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## Sucrose consumption in mice: major influence of two genetic loci affecting peripheral sensory responses

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### Abstract

Individual variability in sucrose consumption is prominent in humans and other species. To investigate the genetic contribution to this complex behavior, we conducted behavioral, electrophysiological, and genetic studies, using male progeny of two inbred mouse strains (C57BL/6ByJ [B6] and 129/J [129]) and their F<sub>2</sub> hybrids. Two loci on Chromosome (Chr) 4 were responsible for over 50% of the genetic variability in sucrose intake. These loci apparently modulated intake by altering peripheral neural responses to sucrose. One locus affected the response threshold, whereas the other affected the response magnitude. These findings suggest that the majority of difference in sucrose intake between male B6 and 129 mice is due to polymorphisms of two genes that influence receptor or peripheral nervous system activity.

### Introduction

The consumption of sucrose and other carbohydrate sweeteners requires a complex integration of peripheral sensory, central nervous system, and post-ingestive events. For example, not only does sucrose taste pleasantly sweet to humans and is highly preferred by many other species (Dobbing 1987; Bolles 1991), but it also stimulates release of endorphins (Melchoir et al. 1991; Blass and Shah 1995) and provides a rich source of calories. It is clear that sensory factors predominate in short-term tests (Pfaffmann 1959; Davis and Smith 1988), but the relative contribution of these factors to long-term sucrose intake is unknown. To test the hypothesis that peripheral sensory factors also play a major role in controlling long-term (that is, daily) consumption, we conducted behavioral, electrophysiological, and genetic studies with two strains of inbred mice (C57BL/6ByJ [B6] and 129/J [129]) that differ greatly in sucrose intake and preference (Lush 1989, Bachmanov et al. 1996a, 1996b).

We determined the chromosomal positions of genes underlying the strain differences by examining sucrose intake and gustatory neural responses to sucrose in the segregating F<sub>2</sub> (B6 × 129) hybrid generation in relation to a dense microsatellite map (Lander and Botstein

1989; Dietrich et al. 1994). We focused on Chromosome (Chr) 4 because two loci, one assigned to the proximal (*dpa*) and one assigned to the distal (*Sac*) end of this chromosome, have been implicated in sweet-taste function. The *dpa* locus, associated with the perception of the sweetness of the amino acid D-phenylalanine, was postulated based on conditioned taste aversion generalization between D-phenylalanine and sucrose in C57BL/6 and BALB/c mice and their hybrids (Ninomiya et al. 1991). The *Sac* locus, which influences preferences for saccharin and some other sweet substances, was suggested based on two-bottle preference tests in C57BL/6 and DBA/2 mice, their hybrids and recombinant-inbred strains (Fuller 1974; Phillips et al. 1994; Lush et al. 1995; Blizard and McClearn 1996).

## Materials and methods

### Animals

Adult male and female C57BL6/ByJ (B6) and 129/J (129) mice obtained from The Jackson Laboratory (Bar Harbor, Me.) were used for breeding. The F<sub>1</sub> was generated by reciprocal crosses with both strains and genders (female 129 × male B6; male 129 × female B6). Two types of the F<sub>2</sub> hybrids were produced by intercrossing the two types of reciprocal F<sub>1</sub> hybrids [(129 × B6 F<sub>1</sub> females) × (129 × B6 F<sub>1</sub> males) and (B6 × 129 F<sub>1</sub> females) × (B6 × 129 F<sub>1</sub> males)]. Mice from the parental strains were bred, raised, and tested simultaneously with the F<sub>2</sub>. Pups were weaned at 21–30 days of age and reared in same-sexed groups of four to six. Because no differences in sucrose intake were found between the hybrids from reciprocal crosses (Bachmanov et al. 1996a), the data for 171 male F<sub>2</sub> mice from both crosses were analyzed together as a single group. We studied only males, not females, in order to reduce gender-related variability and therefore increase power for the detection of quantitative trait loci.

The 21 male F<sub>2</sub> mice were selected for the electrophysiological experiments after genotyping all F<sub>2</sub> hybrids for the *D4Mit4* and *D4Mit42* markers. Mice carrying different combinations of alleles for these markers were included in the group. Because of this selection, the proportion of individuals homozygous for these markers was higher than in the overall F<sub>2</sub> population, which approximated the Mendelian ratios of 0.25 (B6/B6), 0.5 (B6/129), 0.25 (129/129). The selected group included F<sub>2</sub> mice with a broad range of sucrose intakes.

All mice were housed at 23°C on a 12:12 h light:dark cycle and had free access to water and Teklad Rodent Diet 8604. Details of the breeding protocol, the behavioral testing, and the results of biometric analyses are given in Bachmanov and colleagues (1996a).

### Behavioral testing

Taste solution intake was measured in two-bottle tests in individually caged adult mice (~60 days old). Construction of the drinking tubes and other experimental details have been previously described (Bachmanov et al. 1996a, 1996b). Mice were presented with two drinking tubes; one tube contained deionized water and the other tube contained 4% wt/vol (0.12 M) sucrose (Sigma Chemical Company, St. Louis, Mo.) for 4 days. The positions of the tubes were switched every 24 h of the 96-h test to control for side preferences. The body weight of each mouse was measured before and after the 96-h test, averaged, and used to calculate the relative intake of sucrose solution. Fluid intake for the 4-day test was averaged and expressed per 30 g of body weight (the approximate weight of an adult mouse) per day.

The 0.12 M sucrose solution tastes sweet to humans. Both B6 and 129 mice preferred this solution over water in the two-bottle test, with the B6 strain having a higher preference score

than the 129 strain. Genetic analyses of sucrose preference and intake gave similar results, and so only analyses of intake are presented here.

### Electrophysiology

In mice anesthetized with an intraperitoneal injection of sodium pentobarbital (40–50 mg/kg), the right chorda tympani nerve was dissected at the point of its entry to the bulla and placed on an electrode. Test solutions were prepared in deionized water and flowed over the anterior part of the tongue at a flow rate of 0.5 ml/s. Integrated whole nerve responses to chemical stimulation of the tongue were recorded. The magnitude of the integrated response at 15 s after stimulus onset was measured and expressed relative to the response to 0.1 M NH<sub>4</sub>Cl (Ninomiya et al. 1996).

### Genotypic and linkage analysis

Genomic DNA was purified from the mouse tails by phenol/chloroform extraction and precipitation by alcohol (Hogan et al. 1986). Microsatellite markers were amplified by the polymerase chain reaction (PCR) with primers purchased from Research Genetics Inc. (Huntsville, AL), with a protocol modified slightly from that described by Dietrich and coworkers (1992). PCR reaction mixtures included 50-ng template genomic DNA, 100 μM dNTPs, 0.5 μCi <sup>33</sup>P-α-dCTP (Amersham, Arlington Heights, Ill.), 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 1.5 mM MgCl<sub>2</sub>, 1.65 pmol of the forward, 1.65 pmol of the reserve primer, and 1 U of Taq DNA polymerase. PCR conditions were as follows: 35 cycles of 1 min at 94°C, 2 min at 55°C, and 2 min at 72°C, followed by one cycle of 7 min at 72°C. The denatured PCR products were electrophoresed on a 6% polyacrylamide, 8.3 M urea sequencing gel, and the polymorphic sequences were visualized by autoradiography. We have genotyped *D1Mit21*, *D1Mit46*, *D1Mit48*, *D1Mit14*, *D1Mit17*, *D2Mit9*, *D3Mit10*, *D3Mit103*, *D3Mit16*, *D3Mit199*, *D4Mit264*, *D4Mit97*, *D4Mit4*, *D4Mit7*, *D4Mit17*, *D4Mit58*, *D4Mit204*, *D4Mit71*, *D4Mit33*, *D4Mit42*, *D4Mit256*, *D5Mit1*, *D6Mit9*, *D6Mit25*, *D6Mit14*, *D7Mit44*, *D7Mit38*, *D7Mit7*, *D11Mit21*, *D11Mit4*, *D12Mit34*, *D16Mit3*, *D18Mit23*, and *D19Mit11* markers. Because significant linkages were found only for the markers on Chr 4, data for the other markers are not shown in the paper.

Interval mapping based on maximum likelihood estimation was conducted with MAPMAKER/QTL 1.1 software (Lander et al. 1987), which calculated chromosome positions, confidence intervals, and percentage of variance explained by quantitative trait loci. Thresholds for significant linkage were estimated for 2 degrees of freedom, assuming that both additive and dominant components were estimated in the intercross (Lander and Kruglyak 1995). The most likely loci positions were determined at LOD score maximums. Confidence intervals for the loci were estimated as LOD drops of 1.0 proximal and distal to the LOD maximums.

The Pearson correlation coefficients and the analysis of variance (ANOVA) were used to estimate relationships between the F<sub>2</sub> animals' phenotypes and their genotypes expressed as zero, one, or two B6 alleles carried at each marker. When chorda tympani responses were analyzed, one-way ANOVA for each sucrose concentration was conducted. A Bonferroni correction for multiple comparisons requires the level of statistical significance to be set for the matrix of 55 correlation coefficients (5 sucrose concentrations by 11 markers in F<sub>2</sub>) at 0.05/55, or  $p < 0.0009$ , and for 15 one-way ANOVA tests (5 sucrose concentrations by 2 markers in F<sub>2</sub> plus a pair of parental strains) at 0.05/15, or  $p < 0.0033$ , in order to provide  $p < 0.05$  protection against any false relationship in the matrices.

Differences between genotype group means were assessed by post hoc Tukey tests for unequal sample sizes (in two-way ANOVAs, analyzing effects of two marker loci

simultaneously) or by planned a priori comparisons (in one-way ANOVAs, analyzing effect of each marker locus separately). The presence of dominance was assessed by planned comparisons, when a heterozygote mean was compared with (a) each of the homozygote means and (b) an average of the two homozygote means.

## Results

### Sucrose consumption

In a standard two-bottle test with water presented in one drinking tube and 0.12 M (4%) sucrose solution in another, nondeprived adult male B6 mice ( $n = 14$ ) consumed more sucrose than did 129 mice ( $n = 13$ , Fig. 1A). Genotypes for microsatellite markers were determined by PCR in 171  $F_2$  hybrid male mice. Interval mapping with maximum likelihood estimation identified two independently segregating loci influencing sucrose intake in the  $F_2$  mice. One locus with an incompletely recessive B6 allele was mapped to *D4Mit4* on proximal Chr 4 (LOD score = 3.47). The other locus with a dominant B6 allele was mapped to *D4Mit42* on distal Chr 4 (LOD score = 5.82, Fig. 2A). From known marker positions for these loci (respectively 13.55 cM and 81.0 cM from centromere: Mouse Genome Database) and their confidence intervals ( $-5/+8$  cM and  $-3/+7$  cM respectively), the two loci identified in this study are located between 8.55 and 21.55 cM and between 78.0 and 88.0 cM from the centromere on Chr 4. When these two loci were mapped simultaneously, their combined effect accounted for 22% of the phenotypic variation in sucrose consumption. Because total genetic variance in the  $F_2$  (based on difference between phenotypic variance in the  $F_2$  and nongenetic variance calculated from phenotypic variances in the parental strains and  $F_1$ ; Bachmanov et al. 1996a) for sucrose intake was estimated as 41% of phenotypic variance, the proportion of total genetic variance explained by these two loci was 53%.

The modes of inheritance for these two loci were confirmed when the effects of genotypes at *D4Mit4* and *D4Mit42* were analyzed separately. Mice homozygous for the B6 alleles at *D4Mit4* consumed more sucrose than did mice homozygous for the 129 alleles [Fig. 1B;  $F(1,156) = 16.1$ ,  $p = 0.0001$ , planned comparison test]. Heterozygotes at this locus were closer to the 129/129 homozygotes [ $F(1,156) = 1.65$ , ns (not significant)] than to the B6/B6 homozygotes [ $F(1,156) = 11.8$ ,  $p = 0.0008$ ], although their deviation from the average of the two homozygote means did not reach significance [ $F(1,156) = 1.68$ , ns]. B6/B6 homozygotes at *D4Mit42* consumed more sucrose than did 129/129 homozygotes [Fig. 1C;  $F(1,164) = 20.8$ ,  $p = 0.00001$ ]. Sucrose intake by heterozygotes at *D4Mit42* was similar to that of B6/B6 homozygotes [Fig. 1C;  $F(1,164) = 0.09$ , ns] and exceeded both the mean of the 129/129 homozygotes [ $F(1,164) = 23.2$ ,  $p = 0.000003$ ] and an average of the two homozygote means [ $F(1,164) = 8.05$ ,  $p = 0.005$ ].

In order to estimate a possible interaction between the two loci, we analyzed the sucrose consumption of animals of nine genotypes (every combination of 129/129, 129/B6 and B6/B6 genotypes for the *D4Mit4* and *D4Mit42* markers; Fig. 3). The *D4Mit4* genotype did not affect sucrose intake if animals had no B6 alleles at *D4Mit42*, and the *D4Mit4* genotype had a bigger effect on sucrose intake if animals had one or two B6 alleles at *D4Mit42*. Similarly, the genotype for *D4Mit42* did not significantly affect sucrose intake in animals with no B6 alleles at *D4Mit4*, and it did affect it in mice with one or two B6 alleles at *D4Mit4*. Therefore, the two loci closely linked to the *D4Mit4* and *D4Mit42* markers exert nonadditive effects on sucrose intake.

### Gustatory neural response to sucrose

Responses of the whole chorda tympani gustatory nerve to stimulation of the tongue with sucrose solutions were higher and detectable at lower sucrose concentrations in B6 than in

129 mice (Fig. 1D,G). Similar recordings of chorda tympani responses in 21 selected F<sub>2</sub> mice revealed that responses to sucrose solution concentrations below 0.1 M (close to the electrophysiological threshold) correlated significantly with the number of B6 alleles at the markers on the proximal part of Chr 4, whereas responses to 0.1–1.0 M sucrose solutions correlated with the number of B6 alleles at the markers on the distal part of Chr 4 (Fig. 2B). Mice with two B6 alleles at the *D4Mit4* marker (Fig. 1E, H) responded at lower concentrations of sucrose solution than did mice with two 129 alleles, but they did not differ at higher concentrations. Conversely, the responses of the mice with one or two B6 alleles at the *D4Mit42* marker (Fig. 1F, I) did not differ from those of the mice with no B6 alleles at lower sucrose concentrations, but they were higher for 0.1–1.0 M sucrose solutions. In both correlational and ANOVA analyses, levels of significance required by Bonferroni corrections for multiple comparisons were attained: for the correlation between the *D4Mit4* genotype and the response to 1 M sucrose,  $p < 0.0009$ ; for the effect of the *D4Mit4* genotype on response to 0.01 M sucrose, and for the effect of the *D4Mit42* genotype on response to 1 M sucrose,  $p < 0.0033$ .

## Discussion

We have identified two loci on mouse Chr 4 that accounted for over 50% of the genetic variability in sucrose intake and modulated peripheral neurophysiological responses to sucrose in male F<sub>2</sub> hybrids of the B6 and 129 mouse strains. On the basis of our mapping data and other studies (Fuller 1974; Ninomiya et al. 1991; Phillips et al. 1994; Lush et al. 1995; Blizard and McClearn 1996), it is most likely that the two loci on Chr 4 correspond to the previously postulated *dpa* (proximal) and *Sac* (distal) loci. The LOD score attained by the locus on proximal Chr 4 corresponds to the level accepted for “suggestive linkage” (Lander and Kruglyak 1995). However, identification of linkage for sweet taste responses to the *dpa* locus in an independent experiment with a cross between different mouse strains (Ninomiya et al. 1991) provides additional evidence for the existence of this locus. No other obvious candidate genes that could be involved in sweet-taste responses are located near the locus on the proximal portion of Chr 4 (this region shows conserved synteny with the region p22-p32 of human Chr 9). The *Gnb1* gene encoding one of the  $\beta$ -subunits of retinal transducin has been mapped (Danciger et al. 1990) on the distal portion of Chr 4 within 2 cM from the *D4Mit42* marker. Alteration of a G protein  $\alpha$ -subunit affected sweet-taste responses (Wong et al. 1996). Thus, a polymorphism at the  $\beta$ -subunit encoded by the *Gnb1* gene also could possibly affect sweet-taste responsiveness. However, involvement of the *Gnb1* in sweet-taste responses is difficult to evaluate, because no data are available about the *Gnb1* polymorphism among mouse strains and about expression of the corresponding protein in the taste cells. The distal portion of mouse Chr 4, including *Gnb1*, is syntenic to the region p35-p36 of human Chr 1.

Our data indicate that the two loci on mouse Chr 4 influence different components of the neural response to sucrose. The proximal locus affects sensitivity (that is, response threshold), whereas the distal locus affects response magnitude at suprathreshold concentrations. It is possible that response sensitivity depends on properties of the sweet-taste receptor, whereas response magnitude depends on the intensity of the intracellular signal, with both mechanisms contributing to the behavioral response to sucrose. Interaction between the mechanisms encoded by the two loci on Chr 4 is evident from the nonadditive effects of these loci on sucrose consumption. This could be explained by an interaction between the taste receptor(s) and intracellular transduction, as well as by other mechanisms, but this remains to be tested. The non-additive effects of the two loci on sucrose consumption demonstrate that the effect of each locus depends on background genotype and therefore may vary across mouse strains.

Besides peripheral gustatory responsiveness, intake of sweeteners depends also on brain hedonic mechanisms (Gosnell and Majchrzak 1989; Melchior et al. 1991; Blass and Shah 1995; Koch et al. 1995), which may be common to that influencing consumption of alcohol and other euphoria-producing drugs (George et al. 1991). B6 mice consume large amounts of sweeteners (Lush 1989; Bachmanov et al. 1996a, 1996b), alcohol (Belknap et al. 1993; Bachmanov et al. 1996a, 1996b) and morphine (Forgie et al. 1988) compared with the other mouse strains, and genetic correlations have been found between sweetener and alcohol intake in mice (Belknap et al. 1993; Blizard and McClearn 1996; Bachmanov et al. 1996a), suggesting that these behaviors may be affected by a common mechanism. However, quantitative trait loci modulating alcohol and morphine preference of B6 mice have been localized to Chrs 1, 2, 6, 10, and 11, but not to Chr 4 (Berettini et al. 1994; Melo et al. 1996). Although these results do not exclude the existence of loci with pleiotropic effects on these behaviors, they indicate that there are independent mechanisms influencing high consumption of sucrose, alcohol, and morphine by B6 mice.

To summarize, our data demonstrate that a potentially complex behavior—48-h sucrose intake—is directly related to the electro-physiologically measured peripheral responsiveness to sucrose, which in turn is strongly influenced by two genetic loci on Chr 4.

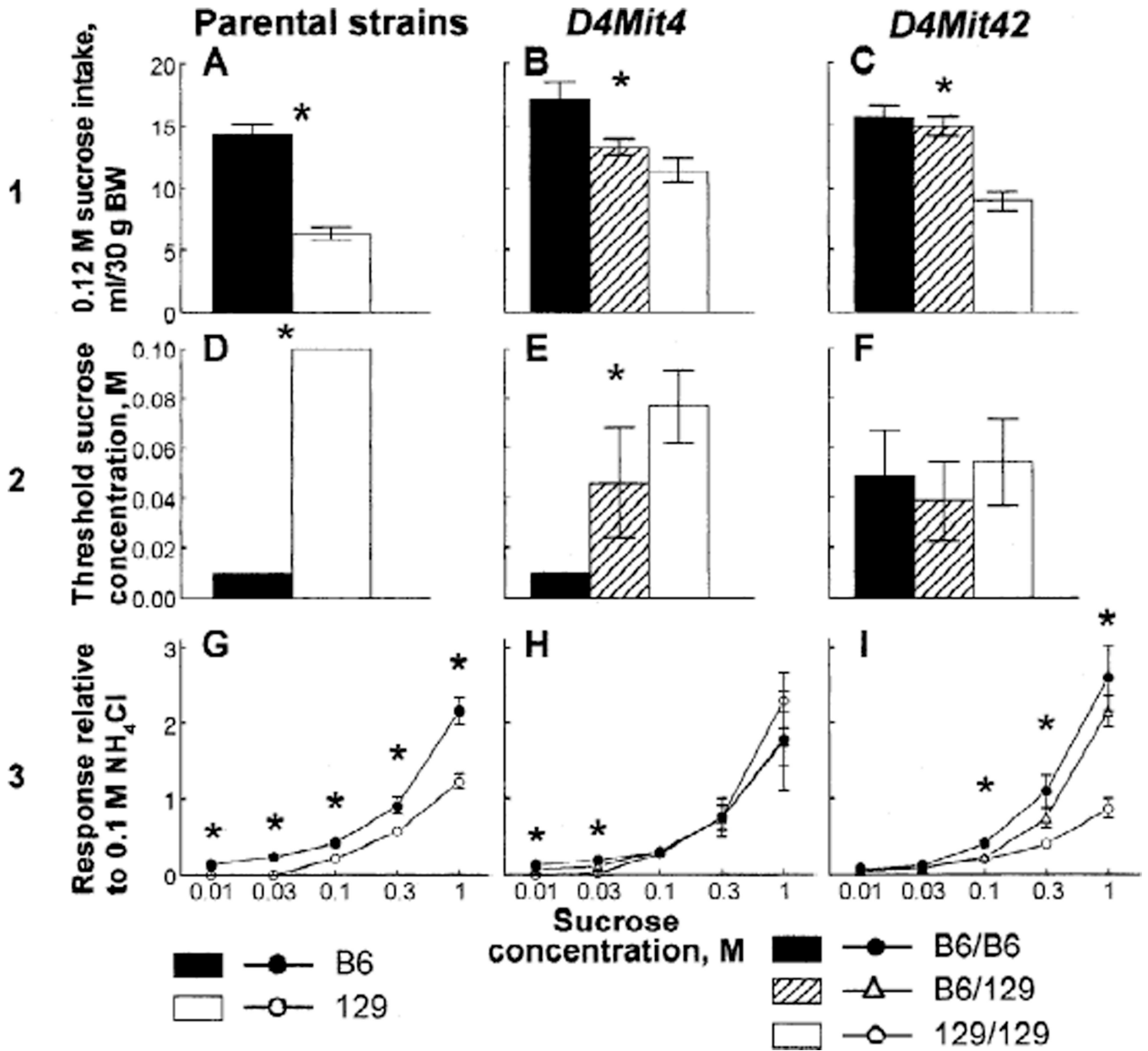
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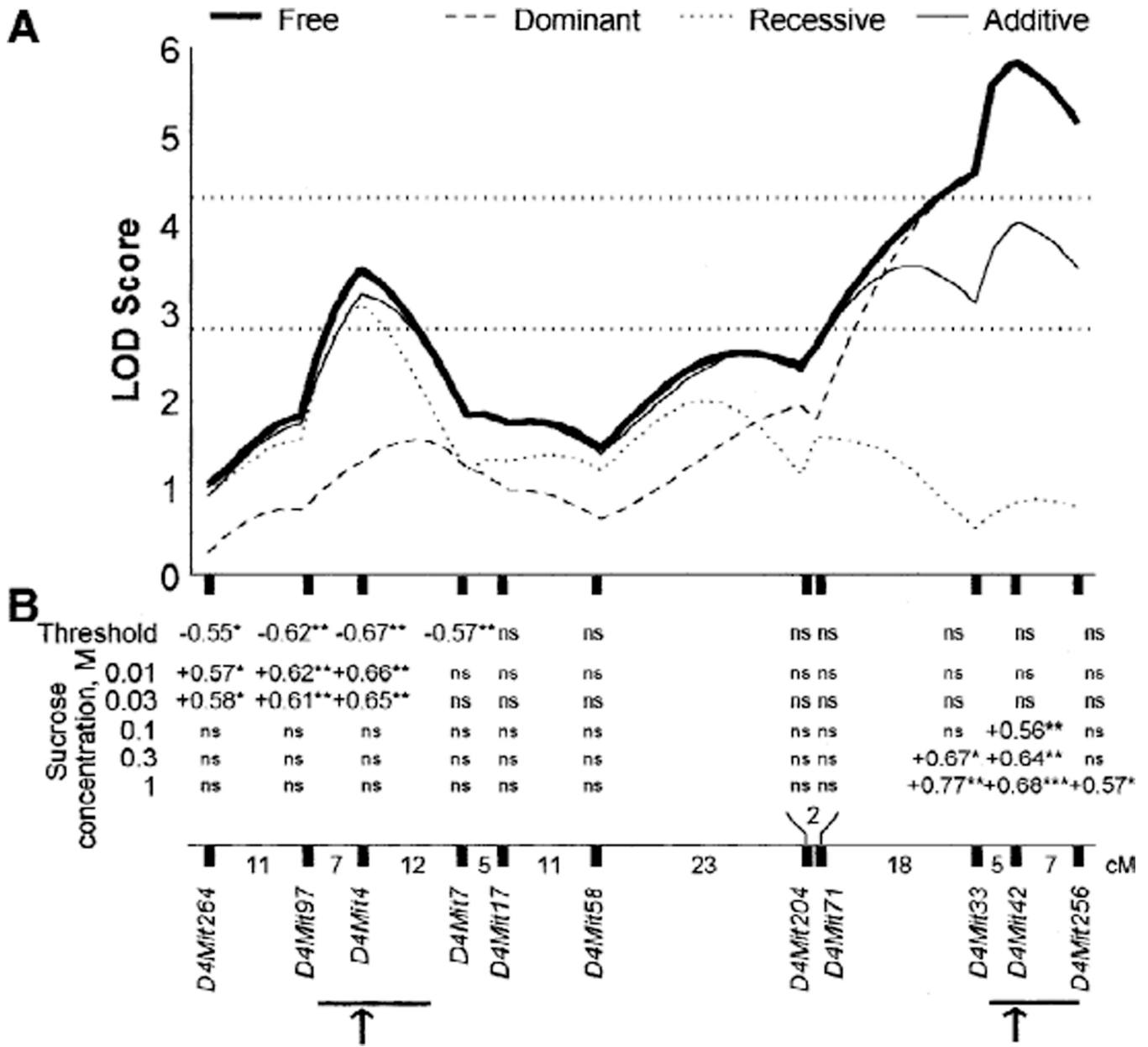
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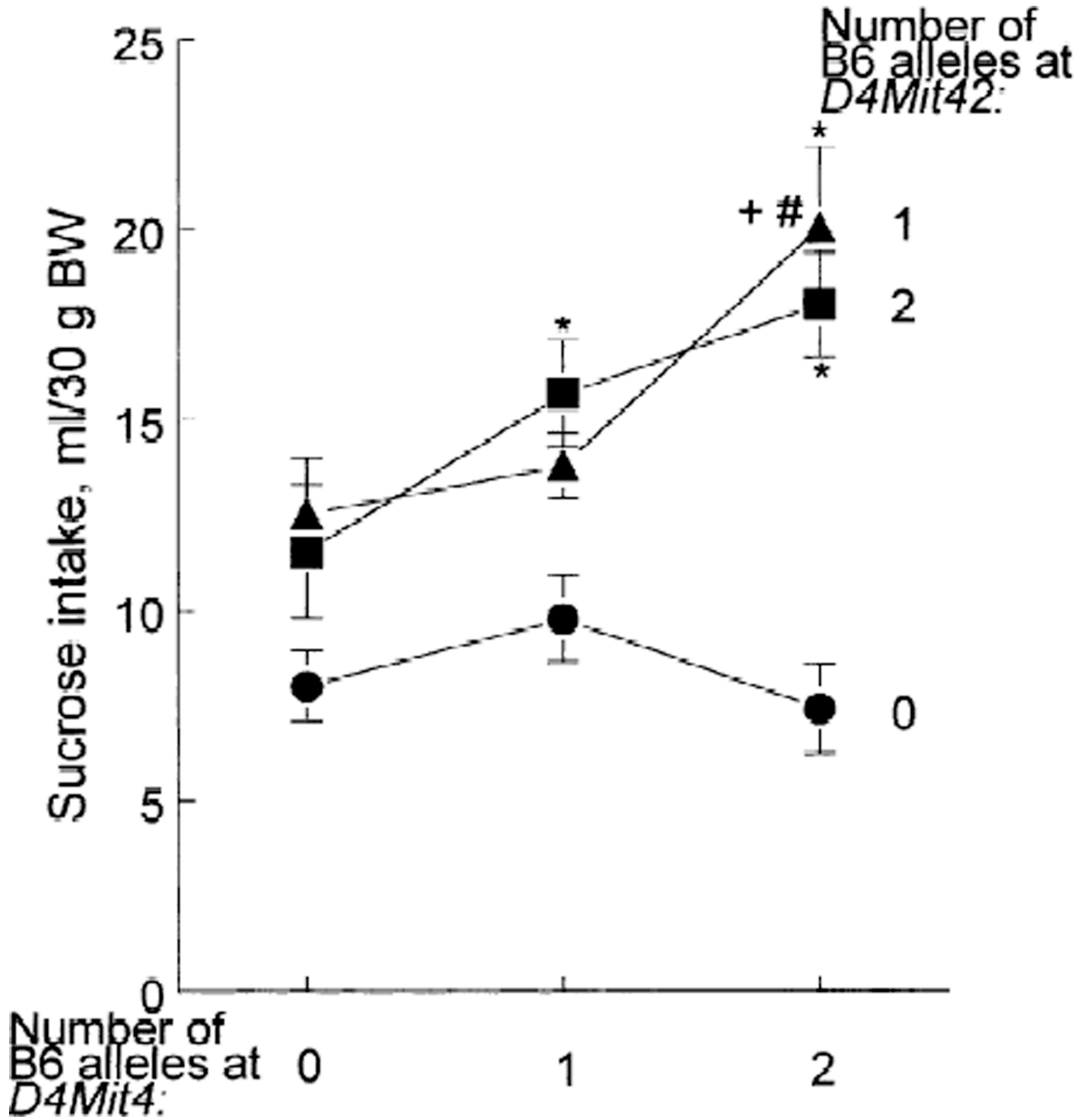
**Fig. 1.** Row 1. Average daily intake of 0.12 M (4%) sucrose solution in 96-h two-bottle tests expressed per 30 g of body weight (BW) in: (A) B6 (n = 14) and 129 (n = 13) mice; (B) their F<sub>2</sub> hybrids with different genotypes for the *D4Mit4* marker (B6/B6, n = 39; B6/129, n = 84; 129/129, n = 37); (C) their F<sub>2</sub> hybrids with different genotypes for the *D4Mit42* marker (B6/B6, n = 45; B6/129, n = 84; 129/129, n = 39). Row 2: Lowest sucrose concentration (threshold) that evoked chorda tympani response for: (D) B6 (n = 5) and 129 (n = 5) mice; (E) their F<sub>2</sub> hybrids with different genotypes for the *D4Mit4* marker (B6/B6, n = 8; B6/129, n = 4; 129/129, n = 8); (F) their F<sub>2</sub> hybrids with different genotypes for the *D4Mit42* marker (B6/B6, n = 7; B6/129, n = 7; 129/129, n = 7). Row 3: Magnitude of chorda tympani responses to different sucrose concentrations relative to 0.1 M NH<sub>4</sub>Cl in the same three groups as in the Row 2. Vertical bars represent standard errors. \*p < 0.05 for effect of genotype by ANOVA.





**Fig. 2.** (A) Chromosome 4 LOD scores for 0.12 M sucrose intake under free (unconstrained), B6 dominant, B6 recessive, and additive models. Dominance of the B6 allele is rejected for the locus on the proximal part of Chr 4 (the LOD score under the dominant model is more than 1.0 lower than that for the free model). The additive and recessive modes of inheritance are rejected for the B6 allele at the locus on the distal part of Chr 4. The dotted horizontal lines show thresholds for significant linkage (LOD = 4.3) and suggestive linkage (LOD = 2.8; Lander and Kruglyak 1995). (B) Correlations between the number of the B6 alleles (0, 1, 2) for the corresponding markers and threshold and relative magnitude of electrophysiological chorda tympani responses to different sucrose concentrations in selected F<sub>2</sub> mice (n = 13 – 21). ns = p > 0.05; \* = p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001. The microsatellite markers and map distances between them in centimorgans (cM) are shown on the X axis. At the bottom

of the figure, arrows show the loci positions at the peak of the LOD scores; the solid horizontal lines show confidence intervals for the loci (LOD Drops of 1.0).



**Fig. 3.** Average daily 0.12 M sucrose intake in 96-h two-bottle tests expressed per 30 g of body weight (BW) in F<sub>2</sub> hybrids with different genotypes for the *D4Mit4* and *D4Mit42* markers (n = 37 in each of the nine groups). Lines correspond to *D4Mit42* genotypes, with numbers of the B6 alleles shown to the right. Each marker genotype affected sucrose consumption [*D4Mit4*: F(2,151) = 4.14, p = 0.018; *D4Mit42*: F(2,151) = 14.4, p = 0.000002; two-way ANOVA]. Although interaction between the *D4Mit4* and *D4Mit42* genotypes was marginally nonsignificant when all nine genotypes were analyzed [F(4,151) = 2.13, p = 0.08], it was significant when analysis of only four homozygous genotypes (combinations of 129/129 and B6/B6 genotypes for the two markers) was conducted [F(1,32) = 5.89, p =

0.02]. The group differences significant at  $p < 0.05$  in the post hoc Tukey tests are shown as \* (compared with  $D4Mit42 = 0$  within the same  $D4Mit4$  group), + (compared with  $D4Mit4 = 0$  within the same  $D4Mit42$  group), and # (compared with  $D4Mit4 = 1$  within the same  $D4Mit42$  group). Vertical bars represent standard errors.