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THE *Shaker* AND *shaking-B* GENES SPECIFY ELEMENTS IN THE PROCESSING OF GUSTATORY INFORMATION IN *DROSOPHILA MELANOGASTER*

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Summary

Mutations that affect the physiological properties of neurones or the development of neuronal circuits are likely to have profound effects on sensory and motor pathways. We have examined the effects of mutations in two loci – the *Shaker* complex and *shaking-B* – on the taste pathway of *Drosophila melanogaster* Meigen at the behavioural and electrophysiological levels. The *Shaker* locus encodes a variety of A-type potassium channels that are likely to be tissue- and stage-specific. Flies containing the different *Shaker* alleles examined exhibit a variety of defects in their gustatory responses to sucrose, NaCl and KCl. The firing patterns of the labellar chemosensory neurones in response to these stimuli are normal. This suggests that the channels encoded by the *Shaker* locus are probably not involved in taste transduction, but affect central gustatory circuits. The *shaking-B* locus affects neuronal connectivity, though its molecular nature is unknown. Mutants at this locus show increased thresholds for detection of sucrose and fructose and lack the attraction response to 0.1 mol l^{-1} sodium chloride that is exhibited by the wild-type fly.

Introduction

An animal perceives the external world through its sensory systems. These transduce external stimuli into electrical information, which is further processed and decoded, leading to a behavioural response. The processing of sensory information involves several steps: stimulus recognition, signal transduction, integration and motor output. The gustatory pathway of *Drosophila melanogaster* is a promising system in which these processes may be analysed at the molecular level.

The fly detects taste stimuli by means of external sensilla located on its proboscis and tarsi (Dethier, 1976). A labellar sensillum in *Drosophila* typically contains

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four functionally distinct chemosensory neurones: an S neurone, which responds to sugars; L1 and L2, which are salt-sensitive; and a W or water neurone (Falk *et al.* 1976; Rodrigues and Siddiqi, 1978; Fujishiro *et al.* 1984). In *Drosophila* and other dipterans, these neurones project to the suboesophageal ganglion (Nayak and Singh, 1985).

The genetic approach to the analysis of gustation involves the isolation and study of single-gene mutations with the aim of pinpointing molecules important for the development and function of the pathway. A number of mutants showing defective responses to taste stimuli have been described (Isono and Kikuchi, 1974; Falk and Atidia, 1975; Rodrigues and Siddiqi, 1978; Tompkins *et al.* 1979; Tanimura *et al.* 1982; Morea, 1985). A complementary approach is to study the function of identified neuronal molecules in the gustatory pathway.

In recent years, several genes that affect nervous system function or development have been studied and their products analysed. The molecules that they encode include channel proteins, for example *Shaker* (Iverson *et al.* 1988; Pongs *et al.* 1988; Timpe *et al.* 1988), potential nuclear regulatory factors, such as *daughterless* (Caudy *et al.* 1988), *hairy* (Rushlow *et al.* 1989) and *achaete-scute* (Ghysen & Dambly-Chaudiere, 1989), transmembrane proteins, such as *Notch* (Wharton *et al.* 1985) and *Delta* (Vässin *et al.* 1987), and synaptic molecules, such as *ard* (Hermans-Borgmeyer *et al.* 1986). These molecules are often expressed in a wide range of tissues, and mutations in genes coding for them are likely to have pleiotropic effects. *daughterless* is a well-known example of a gene that plays a role in sex determination and peripheral neurogenesis (Cline, 1989).

There are fewer examples of molecules specifying neuronal connectivity. The mutations *disconnected* (Steller *et al.* 1987), *bendless* and *Passover* (Wyman and Thomas, 1983) presumably affect genes that encode molecules involved in connectivity.

An analysis of the effects of neurogenic mutations on the function of diverse sensory pathways may provide interesting information on how neural systems are built. The magnitude and specificity of these effects will depend on the nature of the mutation and the requirement of the gene product in the specific circuit. As a first step in this direction, we have examined the effects of mutations in two loci, *Shaker* and *Passover* (*shaking-B*), on the gustatory responses of the fly. The behavioural defects observed are viewed in the light of our current understanding of the function of gustatory circuits in dipterans.

Mutations in the *Shaker* locus affect neuronal excitability, action potential frequency and repolarization, and synaptic transmission (Jan *et al.* 1977; Tanouye *et al.* 1981). These mutations affect a fast, transient outward potassium current, I_A (Salkoff and Wyman, 1983). The *Shaker* locus has been cloned and shown to be the structural gene for the A-subtype of potassium channel (Iversen *et al.* 1988; Timpe *et al.* 1988*b*). The locus is complex, encoding at least 21 different transcripts (Kamb *et al.* 1988). Of these, five that have been analysed by *in vitro* transcription in *Xenopus* oocytes give rise to kinetically distinct populations of A-type potassium channels (Timpe *et al.* 1988*a*).

Mutations giving rise to abnormalities in ion channels could affect gustatory behaviour by altering the peripheral detectors, higher-order neurones or both. There is some evidence for the involvement of potassium channels in gustatory transduction in vertebrates. A voltage-insensitive cyclic-AMP-modulated channel has been implicated in sucrose reception and a voltage-sensitive channel appears to be involved in KCl transduction (Avenet *et al.* 1988; Kinnamon and Roper, 1988).

The properties of neuronal circuits are largely dictated by the cellular localization of channels with different properties. The I_A current is known to play an important role in the modulation of neuronal signals in different circuits (Getting, 1983). The spatial and temporal patterns of expression of the *Shaker* transcripts are therefore likely to have profound effects on the processing of sensory information.

Passover (*Pas*) is an allele of a previously isolated mutation *shaking-B*^{2} (Homyk *et al.* 1980; Baird, 1986). This locus will therefore be referred to as *shaking-B* (*shak-B*) (J. Davies, personal communication). Mutations in the *shaking-B* locus cause a defect in the visually elicited jump response of *Drosophila melanogaster* (Wyman and Thomas, 1983). This response is mediated by a pair of neurones, the giant fibre neurones. The two giant fibres descend from the brain to the thoracic ganglion; there the giant fibre of each side synapses with two neurones – the tergotrochanteral motor neurone (TTMN) and the peripherally synapsing interneurone (PSI). The TTMN drives the TTM or jump muscle, resulting in extension of the mesothoracic leg during jumping. The PSI synapses with the motor neurones innervating the dorsal longitudinal muscles (DLMs) or wing depressors. A sudden light-off stimulus causes the giant fibre (GF) to fire, driving the TTMs and the DLMs, resulting in jumping and flight initiation (Wyman and Thomas, 1983). In *Pas* and *shak-B* strains, the synapse between the GF and TTMN does not form. In addition, the synapses in the GF–PSI–DLMN part of the pathway do not function normally. Our analysis of the gustatory responses of *Pas* and *shaking-B*^{2} strains raises the possibility that this locus is also involved in specifying connectivity in the taste pathway of *Drosophila melanogaster*.

Materials and methods

Strains

The genotypes and sources of the strains used in this study are listed in Table 1. *Drosophila* cultures were maintained on standard corn meal/yeast medium at 23–25 °C.

Chemicals

Sucrose, fructose and sodium chloride were obtained from Sigma, St Louis, MO; potassium chloride was from BDH, India Ltd; agar was from SISCO, Bombay, India, and Carmoisine Red from Anand Dyes and Co., Ltd, Bombay, India. All solutions were made in double distilled water.

Table 1. *List of strains used and their sources*

Strains		Sources and reference
Canton S		D. Gailey, Department of Biology, Brandeis University, USA
Oregon R		K. Gotz, Max Planck Institute, Tübingen, FRG
Sh^5 Sh^{102} Sh^{KS133}		M. Tanouye, CalTech, California, USA (Tanouye *et al.* 1981)
shak-B *Pas*		R. Wyman, Yale University, Connecticut, USA (Homyk *et al.* 1980; Wyman and Thomas, 1983)
$EF535/FM6/y^+Ymal^{106}$ $R\text{-}9\text{-}29/FM6/y^+Ymal^{106}$ $R\text{-}9\text{-}28/FM6/y^+Ymal^{106}$ *y w runt/FM6*		J. Davies, University of Glasgow, Glasgow, UK
Df(1)16-3-22/FM6	19D1;20A2	*Drosophila* Stock Centre, Bloomington, Indiana, USA (Miklos *et al.* 1987)
$Df(1)B57/FM6/y^+Ymal^{106}$	19E1–2;19F1	
$Df(1)16\text{-}3\text{-}35/FM6/y^+Ymal^{106}$	19D3;19E3	
$Df(1)A118/FM6/y^+Ymal^+$	19E4–5;19E8	
Df(1)T2-14A	19E5;19E7–8	
T(X;Y)B55	16F1–4	M. Tanouye, CalTech, California, USA (Tanouye *et al.* 1981)
T(X;Y)W32	16F3–6	
T(X;Y)V7	16F5–8	

The feeding preference test

The test designed by Tanimura *et al.* (1982) was used with minor modifications. Flies to be tested were reared in uncrowded culture bottles at 25±1°C. After emergence, they were placed in fresh culture bottles and allowed to age for 2–3 days. They were then transferred to flasks containing filter paper soaked in distilled water and starved for 18 h. Testing was carried out in 6×10-well microtitre plates (Laxbro, Pune, India) with a well capacity of approximately 0.1 ml. The response to both attractants and repellents can be assayed in this test. When the response to attractants was being assayed, the stimulus was prepared in 1% agar and placed in alternate wells of the plate. The remaining wells were filled with 1% agar containing 0.2% of the food dye Carmoisine Red. For repellents, the stimulus was prepared in agar that also contained the food dye.

Approximately 100 flies were introduced into each plate by means of a hole in the lid. At no stage were the flies anaesthetized in any way. The plates were left undisturbed in the dark at 25°C for 1 h. The flies were scored for the red colour in their abdomens by inspection under a dissection microscope. The response was calculated from the fraction of flies feeding from the stimulus-containing wells. Thus, percentage attraction=number of uncoloured flies/total number of flies; and percentage tolerance=number of coloured flies/total number of flies.

Whenever the response of mutants was being assayed, the wild type was tested in parallel. Means and standard deviations were calculated from data from several independent plates run with different batches of flies on different days. The calculation of acceptance and repulsion responses is affected by the population of 'non-eaters', which appear uncoloured. The number of non-eaters would be expected to vary depending on the concentration of the stimulus being tested. To obtain an estimate of this, a control was carried out at each concentration wherein all wells contained coloured agar, but alternate wells contained stimulus as well.

The proportion of uncoloured flies in these tests indicates the fraction of non-eaters. This proportion has been found to decrease from 20–25 % for no stimulus to 0 % at high concentrations (refer to Fig. 1).

Electrophysiological recordings from the labellar chemoreceptors

The tip recording method of Hodgson *et al.* (1955) was modified for use in *Drosophila* (Rodrigues and Siddiqi, 1978; Fujishiro *et al.* 1984; Arora *et al.* 1987). Three- to four-day-old flies were immobilized by cooling and introduced into plastic micropipette tips (Gilson P-200). The tapered ends of the tips were cut to hold the fly snugly at the thorax, allowing only the head to protrude. The head was held in place using myristic acid wax (melting point 58.5 °C). The proboscis was immobilized by a minute drop of wax applied at the haustellum. The wax was applied with a tungsten wire loop whose temperature could be controlled by varying the current passed through the wire. The flies were allowed 15 min to recover from the treatment.

A Ag/AgCl indifferent electrode filled with *Drosophila* saline was introduced into the thorax of the fly. The recording electrode was a tapered glass capillary connected to a Ag/AgCl electrode and filled with the stimulus. Signals recorded from the labellar hairs were passed through a high-impedance preamplifier and a low-frequency filter (50 Hz) before being displayed on a storage oscilloscope (Tektronix 5113). Four large medial crest bristles and six mediolateral bristles were sampled in each preparation (Falk *et al.* 1976). Each hair was stimulated three times with a 2 min interval between recordings. Spike frequencies were estimated from a 450 ms interval beginning 50 ms after the initiation of the response. The spike frequency of the sensillum was obtained as an average of the three trials. Recordings from different sensilla were pooled to give the mean and standard deviation of the response.

Genetic analysis

Complementation

Reciprocal crosses were set up between the males and virgin females of the different mutant strains. The F1 progeny were tested for their gustatory responses in the feeding preference test. The responses of the heterozygous females were compared with those of both mutants/+ and the homozygous mutants. The mutations were considered to be non-complementing when the percentage

response of the heterozygote was comparable to that of homozygous mutants and significantly different from mutant/+. The males served as internal controls.

Cytological mapping

The mutants were crossed to a set of deficiency-bearing strains with rearrangements in the appropriate regions of the chromosome. Progeny of the relevant genotype were assayed for their taste responses in the feeding preference test.

The response of mutant/deficiency was compared with that of mutant/+ and deficiency/+.

Results

The feeding preference test has been used to characterise the gustatory behaviour of mutant alleles in two loci: *Shaker* and *shaking-B*. We used several criteria to verify that the observed defects were in fact due to lesions in these loci rather than to other mutations arising in the genetic background of the strains. The phenotypes of several independently isolated alleles at each of the loci were examined. Combinations of these alleles were tested for the gustatory behaviour of their phenotypes in complementation tests. The gustatory defects in these strains were mapped to a cytological position using deficiency and duplication strains.

Responses of the wild-type strains: Canton S and Oregon R

The gustatory responses of Canton Special (CS) and Oregon R (OR) are shown in Fig. 1, illustrating the variation in behavioural responses between different wild-type strains. These strains have been chosen since the mutants analysed in this study have been isolated on CS or OR backgrounds. However, the genetic background of the different mutant strains is no longer strictly comparable to that of either wild type, as a result of out-crossing. Thus, CS is used as the standard wild-type strain. Comparisons with OR have been made only to emphasize that the mutant phenotype is detectable even when compared to a less responsive wild-type strain. Differences in gustatory behaviour between these two strains have already been reported (Tompkins *et al.* 1979; Tanimura *et al.* 1982). OR flies showed a reduced response to sugars compared with CS flies (Fig. 1A,B). They were also more salt-tolerant than CS flies (Fig. 1D). The attraction response to low concentrations of NaCl was comparable in the two strains (Fig. 1C). In no case were sex-specific differences in response observed.

The Shaker *complex*

Mutations at this locus cause vigorous leg-shaking under ether anaesthesia (Kaplan and Trout, 1969). *Shaker* has been localized to the 16F region of the X chromosome. 3-Ethylmethanesulphonate (EMS)-induced alleles, Sh^5, Sh^{102} and Sh^{KS133}, and three translocation breakpoints, *T(X;Y)B55*, *W32* and *V7*, all map

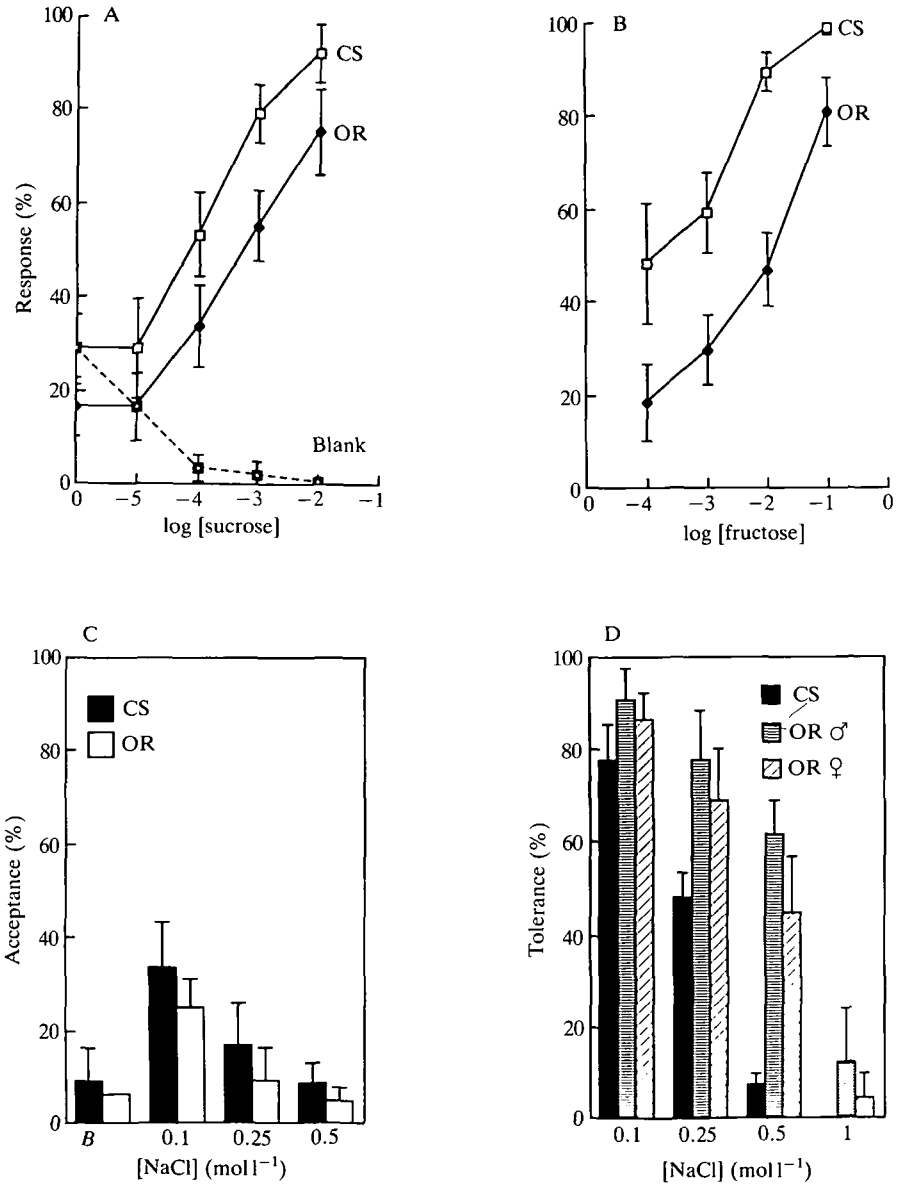

Fig. 1. Gustatory responses of Canton S (CS, □) and Oregon R (OR, ◆) strains. Responses to sucrose (A), fructose (B) and NaCl (C and D) were assayed in the feeding preference test. Acceptance responses to NaCl (C) were measured with the stimulus in the uncoloured wells. A blank plate (*B*) was run where no stimulus was added. For assay of tolerance (D), the salt was added to the wells that also contained the food dye. Tolerance data for males and females are presented separately. In all other tests, there was no sexual dimorphism of the response. The dashed line in A indicates the number of flies that fail to eat and therefore appear uncoloured. To measure non-eaters, all wells were filled with food dye and only alternate wells contained the stimulus. All values are the means and standard deviations of at least 10 readings.

within this complex (Table 1) (Tanouye *et al.* 1981). The translocations show the leg-shaking phenotype typical of the *Shaker* mutants.

Gustatory phenotypes of the Shaker *alleles*

We have tested the responses of the three EMS-induced alleles and the three translocation strains to sugars and salts using the feeding preference test. At 1 mmol l^{-1} sucrose, 85–90 % of wild-type flies eat preferentially from the stimulus-containing wells. Males from each of the six strains showed a reduced preference for sucrose (Fig. 2A). The severity of the defect can be ordered as: $B55 > Sh^5 = Sh^{102} > W32 > V7 > Sh^{KS133}$. Females of Sh^5, Sh^{102} and Sh^{KS133} show comparable responses. Only the males of the translocation strains were tested. The dose–response curve for sucrose suggested that the defect in all six strains is a shift in the threshold of detection (data not shown).

Wild-type flies exhibit a weak attraction to 100 mmol l^{-1} NaCl (Fig. 2B). The *Shaker* strains vary in their NaCl attraction response. Flies containing two alleles, Sh^{102} and Sh^{KS133}, show no attraction to sodium chloride. The response is well below the cut-off values for inherent error in the test due to non-eaters. Sh^5 flies show an enhanced acceptance of NaCl ($P<0.01$). The responses of the translocation strains *B55* and *W32* are not significantly different from those of the wild type ($P>0.01$). *V7* flies shows a small increase in their attraction response ($P<0.01$).

High concentrations of NaCl or KCl are strongly repellent to wild-type flies. Less than 10 % of CS flies are tolerant of 0.5 mol l^{-1} NaCl or 0.2 mol l^{-1} KCl. Strains containing different alleles differed in their degree of tolerance to 0.5 mol l^{-1} NaCl and may be ordered as follows: $B55 > Sh^{102} = Sh^5 > V7 = W32 > Sh^{KS133}$ (Fig. 2C). KCl tolerance may be ordered as: $B55 = Sh^{102} = Sh^{KS133} > V7 > Sh^5$. *T(X;Y)W32* flies behave like the wild type at 0.2 mol l^{-1} KCl. The tolerance to NaCl and KCl observed in these strains is due to an increase in the threshold of repulsion. Repulsion responses can be elicited at high stimulus concentrations (data not shown).

The labellar chemoreceptors of strains containing the Shaker *alleles show normal physiology*

We measured the firing frequency of the labellar chemosensory neurones using the tip recording method (Hodgson et *al.* 1955). Stimulation with NaCl and KCl at 0.1 mol l^{-1} and 0.5 mol l^{-1} results in responses from two salt neurones (L1 and L2). Spikes from L1 are 1.0–1.5 mV in amplitude. The activity of the L1 neurone increases in a dose-dependent manner with a threshold at 1 mmol l^{-1} and saturates at concentrations above 0.1 mol l^{-1} (data not shown). L2 spikes are larger in amplitude, but the responses from this cell are erratic and could not be elicited from all hairs. This neurone exhibits a high-threshold, low-frequency response (Siddiqi *et al.* 1989). The responses of strains containing three *Shaker* alleles, Sh^{102}, Sh^{KS133} and Sh^5, are summarized in Table 2. These strains did not show any alteration in firing frequencies of the labellar chemoreceptors to NaCl or KCl. The spike amplitudes and shapes were comparable to those of the wild-type strain.

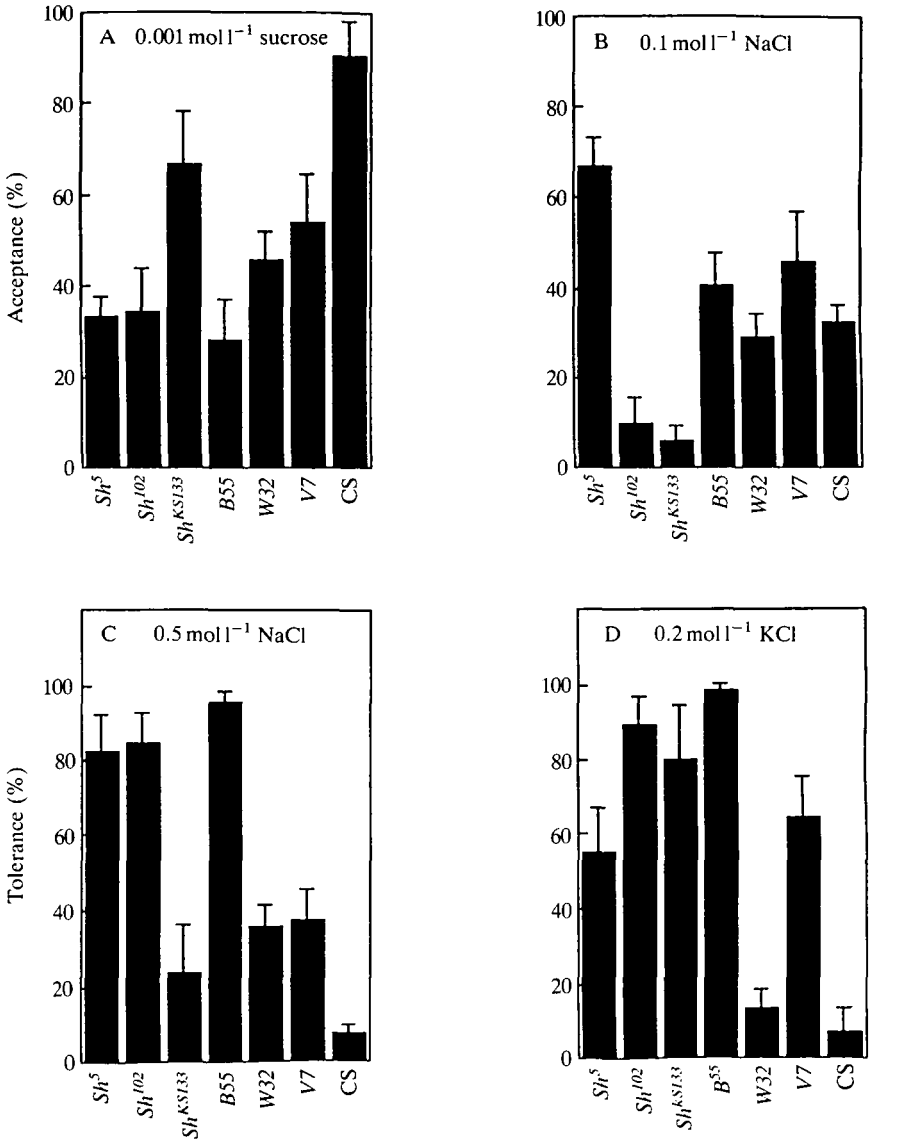

Fig. 2. Responses of the six *Shaker* strains and Canton S (CS) to gustatory stimuli. Acceptance responses to sucrose (A) and NaCl (B) and tolerance responses to NaCl (C) and KCl (D) were assayed in the feeding preference test. Means and standard deviations were calculated from at least 10 readings.

Stimulation of the labellar hairs with sucrose resulted in activity in the S neurone (Rodrigues and Siddiqi, 1978; Fujishiro *et al.* 1984). Neurones from wild-type flies fire at a frequency of 42±3.5 spikes per 450 ms in response to 100 mmol l^{-1} sucrose. The firing frequency of neurones of the *Shaker* alleles is not significantly different from those of the CS strain (data not shown).

Table 2. *Electrophysiological responses to salts of the labellar chemoreceptors of flies containing* Shaker *alleles*

Strain	0.1 mol l^{-1} NaCl	0.5 mol l^{-1} NaCl	0.1 mol l^{-1} KCl	0.2 mol l^{-1} KCl
CS	46.1±7.2 *N*=80	46.2±8.3 *N*=51	11.8±1.9 *N*=82	8.0±1.7 *N*=47
Sh^5/Sh^5	49.5±7.9 *N*=80	47.2±8.8 *N*=42	11.9±2.8 *N*=75	11.8±1.7 *N*=54
Sh^{102}/Sh^{102}	50.6±9.4 *N*=86	42.6±6.1 *N*=54	12.0±2.9 *N*=71	10.4±2.6 *N*=64
Sh^{KS133}/Sh^{KS133}	49.6±6.5 *N*=78	47.3±6.5 *N*=55	11.0±2.9 *N*=79	8.8±1.2 *N*=57

Values are the means±S.D. of the spike frequency per 450 ms interval; *N*=number of hairs sampled.

Complementation analysis of the Shaker *alleles with respect to taste function*

The defects in taste function observed in flies containing the three EMS-induced alleles of *Shaker* are fully recessive when tested with 1 mmol l^{-1} sucrose (Table 3). The ether-induced leg-shaking behaviour is dominant.

We tested the gustatory responses of flies containing *trans* combinations of the *Shaker* alleles. The data are summarized in Table 4. Heterozygotes of Sh^5/Sh^{102}, Sh^{KS133}/Sh^5 and Sh^{102}/Sh^{KS133} all show a reduced response at 1 mmol l^{-1} sucrose compared to the wild-type/*Shaker* flies ($P \ll 0.01$ in all cases). Interestingly, Sh^{KS133}/Sh^5 flies exhibit a response that is significantly lower than that of Sh^{KS133} homozygotes ($P<0.01$).

T(X;Y)B55, *T(X;Y)W32* and *T(X;Y)V7* are independently isolated translocation strains with breakpoints in the 16F region of the X chromosome. Hemizygous males would therefore be null for a small region of the *Shaker* complex (Table 1). The observation that these males exhibit defects in response to taste stimuli maps these lesions to within the *Shaker* complex.

Table 3. *Dominance testing of the* Shaker *alleles*

Genotype	Mean response	S.D.	*N*
CS	89.0	6.5	20
Sh^5	25.9	10.1	8
$Sh^5/+$	79.8	12.7	20
Sh^{102}	33.0	13.0	7
$Sh^{102}/+$	70.4	13.4	6
Sh^{KS133}	64.0	11.2	9
$Sh^{KS133}/+$	78.3	6.9	9

The responses were assayed by the feeding preference test against 1 mmol l^{-1} sucrose.

Table 4. *Complementation analysis between* Shaker *alleles*

Genotype	Mean response	S.D.	*N*
CS	89.0	6.5	20
Sh^5	25.9	10.1	8
Sh^{102}	33.0	13.0	7
Sh^{KS133}	64.0	11.2	9
Sh^5/Sh^{102}	15.7	6.7	15
Sh^5/Sh^{KS133}	29.3	3.0	8
Sh^{102}/Sh^{KS133}	30.3	10.6	10

The responses were assayed in the feeding preference test against 1 mmol l^{-1} sucrose.

The shaking-B *locus*

Pas and *shak-B* are viable alleles at this locus. *Pas* is a semi-dominant allele isolated on a Canton S background (Thomas and Wyman, 1984); *shak-B* is a recessive allele derived from Oregon R (Homyk *et al.* 1980). Both *Pas* and *shak-B* show a connectivity defect in the giant fibre pathway (Wyman and Thomas, 1983; Baird, 1986). *R-9-29* and *EF535* are two of the lethal alleles at this locus.

Gustatory defects in Pas *and* shak-B

The *Pas* and *shak-B* mutations resulted in a reduced attraction response to sucrose and fructose, an abolition of the attraction response to 0.1 mol l^{-1} NaCl and a small increase in tolerance to 0.5 mol l^{-1} NaCl (Figs 3,4). The reduction in the response to sugars was evident at threshold concentrations; at 0.1 mmol l^{-1} and 1 mmol l^{-1} sucrose, there was a significant difference between *Pas* and CS ($P<0.01$) and between OR and *shak-B* flies ($P<0.01$). At saturating concentrations (>10 mmol l^{-1}) there was no significant difference between mutant and wild-type strains ($P>0.01$). Thus, the defect is essentially a shift in the threshold of detection. Fig. 4 shows the lack of an attraction response to 0.1 mol l^{-1} NaCl in the mutants ($P<0.01$). Since the response is indicated by the number of uncoloured flies in the plate, its value will be affected by the number of 'non-eaters'. The acceptance of 10–15 % by mutant flies is below the value normally obtained for non-eaters. *Pas* and *shak-B* flies are slightly more tolerant than CS to 0.5 mol l^{-1} NaCl ($P<0.01$).

Complementation analysis of the shak-B *alleles*

Pas and *shak-B* flies behave as fully recessive alleles when assayed for their acceptance response to 1 mmol l^{-1} sucrose (Table 5). The responses of flies of genotype *Pas*/CS and *shak-B*/OR are comparable to those of CS and OR, respectively ($P=0.5$). These alleles fail to complement one another for their gustatory behaviour (Table 6). We tested the effects of two lethal alleles at the *shak-B* locus, *R-9-29* and *EF535*, on taste function. Both mutations show no behavioural abnormalities in *trans* combination with a wild-type chromosome

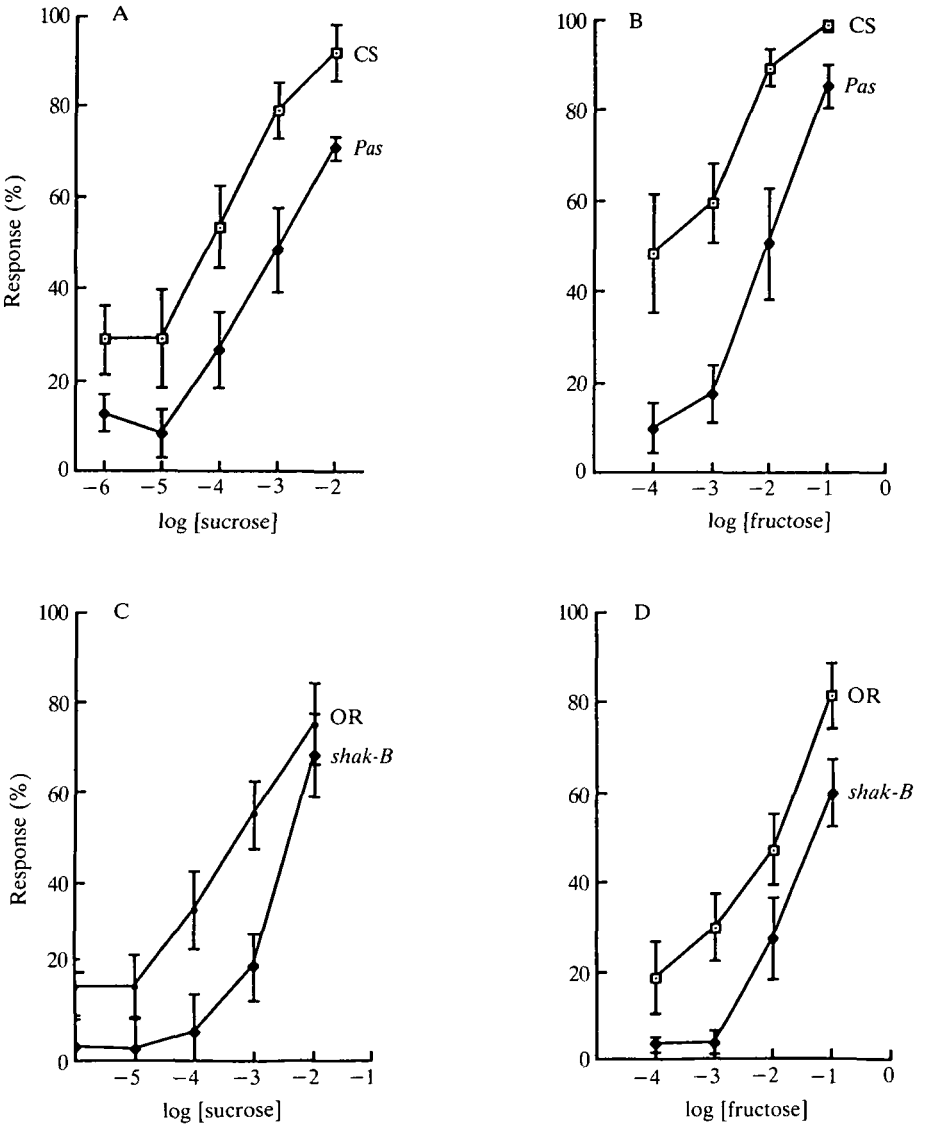

Fig. 3. Stimulus–response curves of *Pas* and *shak-B* strains to sugars. The responses of the *Pas* strain to sucrose (A) and fructose (B) were compared to those of the CS strain, whereas those of the *shak-B* strain (C, sucrose; D, fructose) were compared to those of the OR strain. Values are the means and standard deviations of at least 10 readings. Sucrose and fructose concentration is in mol l^{-1}.

(Table 5). *R-9-29* fails to complement *Pas* and *shak-B* flies for their gustatory defect (Table 6). The phenotypes of *Pas/R-9-29 and Pas/EF535* heterozygotes are more extreme than that of the homozygous strain ($P<0.01$). *shak-B/EF535* i

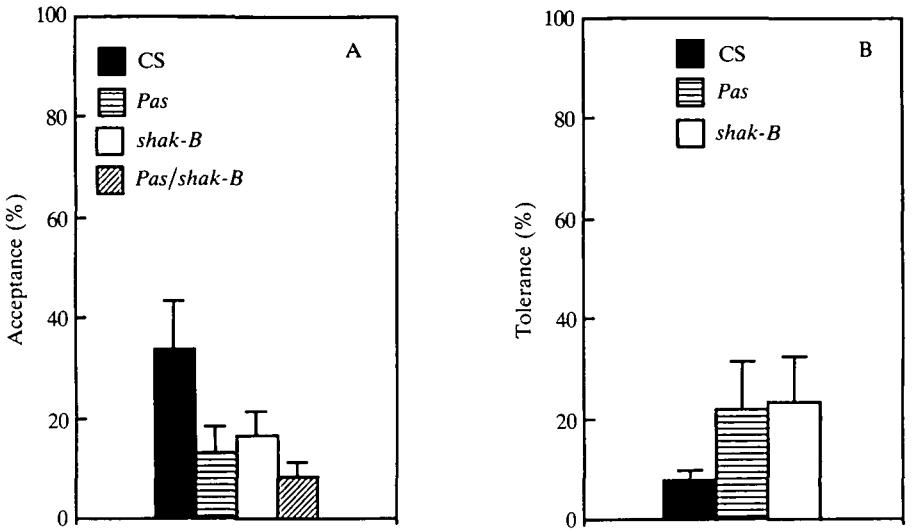

Fig. 4. Responses of *Pas* and *shak-B* strains to NaCl. (A) Acceptance of 0.1 mol l^{-1} NaCl; (B) tolerance to 0.5 mol l^{-1} NaCl. Responses were assayed in the feeding preference test. Values are the means and standard deviations of at least 10 observations.

Table 5. *Dominance testing of the* shaking-B *alleles*

Genotype	Mean response	S.D.	*N*
CS	79.3	6.2	17
Pas/Pas	48.3	9.3	21
Pas/CS	77.7	7.0	16
OR	55.3	7.5	31
shak-B/shak-B	21.2	7.9	30
shak-B/OR	52.7	10.0	6
R-9-29/+	75.4	11.7	7
EF535/+	73.9	13.5	10

The responses were assayed by the feeding preference test against 1 mmol l^{-1} sucrose.

significantly different from *EF535/+* ($P<0.01$), but not from *shak-B/+* ($P=0.4$). The reason for this discrepancy is not clear.

Interactions of shak-B *alleles with members of adjacent complementation groups*

Two complementation groups which flank *shak-B* are *runt*, a segmentation gene in 19E2, and *R-9-28*, a lethal group in 19E4 (D. H. Baird, R. J. Wyman, J. A. Davies and G. L. G. Miklos, unpublished results). We examined the effects of alleles at these loci in *trans* combination with the *shak-B* alleles.

runt fully complements the taste defect of *shak-B* ($P=0.5$) (Table 6). The lethal

Table 6. *Complementation analysis between* shak-B *alleles and their interaction with a neighbouring complementation group* R-9-28

Genotype	Mean response	S.D.	*N*
CS	79.3	6.2	17
Pas/shak-B	8.7	2.1	10
Pas/R-9-29	25.8	9.5	7
Pas/EF535	33.1	10.7	12
shak-B/R-9-29	23.8	11.5	7
shak-B/EF535	51.9	10.1	14
R-9-28/+	80.2	9.7	9
Pas/R-9-28	50.9	7.7	6
shak-B/R-9-28	32.5	11.0	10
y w runt/shak-B	73.5	6.0	12

The responses of the heterozygotes were assayed in the feeding preference test against 1 mmol l^{-1} sucrose.

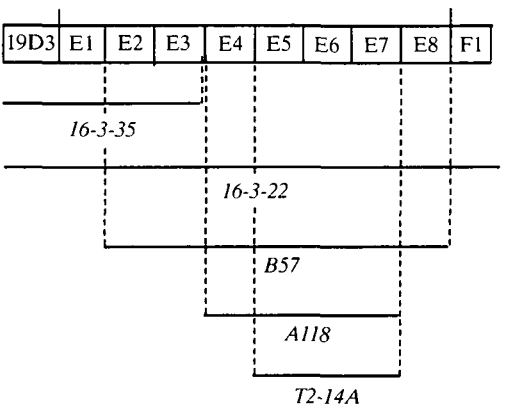

Fig. 5. Cytological map of the 19D3;F1 region of the X chromosome. The deficiency strains used to locate the gustatory lesions in *Pas* and *shak-B* are shown.

allele *R-9-28* interacted with both *Pas* and *shak-B* (Table 6). The response of *Pas/R-9-28* flies to sucrose was comparable to that of the homozygous mutant. The phenotype of *shak-B/R-9-28* was intermediate between that of *shak-B/+* and *shak-B/shak-B*. These results suggest that alleles of *shak-B* interact with *R-9-28* in specifying components of the gustatory pathway. The quantitative effects of this interaction on taste behaviour are allele-specific.

Cytological localization of the taste lesion in Pas *and* shak-B

The *shak-B* locus maps to the 19E3–4 region of the X chromosome (Miklos *et al.* 1987). We used a set of deficiencies in the 19E region (Fig. 5), to map the gustatory lesion on the salivary gland chromosome map. In each case, a

Table 7. *Cytological mapping of the gustatory phenotype in the* Pas *and* shak-B *strains*

Deficiency	+	*Pas*	*shak-B*
16-3-22	76.5±14.7	26.0±11.9	26.0±9.9
	N=6	*N*=7	*N*=7
B57	57.7±12.1	22.3±3.7	26.2±11.4
	N=6	*N*=7	*N*=10
16-3-35	87.1±10.6	10.6±8.2	15.5±3.0
	N=7	*N*=6	*N*=6
A118	84.6±11.6	56.1±14.2	24.4±7.7
	N=6	*N*=7	*N*=7
T2-14A	92.4±5.0	80.4±10.1	79.4±9.2
	N=6	*N*=6	*N*=11

The phenotypes of the relevant genotypes were assayed in the feeding preference test against 1 mmol l^{-1} sucrose.

Values indicate the mean±S.D. of the response; *N*=number of observations.

heterozygote of the deficiency chromosome with *Pas* and *shak-B* was constructed and tested for its response to 1 mmol l^{-1} sucrose in the preference feeding assay. The response obtained was compared to that of deficiency/+ flies.

Results summarized in Table 7 indicate that the gustatory lesions in *Pas* and *shak-B* are uncovered by deficiencies *16-3-22* (19D1;20A2), *B57* (19E1–2;19F1) and *16-3-35* (19D3;19E3). These deficiencies overlap in the 19E3–4 bands, to which the *jump* phenotype has also been mapped (Baird, 1986). The phenotype of *Pas/Df* is more extreme than that of the homozygous mutant strain ($P<0.01$). *Df(1)A118* (19E4–5;19E8) shows an extreme taste phenotype in combination with *shak-B* and a weak phenotype in combination with *Pas*. This deficiency does not uncover the *shak-B* locus but spans the *R-9-28* complementation group with which *shak-B* interacts for its function in the taste pathway. *Df(1)T2-14*A (19E5;19E7–8) does not uncover the taste phenotype of either *Pas* or *shak-B*.

Discussion

We have examined the gustatory properties of mutants in two different loci, *Shaker* and *shaking-B*. Their phenotypes will be discussed in the context of our working model of the gustatory pathway, which is schematically represented in Fig. 6. This simplistic model has been postulated to fit behavioural and electrophysiological data obtained from wild-type and mutant flies.

Adult *Drosophila* are strongly attracted to a variety of different sugars. Sugars are detected by the S neurone, which has been shown to possess independent molecular receptors for pyranose, furanose and trehalose (Tanimura and Shimada, 1981; Rodrigues and Siddiqi, 1981; Tanimura *et al.* 1982). Low concentrations of sodium chloride are accepted by the fly in feeding preference as well as proboscis extension assays (Arora *et al.* 1987). At these concentrations, only one

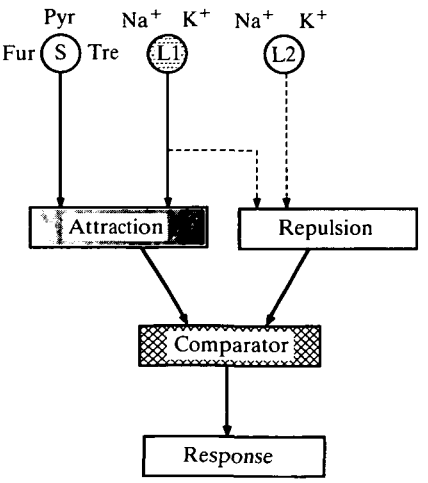

Fig. 6. Comparator model to explain taste function in *Drosophila*. The figure illustrates a simplistic model which attempts to explain gustatory behaviour. The sugar-sensitive neurone (S) has three acceptor sites for pyranose (Pyr), furanose (Fur) and trehalose (Tre). The salt-sensitive cells (L1 and L2) are responsive to Na^+ and K^+.

of the salt-sensitive neurones (L1) is active. This neurone detects NaCl at a threshold of 1 mmol l^{-1} and the response saturates at 100 mmol l^{-1}. The attraction response to this salt is in this concentration range. Since both S and L1 neurones mediate behavioural attraction, we postulate that they are 'wired' to a common centre mediating attraction responses.

At higher concentrations, NaCl as well as KCl are strongly repellent to the fly. At these concentrations, the L1 response is maximal. In addition, activity is also detected from the second salt-sensitive cell (L2). The response of this cell is, however, very erratic and is therefore unlikely to contribute solely to detection of salts at high concentration. Estimation of the concentration of stimulus can be more efficiently achieved by a comparison of activity in two neurones. We postulate wiring of L1 and L2 to a common 'repulsion' centre. Information from the attraction and repulsion centres are compared to give rise to a motor response (Dethier, 1976).

The phenotypes of the *Shaker* and *shaking-B* alleles are compared in Figs 7 and 8 with those of some gustatory mutants isolated in our laboratory (Siddiqi *et al.* 1989; V. Rodrigues, S. Sathe, L. Pinto, R. Balakrishnan and O. Siddiqi, in press). In none of these mutants do the changes in peripheral physiology correlate with the behavioural defects, suggesting that the lesions are at more central steps in the gustatory pathway. In this context, the lack of defects in firing patterns of the labellar chemosensory neurones in flies containing the three *Shaker* alleles is striking in view of their behavioural defects. This result indicates that the family of potassium channels encoded by the *Shaker* locus is not involved in tast

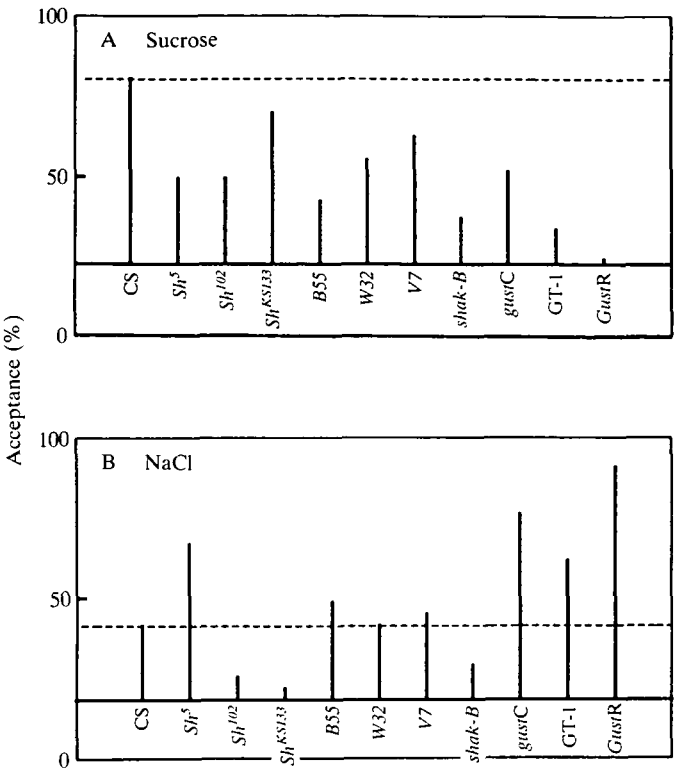

Fig. 7. Schematic representation of the acceptance response of different taste mutants to sucrose ($1\,mmol\,l^{-1}$) and NaCl ($100\,mmol\,l^{-1}$). The vertical lines indicate the percentage acceptance of the stimulus measured by the feeding preference test. The height of the lines is used to compare the responses of the different mutant strains and does not indicate the quantitative value of the response. The response of the wild-type strain (CS) is given by the dotted horizontal line.

transduction; however, these channels affect the central processing of gustatory information.

Several of the mutants, *gust*C, GT-1, *Gust*R and Sh^5, show an increased attraction to $100\,mmol\,l^{-1}$ NaCl together with an increased tolerance to high concentrations of NaCl. This suggests that attraction and repulsion information is compared and integrated, leading to the final response (Fig. 6). In addition, all these mutants also show a decrease in their attraction response to sugars with no correlating electrophysiological defects in the firing of the S neurone. If the information in the S and L1 neurones is indeed compared, this would suggest that an increased 'weight' given to the information content in L1 in the mutants occurs at the expense of that of the S cell.

The comparator model of the taste pathway is further supported by the phenotype of another taste mutant *gust*E (data not shown; Siddiqi *et al.* 1989), in

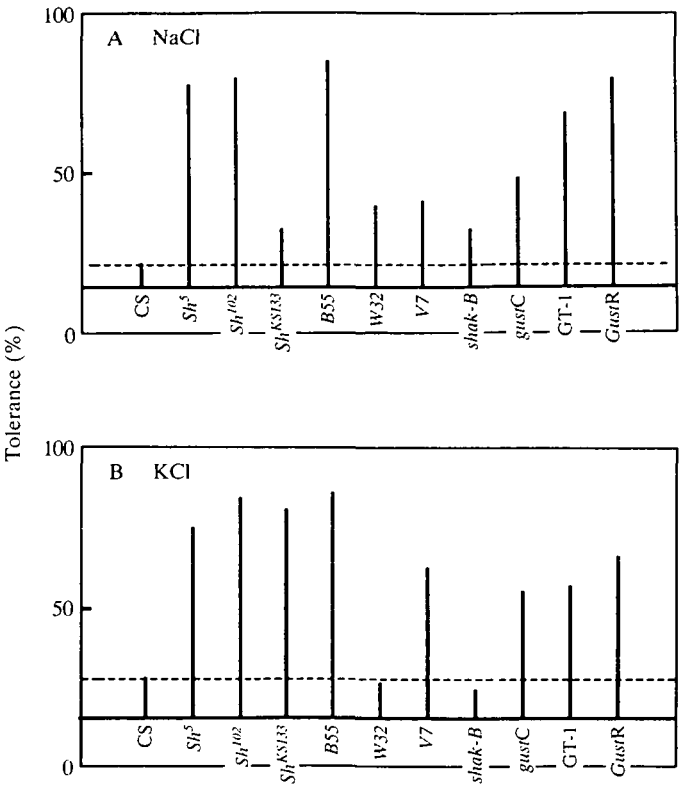

Fig. 8. Schematic representation of the tolerance response of different taste mutants to NaCl ($0.5\,mol\,l^{-1}$) and KCl ($0.2\,mol\,l^{-1}$). The percentage tolerance of each different mutant strain to salts is indicated as vertical lines. The height of the lines qualitatively represents the values obtained from the feeding preference test. The wild-type response (CS) is given by the dotted horizontal line.

which loss of the NaCl attraction response at low concentrations is coupled with an increased repulsion to higher concentrations of NaCl. The defect in this mutation has a peripheral correlate in that the L1 neurone is less sensitive to NaCl.

However, the attraction and repulsion of NaCl is not tightly linked and has been uncoupled in several mutations. Sh^{102} flies have lost the attraction response, but show a greatly increased tolerance to NaCl and KCl. In the translocation strain *B55*, the tolerance response is increased with no change in the attraction to NaCl. These phenotypes are more difficult to explain, since the *Shaker* mutations may be affecting both attraction and repulsion pathways at different points in the circuitry.

The comparator model also suggests that NaCl and KCl are detected by common receptor cells and predicts that defects in response to these salts should be linked. Most of the mutations shown in Figs 7 and 8 satisfy this prediction. Sh^{KS133} and *T(X;Y)W32* are notable exceptions to this rule. *T(X;Y)W32* causes a small increase in NaCl tolerance with no significant effect on KCl. Sh^{KS133} causes

an increased threshold of repulsion for KCl with little effect on that for NaCl. The firing patterns of the labellar taste neurones in response to both stimuli are unaltered. This suggests that the nervous system must receive information about KCl that is distinct from that for NaCl. The mechanism by which this information is conveyed needs to be examined in detail. There is behavioural evidence to suggest that these two salts are discriminated by the fly; although NaCl at low concentrations is accepted by CS flies, KCl is not attractive at any concentration.

The two viable alleles at the *shaking-B* locus, *Pas* and *shak-B*, cause an increased threshold of detection of sugars and a loss in the attraction response to NaCl. We speculate that the lesion in these strains could be in the 'attraction' centre. Genetic analysis indicates that *shak-B* interacts with an independent complementation group *R-9-28* to specify components of the gustatory pathway. This observation is preliminary and the interaction of other alleles at *R-9-28* with *shak-B* needs to be examined. The molecular nature of the *shak-B* locus is unknown. Alleles at this locus have been shown to cause a defect in the ability of the giant fibre pathway to establish synaptic connections (Wyman and Thomas, 1983). The neuronal basis of the taste defect in these mutant strains is still to be investigated. The various *Shaker* alleles cause diverse behavioural abnormalities. This locus generates a variety of different transcripts which are translated into distinct protein products (Schwartz *et al.* 1988). Mutations that affect specific *Shaker* isoforms could result in the behavioural patterns seen in flies containing the alleles at this locus. Antibodies directed against the variable and constant regions of the *Shaker* protein have provided evidence for the differential distribution of the different splice variants in different regions of the brain (Schwartz *et al.* 1990). Deletion of the *Shaker* gene affects the transient K current in only a minority of neurones (Baker and Salkoff, 1990). However, the properties of a particular cell in a circuit are determined not only by the presence or absence of a particular type of channel but also its topography on that cell.

An analysis of the effects of mutations in genes that encode channel or synaptic components on behaviour and physiology can allow dissection of circuits involved in gustation and other functions. Many such loci have been defined and thus allow such a study (Ganetzky and Wu, 1986). Analysis of suppressors and enhancers of gustatory phenotypes will also serve to define new interacting genes. Our model, therefore, serves as a starting point for unravelling processing circuits and its simplistic features will be modified as we learn more about how the fly analyses its taste inputs.

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