

Single-Locus Control of Saccharin Preference in Mice

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EVIDENCE for single-locus control of behavioral differences among inbred strains of mice is accumulating; examples include proneness to audiogenic seizure³, avoidance learning⁸, various activity measures¹¹, and vocalization^{12,13}. The demonstration of single-locus control is often an important first step toward investigating the physiological bases of behavioral phenomena. Such single-locus systems are more amenable to reductive analysis than polygenic ones, since in principle a behavioral variation must be traceable back to the synthesis of different polypeptides. It must be remembered, however, that positive findings in one pair of mouse strains do not prove the universality of such control in the species as a whole, nor do they indicate that the demonstrated locus is the center of organization for the observed behavior. While behavioral phenotypes (psychophenes) involve the total organism and are, in a broad sense, polygenic, our best hope for explaining intraspecific genetic variation lies in the detection of differences attributable to gene substitution at single loci.

This paper presents evidence of the involvement of a single locus in the difference between mouse strains C57BL/6J (B) and DBA/2J (D) in their preference for dilute solutions of sodium saccharin. Saccharin is a nonnutritive sweet-tasting substance that psychologists often employ in studies of the nature of motivation. These strains were selected for the test of the single-locus hypothesis on the basis of an extensive study by Cooper⁴, who compared strains B and D and their F₁ hybrid on the following four measures related to saccharin intake: 1) acuity threshold (minimal concentration of saccharin effective in cueing avoidance of shock); 2) preference threshold (minimal concentration eliciting significant preference over water); 3) aversion threshold (minimal concentration eliciting significant preference for water); and 4) preferred concentration (the simultaneous presentation of six different concentrations of saccharin,

beginning with .096 percent [W/V] and doubling successively to 3.08 percent [W/V]).

No differences were found between strains B and D in acuity threshold or aversion threshold. The B mice had a lower threshold for preference and drank more from the higher concentrations when the six solutions were simultaneously presented. The F₁ hybrids responded like the B mice. The preference and avoidance thresholds are shown in Figure 1. Note that in comparison with the two other genotypes the area of preference for the D mice is restricted both with respect to range of concentrations (horizontal dimension) and to intensity of preference when it occurs (vertical dimension). This pattern suggests the complete dominance of the B strain's preference for saccharin. The hypothesis was further supported by the response to saccharin of both strains and their hybrid during food deprivation⁵. Pelz *et al.*⁹ also found large differences between inbred strains and feral mice in their preference for saccharin, and although they reported no data from crosses beyond the F₁, their results are compatible with the hypothesis of single-locus regulation of preference for saccharin at moderate concentrations. This paper discusses the results of an experiment in which the genetics of preference were investigated in hybrids between B and D mice.

Method

Subjects

The mice used in the experiment included 33 B, 30 D, 28 F₁ (pooled reciprocals), 62 F₂, 14 F₁B, 62 F₁D, and 2 second backcrosses to D, designated HD and LD. These latter two matings were made by separating the offspring of F₁D into a high-preference (H) and a low-preference (L) group and backcrossing each to the presumptive homozygous recessive strain. Thirty-eight offspring of HD were tested; 44 of LD.

The distribution of the phenotypes of the segregating groups were predicted under the assumption of single-locus control based on the empirically determined distribution function (f_x) of B, D, and F₁¹. The distributions were:

$$\begin{aligned} f_x F_1 B &= \frac{1}{2} f_x B + \frac{1}{2} f_x F_1 \\ f_x F_1 D &= \frac{1}{2} f_x D + \frac{1}{2} f_x F_1 = f_x HD \\ f_x F_2 &= \frac{1}{4} f_x B + \frac{1}{2} f_x F_1 + \frac{1}{4} f_x D \\ f_x LD &= f_x D. \end{aligned}$$

The mice were 8 to 12 weeks old when tested.

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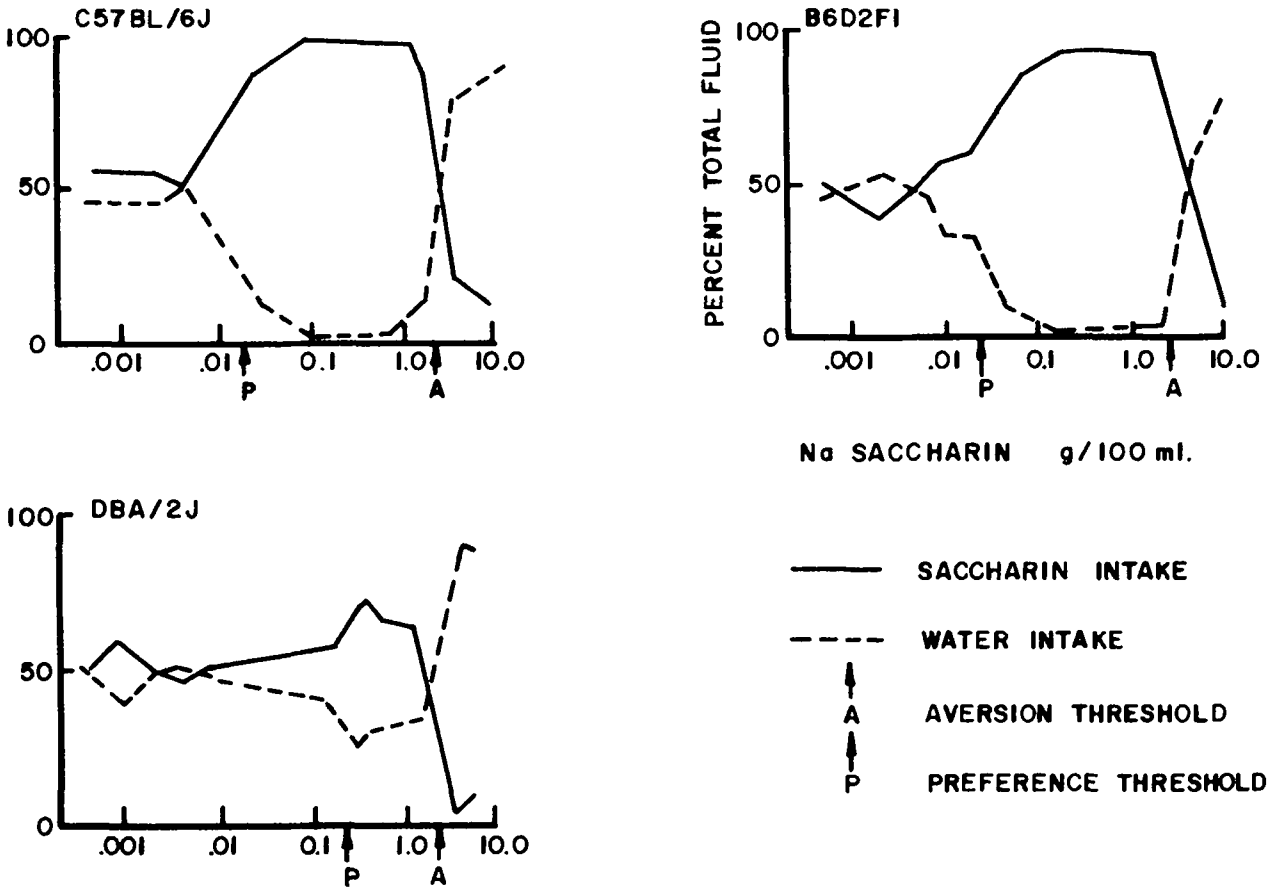


FIGURE 1—Distribution of total intake of water and various concentrations of sodium saccharin in mouse strains C57BL/6J

(strain B), DBA/2J (strain D), and their F₁ hybrid (from Cooper⁴).

Test procedure

Preference tests were conducted in solid aluminum cages 22.5 × 12.5 × 9 cm high. Two holes in the top permitted the insertion of steel drinking tubes in which the tip openings were reduced to 1.5 to 2.0 mm to reduce leakage. The test fluids were contained in graduated 25-ml cylinders that could be read to the nearest 0.1 ml. Food was present at all times.

In groups B, D, F₁, F₂, F₁B, and F₁D observations were made for 3 consecutive days. On day 1 both tubes contained water (condition W-W); on day 2 both contained 0.1 percent sodium saccharin (W/V) (condition 0.1S-0.1S); and on day 3 water was paired with 0.1 percent saccharin (condition W-0.1S). On each test day the positions of the tubes were reversed midway, and observations were taken after 23 hours of exposure to the solutions. Preference ratios were computed from the formula $S/(S + W)$, where S equals the volume of saccharin solution the mice drank and W, the volume of water.

In groups HD and LD, observations were limited to the W-0.1S condition since pilot studies indicated that mean preference ratios did not change when there had been no previous experience with saccharin. After the second day, the test was extended to 4 days with position reversal, with the objective of improving the stability of preference ratios.

Results

Data from males and females were pooled since no differences were found between the sexes. Total fluid intakes in the W-W condition were essentially the same in the six groups tested. For all except D, intake increased significantly in the 0.1S-0.1S condition. Mean increments and tests for reliability are given in Table I. Some mice are not included in Table I since data were available only for their preference tests.

Each individual with full intake data was assigned to one of two classes: 1) those increasing their total fluid intake by 1 ml or more, and 2) those not showing such a

Table I. Mean increase in fluid intake on shifting from water to 0.1 percent saccharin

Group	No.	Change (ml/day)	t	P
B	21	2.96	6.73	<.01
D	18	0.05	0.63	>.50
F ₁	16	2.41	6.05	<.01
F ₁ B	15	1.90	6.84	<.01
F ₁ D	50	1.60	4.37	<.01
F ₂	49	1.77	5.62	<.01

gain. These criteria produced the least overlap between the two inbred strains. The distribution of these two phenotypes is shown in Table II. It is clear that the groups differ significantly ($\chi^2 = 37.14$; $df = 5$; $P < .001$). The table also gives predicted values for the segregating generations based on the hypothesis that an increase of 1 ml or more is indicative of the presence of a dominant gene from B. None of the observed values deviates significantly from the predicted ones.

A similar analysis was conducted using preference ratio as the basis of phenotypic classification. An examination of the preference ratios of strains B and D indicated that the best separation between them was obtained with a cutting point of .75. Most B mice in the W-0.1S condition scored .75 or higher; most D mice scored below this level. The data from W-W and 0.1S-0.1S conditions were inspected to see how frequently pseudopreference might be demonstrated using the .75 criterion. In 338 observations there were 65 such cases (19 percent) with no indication of differences in the proportion of position preference between the groups. This value is almost certainly greater than the error rate in actual preference tests. When both cylinders contain the same fluid, spatial preferences and other disturbing factors will have a relatively greater influence on intake than in cases in which fluid preference or aversion is

involved. Nevertheless, the data indicate that in the absence of aversion for 0.1S one might expect as many as one-tenth of the D mice to "prefer" the 0.1S tube over the W tube.

Under the assumption that high saccharin preference ratios (75 percent or more) usually reflect the presence of a dominant gene of B-strain origin, there are three groups—B, F_1 , and F_1B —that should have similar psychophenes. The proportions of high preferers (phenotype H) were found to be: B, 32/33; F_1 , 25/28; and F_1B , 14/15. Since these values are not significantly different, a pooled estimate for the probability (P) of phenotype H in homo- and heterozygotes for the postulated dominant allele is .934. Similarly, the value of P for the homozygous carriers of the recessive allele was computed from the incidence of H in the D and LD groups. The inclusion of the LD subjects for this estimate assumes that no errors were made in the genotypic classification of the hybrid parents of this group. The observed proportions of high preferers were D, 5/30 and LD, 7/44, yielding an estimate for the homozygous recessive genotype of $P = .162$.

Using these estimates, the frequencies of H phenotypes in the pooled F_1D and HD groups, which did not differ significantly, were compared with values predicted from the dominant one-locus model. The results for the observed frequency of H were 45/99 and for the predicted frequency, 45.2/99. Using a similar procedure for the F_2 , the results for the observed frequency of H were 50/62 and for the predicted frequency, 45.9/62 ($\chi^2 = 1.448$, $df = 1$, $P > 0.25$).

A more detailed analysis of the predicted and observed distributions was made by comparing the total arrays of the preference ratios of the segregating generations with those computed from the dominant one-locus model¹. The values are presented in Table III. For each segregating generation the two distributions were compared by the Kolmogorov-Smirnoff test¹⁰. The values of D_{max} for the comparison of observed and expected cumulative distributions and the critical values at the .05 level (in parentheses) were: F_1D , .102 (.248); F_2 , .081 (.224); HD, .123 (.254); and LD, .174 (.272). None of the observed distributions deviates significantly from those predicted by the single-locus model with complete dominance of the B allele.

Table II. Individuals showing fluid intake increases of at least 1 ml when shifted from water to 0.1 percent saccharin

Group	Observed increase				Predicted increase*	
	1 ml or more		<1 ml		1 ml or more	<1 ml
	No.	%	No.	%		
B	18	86	3	14		
D	1	6	17	94		
F_1	13	81	3	19		
F_2	32	65	17	35	31.1	17.9
F_1B	13	87	2	13	12.6	2.4
F_1D	27	54	23	46	22.8	27.2

* Predicted on the assumption that an increase of at least 1 ml is associated with a single dominant gene from B

Table III. Cumulative frequencies of preference ratios for 0.1 percent saccharin vs. water

Group	No.	0-40	41-60	61-65	66-70	71-75	76-80	81-85	86-90	91-95	96-100
B	33	.000	.030	.030	.030	.030	.030	.130	.400	.850	1.000
D	30	.230	.500	.560	.700	.810	.880	.980	1.000	1.000	1.000
F_1	28	.000	.000	.000	.050	.090	.230	.390	.720	.910	1.000
F_1B	15	.000	.000	.000	.000	.000	.070	.360	.790	1.000	1.000
F_1D	62	(.000)	(.015)	(.015)	(.040)	(.060)	(.130)	(.260)	(.560)	(.925)	(1.000)
	(58)	.097	.306	.339	.355	.435	.567	.597	.758	.952	1.000
	(58)	(.115)	(.250)	(.280)	(.375)	(.450)	(.555)	(.685)	(.860)	(.955)	(1.000)
F_2	62	.000	.081	.129	.161	.193	.322	.387	.645	.871	1.000
	(91)	(.051)	(.133)	(.148)	(.208)	(.256)	(.338)	(.468)	(.708)	(.918)	(1.000)
HD	38	.131	.342	.368	.474	.526	.553	.658	.737	.974	1.000
	(58)	(.115)	(.250)	(.280)	(.375)	(.450)	(.555)	(.685)	(.860)	(.955)	(1.000)
LD	44	.364	.674	.704	.750	.841	.841	.886	.909	.932	1.000
	(30)	(.230)	(.500)	(.560)	(.700)	(.810)	(.880)	(.980)	(1.000)	(1.000)	(1.000)

* Predicted frequencies for segregating generations in parentheses

The observed values for HD and LD were also compared with predictions based on a two-locus additive model. Whitney and Klein¹⁴ have argued that the method described above cannot distinguish between a single-locus and an *n*-locus model in the absence of epistasis. Collins² has contested this point. Regardless, the predictions for the *second* backcrosses with a two-locus model with dominance at both loci but no epistasis do differ from the one-locus model. Assuming that the single heterozygotes are intermediate between the F₁ (double heterozygote) and the homozygous recessive strain (D), and thus have an equal probability of falling within the phenotypic range of either, one obtains the following estimates:

$$\begin{aligned} f_x \text{HD} &= (3 f_x F_1 + 5 f_x D)/8 \\ f_x \text{LD} &= (f_x F_1 + 7 f_x D)/8. \end{aligned}$$

D_{max} was considerably higher for these predictions than for the single-locus model; i.e., for HD it was .180 and for LD, .214. The two-locus model is not excluded with the size of the sample available, but it is distinctly a second choice.

Discussion

This experiment was planned to investigate the genetics of preference for saccharin in a situation involving choice. After all data had been collected, a comparison was made in F₁D between the two methods used in this study of classifying individuals as high preferers, specifically: (a) a preference ratio of .75 or more, and (b) an increase of 1 ml or more in total fluid intake when mice were shifted from W to 0.1S in a no-choice situation. Classifications were concordant in 38 instances and discordant in 12. The proportion of high preferers by method (a) was .62 and by method (b), .54. Thus, the minimum possible number of discordances would be 4. It appears that the two methods are measuring the same underlying phenotype. Since method (b) yields results closer to Mendelian expectations, it is probably the more accurate indicator of genotype and should be employed in future work in place of or in addition to method (a). Nevertheless, preference ratios are stable enough to permit evaluation of genetic hypotheses.

The evidence is strong that a single locus in the house mouse has a major effect on the threshold for saccharin preference. The strongest basis for accepting this hypothesis is the sharp distinction between the outcomes of the two second backcrosses to DBA/2J when the hybrid parents were separated according to their individual behavioral phenotypes. The designation *Sac* is proposed for this locus, with the C57BL/6J allele, *Sac*^b, and the DBA/2 allele, *Sac*^d. The gene seems to operate by affecting the incentive value of dilute saccharin solutions, leading to greater consumption in both single-tube and double-tube (choice) situations. The evidence from Cooper's⁴ study is that the detection threshold for saccharin is not impaired in strain DBA/2J, and the degree of preference is reduced even at higher concen-

trations that are clearly preferred over water (Figure 1). In addition, the enhancement of saccharin intake induced by food deprivation in strain C57BL/6J is present in strain DBA/2J, but to a significantly reduced degree⁵. It is possible that the place of gene action is central rather than peripheral, thus distinguishing this character from other taste polymorphisms in humans⁶ and in mice⁷.

Summary

The preference of mice for a 0.1 percent solution of saccharin in a situation involving free choice seems to be primarily regulated at a single locus for which the designation *Sac* is proposed. The allele present in the C57BL/6J strain of mice, *Sac*^b, is dominant over *Sac*^d, found in the DBA/2J strain and results in higher preference scores. The mechanism of preference is probably related to the incentive value of the taste of saccharin rather than to the threshold for detection.

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