Genet. Res., Camb. (1986), **47**, *pp.* 117–123 *With 4 text-figures Printed in Great Britain*

The genetics of tasting in mice

IV. The acetates of raffinose, galactose and β-lactose

IAN E. LUSH

Department of Genetics and Biometry, University College London, Wolfson House, 4 Stephenson Way, London, NW1 2HE

(*Received 15 July 1985 and in revised form 2 September 1985*)

Summary

Thirty strains of mice were tested for their ability to taste a 0·4 mM solution of raffinose undecaacetate (RUA). There were large strain differences. Some strains showed little or no ability to taste the RUA. Two strains, SWR and Schneider, could taste RUA because they possess the *Soa*^{a} allele which enables them to taste a variety of acetylated monosaccharides. Three other strains, BALB/c, DBA/2 and C3H, could taste RUA because they possess the *Rua*^{a} allele which enables them to taste some larger structure which is a feature of the molecule as a whole. The gene *Rua* is tightly linked to the gene for quinine tasting. *Qui*, but the distribution of their alleles among the strains shows that they are different genes. It is suggested that there is in the mouse a cluster of tightly-linked genes, each one determining a taste receptor for a different bitter substance or chemical group. The relevance of these findings to the physiology of tasting is discussed.

1. Introduction

Previous papers in this series (Lush, 1981*a*, 1982, 1984) have shown that the sensitivity of a mouse to the bitter taste of sucrose octaacetate and of strychnine is determined by the gene *Soa*. Sensitivity to the bitter taste of quinine is determined by a different gene, *Qui*. Since there is a very large number of different substances which taste bitter to humans (and possibly also to mice) it seemed worthwhile to continue this line of work a few steps further to see if it makes possible an estimate of the total number of tasting genes which exist in the mouse. It may also shed some light on the nature of the bitterness receptors – a subject which is almost completely obscure at present.

While studying a number of acetylated sugars whose bitterness to mice is influenced by the *Soa* gene, it was noticed that some strains which are non-tasters for sucrose octaacetate showed differences in their ability to taste raffinose undecaacetate. This variation was systematically investigated and the results, which are described in this paper, show that the variation is due to a gene, *Rua*, which is closely linked to *Qui*.

2. Materials and Methods

The strains of mice are those which were used previously (Lush, 1981*a*, 1982, 1984) with the addition of STS/A and BALB/cA which came from Dr J. Hilgers, Netherlands Cancer Institute, Amsterdam. Schneider is the only strain which is not fully inbred. To supplement the seven BALB/cBy × C57BL/6By (CXB) RI strains developed by Dr Donald Bailey (Jackson Laboratory, Bar Harbor) two more have been developed in this laboratory using BALB/cGr and C57BL/Gr as the founder strains (Lush, 1981*b*). These two new RI strains are called CXBP and CXBQ and have now undergone 20 generations of inbreeding by sib-mating. The other RI strains used in this work were the C57BL/6 × DBA/2 (BXD) set developed by Dr Benjamin Taylor (Jackson Laboratory, Bar Harbor). For a general account of the use of RI strains see Green (1981). Mice were normally kept on softwood sawdust, but this was changed to beech sawdust for mice which were being tested.

The tastants α-D-galactose pentaacetate, β-D-galactose pentaacetate, sucrose octaacetate, β-lactose octaacetate and raffinose undecaacetate were all obtained from Sigma Ltd. The chemical relationship between raffinose, sucrose and β-lactose is shown below.

Raffinose
O-α-D-Galactopyranosyl-(1 → 6)-*O*-α-D-Glucopyranosyl-(1 → 2)-β-D-Fructofuranoside

Sucrose
O-α-D-Glucopyranosyl-(1 → 2)-β-D-Fructofuranoside

β-Lactose
O-β-D-Galactopyranosyl-(1 → 4)-β-D-Glucopyranose

Each substance was dissolved in a small quantity of ethanol before being diluted with distilled water to the

final concentration. The final concentration of ethanol in the experimental and the control solutions did not exceed 5·0% and was usually less than this. The technique for measuring the degree of aversion of a mouse towards a tastant was described in detail in the previous paper (Lush, 1984). Adult mice of both sexes were used.

3. Results

(i) *Raffinose undecaacetate (RUA) and sucrose octaacetate (SOA)*

All thirty strains and substrains were tested with 0·4 mM-RUA as the tastant. Two of the strains, SWR and Schneider, gave unsatisfactory results at this concentration because the mice often refused to drink either the tastant or the control liquid. However with 0·2 mM-RUA the SWR and Schneider mice behaved more consistently and they were therefore tested at this concentration. The results of the strain survey are shown in Table 1 and in Fig. 1 *A*. It is clear that some strains, including all four substrains of BALB/c, avoid drinking RUA at this concentration. Several other strains, including the three C57BL substrains, show little or no aversion to drinking the RUA. Some strains from each end of the range were tested with several other concentrations of RUA in order to produce the concentration-response curves shown in Fig. 2. All the other concentrations had to be lower than 0·4 mM because at higher concentrations the RUA tended to come out of solution.

There is an obvious similarity between the chemical structures of RUA and SOA. Before the next step could be taken it was therefore necessary to know which strains are tasters of SOA. Previous work (Lush, 1981*a*) had shown that SWR is a taster strain, but some of the other strains used in the present work had not previously been tested with SOA. The result of a survey, with 0·4 mM-SOA, of all the currently used strains is shown in Fig. 1 B. Schneider clearly resembles SWR and it is therefore a second taster strain. All the other strains are non-tasters.

Because of the chemical similarity of RUA and SOA

Table 1. *Consumption of RUA and β-lactose acetate by mice from thirty strains: each cage contained up to 4 mice*

Tastant				
RUA (0·4 mM)*			β-lactose acetate (0·3 mM)	
Strain	Cages tested	Mean tastant consumed (%)	Cages tested	Mean tastant consumed (%)
PJ	3	54	2	46
C57BL/Gr	2	51	2	48
Au	3	50	2	53
NMRI	3	50	2	48
C57BL/6By	5	48	1	56
C57BL/6Past	2	47	2	51
C57L	2	47	2	56
129/Sv	8	45	2	48
C57/BL/10	2	44	2	50
A/J	4	43	4	36
CE	3	42	2	58
129/Rr	3	41	2	47
CBA	9	41	3	39
A2G	5	40	6	38
DBA/1	5	40	2	43
SEA	6	39	7	35
STS/A	3	37	4	48
AKR	7	32	3	41
Is/Cam	6	32	2	56
TO	6	31	2	49
ST/bJ	12	29	2	51
SM	9	29	2	48
BALB/cGr	6	18	8	45
BALB/cA	6	12	4	18
Schneider	8	11	4	5
SWR	8	10	4	6
DBA/2	4	5	3	42
BALB/cBy	3	3	7	22
BALB/cPast	2	3	5	19
C3H	3	3	6	36

* The RUA concentration for SWR and Schneider was 0·2 mM.

https://doi.org/10.1017/S0016672300022941 Published online by Cambridge University Press

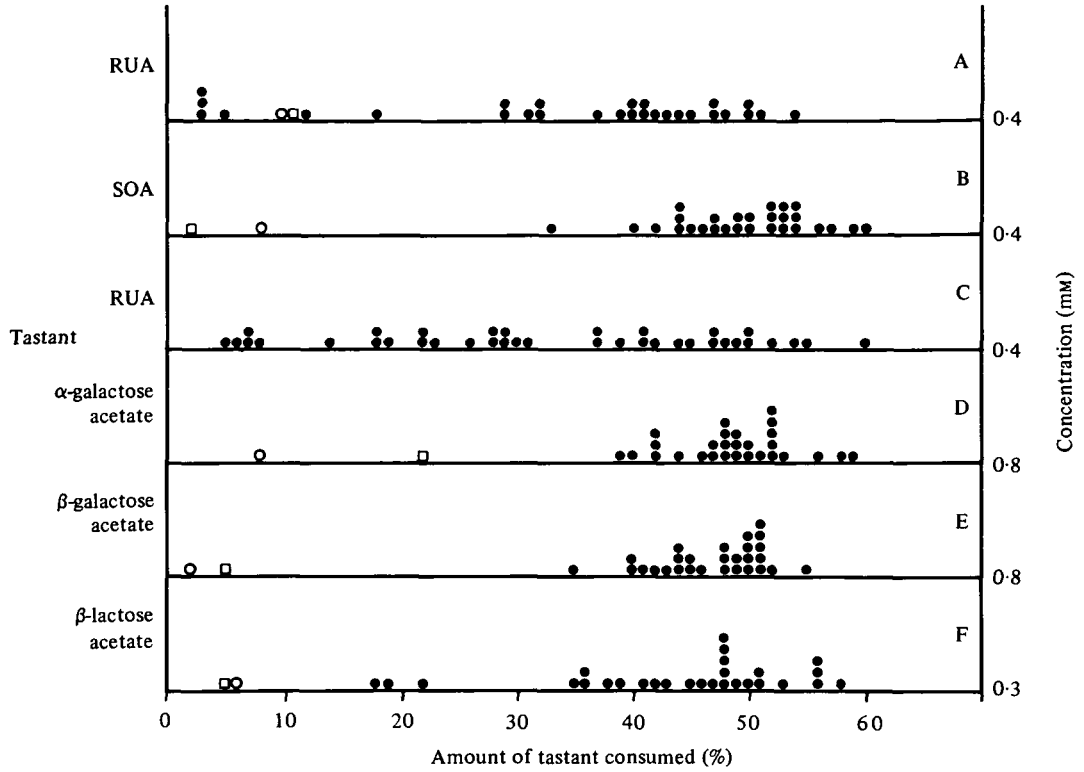

Fig. 1. Consumption of several different tastants. Each symbol is the mean value of a strain except in (C) where the symbols are the 37 individual progeny from the cross (A2G × C3H) × 129/Sv. In (A) and (F) the data are taken from Table 1 to show the range of responses by different strains to RUA and β-lactose acetate respectively. Strains SWR (○) and Schneider(□) are identified throughout by the symbols shown.

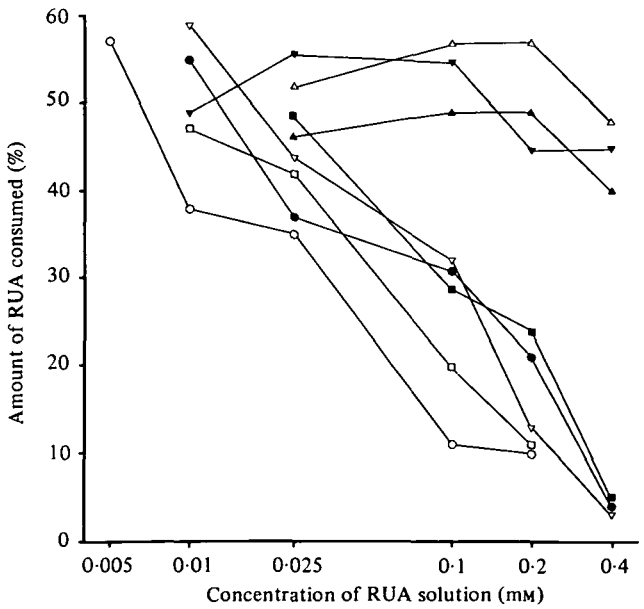

Fig. 2. Concentration-response curves of eight strains with RUA. △, C57BL/By; ▼, 129/Sv; ▲, A2G; ▽, BALB/cBy; ●, C3H; ■, DBA/2; □, Schneider; ○, SWR. Each point is the mean of between two and eight experiments.

it is possible that SWR and Schneider mice might taste RUA using the same type of taste receptor that enables them to taste SOA. This is the receptor determined by the *Soa*^{a} allele (Lush, 1981 *a*). If this were so, the ability to taste RUA and the ability to taste SOA would segregate together in the progeny of any crosses derived from these strains. To test this hypothesis, three F_1 mice from an SWR × C57BL/By cross were first tested with RUA and SOA and were found to be tasters of both substances. These mice were then backcrossed to C57BL/By and sixty-six progeny from this cross were tested with SOA and (twice) with RUA. The results are shown in Fig. 4. With respect to SOA the progeny showed a clear segregation which was not significantly different from the expected 1:1 ratio since twenty-five were tasters and forty-one were non-tasters. ($\chi^2 = 3{\cdot}41$, $P > 0{\cdot}05$). With respect to RUA the progeny do not show a clear-cut segregation into tasters and non-tasters, but there are no obvious exceptions to the hypothesis that the mice which can taste SOA can also taste RUA.

Turning now to the other three strains (BALB/c, C3H and DBA/2) which are tasters for RUA but non-tasters for SOA; clearly these strains must use some other receptor to taste RUA. To investigate the genetics of the difference between these strains and the RUA non-tasters (e.g. C57BL, 129/Sv and A2G) an A2G × C3H cross was made and ten F_1 progeny were tested with 0·4 mM-RUA. They gave a mean response of $23 \pm 5{\cdot}5\%$. This result, and its rather large standard error, seemed to indicate that a clear segregation into two classes was unlikely to be obtained in the progeny of a backcross to either a taster or a non-taster strain.

https://doi.org/10.1017/S0016672300022941 Published online by Cambridge University Press

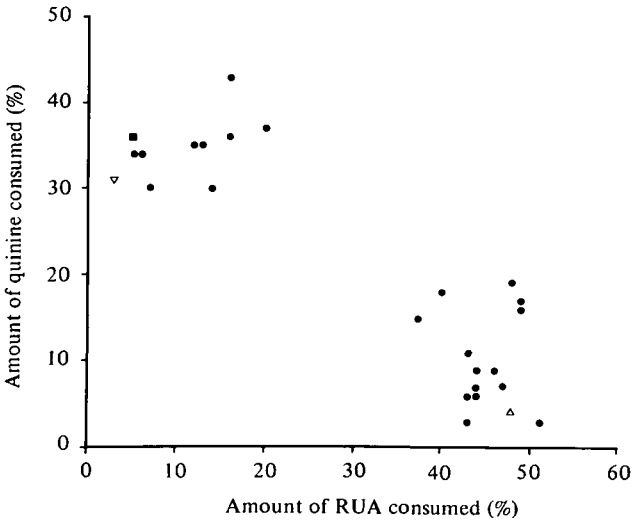

Fig. 3. Consumption of RUA (0·4 mM) and quinine (0·8 mM) by: ●, the 23 RI strains; △, C57BL/By; ■, DBA/2; ▽, BALB/c.

Nevertheless, some F_1 mice were backcrossed to the non-taster strain 129/Sv and 37 progeny were tested. The results are shown in Fig. 1*C* where it can be seen that although the progeny covered the whole range of responses they did not segregate into two classes. Thus a demonstration of a single gene with major effect could not be achieved by means of a conventional backcross.

A further attempt to demonstrate the presence of a single gene with major effect on RUA tasting was therefore made using the two sets of RI strains. C57BL is a non-taster strain and both BALB/c and DBA/2 are taster strains. If the difference between taster and non-taster is largely due to a single gene, each of the CXB and BXD RI strains should be either a taster or a non-taster, and none of them should be intermediate. Twenty-three RI strains (nine CXB and fourteen BXD) were tested with RUA and the results are shown in Table 2. Fourteen of the strains were non-tasters and nine were tasters, although the taster strains did not show quite such a high degree of aversion as was shown by BALB/c and DBA/2. Thus

Table 2. *Consumption of RUA and quinine by the CXB and BXD RI strains*

	Tastants					
	RUA (0·4 mM)			Quinine (0·8 mM)		
	Cages tested	Mean tastant consumed (% ±SEM)	SDP	Cages tested	Mean tastant consumed (% +SEM)	SDP
CXB strains						
D	3	46±0·9	B	3	9±4·7	B
E	3	51±2·0	B	2	3	B
G	7	16±6·4	C	2	43	C
H	8	20±7·2	C	2	37	C
I	4	14±9·2	C	2	30	C
J	10	47±2·4	B	2	7	B
K	3	13±8·9	C	2	35	C
P	4	44±3·5	B	4	9±0·6	B
Q	4	12±5·5	C	4	35±6·9	C
BXD strains						
2	4	42±4·5	B	4	6±2·4	B
6	4	40±4·2	B	5	18±8·5	B
9	3	7±2·2	D	8	30±6·4	D
12	3	44±11·4	B	4	7±1·6	B
16	3	43±3·8	B	4	11±5·5	B
18	3	43±2·0	B	4	3±0·8	B
19	3	5±0·3	D	4	34±10·2	D
22	3	49±3·8	B	4	16±8·2	B
27	4	48±2·6	B	8	19±5·1	B
28	4	49±2·9	B	4	17±4·7	B
29	6	16±6·3	D	4	36±4·3	D
30	3	44±5·5	B	4	6±1·0	B
31	7	37±2·9	B	5	15±4·0	B
32	5	6±0·7	D	4	34±4·6	D

The quinine data for CXB strains D to K are taken from the previous paper (Lush, 1984). The strain distribution pattern (SDP) symbols are as follows: B = like C57BL, C = like BALB/c, D = like DBA/2.

https://doi.org/10.1017/S0016672300022941 Published online by Cambridge University Press

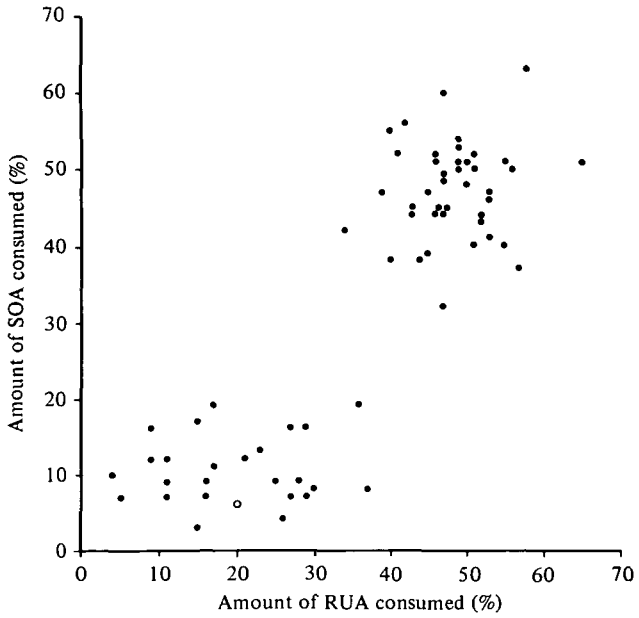

Fig. 4. Consumption of RUA (0·4 mM) and SOA (1·0 mM) by the 66 progeny of the backcross (SWR × C57BL/By) × C57BL/By. Each filled circle is an individual mouse. The open circle is the mean of the three (SWR × C57BL/By)F_1 mice.

the RI strains provided good evidence for a gene with a major effect on RUA tasting. This gene will be given the symbol *Rua*, with the allele Rua^a being present in strains BALB/c, C3H and DBA/2; and the allele Rua^b being present in C57BL.

It so happens that both sets of RI strains can also be classified with respect to their sensitivity to the taste of quinine – a characteristic which is determined by the *Qui* gene (Lush, 1984). Strains BALB/c and DBA/2 are non-tasters of quinine and C57BL is a taster strain. The results obtained by testing all the RI strains with quinine are included in Table. 2. It is immediately apparent that the strain distribution patterns (SDPs) for quinine and for RUA are completely concordant. Since C57BL is a taster for quinine and a non-taster for RUA this means that the two measurements are inversely related, as shown in Fig. 3. The complete concordance of the SDPs for RUA and for quinine is almost certainly the result of close linkage between *Qui* and *Rua*, as will be explained more fully in the Discussion.

(ii) *α-galactose acetate, β-galactose acetate and β-lactose acetate*

It seems likely that the gene *Rua* determines the sensitivity of a particular receptor in the taste cells of the tongue to RUA. Since this receptor is not sensitive to SOA, the bitter taste of RUA must be due to the presence of the α-galactose acetate part of the molecule. The bitter taste could be due solely to the attached α-galactose acetate, or it could be due to the α-galactose acetate acting synergistically with some other part of the molecule. To decide this question, all the strains were tested with 0·8 mM α-galactose acetate. The results are shown in Fig. 1 *D*. It can be seen that the only strains which taste the α-galactose acetate are SWR and Schneider. The fact that the Schneider mice drank 22% of tastant may be because this strain is not inbred and therefore some Schneider mice may not be homozygous for the Soa^a allele. The three RUA taster strains BALB/c, C3H and DBA/2 resemble all the other strains in their inability to taste α-galactose acetate. The taste of RUA for BALB/c, C3H, and DBA/2 must therefore be a function of more than just the α-galactose acetate part of the molecule.

It was thought that testing with β-lactose acetate might also help to identify those features of the RUA molecule which make it taste bitter to the strains with the Rua^a allele. For example, if β-lactose acetate were found to be bitter to these strains, then at least one would know that the presence of a terminal fructose acetate is not necessary for bitterness. All the strains were first tested with β-galactose acetate since this is the anomeric form present in β-lactose acetate. The results in Fig. 1 *E* show that SWR and Schneider are again the only two taster strains. The results of testing with β-lactose acetate are given in Table 1 and are also shown in Fig. 1 *F*. Strains SWR and Schneider are once again clearly tasters. Strains BALB/cBy, BALB/cPast and BALB/cA also show some degree of tasting ability, but the other strains with the Rua^a allele (BALB/cGr, C3H and DBA/2) are no different from the main group of non-taster strains.

4. Discussion

It is conventional wisdom in mouse genetics that before the existence of a new gene can be accepted the gene must be shown to segregate in the progeny of a cross. This normally presents no problems when working with genes which express morphological or biochemical phenotypes. However with behavioural phenotypes the measurements are inevitably less precise, and therefore the classification of individual offspring is more difficult. The results of the backcross shown in Fig 1 *C* illustrate this problem. Even though each mouse was tested twice with RUA the mean values did not clearly segregate into two classes. The use of RI lines alleviates this problem because instead of trying to distinguish between heterozygous and homozygous phenotypes it is only necessary to distinguish between the two homozygous phenotypes. The clear segregation of the twenty-three RI strains is therefore the best available evidence for the existence of the *Rua* gene.

The complete concordance between the RI strain distribution patterns of *Qui* and *Rua* must mean that the two genes are tightly linked. If the two genes were unlinked then the chance of them having the same SDP in twenty-three RI strains would be $(\frac{1}{2})^{23}$, which is less than one in eight million. It could be argued that the complete concordance between the RI strain

https://doi.org/10.1017/S0016672300022941 Published online by Cambridge University Press

distribution patterns of *Qui* and *Rua* is the result of the segregation of one gene rather than two genes. For example there could be one taste receptor which exists in two genetically-determined forms. In one form it might be sensitive to quinine but insensitive to RUA; in the other form it might be insensitive to quinine but sensitive to RUA. However if this were true one would expect to find a similar inverse relationship among inbred strains in general, and no such relationship is found. Thus strains A2G, DBA/1, SEA and A/J are all non-tasters for both RUA and quinine. It is true that BALB/c, DBA/2 and C3H, which are all tasters for RUA are also all non-tasters for quinine, but this may be a chance result due to the relatively small number of RUA taster strains among strains in general.

The idea of *Rua* and *Qui* being two closely-linked genes rather than one gene is supported by unpublished results with another bitter substance, cycloheximide. The SDP for cycloheximide tasting in the BXD RI strains is identical to that for RUA and quinine except for one strain, BXD 2, which is D instead of B. It seems that in the early stages of the formation of this strain a cross-over took place between the gene for cycloheximide tasting (*Cyx*) and the *Rua-Qui* pair of genes. Thus the picture which is beginning to emerge is of several tightly-linked genes, each one probably determining a receptor for a different bitter substance or chemical group.

What is the chemical group that makes RUA taste bitter to mice? The results now show that to put the question in this way is misleading. RUA tastes bitter to SWR and Schneider mice because they have a receptor (determined by the Soa^a allele) which is sensitive to galactose acetate, and to SOA. Thus to SWR and Schneider mice RUA is bitter because its component parts are bitter. On the other hand RUA is bitter to BALB/c, DBA/2 and C3H because of some larger structure which probably involves all the component parts of the molecule acting together. If the galactose acetate part is removed the molecule loses its bitterness. Similarly if the fructose acetate part is removed the molecule loses its bitterness, although this second statement requires some qualification. This is because β-lactose is not simply RUA minus its fructose acetate moiety. The anomeric forms of the galactose and glucose acetates, and the nature of the linkage between them, are different in β-lactose acetate. These differences may play some part in the loss of bitterness.The three BALB/c substrains which show some ability to taste β-lactose do not invalidate the above conclusions although their behaviour cannot at present be explained.

Physiologists and psychologists have been arguing for many years about the sensory and neural mechanisms of the sense of taste (Scott & Chang, 1984). One view, which has the virtue of simplicity, is that each taste sensory cell in the tongue responds only to a single type of chemical stimulus and mediates only a single type of sensation, e.g. sweet, sour, salt or bitter. The problem with this theory is that electrophysiological readings from single taste cells seem to show that they respond to more than one type of chemical stimulus, sometimes even to all four types of stimulus. Thus a single cell may respond to sucrose, HCl, NaCl and quinine. This problem has led to the promotion of an alternative theory according to which each sensory cell has only partial selectivity in its response to chemical stimuli. This means that even with a single chemical stimulus, e.g. quinine, the tongue sends to the brain a complicated pattern of nerve impulses involving many different sensory cells and neurones and the brain is able to interpret this pattern as bitterness. When the tongue is stimulated with sucrose the same neurones send to the brain a different pattern of impulses which is interpreted as sweetness. One difficulty with this theory is that it is hard to see how bitterness and sweetness and other tastes could be sensed at the same time (as they undoubtedly can) without the various patterns of neural activity interfering with each other and presenting the brain with an uninterpretable confusion of impulses.

Does the identification of the mouse tasting genes *Soa*, *Qui*, *Rua* (and *Cyx*) have any bearing on these theories? It does not provide a critical test between them but it does seem to tilt the balance of probability in favour of the first theory. SOA, RUA, quinine and cycloheximide all taste bitter to humans and we can assume that they all taste bitter to mice, at least to those mice that are able to taste them. Therefore several different kinds of receptor molecules, stimulated by different chemicals, all cause the same sensation. If all the different kinds of bitterness receptors are located together in one type of sensory cell the neurones from those cells could all project to one area of the brain and produce the sensation of bitterness. Presumably a similar arrangement could exist for sweetness. This is essentially the situation envisaged by the first of the two theories discussed above, sometimes called the 'labelled-line' theory. However the second theory, sometimes called the 'across-neurone pattern' theory, envisages the sensation of bitterness as the result of a particular pattern of activity extending across a heterogeneous population of sensory cells. In order for SOA and RUA to both produce an identical bitter taste the receptor molecules determined by the *Soa* and *Rua* genes must be identically distributed across the population of sensory cells, or at least they must produce an identical pattern of activity when stimulated by their respective tastants. The same applies to quinine and to cycloheximide and to any other bitter substances which may be detected by different receptor molecules determined by different genes. The situation is made more complicated by the fact that each sensory cell has a very short life span. In rats their average life is ten days (Beidler & Smallman, 1965; Beidler, 1984). Thus the whole population of cells is in a continual state of flux. It is

difficult to see how any stable and reproducible pattern of neural activity could be produced by such an unstable system. The rapid turnover of cells presents no problem for the labelled-line theory because the death of some sensory cells does not alter the significance of the information transmitted by the remaining sensory cells. Therefore the existence of multiple receptors for bitterness is more easily accommodated by the labelled-line theory than by the across-neurone pattern theory. Perhaps the difficulty in confirming the labelled-line theory by electrophysiological measurements on single taste cells is due to inadequate techniques rather than to erroneous theory.

I am grateful to Dr Gordon Birch for advice on chemical nomenclature and to Mrs Gail Holland for her assistance with the experiments and with the care of the mice.

References

Beidler, L. M. (1984). A personalised history of taste biophysics. In *Fundamentals of Sensory Sciences* (ed. W. W. Dawson and J. M. Enoch). Berlin: Springer-Verlag.

Beidler, L. M. & Smallman, R. L. (1965). Renewal of cells within taste buds. *Journal of Cell Biology* **27**, 263–272.

Green, E. L. (1981). *Genetics and probability in animal breeding experiments*. London: MacMillan.

Lush, I. E. (1981*a*). The genetics of tasting in mice. I. Sucrose octaacetate. *Genetical Research* **38**, 93–95.

Lush, I. E. (1981*b*). Hypothermia induced by dopamine agonists in mice: evidence for a gene with major effect. *General Pharmacology* **12**, 397–403.

Lush, I. E. (1982). The genetics of tasting in mice. II. Strychnine. *Chemical Senses* **7**, 93–98.

Lush, I. E. (1984). The genetics of tasting in mice. III. Quinine. *Genetical Research* **4**, 151–160.

Scott, T. R. & Chang, F. C. T. (1984). The state of gustatory neural coding. *Chemical Senses* **8**, 297–314.