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4 # Genetics of mouse behavioral and peripheral neural
5 # responses to sucrose
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22 **Acknowledgments**

23 We gratefully acknowledge Maria L. Theodorides, Zakiyyah Smith, Mauricio Avigdor,
24 and Amy Colihan for assistance with animal breeding. We also acknowledge Richard Copeland
25 and the consistent high-quality assistance of the animal care staff at the Monell Chemical
26 Senses Center and thank them for their service. Yutaka Ishiwatari assisted with genotyping
27 markers.

28 **DECLARATIONS**

29 **Conflict of interest statement:** On behalf of all authors, the corresponding author states that
30 there are no conflicts of interest.

31 **Funding:** National Institutes of Health grants R01 DC00882, R03 DC03854 (AAB and GKB),
32 R01 AA11028, R03 TW007429 (AAB), R03 DC03509, R01 DC04188, R01 DK55853, R01
33 DK094759, R01 DK058797, P30 DC011735, S10 OD018125, S10 RR025607, S10 RR026752,
34 and G20 OD020296 (DRR) and institutional funds from the Monell Chemical Senses Center
35 supported this work, including genotyping and the purchase of equipment. The Center for
36 Inherited Disease Research through the auspices of the National Institutes of Health provided
37 genotyping services.

38 **Ethics approval:** All animal procedures in this study were approved by the Institutional Care
39 and Use Committee of the Monell Chemical Senses Center.

40 **Consent to participate:** Not applicable.

41 **Consent for publication:** Not applicable.

42 **Availability of data and material:**

43 **Code availability:** Not applicable.

44 **Accession IDs:**

45

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46 **Author contributions**

47 AB and DR designed the study. CL conducted the study. CL and DR analyzed data. MI conduct
48 electrophysiological experiment. XL and NB genotyped mice. CL and DR wrote the paper. AB,
49 MT and GB commented and edited the paper. All authors read the paper and approved its
50 contents.

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52 **Abstract**

53 Mice of the C57BL/6ByJ (B6) strain have higher consumption of, and stronger peripheral neural
54 responses to, sucrose solution than do mice of the 129P3/J (129) strain. To identify quantitative
55 trait loci (QTLs) responsible for this strain difference and evaluate the contribution of peripheral
56 taste responsiveness to individual differences in sucrose intake, we produced an intercross (F_2)
57 of 627 mice, measured their sucrose consumption in two-bottle choice tests, recorded the
58 electrophysiological activity of the chorda tympani nerve elicited by sucrose in a subset of F_2
59 mice, and genotyped the mice with DNA markers distributed in every mouse chromosome. We
60 confirmed a sucrose consumption QTL (*Scon2*, *or Sac*) on mouse chromosome (Chr) 4,
61 harboring the *Tas1r3* gene, which encodes the sweet taste receptor subunit T1R3 and affects
62 both behavioral and neural responses to sucrose. For sucrose consumption, we also detected
63 five new main-effect QTLs *Scon6* (Chr2), *Scon7* (Chr5), *Scon8* (Chr8), *Scon3* (Chr9) and a sex-
64 specific QTL *Scon9* (Chr15), and an interacting QTL pair *Scon4* (Chr1) *and Scon3* (Chr9). No
65 additional QTLs for the taste nerve responses to sucrose were detected besides the previously
66 known one on Chr4 (*Scon2)*. Identification of the causal genes and variants for these sucrose
67 consumption QTLs may point to novel mechanisms beyond peripheral taste sensitivity that
68 could be harnessed to control obesity and diabetes.

69 Key words: sucrose intake; sweet taste; genetics; QTL; chorda tympani gustatory nerve
70 response

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71 **Introduction**

72 Inbred mouse strains C57BL/6ByJ (B6) and 129P3/J (129) differ in sucrose consumption
73 (Bachmanov, Reed et al. 1996, Bachmanov, Tordoff et al. 1996, Bachmanov, Reed et al. 1997,
74 Bachmanov, Tordoff et al. 2001, Inoue, Reed et al. 2004, Sclafani 2004, Sclafani and Ackroff
75 2004, Inoue, Glendinning et al. 2007, de Araujo, Oliveira-Maia et al. 2008, Bachmanov, Bosak
76 et al. 2011), with heritability ranging from 36% to 78%, depending on the measure of
77 consumption used. There is a high heritability for sucrose intake but relatively low heritability for
78 sucrose preference score owing to a ceiling effect (i.e., preference scores are nearly 100% for
79 concentrated sucrose). The heritability of sucrose intake is partially determined by a genetic
80 locus (*Scon2,* or *Sac*) on distal Chr4, which corresponds to the “sweet taste” gene (*Tas1r3*)
81 coding for the T1R3 protein (Bachmanov, Li et al. 2001, Li, Staszewski et al. 2002, Reed, Li et
82 al. 2004). Allelic variation in this gene affects consumption of sucrose and the other sweeteners
83 (Inoue, Reed et al. 2004, Reed, Li et al. 2004, Inoue, Glendinning et al. 2007).

84 However, the *Scon2*/*Sac* locus explains only a part of the phenotypic variation in
85 sucrose consumption. Knocking out the *Tas1r3* gene eliminates most behavioral responses to
86 some sweeteners (Damak, Rong et al. 2003, Zhao, Zhang et al. 2003), but these mice still
87 prefer concentrated sucrose, which suggests that genetic loci other than *Scon2*/*Sac* affect
88 sucrose intake (Damak, Rong et al. 2003, Zhao, Zhang et al. 2003).

89 It is unknown whether these additional genetic loci act via peripheral taste mechanisms
90 or postoral mechanisms (for example, by influencing sucrose metabolism). To assess whether
91 genetic loci affect the peripheral taste mechanisms, investigators measure responses in the
92 chorda tympani gustatory nerve to oral stimulation with sucrose (Ninomiya, Kajiura et al. 1993,
93 Bachmanov, Reed et al. 1997, Inoue, McCaughey et al. 2001, Damak, Rong et al. 2003,
94 Danilova and Hellekant 2003).

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95 To detect novel quantitative trait loci (QTLs) that affect sucrose consumption (measured as
96 sucrose solution intakes and preference scores in two-bottle choice tests), we intercrossed the
97 B6 and 129 strains and conducted linkage analyses of the F_2 mice; neural responses to sucrose
98 were recorded in a subset of these F2 mice. To describe the genetic architecture of behavioral
99 and neural responses to sucrose, we included epistasis and sex-specific effects in our statistical
100 analysis strategy. We detected seven QTLs and named them **s**ucrose **con**sumption QTL 2-4, 6-
101 9 (symbols *Scon2-4, 6-9*), which are mapped to Chr4, 9, 1, 2, 5, 8 and 15, respectively (*Scon1*
102 and *Scon5* QTLs were detected in previous studies). We considered linkages for intakes and
103 preference scores across sucrose concentrations as the same QTL if confidence intervals for
104 different phenotypic measures overlapped and QTLs with the same effect direction. Our
105 previous studies (Bachmanov, Li et al. 2001, Inoue, Reed et al. 2004, Reed, Li et al. 2004,
106 Inoue, Glendinning et al. 2007) have shown that *Scon2*, *Sac* (saccharin preference locus) and
107 *Tas1r3* (taste receptor, type 1, member 3 gene) are identical, and so here we use *Scon2* to
108 describe linkages to the distal Chr4 (154.4-156.5 Mb) for 120 mM and 300 mM sucrose intakes
109 and preferences as well as neural responses to sucrose (100-1000 mM).

110 **Materials and methods**

111 ***Animals and breeding.*** The B6 (stock no. 001139) and 129 (stock no. 000690) inbred mice
112 were obtained from The Jackson Laboratory (Bar Harbor, ME, USA) and intercrossed to
113 produce F_1 and F_2 hybrids in the Animal Facility of Monell Chemical Senses Center, located in
114 Philadelphia, Pennsylvania (USA). Pups were weaned at 21-30 days of age and reared in
115 groups of the same sex. The mice were housed in a temperature-controlled vivarium at 23°C on
116 a 12/12-hr light-dark cycle, with lights off at 7 pm, barring unusual circumstances (e.g., power
117 outages), and had ad lib access to tap water and food (Rodent Diet 8604, Harlan Teklad,
118 Madison, WI, USA). We bred F_2 mice for two separate experiments (**S1 Table**). All animal

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119 procedures in this study were approved by the Institutional Care and Use Committee of the
120 Monell Chemical Senses Center.

121 ***Phenotyping.***

122 *Two-bottle taste preference tests.* All F_2 mice were tested for their intake and preference for
123 sucrose and other solutions using two-bottle taste preference tests. Mice from the parental B6
124 and 129 strains (20 of each strain) were also included in the two-bottle tests in Experiment 2
125 (described below).

126 Procedure details for the two-bottle taste test have been described elsewhere (Tordoff
127 MG 2001, Bachmanov, Reed et al. 2002, Bachmanov, Reed et al. 2002, Bachmanov, Reed et
128 al. 2002). In brief, individually caged mice were presented with one tube containing a taste
129 solution in deionized water and another tube containing deionized water only. Daily
130 measurements were made in the middle of the light period by reading fluid volume to the
131 nearest 0.1 ml. The positions of the tubes were switched every day to control for positional
132 preferences (some mice prefer to drink from one side regardless of the contents of the tube).
133 For 2 days before testing, mice were offered both tubes with deionized water.

134 Here, we present data for 96-hr (4-day) two-bottle tests with 3, 120, and 300 mM
135 sucrose obtained in two experiments. In addition to sucrose, mice were also tested with other
136 solutions (data are not shown). In **Experiment 1** (F_2 males only) mice were offered the following
137 taste solutions in this order: 300 and 75 mM NaCl, 120 mM sucrose, 0.1 mM citric acid, 10%
138 ethanol, and 0.03 mM quinine hydrochloride. In **Experiment 2** (B6, 129, and F_2; females and
139 males) mice were offered the following taste solutions in this order: 30 mM glycine, 30 mM D-
140 phenylalanine, 20 and 1 mM saccharin, 120, 300, 3 mM sucrose, 1 and 300 mM monosodium
141 salt of L-glutamic acid (MSG), 3% and 10% ethanol, and 0.03 mM quinine hydrochloride. We
142 purchased all taste compounds from Sigma Chemical Co. (St. Louis, MO, USA), except the
143 ethanol, which was purchased from Pharmco Products (Brookfield, CT, USA).

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144 Each concentration of a solution was presented for 4 days (Tordoff and Bachmanov
145 2002). Between tests of each taste compound, mice had water to drink from both tubes for at
146 least 2 days to reduce potential carryover effects. This procedure was also used with sucrose
147 between 300 and 3 mM (but not for other concentrations of sucrose and within other
148 compounds). No measures of water intakes were available for Experiment 2, so we used total
149 fluid intake of quinine hydrochloride (sum of water intake and quinine solution intake) as mouse
150 water intakes, because almost all of mice avoid the quinine solution, and thus the measure of
151 total fluid intake of quinine solution approximately equals water intake.

152 *Electrophysiology*. Electrophysiological experiments were conducted with a subset of 84
153 F_2 mice selected from Experiments 1 and 2 and an additional F_2 cross (Inoue, Reed et al. 2004)
154 (**S1 Table**). The activity of the whole chorda tympani nerve in response to lingual application of
155 taste solutions was recorded electrophysiologically for the following stimuli for each group of
156 mice separately: for mice from Experiment 1 (19 males), 10, 30, 100, 300, and 1000 mM
157 sucrose; for mice from Experiment 2 (58 mice: 29 males, 29 females), 3, 10, 30, 100, 300, 500,
158 and 1000 mM sucrose; for the 7 additional F_2 mice (2 males, 5 females), 500 mM sucrose. For
159 all mice, 100 mM NH_4Cl solution was presented at regular intervals to serve as a reference
160 stimulus. During chemical stimulation of the tongue, the test solutions flowed for 30 sec (Inoue,
161 Li et al. 2001, Inoue, McCaughey et al. 2001, Inoue, Reed et al. 2004). Between taste stimuli,
162 the tongue was rinsed with deionized water for at least 1 min to offset carryover effects. The
163 magnitude of the integrated response at 20 sec after stimulus onset was measured and
164 expressed as a proportion of the average of the previous and following responses to 100 mM
165 NH_4Cl (Inoue, Li et al. 2001, Inoue, McCaughey et al. 2001).

166 *Body weight and composition.* Body weights were measured before and after each taste
167 compound was offered in the two-bottle taste preference tests. Body weight did not change
168 appreciatively during the 4-day taste tests, so we averaged the before and after body weights

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169 for some analyses. In addition, we performed body composition analyses for all Experiment 2
170 mice by necropsy, weighing the epididymal and retroperitoneal adipose depots to the nearest
171 0.01 g and summing these values as a proxy for total amount of dissectible fat per mouse. At
172 necropsy we also used a ruler (±1 mm precision) to measure body length from the base of the
173 teeth to the anus.

174 ***Genotyping.*** Genomic DNA was extracted and purified from mouse tails by a sodium hydroxide
175 method or by proteinase K digestion followed by high-salt precipitation (Gentra/Qiagen,
176 Valencia, CA, USA). In total, 627 mice were genotyped with 429 polymorphic markers (see **S2**
177 **Table**), with control DNA samples from the F_1 129 and B6 inbred strains. Genotyping was
178 conducted in two steps to achieve an average distance of 5.9 Mb between markers and no gap
179 greater than 40 Mb. First, simple-sequence repeat markers known to be polymorphic between
180 the parental strains were genotyped to cover all 19 autosomes and the X chromosome (simple
181 sequence-length polymorphisms). Fluorescently labeled microsatellite primers were amplified by
182 PCR, and the products were scanned by an ABI 3100 capillary sequencer (Applied Biosystems,
183 Foster City, CA, USA). Second, single-nucleotide polymorphisms (SNPs) were added to fill gaps
184 (KBiosciences, Herts, UK). A few SNPs were also genotyped in our laboratory, using primers
185 and fluorescently labeled probes designed to discriminate alleles using an ABI Prism 7000 real-
186 time PCR system (ABI Assay-by-Design, Applied Biosystems, Foster City, CA, USA). In a few
187 cases, we typed an SNP marker and a simple sequence-length polymorphism marker that were
188 adjacent, in nearly identical physical locations, and pooled these data into a single maker for
189 linkage analysis. In addition, genotypes associated with coat and eye color were included in the
190 linkage analysis.

191 ***Data analyses.***

192 *Taste solution intake and preference.* For each concentration of taste solution, individual
193 average solution and water intakes were calculated based on daily intake values. Preference

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194 scores were calculated for each mouse as the ratio of the average daily solution intake to
195 average daily total fluid (solution + water) intake, in percent. Correlations among taste intakes
196 and preferences for all solutions were evaluated using Pearson's correlation coefficients. For
197 correlational analyses among different taste solutions, we imputed missing data using an
198 algorithm implemented in the MICE package (Multivariate Imputation by Chained Equations)
199 (Resche-Rigon and White 2018) (**S1 Figure**).

200 *Sex, genotype, and dominance effects.* We pooled the data (129, B6, and F_2) and tested
201 whether males and females differed by sex and genotype using a two-way ANOVA with sex and
202 genotype as fixed factors. We tested for dominance effects using with a *t*-test to compare the
203 mean trait values in the F_2 mice relative to the mean values from the parental strains
204 (Bachmanov, Reed et al. 1996).

205 *Preparing data for linkage analysis.* Distributions (**S2 Figure**) and sex differences and
206 normality (**S3 Table**) of the sucrose intakes and preferences of F_2 mice and mice from each
207 parental strain were analyzed using the R package *fitdistrplus* (Delignette-Muller).

208 To assess whether data need to be adjusted (standardized), we used observed
209 (unadjusted) data to evaluate the effect of covariates (habitual water intake, sex, age, body fat,
210 body length, and body weight) on sucrose intake and preference and found that habitual water
211 intake, age, and sex were bona fide covariates but that body weight, body length, and body
212 composition were not. For age, the range of age differed between Experiments 1 and 2, but the
213 ages were similar within each experiment, and therefore standardizing sucrose intake and
214 preference within each experiment eliminated the variation due to age. Thus, for the linkage
215 analyses described below, we used data that were adjusted (standardized) separately for each
216 sucrose concentration tested and for each index (intakes and preferences): for intakes, we
217 calculated standardized residuals (residual values of the sucrose intake relative to habitual

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218 water intake, standardized within sex), and for preference scores, we calculated standardized
219 scores (sucrose preference scores standardized within sex and experiment, if applicable).

220 We conducted similar analysis for the electrophysiology data. There were effects of sex
221 but no other *bona fide* covariates. Therefore, we standardized the data within sex and
222 experiment and used these adjusted (standardized) data for linkage analyses described below.

223 *Linkage analyses.* We first screened the genotype data for errors by determining
224 whether genotypes were compatible with the preexisting haplotypes; genotypes such as those
225 that created double recombinants were re-assayed.

226 Genome-wide scans of the F_2 mice for the sucrose consumption and
227 electrophysiological data were conducted using markers from 19 mouse autosomes and the X
228 chromosome. The degree of linkage between individual traits and genotypes was computed
229 using algorithms implemented by the R/QTL1.42 package of R (Broman, Wu et al. 2003).
230 Genotype probabilities and genotype errors were estimated using the *calc.genoprob* function,
231 and the missing genotypes were imputed using the *sim.geno* function. Interval mapping by
232 maximum likelihood estimation (EM algorithm) was conducted to screen for main effect QTLs
233 using the *scanone* function. The significance of each marker regression result was established
234 by comparison with 1000 permutations of the observed data using the *n.perm* function. The
235 main-effect confidence intervals were defined by a drop of 1 LOD (logarithm of the odds) from
236 peak linked marker and were calculated by applying the *lodint* function and expanded to the
237 outside markers.

238 We also assessed sex-specific effects using R/QTL to calculate LOD scores for two
239 models, “sex additive” and “sex additive with interactions” (Solberg, Baum et al. 2004), and we
240 calculated the difference in LOD scores between the genome scan results obtained by including
241 these two models separately using the *arithscan* function. To quantify sex-by-genotype
242 interactions, we compared the fit of the two models using the difference in LOD scores as a

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243 metric (ΔLOD) and using a cutoff ΔLOD 2.0 as a criterion (Lin, Theodorides et al. , Lin,
244 Theodorides et al. 2013).

245 Two-dimensional genome scans were used to estimate marker pair interactions
246 (epistasis) using the *scantwo* function. The LOD score cutoff for significant epistasis was
247 determined by a comparison with 1000 permutation tests at the $p<0.05$ level and confirmed in a
248 general linear model using marker pairs as a fixed factor and habitual water intake as a
249 covariate.

250 To assess dominant/additive interactions between the QTL alleles, the trait mean for the
251 129/B6 heterozygotes for the marker at peak linkage was compared with the collapsed trait
252 value for the 129/129 and B6/B6 homozygotes; these tests were conducted using planned
253 comparisons.

254 *Statistical models and programs.* For all statistical models, we used a type 1 (sequential)
255 sum-of-squares model, and when testing for group differences, we used Tukey's HSD tests. We
256 computed statistical tests with R (version 3.3.3) and RStudio (version 1.0.136) and graphed the
257 results using either R or Prism 6 (version 6.05; GraphPad Software, La Jolla, CA, USA).

258 **Candidate gene analyses.** We defined the QTL critical regions by 1-LOD drop from the peak
259 linked marker and expanded them to the most adjacent outside marker. We merged regions for
260 sucrose QTLs if they were on the same chromosome with overlapping confidence intervals. The
261 resulting chromosome regions were used to evaluate candidate genes, as follows: *Scon6* @
262 chr2:100122595-128989913, *Scon2* @ chr4:154415509-1558633334, *Scon7* @ chr5:22747915-
263 66662082, *Scon8* @ chr8:72545028-129008800, *Scon3* @ chr9:87885544-110762590, *Scon9*
264 @ chr15: 40369844-71152967. For the critical region of *Scon4* on Chr1 (epistatic QTL), we
265 defined the region using the peak linked marker *rs3714728* (9.0 Mb) with a 20-Mb flanking
266 region (chr1:1-19023602). Within these regions, we listed the known genetic variants and
267 evaluated them in several ways. First, we used information in the Mouse Genomes Project-

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268 Query SNPs, indels, or structural variations (Anonymous Mouse Genomes Project - Query
269 SNPs, indels or SVs. 2011: Wellcome Trust Sanger Institute) to make the list. This database
270 contains the results of a large-scale genome sequencing project of many inbred mouse strains,
271 including the 129P2/OlaHsd and C57BL/6J strains (Keane, Goodstadt et al. 2011, Yalcin, Wong
272 et al. 2011), which are closely related to the 129 and B6 parental inbred strains used in our
273 study (Yang, Wang et al. 2011). From this list, we identified sequence variants with the potential
274 to cause functional changes using the PROVEAN web server (Choi and Chan 2015). Next,
275 using the Mouse Genome Data Viewer (Anonymous), we identified all genes residing within the
276 chromosome critical regions and classified these candidate genes using the PANTHER system
277 (Mi, Muruganujan et al. 2013).

278 Using results from the two-dimensional scan, we determined if there were candidate
279 genes from the interacting QTLs *Scon3* and *Scon4* that had the same or similar biological
280 function, reasoning that if the two genes have epistatic interaction, they likely act in the same
281 pathway to regulate sucrose intake. To that end, we identified interacting gene pairs and probed
282 their protein-protein associations by searching the STRING database (von Mering, Jensen et al.
283 2005, York, Reineke et al.). We exported gene pairs for protein-protein interactions with
284 association scores > 0.9, which were computed by benchmarking them against the training set
285 (von Mering, Jensen et al. 2005), and identified those pairs that fit our criterion (confidence
286 score = 0.9).

287 Finally, for the genes (*N*=2565) within the QTL confidence intervals, we used publicly
288 available GEO data (Davis and Meltzer 2007) to compare patterns of these gene expression at
289 three tissues (striatum, brown and white fat) between the 129S1/SvImJ and C57Bl/6J inbred
290 strains, which are closely related to the 129 and B6 strains used in our study. For mouse
291 striatum, we downloaded microarray data for three available samples from each strain (129P3/J
292 and C57BL/6J; GSE7762 (Korostynski, Piechota et al. 2007)) and conducted gene expression

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293 analysis with the web tool GEO2R (http://www.ncbi.nlm.nih.gov/geo/geo2r). We calculated the
294 distribution of fluorescence intensity values for the selected samples and then performed a
295 linear model for microarray data (LIMMA) statistical test (Smyth 2005) after applying log
296 transformation to the data. For fat, we downloaded bulk RNASeq data for four available samples
297 from each strain (129S1/SvImJ and C57Bl/6J; GSE91067 (Raymond E Soccio 2017) and each
298 tissue (brown and white fat) and transformed the transcript count data. We created a matrix for
299 the two groups (129 vs B6) and fitted data with the genewise glmFit function of the edgeR
300 package (Robinson, McCarthy et al. 2010); we conducted likelihood ratio tests for 129 vs B6 for
301 brown and white fat tissue separately. The differentially expressed genes for the two types of
302 gene profiling data sets were reported as one log_2-fold change (corresponding to a twofold
303 change) with an associated p-value corrected for false discovery (FDR<0.05) for multiple tests
304 (Benjamini 1995).

305 **Results**

306 ***Phenotypes***

307 *Sucrose intake; preference in B6, 129, and F_2 mice; and sex-specific hereditary model.*
308 On average, B6 mice drank significantly more sucrose and had significantly higher preferences
309 for 3, 120, and 300 mM sucrose than did 129 mice (**Figure 1**; **S3 Figure**), with F_2 mice falling
310 between the means of the parental groups. Female mice drank more sucrose on average and
311 had a higher preference for sucrose compared with male mice of the same genotype (**Figure 1**;
312 **S3 Figure**, **S3 Table**). Significant interactions between sex and genotype were observed for
313 120 and 300 mM sucrose intake and preference but not for 3 mM sucrose (**S4 Table**). For 120
314 and 300 mM sucrose, all female mice regardless of genotype had similarly high sucrose intakes
315 and preferences, but B6 males had higher sucrose intakes and preferences than did 129 males
316 (**Figure 1**). The inheritance pattern was dominant (as measured by the significant difference

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317 between the observed mean of the F_2 mice and the theoretical midparent mean) except for 3
318 and 300 mM sucrose preference in females (**S5 Table**).

319 *Correlations between indexes of consumption of taste solutions.* In the F_2 mice, sucrose
320 intake correlated with sucrose preference score, but this relationship was weaker at the higher
321 concentrations (**S6 Table;** observed data), most likely due to a ceiling effect, with truncated
322 ranges of preference scores for concentrated sucrose (near 100%; **Figure 1**). Within sucrose
323 intakes and preferences, traits also correlated among concentrations for intakes and preference
324 scores (**S4 Figure**; imputed data).

325 *Body size and composition*. There were no significant correlations between sucrose
326 intake and body weight, body length, or body fat in F_2 mice (**S5A-F Figures**) except a weak
327 correlation between 3 mM sucrose intake and body weight in females (r=0.15, p=0.02) that did
328 not extend to the adjusted residual scores of sucrose intakes (r=0.09, p=0.19, **S5B Figure**).
329 There were similar correlations between sucrose intake and body length in males (3 mM:
330 r=0.15, p=0.02; 120 mM: r=0.16, p=0.02; 300 mM: r=0.16, p=0.02) that did not extend to the
331 adjusted residual scores of sucrose intakes (**S5E Figure**). There was no correlation between
332 sucrose preference and body weight, body fat, or body length (**S6A-F Figures**) except for a
333 weak correlation between 3 mM sucrose preference and body length in females (r=0.17,
334 p=0.01; **S6F Figure**).

335 *Habitual water intake.* There was a significant mouse strain effect on habitual water
336 intake ($F_{(1, 654)}$ = 18.9, p<0.0001, two-way ANOVA; **S4 Table**). In the F_2 mice, sucrose intakes
337 significantly correlated with habitual water intakes, but as expected, the adjusted residual scores
338 of sucrose intakes did not correlate with habitual water intake(**S5G Figure**). There was no
339 correlation between habitual water intakes and sucrose preferences, using either unadjusted or
340 adjusted scores (**S6G Figure**).

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341 *Sex*. In addition to the genotype effects, we observed significant sex and/or sex-by-
342 genotype interaction effects on sucrose intake, especially for the concentrated sucrose solutions
343 (120 and 300 mM; **S4 Table**). Significant sex effects on water intake and body weight were
344 observed (**S4 Table**).

345 *Age*. When the two-bottle tests began in Experiment 1, the F_2 mice were 66 ± 0.6 days
346 old (range, 60-90 days); for Experiment 2, the F_2 mice were 162.9 ± 1.2 days old (range, 117-
347 204 days), and the B6 and 129 mice were 120 ± 3 days old (range, 90-147 days). At the time of
348 conducting electrophysiological experiments, the F_2 mice from Experiment 1 were 81 ± 0.6 days
349 old (range, 75-105 days), and those from Experiment 2 were 279 ± 3 days old (range, 234-351
350 days). The 7 mice from the additional cross were adult (but the exact ages were not recorded).
351 At the time of performing the body composition analyses, the F_2 mice from Experiment 2 were
352 267 ± 1.5 days old. There was no significant correlation (r<0.1, p>0.05) between sucrose
353 consumption (intake and preference) and age (**S5H** and **S6H Figures**), except a weak
354 correlation between unadjusted but not adjusted 120 mM sucrose preference and age
355 (unadjusted, r=0.09, p=0.02; adjusted, r=0.01, p=0.88; **S6H Figure**).

356 **Linkages**

357 *Main-effect QTLs.* For 120 mM sucrose solution, chromosome mapping identified QTLs
358 on Chr4 (*Scon2*) and Chr9 (*Scon3*) for both intake (*Scon2*: LOD = 27.1; *Scon3*: LOD = 5.3) and
359 preference (*Scon2*: LOD = 59.2; *Scon3*: LOD = 4.1), with the B6 allele increasing the traits
360 (**Figure 2**; **S7 Table**); because these QTLs for sucrose intake and preference were detected in
361 the same chromosomal location with overlapping confidence intervals, we named them as the
362 same QTL symbols (**S7 Table).** There was a QTL on Chr5 (*Scon7*) for 120 sucrose preference
363 (LOD = 4.1), with the 129 allele increasing the phenotype (**Figure 2**; **S7 Table**). The LOD
364 scores of the peak marker, confidence intervals of these QTLs, and fractions of genetic variance
365 they explain (4-90%) are summarized in the **S7 Table**.

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366 For 300 mM sucrose solution, we identified four main effect QTLs for intakes (*Scon6,*
367 *Scon2, Scon8 and Scon3* on Chr 2, 4, 8, and 9) and *Scon3* (on Chr9) for preference (**Figure 3**;
368 **S7 Table**), with the B6 allele increasing the trait for all QTLs except for *Scon6*, for which the 129
369 allele increased the trait. The LOD scores of the peak marker, the confidence intervals of these
370 QTLs, and fractions of genetic variance they explain (4-26%) are summarized in the **S7 Table.**

371 *Sex-specific QTL.* For 300 mM sucrose intake, there was a male-specific QTL (*Scon9)*
372 on Chr15 with the peak LOD score of 4.73 at peak marker *rs4230721* (54.7 Mb), with a
373 confidence interval of 40.3-71.1 Mb (between *rs6396894* and *rs3088560*); it explained 7% of the
374 genetic variance in sucrose intake, with the B6 allele increasing the phenotype (**Figure 4**). This
375 male-specific effect was confirmed in a general linear model using habitual water intake as a
376 covariate (sex: $F_{(1,444)}$=3.59, p=0.058; genotype of *rs4230721*: $F_{(2, 444)}$=5.08, p=0.006, sex ×
377 genotype of *rs4230721*: $F_{(2,444)}$ =4.05, p=0.018).

378 *Epistasis QTL*. Only for 300 mM sucrose intake (but not for other sucrose consumption
379 measures), the two-dimensional QTL scan identified a significant interaction between markers
380 of on Chr1 (*rs3714728*, 9.0 Mb; *Scon4*) and Chr9 (*rs4227916*, 108 Mb; *Scon3*; **Figure 5A**). This
381 epistatic interaction was confirmed by a generalized linear model analysis (**Figure 5B**).

382 *QTL for the electrophysiological taste responses to sucrose.* A QTL for the peripheral
383 neural responses to 100-1000 mM sucrose solutions was mapped to 155.2-156.5 Mb on Chr4
384 (*Scon2*, corresponding to the *Sac* locus and the *Tas1r3* gene), with the B6 allele increasing the
385 phenotype (**Figure 6**). This locus explained 31-69% of the genetic variance, depending on
386 sucrose concentration (**S7 Table**). There was no effect for lower concentrations (3 and 30 mM)
387 of sucrose (**S7 Figure**).

388 **Candidate genes and their targeted variants**

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389 Within the seven critical chromosome regions (*Scon4* on chr1:1-19023602, *Scon6* on
390 chr2:100122595-128989913, *Scon2* on chr4:154415509-1558633334, *Scon7* on chr5:22747915
391 -66662082, *Scon8* on chr8:72545028-129008800, *Scon3* on chr9:87885544-110762590, and
392 *Scon9* on chr15: 40369844-71152967), there are 2565 genes (**S8 Table**), including the taste
393 receptor gene *Tas1r3* in the Chr4 QTL region, known to be associated with behavioral and
394 neural sweet taste responses to sucrose (Inoue, Reed et al. 2004, Bachmanov, Bosak et al.
395 2011). In total, 1373 missense and stop codon gain or loss variants between parental strains B6
396 and 129 (**S8 Table**) are within these genes, which include six missense variants, *rs32766606,*
397 *rs13478071, rs13459081, rs13478079, rs13478082,* and *rs13478081*, within the sweet taste
398 receptor gene *Tas1r3.* All these genes are grouped based on their molecular functions,
399 biological processes, cellular components, protein classes and pathways, and more than 30%
400 genes are categorized into catalytic activity (GO:0003824), about 30% categorized into
401 metabolic process (GO: 0008152), about 17% categorized into cellular membrane (GO:
402 0016020), about 2% categorized into membrane traffic protein (PC00150), and about 3%
403 categorized into opioid related pathways (P05915, P05916, P05917), and 3% categorized into
404 oxytocin receptor mediated signaling pathway (P04391) (**S9 Table**).

405 Using results from the two-dimensional scan, we determined if there were candidate
406 genes from the interacting regions that had the same or similar biological function, reasoning
407 that if the two genes have epistatic interaction, they likely act in the same pathway to regulate
408 sucrose intake. We identified 15 gene pairs with the association confidence scores > 0.9 and
409 that contained one gene from the *Scon4 (*on Chr1) QTL region and another from the *Scon3* (on
410 Chr9) QTL region (**S8 Figure**, **S10 Table**). The interaction of these gene pairs supported the
411 presence of an epistatic interaction effect on mouse sucrose intake.

412 Gene expression profiling of three mouse tissue sites (striatum, brown and white fat)
413 revealed differential expression of candidate genes (stratum: *n*=15; brown fat: *n*=37, white fat:

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414 *n*=171) within the QTL regions (**S11 Table**) between 129 and B6 inbred mouse strains (twofold
415 change with FDR<0.05); the top differentially expressed genes are shown in **Figure 7.**

416 **Discussion**

417 In this study, we identified a complex genetic architecture underlying sucrose intake
418 consisting of seven loci, including one sex-specific locus and an epistatically interacting locus
419 pair (*Scon3* and *Scon4*). One locus (*Scon2*) on distal Chr4 harbors the *Tas1r3* gene, which
420 codes for sweet taste receptor subunit T1R3, a major component contributing to behavioral and
421 neural responses to sucrose in mice (Inoue, Reed et al. 2004, Reed, Li et al. 2004). The other
422 loci had significant effects on sucrose consumption, but they apparently do not act at the
423 peripheral level because they did not affect chorda tympani electrophysiological taste responses
424 to sucrose. Several hormones and neuromodulators act as peripheral sweet taste modulators,
425 such as leptin (Kawai, Sugimoto et al. 2000, Nakamura, Sanematsu et al. 2008), oxytocin
426 (Sinclair, Perea-Martinez et al. 2010), glucagon-like peptide-1 (Shin, Martin et al. 2008),
427 cholecystokinin (Hajnal, Covasa et al. 2005), and the central nervous system peptides orexin
428 neuropeptide Y, and opioids (Yirmiya, Lieblich et al. 1988, Marks-Kaufman, Hamm et al. 1989,
429 Dym, Pinhas et al. 2007, Olszewski and Levine 2007), as well as the dopamine D2 receptor
430 (Bulwa, Sharlin et al. , Schneider 1989, Dym, Pinhas et al. 2009, Eny, Corey et al. 2009).
431 Furthermore, sucrose intake is controlled by mechanisms beyond the peripheral taste for caloric
432 and sweet substances (Sclafani 2001, de Araujo, Oliveira-Maia et al. 2008, de Araujo 2012); for
433 instance, fibroblast growth factor 21 mediates endocrine control of sugar intake through a
434 hormonal liver-to-brain feedback loop (von Holstein-Rathlou, BonDurant et al. 2016, Soberg,
435 Sandholt et al. 2017) independent of peripheral taste (Dushay, Toschi et al. 2015). Except for
436 *Tas1r3,* none of these genes is located in the seven sucrose QTL regions, so they cannot
437 account for their contribution to sucrose consumption.

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438 We revealed the male-specific QTL (*Scon9*) on Chr15 that affects concentrated sucrose
439 intake (**Figure 1**). Several studies in mice and other species point to sex-specific QTLs
440 (Nuzhdin, Pasyukova et al. 1997, Lionikas, Blizard et al. 2003, Jerez-Timaure, Kearney et al.
441 2004). For instance, there are male-specific QTLs for alcohol consumption (Melo, Shendure et
442 al. 1996, Peirce, Derr et al. 1998), a sex-specific effect on memory performance when rats drink
443 10% sucrose daily (Abbott, Morris et al. 2016), and a female-specific QTL (*Dpml*;
444 chr15:51141298-76910293) for dopamine loss in the neostriatum (which serves as a marker of
445 nigrostriatal dysfunction) (Sedelis, Hofele et al. 2003).

446 In the genome scan for peripheral neural taste responses to sucrose using F_2 mice, we
447 detected QTL only to distal Chr4 (*Scon2;* encompassing the sweet taste receptor gene *Tas1r3*).
448 However, but we cannot rule out linkages for the neural taste responses to sucrose to other
449 chromosomes: compared with number of mice used for sucrose consumption phenotyping, we
450 measured neural responses in a much smaller number of F_2 mice, and so there may be lack of
451 statistical power to detect QTLs with smaller effect sizes in other genome regions. We conclude
452 that *Tas1r3* is a major factor contributing to the peripheral taste responses to sucrose and that
453 other sucrose consumption loci are likely independent of peripheral sweet taste.

454 *Slc5a1* (solute carrier family 5, sodium/glucose cotransporter, member 1), harbored in
455 the *Scon7* (Chr5) QTL region, is expressed in the taste cells and may serve as a mediator of the
456 T1R-independent sweet taste of sugars (Yee, Sukumaran et al. 2011). Thus, *Slc5a1* can be
457 considered a candidate gene for *Scon7.* However, there is no significant linkage to this QTL
458 region for peripheral taste responses to sucrose, suggesting that *Slc5a1* is unlikely a peripheral
459 taste contributor to sucrose intake. Nevertheless, it is possible that we did not detect linkage of
460 the neural responses to sucrose to *Scon7* because of the lack of statistical power. We did not
461 observe any missense variants within this gene between the 129P2/OlaHsd and C57BL/6J
462 strains (closely related to the 129 and B6 parental strains used in our study), but it is possible

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463 that there are functional variants in *Slc5a1* between the C57BL/6ByJ and 129P3/J strains that
464 are absent between the 129P2/OlaHsd and C57BL/6J strains (that are not genetically identical
465 with the 129P3/J and C57BL/6ByJ strains).

466 Excess sugar consumption is a public health concern, which is indicated as a major
467 factor for such human diseases as cardiovascular disease, fatty liver disease, metabolic
468 syndrome, obesity, and diabetes (Ruff, Suchy et al. 2013). This study identified several novel
469 loci that control sucrose intake, which may point to novel pathways beyond peripheral taste
470 sensitivity that could help in understanding the genes involved in sucrose's effects in other
471 model systems (May, Vaziri et al. 2019), and we may be able to harness these novel
472 mechanisms to control obesity and diabetes and to complement similar efforts to understand the
473 genetic effects on human sucrose intake and preference (von Holstein-Rathlou, BonDurant et
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695 **Figure legends**

696 **Figure 1. Dominance effects for sucrose intake and preference by sex**

697 Data are mean and standard error for intake (left) and preference (right) of 120 mM (top) and
698 300 mM (bottom) sucrose solutions by male and female parental strains (B6, 129) and F_2 mice.
699 Values for the F_2 mice (data points with center bar) are displayed at the parent midpoint to show
700 the direction of dominance, if any. Asterisks indicate a dominant mode of inheritance (as defined
701 by a significant difference between the F_2 group average and the parental midpoint).

702 **Figure 2. Genome-wide scan of main-effect QTLs for 120 mM sucrose intake and**
703 **preference**

704 The x-axis shows chromosome numbers and chromosomal locations (in Mb). The y-axis shows
705 the logarithm of the odds (LOD) scores calculated for adjusted intakes and preferences. The
706 horizontal dashed lines represent significant linkage thresholds that correspond to an LOD of
707 3.84 for intake (black) and 3.68 for preference (red). Boxed insets show least-square means
708 (LSM) and standard errors (SE) for sucrose intakes (black frames) and preferences (red frames)
709 of the F_2 mice, grouped by genotype of the markers nearest to the linkage peak. Letters (a, b),
710 when different, indicate statistically significant differences between genotypes (the results of
711 post hoc tests in a general linear model with habitual water intake as a covariate).

712 **Figure 3. Genome-wide scan of main-effect QTLs for 300 mM sucrose intake and**
713 **preference**

714 The horizontal dashed lines represent significant linkage thresholds that correspond to an LOD
715 of 3.61 for intake (black) and 2.90 for preference (red). Other details are the same as for **Figure**
716 **2**.

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717 **Figure 4. Genome-wide scan of sex-specific QTLs for 300 mM sucrose intake**
718 **Bottom panel**, Plots of LOD scores (right y-axis) for genome scans of 300 mM sucrose with sex
719 as additive covariate (black curve; black horizontal dashed line shows significant linkage
720 thresholds that correspond to an LOD of 3.61) and sex as additive plus interactive covariate
721 (blue curve; blue horizontal dashed line shows significant linkage thresholds that correspond to
722 an LOD of 4.65). Significant linkages were detected on Chr2, 4, 8, and 9 using sex as additive
723 covariate and on Chr2, 4, 8, 9, and 15 using sex as additive and interactive covariate. The x-
724 axis shows chromosome numbers and chromosomal locations (in Mb). **Top panel,** Absolute
725 difference in LOD scores (left y-axis) for each marker. The red horizontal dashed line represents
726 the threshold for significant sex-specific linkage that corresponds to 2 ΔLOD. The boxed inset
727 shows LSM and SE for sucrose intakes of the F_2 mice grouped by sex and genotype of the
728 markers nearest the linkage peak. Other details are the same as for **Figure 2**.

729 **Figure 5. Genome-wide scan of interacting QTLs *Scon3* and *Scon4* for 300 mM sucrose**
730 **intake**
731 (A) Heat map for a two-dimensional genome scan with a two-QTL model. The maximum LOD
732 score for the full model (two QTLs plus an interaction) is indicated in the lower right triangle. The
733 maximum LOD score for the interaction model is indicated in the upper left triangle. A color-
734 coded scale displays values for the interaction model (LOD threshold = 6.2) and the full model
735 (LOD threshold = 9.1) on the left and right, respectively. A red circle in the upper left section
736 shows significant interaction (LOD = 7.01) between QTLs *Scon3* on Chr9 (108 Mb) and *Scon4*
737 on Chr1 (9 Mb). (B) LSM ± SE for sucrose intakes of the F_2 mice grouped by genotypes of the
738 markers with the highest epistatic interaction (*rs3714728* and *rs4227916*). The letters (a, b, c),
739 when different, indicate statistically significant differences between genotypes (Tukey's HSD
740 test: $p<0.05$; for details, see the Methods section).

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741 **Figure 6. Genome-wide scan of main-effect QTL for peripheral taste responses to**
742 **sucrose**
743 The horizontal dashed lines represent significant linkage thresholds that correspond to an LOD
744 of 3.96, 4.17, 4.27, and 3.95 for sucrose concentrations of 100 mM (**A**), 300 mM (**B**), 500 mM
745 (**C**), and 1000 mM (**D**), respectively. Boxed insets show means and standard errors for
746 normalized integrated chorda tympani nerve responses to oral stimulation with sucrose of the F_2
747 mice grouped by genotype of the markers nearest the linkage peak. Other details are as for
748 Figure 2.

749 **Figure 7. Gene expression profiling shows differential expression of candidate genes**
750 **(*n*=2675) within the QTL regions between 129 and B6 inbred mouse strains**
751 Microarray-based gene expression analysis data are shown for mouse striatum (**A**), and bulk
752 RNASeq analyses for brown (**B**) and white (**C**) fat. Red dots show differentially expressed genes
753 (twofold changes with FDR<0.05) between two inbred strains, but only the top 10 genes are
754 labeled in B and C. The horizontal dash lines show the corrected significance threshold
755 (FDR=0.05).

756 **S1 Figure. Imputation of missing data of behavioral phenotypes**
757 (**A**) Missing data portions of each trait for intake (Int) and preference (Pre) of 3, 120, or 300 mM
758 sucrose. (**B**) Summary of missing phenotype data pattern: red, missing data; blue, observed
759 data. (**C**) Plausible values for the imputed data points (magenta; imputed by multiple imputation
760 by chained equations) and observed data points (blue). The x-axis shows imputation numbers:
761 0 = observed data; 1-5 = data of 1-5 imputations. The y-axis shows intake (ml) or preference
762 (%).

763 **S2 Figure. Distribution of sucrose intake and preference in female and male F_2 mice**
764 The y-axis shows density, the fraction of number of animals with sucrose intake or preference

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765 (x-axis). Colors show different sucrose concentrations, and the white dashed lines indicate
766 means for each concentration. **Top row,** Unadjusted sucrose intake and preference data.
767 **Bottom row,** Sucrose intake residual scores standardized to habitual water intakes calculated
768 within each sex, and sucrose preferences standardized within sex and experiment.

769 **S3 Figure. Dominance effects for 3 mM sucrose intake and preference by sex**
770 Data are mean ± SE for sucrose solution intake (left) and preference (right) by male and female
771 parental strains (B6, 129) and F_2 mice. Values for the F_2 mice (data points with center bar) are
772 displayed at the parent midpoint to show the direction of dominance, if any. Asterisks indicate a
773 dominant mode of inheritance (as defined by a significant difference between the F_2 group
774 average and the parental midpoint).

775 **S4 Figure. Heat map of Pearson correlations for sucrose intakes and preference scores**
776 **of the F_2 mice (*N*=623)**
777 Missing and imputed data for each trait are summarized in **S1 Figure**. For correlation analyses
778 shown here, we used the first imputation (imputation number 1 in S1 Figure).

779 **S5 Figure. Correlations between sucrose intake (ml/d) and phenotypes of F_2 mice**
780 Sucrose intake data are shown for unadjusted data (blue) and adjusted residual scores (red).
781 Phenotypes were sex (M, F), total body weight (BW; g), total body fat (g), body length (cm),
782 daily habitual water intake (Wat; ml/d), and age (months). N = 452 for 3 and 300 mM sucrose; N
783 = 623 for 120 mM sucrose.

784 **S6 Figure. Correlations between sucrose preference (%) and phenotypes of the F_2 mice.**
785 Sucrose preference data are shown for unadjusted (blue) and adjusted standardization scores
786 (red). For details, see **S5 Figure**.

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787 **S7 Figure. Chorda tympani responses to sucrose (relative to 100 mM NH_4Cl) in F_2 mice**

788 Data are standardized within sex and experiments, grouped by *Tas1r3* genotype. Values are

789 means ± standard errors. *p<0.01, one-way ANOVA.

790 **S8 Figure. Protein-protein associations for genes within the sucrose epistasis QTL**

791 ***Scon3* and *Scon4* confidence interval regions**

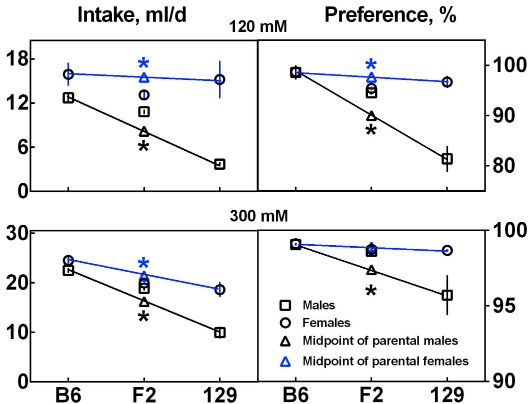
792 Protein-protein associations for genes within QTL confidence intervals of *Scon4* (on Chr1)

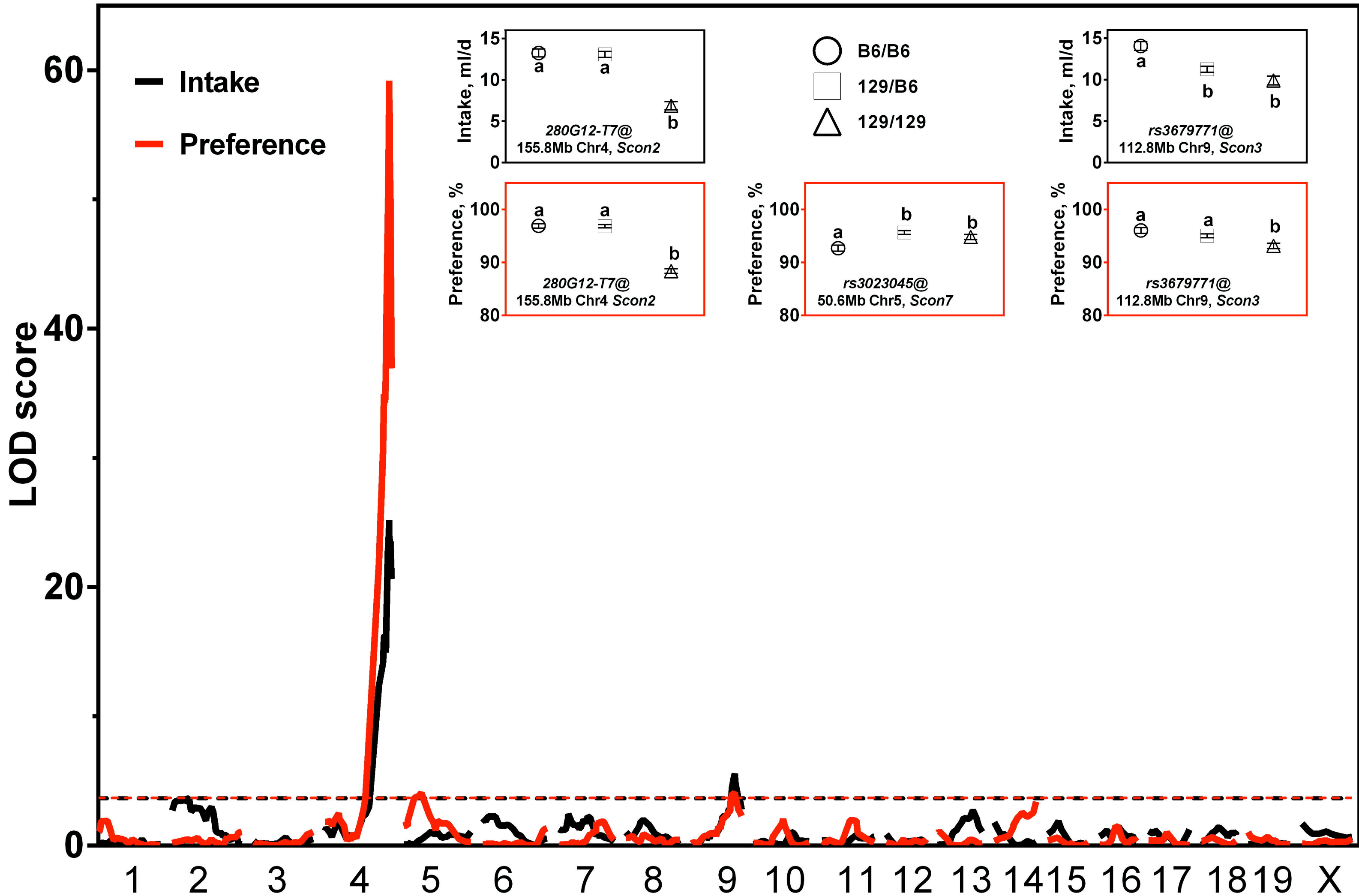
793 *Scon3* (on Chr9) (see **S11 Table**) identified by searching protein names using information from

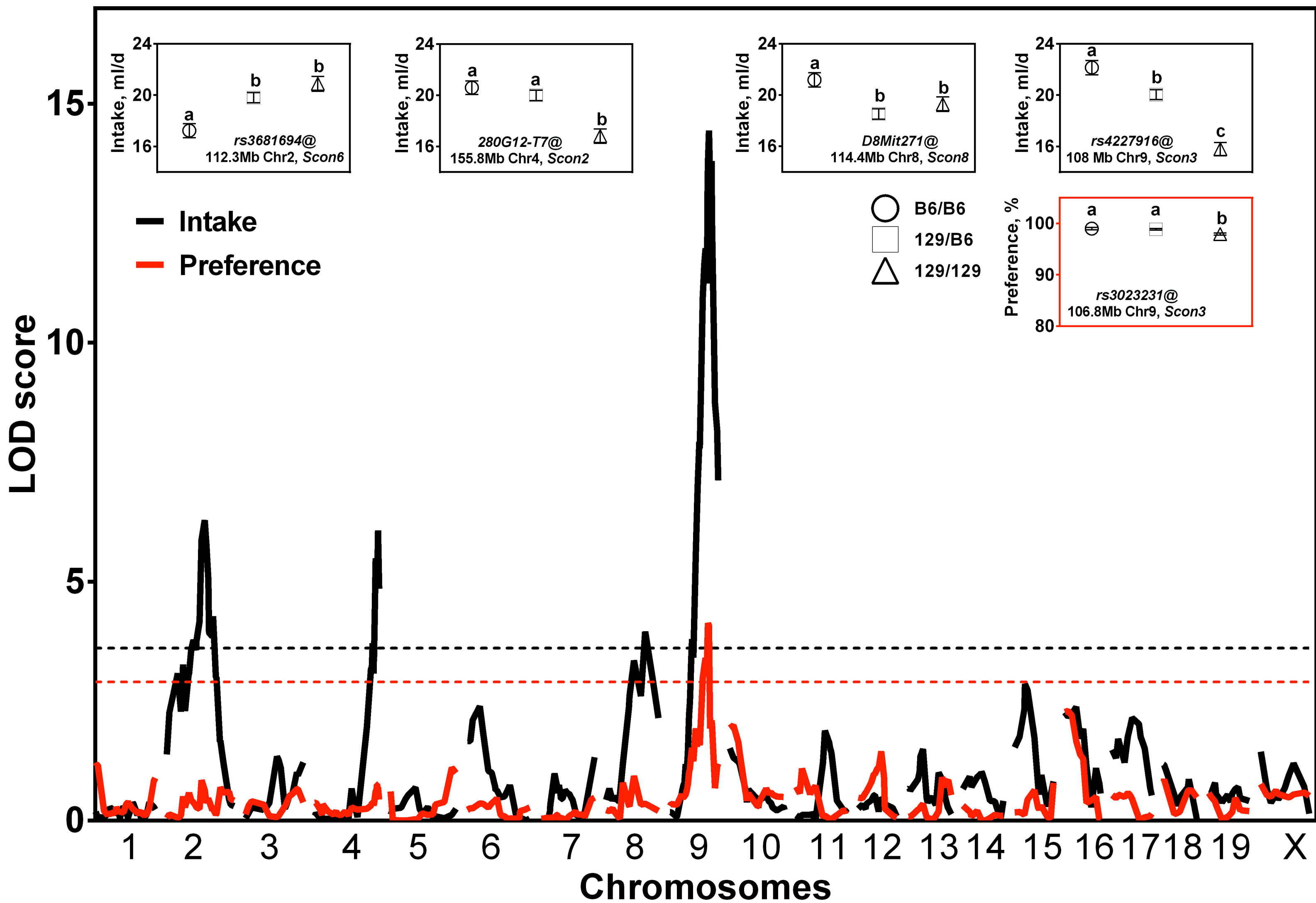
794 high-throughput experimental data, mining of literature databases, and predictions based on

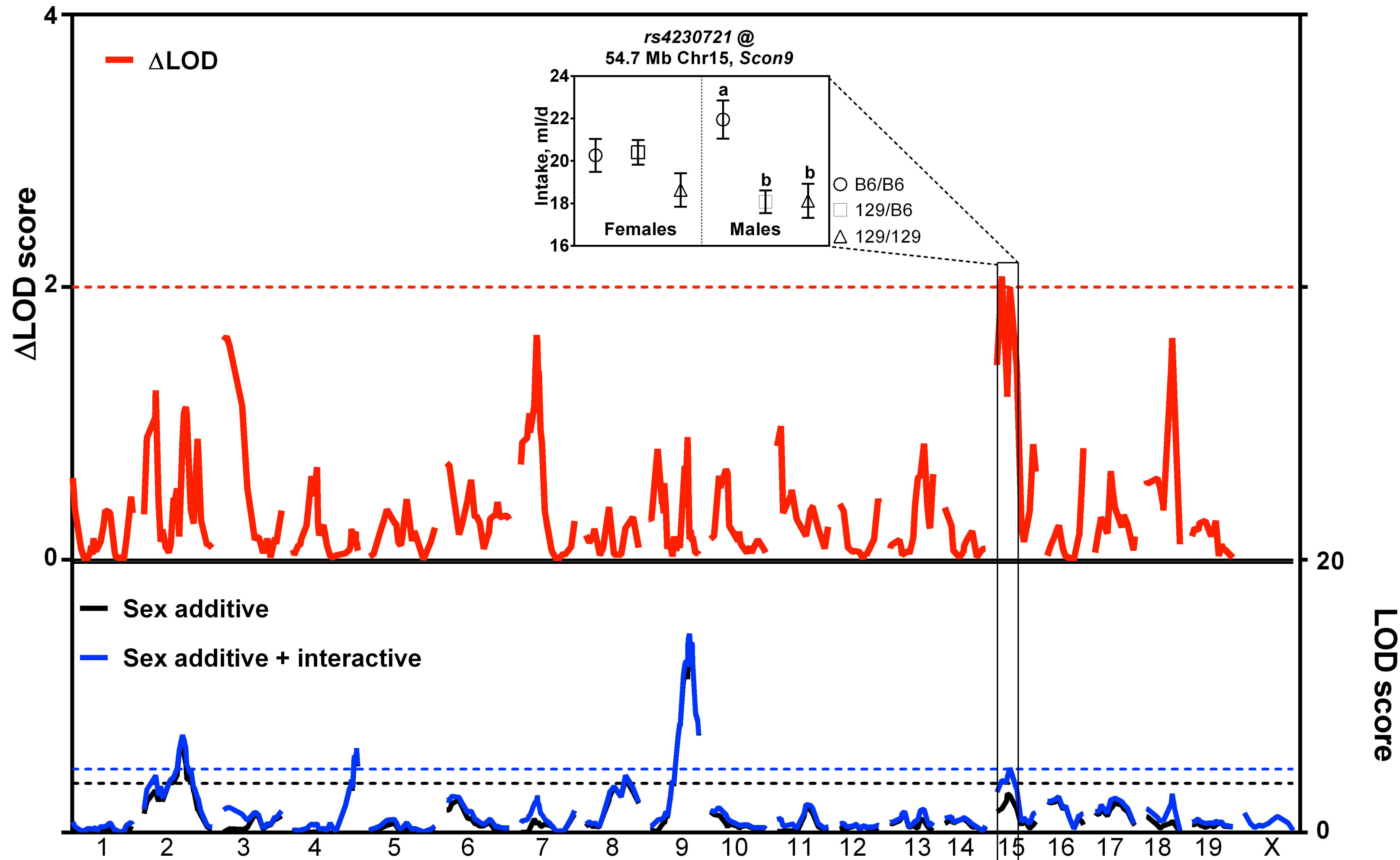
795 genomic context analysis.

796
797


Intake, ml/d
Preference, %
120 mM
300 mM
18
12
6
0
30
20
10
0
100
90
80
100
95
90
*
*
*
*
*
*
Males
Females
Midpoint of parental males
Midpoint of parental females
B6
F2
129
B6
F2
129

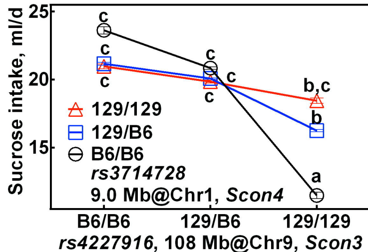

LOD score
Intake
Preference
0
20
40
60
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 X
B6/B6
129/B6
129/129
Intake, ml/d
280G12-T7@
155.8Mb Chr4, Scon2
rs3679771@
112.8Mb Chr9, Scon3
Preference, %
280G12-T7@
155.8Mb Chr4 Scon2
rs3023045@
50.6Mb Chr5, Scon7
rs3679771@
112.8Mb Chr9, Scon3

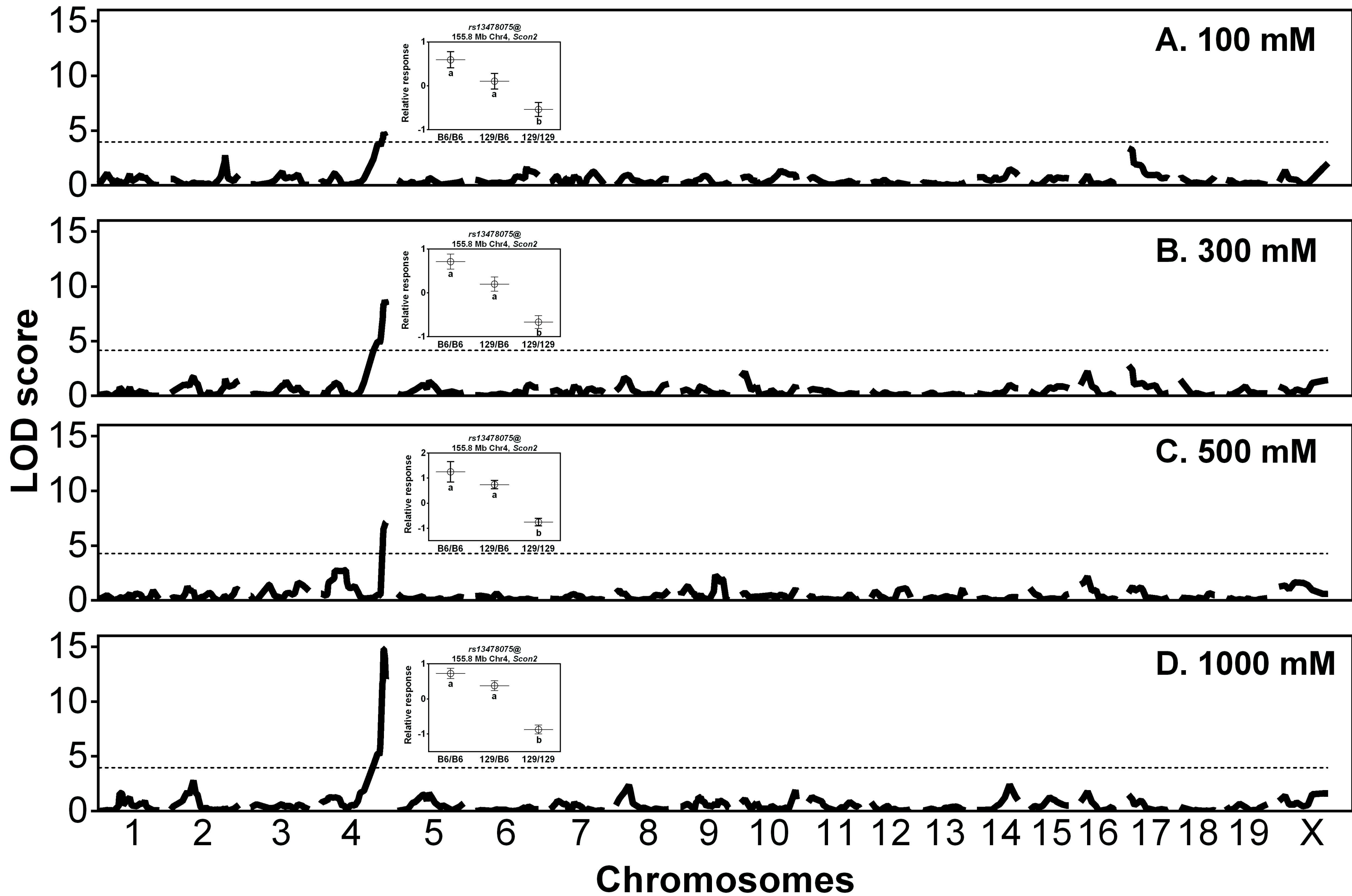

LOD score
Chromosomes
Intake
Preference
B6/B6
129/B6
129/129
Intake, ml/d
rs3681694@
112.3Mb Chr2, Scon6
280G12-T7@
155.8Mb Chr4, Scon2
D8Mit271@
114.4Mb Chr8, Scon8
rs4227916@
108 Mb Chr9, Scon3
Preference, %
rs3023231@
106.8Mb Chr9, Scon3
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 X


ΔLOD
ΔLOD score
rs4230721 @
54.7 Mb Chr15, Scon9
Intake, ml/d
a
b
b
Females
Males
B6/B6
129/B6
129/129
Sex additive
Sex additive + interactive
LOD score
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
X

A

B





A. 100 mM
B. 300 mM
C. 500 mM
D. 1000 mM
rs13478075@
155.8 Mb Chr4, Scon2
Relative response
B6/B6
129/B6
129/129
LOD score
Chromosomes
1
2
3
4
5
6
7
8
9
10
11
12
13
14
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16
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18
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X

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