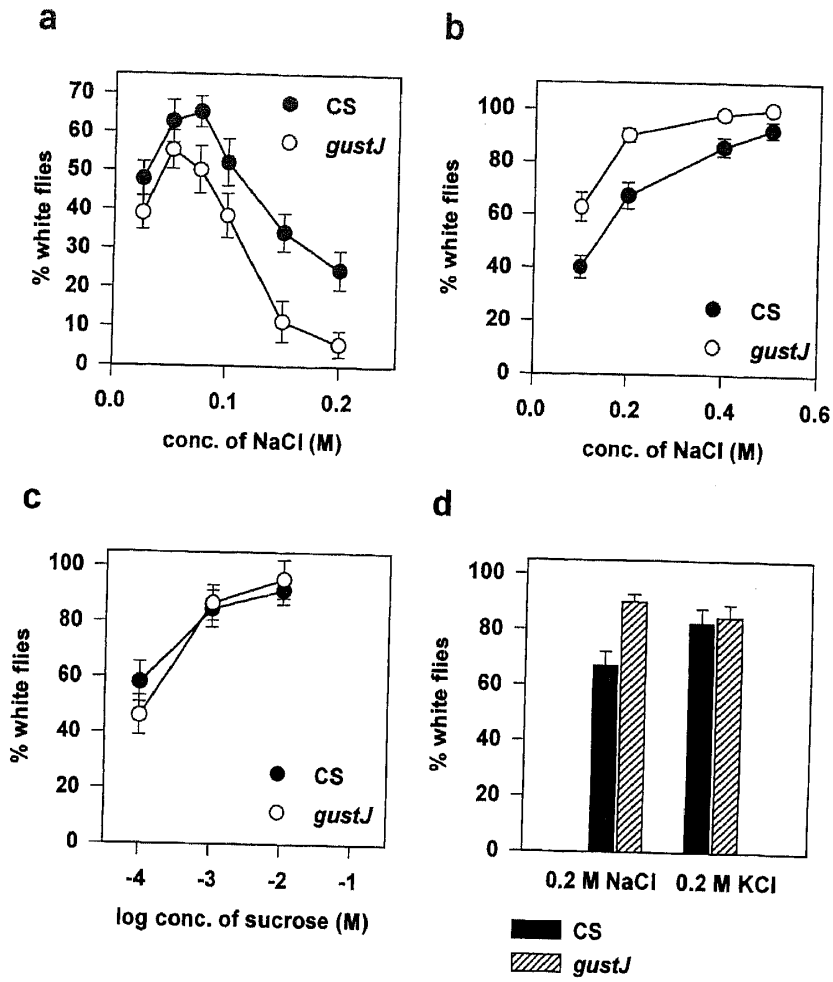

Figure 3. Behavioural responses of *gustJ* and CS adults to taste stimuli. The Canton-S strain (CS) was used as the wild type. All responses were measured in the feeding preference test. In the case of attractants the stimulus was placed in the uncoloured wells and the percentage white flies indicates the proportion of flies that were attracted to the stimulus. Repellents were placed in the coloured wells, and in this case percentage white signifies the proportion of flies that avoided the stimulus. (*a*) Per cent attraction response to different concentrations of NaCl; (*b*) per cent avoidance to different concentrations of NaCl; (*c*) attraction response to sucrose; (*d*) avoidance response to 0·2 M KCl. Each point shows the mean and standard deviations for at least 10 experiments.

mutant *gustJ*, there is a loss of attraction and an increase in the avoidance of NaCl. The responses of *gustJ* to sucrose and KCl are indistinguishable from those of the wild type (figure 3, c & d).

3.4 *Genetic characterization*

The P insert was localized to 17 C/D by *in situ* hybridization with the ry^+ gene probe. The mutation *gustJ* is recessive; the electrophysiological responses to NaCl of the heterozygotes with wild type show a response similar to that of the wild-type flies (figure 4a). Electrophysiological responses were also measured in heterozygotes with

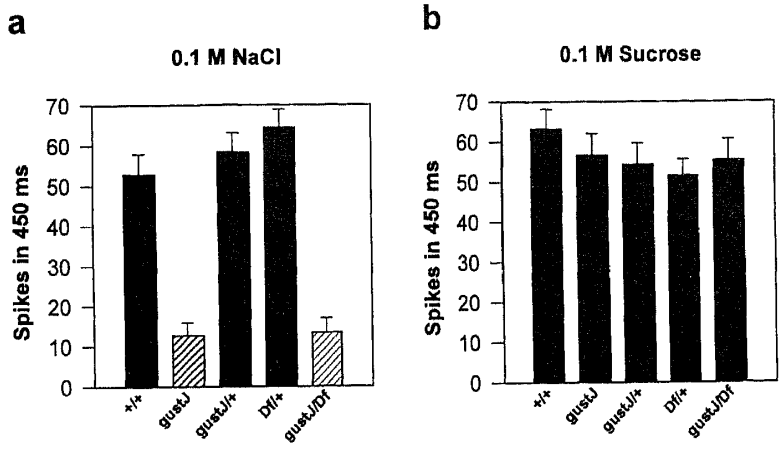

Figure 4. Electrophysiological responses of deficiency heterozygotes of *gustJ*. The responses of heterozygotes of the deficiency Df(1)N19 with CS and *gustJ* to 0·1 M NaCl (*a*) and 0·1 M sucrose (*b*) were measured as spikes arising in 450 ms, 50 ms after initiation of the response. Each bar shows the mean and standard deviations of recording s from 50 chemosensilla.

Table 1. The electrophysiological phenotype of *gustJ* is linked to the P[ry^+] insert. The male progeny of the F_2 of the cross between *f car*;*ry*/*ry* males and homozygous *gustJ* females are classified. The responses of the labellar chemoreceptors of the recombinants to 0·1 M NaCl were measured. In each of the parental types, the responses were scored from 12 flies. The phenotype is indicated as normal when the spikes in 450 ms are more than 45 spikes and considered mutant when they are fewer than 13 spikes. All flies carrying the P[ry^+] insert were found to be mutant.

Genotype	No. of flies	Electrophysiological phenotype
f car;*ry*/*ry*	278	Normal
P[ry^+]	329	Mutant
f P[ry^+];*ry*/*ry*	12	Mutant
car;*ry*/*ry*	15	Normal
P[ry^+] *car*;*ry*/*ry*	6	Mutant
f;*ry*/*ry*	8	Normal
f P[ry^+] *car*;*ry*/*ry*	1	Mutant
+ + +;*ry*/*ry*	0	—

the deficiency Df(1)N19 (17A3–6; 17F2–18A3). The deficiency uncovers the mutant phenotype (figure 4).

A cross was made between *gustJ* and a strain carrying two closely linked markers *f* (1–56.7) and *car* (1–62.5) that flank the insert on either side. The male progeny in the F_2 were classified as parental and recombinant genotypes and the electrophysiological responses of the labellar hairs to NaCl was measured in these flies (table 1). All flies that carried the P[ry^+] insert had a mutant phenotype and vice versa. *gustJ* segregates as a single gene. There was no recombination between the P element and the gustatory phenotype.

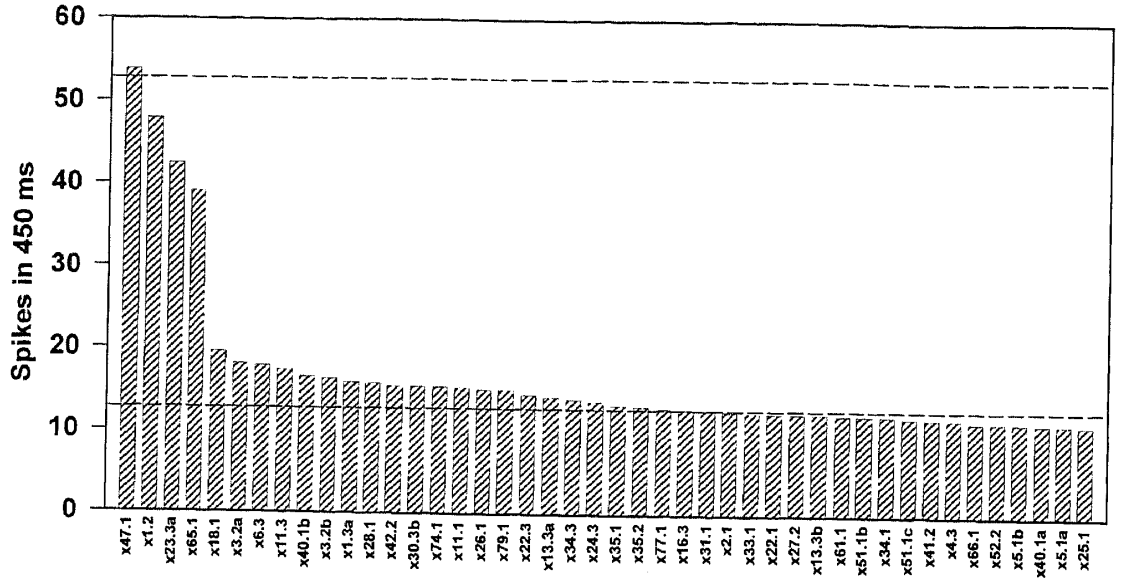

Figure 5. Responses from the labellar chemoreceptors of flies with excisions derived from *gustJ* to 0·1 M NaCl. The frequency of spikes in a 450-ms interval 50 ms after initiation of the spikes was counted in each case. The lines are arranged in the decreasing order of their L1-neuron responses. The lower broken line corresponds to the response elicited by the P insert mutant *gustJ* and the upper broken line to that of the wild type, CS. Each bar is the mean response for approximately 25 chemosensilla.

3.5 *Generation of alleles of gustJ by excision of the P element*

To excise the P element, *gustJ* females were crossed to FM6; TM2 *Ubx*[(Δ2–3)ry^+] males. *Ubx* females in the F_1 were pair-mated to FM6; ry males and, from the progeny, flies which had lost the ry^+ marker were picked to set up independent excision lines. In 180 lines examined there were no lethals. A selected set of 44 lines were characterized by electrophysiology. The results are presented in figure 5. A few of the excision lines have reverted to the wild-type firing frequency against NaCl. This suggests that the insertion of the P element is the likely cause of the mutant phenotype. The majority exhibit mutant phenotypes similar to that of the original insert. The excision experiment thus generated multiple alleles of *gustJ*. Eight of these, viz. x47.1, x1.2, x23.3a, x65.1 (phenotype close to wild type), and x42.2, x16.3, x4.3 and x66.1 (phenotype like *gustJ*) were analysed further.

The salt avoidance response of the selected lines is shown in figure 6a. The mutants are arranged in the order of decreasing L1 responses (see figure 5). It may be seen that the avoidance response is approximately inversely correlated with the NaCl-induced firing in the excision mutants (figure 6b; $r = 0.67$; $p < 0.01$).

The three mutant alleles x16.3, x4.3 and x66.1 were tested as heterozygotes with the wild type, the original P insert *gustJ*, and the deficiency Df(1)N19 (figure 7). All three excision alleles are recessive and are uncovered by the deficiency.

gustJ was also tested for complementation against two previously isolated mutants, *gustE* and *BE1323* (Siddiqi *et al.* 1989), which exhibit reduced NaCl-induced L1 firing. *gustJ* complements both these mutants and there is no observable interaction between *gustJ* and these two mutants (figure 8).

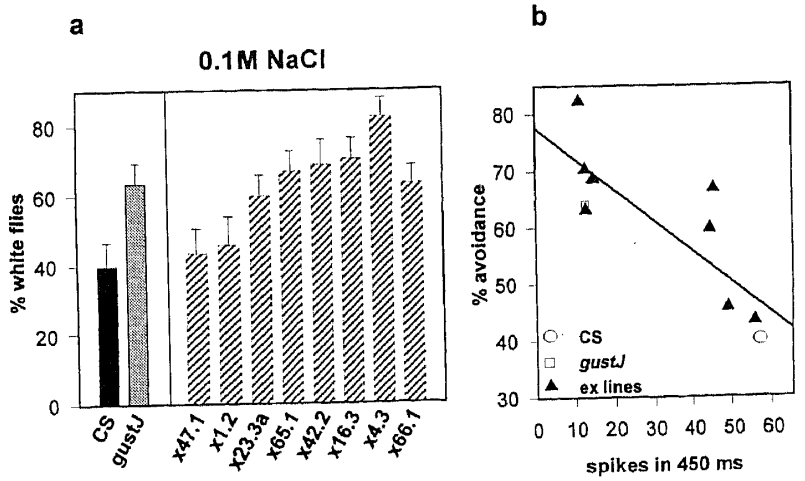

Figure 6. Behavioural responses of flies with excisions derived from *gustJ* measured by the feeding preference test. (*a*) The eight excision lines are arranged in the order of decreasing strengths of L1-neuron responses (same as in figure 5). The percentage white flies indicates the proportion of flies that avoid the stimulus. The bars show the mean and one standard deviation of the responses from at least 10 experiments. (*b*) Regression of the per cent avoidance to 0·1 M NaCl and the frequency of firing in response to 0·1 M NaCl stimulation in CS, *gustJ* mutants and flies with excision alleles of *gustJ* (linear correlation coefficient $r = 0{\cdot}67$; $P < 0{\cdot}01$).

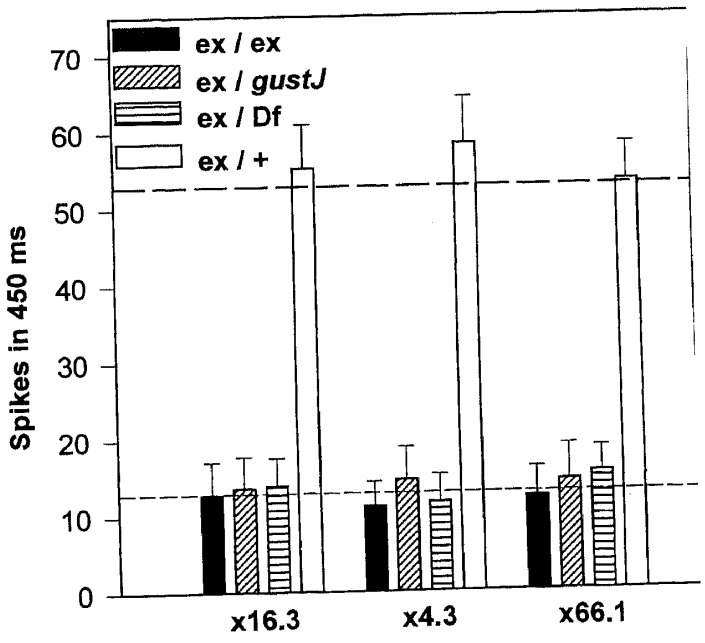

Figure 7. Genetic characterization of the excision alleles of *gustJ*. The electrophysiological responses of the labellar sensilla of the excision strains to 0·1 M NaCl were measured in homozygotes (filled bars), heterozygotes (open bars), and in *trans* heterozygotes with *gustJ* (slanted-hatched bars) and deficiency Df(1)N19 (horizontal-hatched bars) flies. The lower broken line indicates the response of the homozygous mutant and the upper broken line the response of the wild type, CS. Each bar shows the mean and one standard deviation of recordings from at least 50 sensilla.

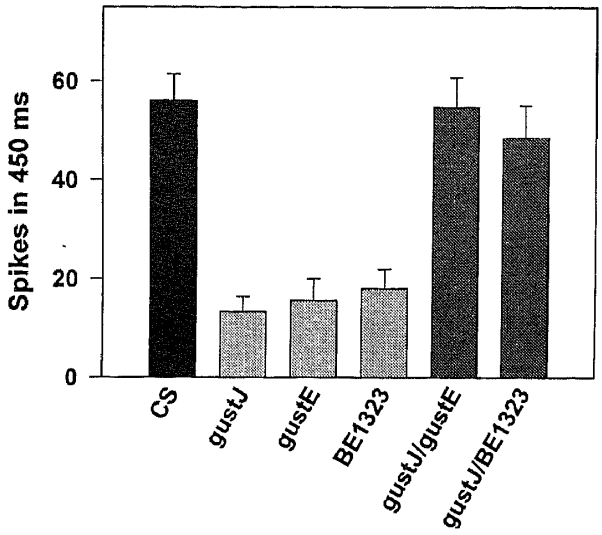

Figure 8. Complementation analysis of *gustJ* with other sodium-specific mutants. The electrophysiological responses to 0·1 M NaCl were measured in *trans* heterozygotes with *gustE* and *BE1323* mutants. The spikes were measured in a 450-ms interval 50 ms after initiation of the response. Each bar shows the mean and one standard deviation of recordings from at least 60 sensilla.

4. Discussion

We have screened P insert lines for mutants that are specifically altered in the firing of the neurons of the labellar chemoreceptors. The mutant line *gustJ* that was isolated in this screen has Na^+-specific electrophysiological deficits. In these flies the firing of the salt-sensitive L1 neuron of the proboscis is greatly reduced while the responses of the other neurons are unchanged. In feeding assays these flies have an altered feeding response only to Na^+. Wild-type flies are attracted to low concentrations of NaCl and they avoid higher concentrations (greater than 0·075 M NaCl) of the salt. In the mutant *gustJ* the attraction to low concentrations of NaCl is decreased, while the avoidance to higher concentrations of NaCl is greater than that of the wild-type flies. The feeding responses to potassium and sucrose are unaffected. This suggests a role for this gene in sodium sensing in the fly.

In the mutant *gustJ* the reduction in the NaCl-induced L1 neuron firing is accompanied by an increase in the avoidance or a decrease in attraction to NaCl in the feeding preference test. As attraction and avoidance are antagonistic behaviours, the directionality of the feeding defect in *gustJ* as measured by the two tests is the same. In the eight excision lines of *gustJ* whose feeding behaviour has been examined, a decrease in the L1-neuron firing in response to NaCl leads to an increased avoidance of this salt by the flies; the strong allele x4.3 shows maximum avoidance (figure 6). Thus the frequency of firing of the L1 neuron seems to have an inverse relation to the NaCl avoidance response of the fly. Studies with various mutants of *Drosophila* have indicated that the normal role of the L1 neuron of the labellum might be to mediate attraction to salt. In the mutant *gustB* (Arora *et al.* 1987) and the plieotropic mutants *gustC* and *gustR* (Siddiqi *et al.* 1989; Rodrigues *et al.* 1991) an increased firing of the L1 neuron in response to NaCl is correlated with an increase in the fly's attraction to this salt. On the other hand, in the mutant *gustE* (Joshi *et al.* 1989), which has a reduced L1-neuron

firing, the attraction to NaCl is completely abolished. It is therefore likely that the L1-neuron firing signifies attraction in the feeding behaviour.

Interestingly, the reduction in the NaCl-induced firing of the salt cell is seen only in the proboscis chemoreceptors. The responses of the salt cell of the tarsal chemosensilla to Na^+ are normal. When a fly sits on a food substance, its tarsal sensilla are the first to contact the food. If the inputs from these are favourable, it extends the proboscis, which may ultimately lead to feeding. In behavioural tests, if the tarsal sensilla are stimulated with sucrose, for instance, the fly extends its proboscis. Water-satiated flies are indifferent to stimulation by low concentrations of NaCl, and high concentrations inhibit the extension of the proboscis induced by sucrose (Dethier 1976). Looking at the firing dose response curve of the tarsal salt neuron, one might suggest that there exists a threshold value of response of this neuron such that any firing above this threshold causes an inhibition of the proboscis extension even in thirsty flies. If the firing response is lower than this value, the extension of the proboscis is not inhibited, and depending on the inputs from the labellar salt neuron the fly either accepts or rejects the particular concentration of salt. If this is true, then the tarsal salt neuron would have no role in the behavioural attraction to salt, which would be governed only by the labellar inputs.

Due to the specificity of the defect in *gustJ* it is tempting to suggest that the primary effect of the mutation is on the sensory neuron itself, i.e. on the Na^+ acceptor or the other components involved in Na^+ reception. By competition studies between different cations, Arora (1985) has shown that there are at least two types of acceptor sites for cations on the L1 neuron; the 'B site' is relatively nonspecific and responds equally to cations like Li^+, Na^+ and K^+, while the 'A site' responds selectively to sodium. The response of the L1 neuron due to the 'B site' alone saturates at about 15 spikes in 450 ms, while the overall response of this neuron saturates at over three times this value. The reduction in the firing in *gustJ* flies as well as in flies with its excision alleles corresponds to the portion contributed by the 'A site'. It is plausible that this mutation affects the Na^+-specific site. Alternatively, it is possible that *gustJ* modulates the L1-neuron firing indirectly. In studies done on the taste buds of vertebrates and frogs, it has been shown that the transport of Na^+ ions through epithelial membranes outside the taste buds can modify the taste receptor cell (TRC) membrane potentials (Li *et al.* 1994). Also, the paracellular diffusion and current flow within the taste buds might have a modulatory effect on the TRC responsiveness to Na^+ (reviewed in Lindemann 1996). changes in the levels of certain hormones like antidiuretic hormone (ADH) or aldosterone (Herness 1992; Gilbertson *et al.* 1993) in the blood have been shown to enhance the amiloride-blockable component of the NaCl-induced response in rats. ADH levels increase either due to water deprivation or due to NaCl ingestion (i.e. leading to an increase in blood osmolarity). The increase in perceived intensity of salty taste in food due to increase in levels of ADH may thus lead to decrease in further intake of salts (Herness 1992; Gilbertson *et al.* 1993). The reverse effect of 'blunting' of Na^+ taste is seen in rats that had been fed on a NaCl-restricted diet during embryonic growth (Hill and Przekop 1988). Thus, at least in vertebrates, the ionic predisposition of the animal seems to have a bearing on the sensitivity of TRCs. In locusts Abisgold and Simpson (1988) have shown that an increase in the amino acid levels in the haemolymph reduces the responsiveness of the chemoreceptors to amino-acid stimulation. An increase in blood osmolality depresses the NaCl responses. It is possible that in the *gustJ* mutant the effect on L1-neuron firing is by modulation of the sensitivity of this neuron to salt

stimulation. Thus, although a mutation in *gustJ* specifically affects the Na^+-induced firing of the L1 cell, whether this is due to a direct effect on the molecules involved in salt sensing is not clear, and the answer must await molecular characterization of this gene.

Acknowledgements

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