Published in final edited form as:

Behav Genet. 2008 July ; 38(4): 417–423.

Genetic Mapping of Vocalization to a Series of Increasing Acute Footshocks Using B6.A Consomic and B6.D2 Congenic Mouse Strains

Douglas B. Matthews^{1,2}, Elissa J. Chesler³, Melloni N. Cook¹, Jody Cockroft⁴, Vivek M. Philip⁵, and Dan Goldowitz⁴

¹ Department of Psychology, University of Memphis, Memphis, TN 38152, USA

² Department of Psychology and Neuroscience, Baylor University, Waco, TX 76706, USA, e-mail: doug_matthews@baylor.edu; d.matthews@mail.psy.memphis.edu

³ Systems Genetics Group, Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, USA

⁴ Department of Anatomy and Neurobiology, University of Tennessee Health Science, Center, Memphis, TN 38163, USA

⁵ Genome Science and Technology Program, University of Tennessee, Knoxville, TN, USA

Abstract

Footshock response is used to study a variety of biological functions in mammals including drug self-administration, learning and memory and nociception. However, the genetics underlying variability in footshock sensitivity are not well understood. In the current studies, a panel of B6.A consomic mouse strains, two B6.D2 genome-tagged mouse lines, and the progenitor strains were screened for footshock sensitivity as measured by audible vocalization. It was found that A/J (A) mice and C57BL/6J (B6) mice with an A Chromosome 1 (Chr 1) were less sensitive to footshock compared to B6 animals. Furthermore, the offspring of Chr 1 consomic mice crossed with B6 mice had vocalization levels that were intermediate to A/J and B6 animals. A F2 mapping panel revealed two significant QTLs for footshock vocalization centered around D1Mit490 and D1Mit206 on Chr 1. The role of these Chr 1 loci in footshock sensitivity was confirmed in B6.D2 genome-tagged mouse lines.

Keywords

Stress; Footshock; Vocalization; Mice; QTL; Chromosome 1

Footshock stress is a procedure often used to investigate a variety of biological functions in mammals. For example, footshock stress has been used to study reinstatement of ethanol self-administration (Le et al. 1999; Liu and Weiss 2002; Martin-Fardon et al. 2000), in ethanol drinking (Vengeliene et al. 2003), HPA axis functioning (Harbuz et al. 2002), learning and memory (Carran et al. 1964; de Quervain et al. 1998), and nociception and skin resistance (Roberts 1967; Swedberg 1994; Weller and Sulman 1970).

Sensitivity to footshock stress can be ascertained by monitoring a variety of behaviors including audible vocalization to footshock. Specifically, if mice are administered an ascending series

of mild footshocks through the grid floor of an experimental chamber, subjects' response patterns usually include an initial flinch followed by some movement (for e.g. jumping), then an audible vocalization. Vocalization to footshock stress has also been useful in ascertaining the effects of analgesia (Swedberg 1994; Weller and Sulman 1970) and as a measure of "wildness" (Coburn 1922). These multiple applications for footshock stress (vide infra) suggest that it is a good biological activator.

Despite its multiple applications, the genetic mechanisms influencing the sensitivity to footshock and related phenotypes (i.e., vocalization) are not well characterized. For example, an intermediate mode of inheritance for sensitivity to footshock stress has been suggested, implying that several genes influence the response (Wahlsten 1972).

Early studies investigating the genetics of vocalization in mice indicated a single dominant gene influenced handling-induced vocalization (Whitney 1969, 1973). Because sensitivity to footshock appears to be influenced by several genes, it is likely that vocalization to footshock is also a complex trait, influenced by several genes. Several recently developed and powerful genetic tools are now available to investigate this behavioral phenotype in mice. Novel approaches include use of the recently constructed chromosome substitution (Singer et al. 2004), consomic (Santos et al. 2002), and congenic (Davis et al. 2005; Iakoubova et al. 2001) mouse strains. These mice have a single chromosome, or segments of a chromosome, from one strain of mouse introgressed into the genetic background of another strain (e.g. in the case of Singer et al. a chromosome from the A/J strain is backcrossed into a B6 genome using marker-assisted breeding). In as much as a contiguous set of genes may contribute to a phenotype (as most dramatically seen in contiguous gene syndromes in humans, e.g. Yingling et al. 2003), consomic and congenic mice offer another to genetically map complex traits. These models allow one to assess the contribution of one chromosome, or chromosomal region at a time, against a stabilized genetic background, thereby boosting the power with which genetic effects can be detected. This provides a very well defined model for the genetic analysis of behavior and a complement to the conventional use of recombinant inbred lines or F2-intercrosses.

It is likely that the sensitivity to footshock stress and its effect on behavior is a complex biological response which will only be understood by the integrative use of multiple, convergent, genetic mouse models. In this study, we set about to identify the genetic mechanisms involved in audible vocalization response to ascending footshock (and differential footshock sensitivity between B6, A/J, and D2 mice) using two different genetic animal models, consomic and congenic mouse strains. These data are the first to identify QTLs for vocalization to ascending footshock. Finally, these data demonstrate the strength of using multiple mouse genetic tools to study complex traits.

Methods

Subjects

Male and female mice from The Jackson Laboratory of the C57BL/6J (B6), A/J (A), DBA/2J (D2), and seven B6.A consomic strains (consomic strains for A Chr 1, 2, 4, 9, 11, 14, and 16; whose official nomenclature is C57BL/6J-Chr #^A/NaJ and two B6.D2 congenic strains (termed Genome-tagged lines or GTM; official nomenclature of B6.D2)/LusJ; Iakoubova et al. 2001) were used in these experiments. The two B6.D2 congenic lines were targeted to Chr 1 and termed 1M and 1D for medial and distal portions of the chromosome, respectively (between 25–85 cM for 1M and 80–115 for 1D; Iakoubova et al. 2001). Consomic mice were originally obtained from Dr. Joe Nadeau or the Jackson Laboratory. GTM mice were obtained from Richard Davis and Aldon Lusi at UCLA. F1 ($n = 10$ males and 10 females) and F2 ($n = 94$ males and 91 females) mice were produced by mating females of a consomic line to a B6 male

and then intercrossed, respectively. All experimental mice were bred in the animal facilities at The University of Tennessee Health Science Center. Animals were weaned when they were approximately 25-days old and housed in groups of 3–5 of the same sex until 45 days of age. At that time animals were ear clipped, transferred to the animal facility at the University of Memphis and individually housed until 56 days of age when data collection was conducted. All mice received food and water ad libitum and were housed on a 12:12 h light dark cycle. For the B6, A, and D2 progenitor strains, 15 males and 15 females of each strain were used. Five males and five females from each of the seven different B6.A consomic strains as well as the two B6.D2 congenic strains were used.

Apparatus and test method

The thresholds (mA) to which animals audibly vocalized were assessed following the generation of a mild footshock by a Med Associates, Inc Shock Titration Package for Mice (model ENV-307 W). Specifically, on each test day, animals ($n = 5$ male and $n = 5$ female per strain) were moved from the mouse colony room to a holding room adjacent to the footshock chambers. Following at least a 25-min wait period (to allow for acclimation to the move), audible vocalization thresholds were assessed. Mice were placed, individually, in a shock chamber and allowed to adapt to the chamber for 5 min. Animals then received a mild footshock via the floor grid every 30 s for 500 ms. The intensity of the first footshock was 0.05 mA and each subsequent footshock increased in increments of 0.05 mA until the animal vocalized as determined by a technician positioned within 1 m of the shock chamber. Once the mouse vocalized, the experiment was terminated and the animal was removed from the chamber. Each chamber was cleaned between test subjects. To control for experimenter related variation in audible vocalization detection, the same technician collected every data point for the current manuscript and was blind to the subjects' genotype. Naive animals were held in the adjacent room during the time other subjects were being tested so that they could not hear or otherwise be influenced by the vocalizations of other subjects. Data collection was not counterbalanced across strain or sex. We saw no significant differences in response values in *t*-test comparing the first and last 25% of the observations, and the first and last 50% of observations in the F2 cross, indicating adequate stability of environmental variability for this phenotype.

In the previous experiment, a series of ascending foot-shocks were used to determine footshock intensities to which animals vocalized. If an animal did not vocalize in response to the initial shock, then it is possible that exposure to this shock level, and any subsequent shocks to which the animals were non-responsive, may have influenced the threshold at which animals eventually vocalized. Typically, the vocalization response did not occur until a current level of 0.2 mA was reached, resulting in an apparent left censored distribution in the F2 cross. To determine if the administration of multiple footshocks influenced the intensity (mA) at which animals vocalized, separate groups of B6, A, and D2 animals (10 males and females from each strain) received either a single footshock (if they responded to the initial footshock) or a maximum of three footshocks separated by 30 s if the animal did not vocalize to the second footshock. The footshock level used was the mean footshock level, specific to strain and sex, which evoked an audible vocalization in the initial experiments.

Tissue collection in F2 animals

Following completion of vocalization threshold determination, animals were sent to the University of Tennessee Health Science Center and euthanized, tissue was harvested, and DNA was isolated using a high-salt protocol (Reeves et al. 1989) for genotyping of mice for markers on the target chromosome. Evenly spaced MIT markers for Chr 1 (D1Mit213 at 25.7 cM, 43.276 Mb; D1Mit7 at 41 cM, 74.886 Mb; D1Mit490 at 59.5 cM, 105.896 Mb; D1Mit139 at 65 cM, 128.344 Mb; D1Mit200 at 80 cM, 152.080 Mb; D1Mit206 at 95.8 cM, 174840 Mb;

and D1Mit511; Research Genetics, now Invitrogen) that were polymorphic between A and B6 were used to genotype animals.

Statistical analysis

Average vocalization thresholds were determined by sex and strain and analyzed by two-way analysis of variance for the initial screen and the F1 comparison. Dunnett's post-hoc tests were conducted comparing groups to the B6 animals when statistical significance ($P < 0.05$) was obtained. The likelihood of vocalizing to a single footshock or three footshocks was analyzed by Chi-square analysis. One female F2 animal did not vocalize and was assigned a score of 0.70 mA, the highest footshock used in the current study.

MapManager was used to run a simple one-way scan for QTL effects in the F2 data. The data were also imported into R/QTL (Broman et al. 2003), which enables multiple locus modeling and analysis of the sex covariate. Sex was included in this analysis because there is a significant sex difference in the F2 mice, and a potential sex $\times$ genotype interaction evident in the progenitor data. Data were log transformed to satisfy the distributional assumptions of the mapping model, including normality and homoscedasticity.

To determine whether a multiple locus model explained variation in footshock vocalization, we repeated the one-way scan with a sex covariate, and performed a QTL scan of each pair of loci with the sex covariate. Each mapping analysis was permuted 1,000 times. The significant loci and sex effect were then analyzed using QTL model fitting procedures in R/QTL.

To further assess the effects of multiple loci, and to confirm whether the results were robust to violation of the distributional assumptions due to the thresholding effect observed in these data (14.13% of the observations had a value of 0.20 mA, the minimum value to elicit an audible response), we performed a proportional hazards regression using SAS v 9.1. Briefly, all of the genotype markers, sex, and a series of sex $\times$ genotype interaction effect code variables were entered into a stepwise proportional hazards regression with an entry criteria of 0.15 and a stay criteria of 0.10.

Results

Vocalization thresholds were significantly influenced by the strain and sex of the animals in the B6, A, B6.A consomic, and B6.A Chr 1 F1 comparison [Two-way ANOVA, main effect of strain, $F = 5.36$, $df (9, 90)$, $P < 0.0001$, main effect of sex, $F = 3.95$, $df (1, 90)$, $P < 0.05$]. Female mice had significantly higher vocalization thresholds compared to male mice (mean female vocalization threshold was 0.276 ± 0.01 mA while mean male vocalization threshold was 0.258 ± 0.01 mA). Chr 1 consomic, A, and B6.A Chr 1 F1 mice had significantly higher vocalization thresholds compared to B6 mice [One-way ANOVA, $F = 4.937$, $df (9, 109)$, $P < 0.0001$; Dunnett's post-hoc tests Chr 1 $t = 3.18$, $P < 0.05$; A/J $t = 5.13$, $P < 0.01$, B6.A Chr 1 F1 $t = 3.17$, $P < 0.05$]. None of the other B6.A consomic lines differed significantly from the B6 progenitor strain. (see Fig. 1).

We produced a mapping panel of F2 mice where Chr 1 consomics were crossed to the B6 mice, F1 progeny intercrossed, and the resultant F2 animals phenotyped for audible vocalization to footshock, and genotyped using Chr 1 markers. The mapping results obtained from 185 F2 progeny, using MapManagerQTX, revealed a significant QTL (LRS = 17.31; LOD = 3.76; $P = 0.003$) centered around D1Mit490, at 59.5 cM or 105.97 Mb on Chr 1 (Fig. 2a). This QTL is termed Voc1.

The simple one-way QTL scan performed using Map-Manager revealed a potential second locus on Chr 1 at the distal most region, near marker D1Mit206 at 174.93 Mb, that is termed

Voc2 (Fig. 2a). A test of recombination across loci did not reveal significant linkage of D1Mit490 and D1Mit206, indicating that the two loci may have independent effects on the phenotype. An effect scan revealed that the proximal QTL has a positive dominance deviation, and the distal QTL has a negative dominance deviation (Figs. 2b, c).

Data were then imported into R/QTL for multi-locus QTL analysis. A pair-wise QTL analysis tested all possible marker pairs for additive and interaction effects on phenotype. A sex covariate was included in these analyses. This revealed a significant two locus additive model with loci at 59.5 cM (105.9 Mb) and 95.8 cM (174.9 Mb) corresponding to the markers D1Mit490 and D1Mit206. The model consisted of independent effects of the two loci and sex with a LOD score of 7.4, $P = 3.7 \times 10^{-6}$. This additive model accounts for 16.72% of the phenotypic variance. An empirical significance threshold was determined for this model using a permutation test with 1,000 permutation runs over all marker pairs. The permutation P -value for this model was < 0.05 . P -values for the individual effects, based on dropping them one at a time from the model were obtained. For D1Mit490, LOD = 3.02, $P < 0.001$, percent variance accounted for (VAF) = 6.5; for D1Mit206, LOD = 1.49, $P = 0.0322$, % VAF = 3.15; and for sex, LOD = 1.37, $P = 0.0118$, % VAF = 2.90.

This result is subject to both conservative and liberal bias due to the thresholding at the low end of phenotypic values, which may deflate estimates of within group variance, while simultaneously reducing the magnitude of phenotypic difference observed among allele classes. We therefore ran a proportional hazards regression to confirm the robustness of the QTLs to these biases. The main effects of D1Mit490 and sex remained in the stepwise proportional hazard regression model. The likelihood ratio test for the proportional hazards regression revealed significant non-zero regression weights ($\chi^2_{df=3} = 21.289$, $P < 0.0001$). The D1Mit490 main effect was significant ($\chi^2_{df=1} = 6.93$, $P < 0.01$), as was the main effect of sex ($\chi^2_{df=1} = 5.1571$, $P < 0.05$). The main effect was also retained at a confirmatory threshold, D1MIT206 ($\chi^2_{df=1} = 2.72$, $P = 0.098$). The hazard ratios for a threshold response for sex = 1.45 indicating that females were 1.45 times more likely to respond to the minimum effective number of stimuli, D1Mit206 = 1.24, and D1Mit490 = 1.35. Other effects were eliminated including the additional loci, sex interactions, and multi-locus interactions.

To further investigate if these regions on Chr 1 modulate vocalization to a mild footshock, two strains of B6.D2 Chr 1 mice (Chr 1M and Chr 1D; see Iakoubova et al. 2001; see Methods) were assessed for vocalization. Use of these congenic strains confirmed that a region on Chr 1 modulated vocalization to a mild footshock (Two-way ANOVA, strain $\times$ sex; main effect of strain, $F = 29.35$, $df (3, 32)$, $P < 0.0001$). Of special note, it was found that the vocalization threshold of B6.D2 Chr 1M (Dunnett's post-hoc, $t = 3.101$, $P < 0.05$), the B6.D2 Chr 1D mice (Dunnett's post-hoc, $t = 8.391$, $P < 0.01$) and the D2 animals (Dunnett's post-hoc, $t = 6.931$, $P < 0.01$) all differed significantly from the vocalization threshold of the B6 mice (See Fig. 3).

To determine if the ascending series of footshock currents used in the previous experiments affected the shock level at which mice vocalized, naive male and female B6, A, and D2 mice received one, or up to three footshocks at the previously determined shock intensity. There was not a significant number of B6, A, or D2 animals that vocalized to the first footshock ($\chi^2 = 4.21$, $P > 0.05$). In fact, only 10% of B6 mice, 5% of A mice, and 25% of D2 mice vocalized following the first footshock. Following the third footshock, a greater percentage of animals in all strains vocalized: 35% of B6 mice, 15% of A mice, and 35% of D2 mice produced an audible vocalization. This is compatible with our observations in the F2 cross that a minimum footshock threshold of 0.20 is required to induce vocalization because this response typically follows three other shocks at intensities of 0.05, 0.1, and 0.15 mA.

Discussion

Footshock has often been used as a stimulus in many types of research paradigms, but the genetic mechanisms underlying sensitivity to footshock are not well understood. We have identified two genomic regions on Chr 1 that house QTLs, termed Voc1 and Voc2, which influence the resistance of mice to the induction of audible vocalization by a series of ascending footshocks. Thus, the current work is the first to identify significant quantitative trait loci associated with sensitivity to an ascending series of foot-shocks using B6.A consomic mice. Furthermore, a congenic set of B6.D2 mice and a mapping panel were used to provide not only confirmation of the QTL, but finer localization of the chromosomal regions that modulate sensitivity to footshock stress.

Previous research has indicated that initial sensitivity to footshock has a genetic component, that is an intermediate mode of inheritance (Wahlsten 1972) and early studies suggested that sensitivity to footshock may be modulated by a single gene (Whitney 1969, 1973). Analysis of consomic strains demonstrated that insertion of A Chr 1 on a B6 background produced an A phenotype and the F1 analysis revealed this phenotype was of intermediate dominance. The pattern of inheritance is in agreement with those of Wahlsten (1972).

The present study reveals that the vocalization to footshock is likely regulated by genes in at least two loci. The presence of the second locus is confirmed by our converging data from two independent studies, which include the B6.D2 Chr 1D lines, and the QTL mapping study. To account for this intermediate dominance, both QTLs, with their opposing dominance deviations, are required. Neither locus alone accounts for the intermediate phenotype of the F1s because each shows dominance effects. Another indicator of the two loci is that, both of the B6.D2 congenic strains differed significantly from the B6 progenitor strain; however, the region of overlap between these two strains is a 5 cM region that was not identified in our QTL mapping analysis. The B6.D2 1M line ($P < 0.05$) has a region of D2 Chr 1 that goes from about 25 to 85 cM (~50–170 Mb) while the 1D line ($P < 0.01$) has a region of D2 Chr 1 from 80 to 115 cM (~160–230 Mb). Mapping results revealed a significant peak around D1Mit490 which is at 106 Mb, with a rather broad peak that extends from 75 to 150 Mb with the markers we used, and a second locus at D1Mit206 which is at 175 Mb. Together, these results confirm the presence of two QTLs on Chr 1.

A potential interpretive issue in the current work is that our phenotype reflects the combined sensitivity to repeated shock and intensity of shock. In our test paradigm, foot-shock intensity increased every 30 s until the animal produced an audible vocalization. Therefore, animals from strains with higher mean vocalization thresholds received more footshocks than animals from strains with lower mean audible vocalization thresholds. Differences in the number of footshocks received might have affected the overall vocalization score for each strain. Recently, we have generated data demonstrating that the use of the identified mean audible vocalization shock thresholds in B6, A, and D2 animals produces similar increase in plasma corticosterone levels regardless of the differences in shock thresholds for the three strains (Matthews et al. in press). As such, the different number of footshocks received by animals in different strains in the current studies likely affects behavior but does not appear to affect stress-induced plasma corticosterone levels. However, a related concern exists. Specifically, the previous footshocks in the ascending intensity could have produced sensitization to footshock stress such that animals actually produce an audible vocalization at lower footshock levels than they would have produced if a single footshock was used to determine the audible vocalization threshold. This would introduce further conservative bias in our detection of group differences. To investigate this possibility, we determined the percentage of B6, A, and D2 male and female mice that produced an audible vocalization when administered a single, or a series of three footshocks at the previously determined level. Interestingly, few naive animals vocalized to

the single footshock while a greater percentage, but less than 50%, vocalized following three footshocks. These data strongly suggest that the previous footshocks given during the initial determination series produced a greater sensitivity in animals such that they vocalized at lower shock intensities than might otherwise occur following a single footshock.

An additional confound is the possibility that different strains of mice may respond differently to similar levels of footshock. For example, strains might first jump then vocalize to footshock while other strains might vocalize first and then jump afterwards. In fact, such idiosyncratic strain-specific responses to footshock have been previously reported (Mori and Makino 1994; Zerbollo 1967). Such differential expression patterns in response to footshock could confound the interpretation of strain-specific response to footshock when only one behavior, like audible vocalization, is measured. Future studies would be well-served to record a time sequence of behavioral responses to better understand how strain affects footshock sensitivity, specifically audible vocalizations.

As previously mentioned, footshock stress is used in a variety of experimental questions including reinstatement of ethanol self-administration (Le et al. 1999; Liu and Weiss 2002; Martin-Fardon et al. 2000) and increases in ethanol drinking (Vengeliene et al. 2003). Stress is an often-reported cause for the consumption of alcohol in humans (Brown et al. 1995; Cooper et al. 1992; Dawson et al. 2005, 2007). In fact, the stress reduction hypothesis posits that alcohol may reduce the symptoms of stress, thus promoting its consumption (Conger 1956; Volpicelli 1987; for review see Goeders 2003). The current work increases the understanding of sensitivity to physical stress with evidence that a gene or genes on Chr 1 are critical to modulating such sensitivity. Although footshock has been correlated with several alcohol-related traits (vide infra), there are no reports of alcohol-related QTLs on Chr 1 (for example, Chr 2 see Fehr et al. 2005). As such, the currently identified QTLs, *Voc1*, and *Voc2*, are not likely to affect ethanol self-administration unless new QTLs for ethanol self-administration are identified in this region of Chr 1. To investigate potential interactions between genes in these QTL regions, future work can manipulate the availability of genes in the identified QTL to assess if such changes alter the effect of footshock stress on ethanol reinstatement and consumption.

Acknowledgements

This manuscript was supported by the following grants: NIMH grant MH61971, R25 MH-066890, U01-AA-13503, AA014588, AA13509, DA020677, and AA016662 to the authors.

References

- Broman KW, Wu H, Sen S, Churchill GA. R/qtl QTL mapping in experimental crosses. *Bioinformatics* 2003;19:889–890.10.1093/bioinformatics/btg112 [PubMed: 12724300]
- Brown SA, Vik PW, Patterson TL, Grant I, Schuckit MA. Stress, vulnerability and adult alcohol relapse. *J Stud Alcohol* 1995;56:538–545. [PubMed: 7475034]
- Carran AB, Yeudall LT, Royce JR. Voltage level and skin resistance in avoidance conditioning of inbred strains of mice. *J Comp Physiol Psychol* 1964;58:427–430.10.1037/h0046774 [PubMed: 14241059]
- Coburn CA. Heredity of wildness and savageness in mice. *Behav Monog* 1922;4:1–71.
- Conger JL. Alcoholism: theory, problem and challenge. II. Reinforcement theory and the dynamics of alcoholism. *Q J Stud Alcohol* 1956;17:296–305. [PubMed: 13336262]
- Cooper ML, Russell M, Skinner JB, Frone MR, Mudar P. Stress and alcohol use: moderating effects of gender, coping, and alcohol expectancies. *J Abnorm Psychol* 1992;101:139–152.10.1037/0021-843X.101.1.139 [PubMed: 1537960]
- Davis RC, Schadt EE, Smith DJ, Hsieh EW, Cervino AC, van Nas A, et al. A genome-wide set of congenic mouse strains derived from DBA/2J on a C57BL/6J background. *Genomics* 2005;86:259–270.10.1016/j.ygeno.2005.05.010 [PubMed: 16039824]

- Dawson DA, Grant BF, Ruan WJ. The association between stress and drinking: modifying effects of gender and vulnerability. *Alcohol Alcoholism* 2005;40:453–460.10.1093/alcac/agh176
- Dawson DA, Grant BF, Li TK. Impact of age of first drink on stress-reactive drinking. *Alcohol Clin Exp Res* 2007;31:69–77.10.1111/j.1530-0277.2006.00265.x [PubMed: 17207104]
- de Quervain DJ, Roozendaal B, McGaugh JL. Stress and glucocorticoids impair retrieval of long-term spatial memory. *Nature* 1998;394:787–790.10.1038/29542 [PubMed: 9723618]
- Fehr C, Shirley RL, Crabbe JC, Belknap JK, Buck KJ, Phillips TJ. The syntaxin binding protein 1 gene (Stxbp1) is a candidate for an ethanol preference drinking locus on mouse chromosome 2. *Alcohol Clin Exp Res* 2005;29:708–720.10.1097/01.ALC.0000164366.18376.EF [PubMed: 15897714]
- Goeders NE. The impact of stress on addiction. *Eur Neuropsychopharmacol* 2003;13:435–441.10.1016/j.euroneuro.2003.08.004 [PubMed: 14636959]
- Harbuz MS, Chover-Gonzalez A, Gibert-Rahola J, Jessop DS. Protective effect of prior acute immune challenge, but not footshock, on inflammation in the rat. *Brain Behav Immun* 2002;16:439–449.10.1006/brbi.2001.0658 [PubMed: 12096889]
- Iakoubova OA, Olsson CL, Dains KM, Ross DA, Andalibi A, Lau K, et al. Genome-tagged mice (GTM): two sets of genome-wide congenic strains. *Genomics* 2001;74:89–104.10.1006/geno.2000.6497 [PubMed: 11374905]
- Le AD, Poulos CX, Harding S, Watchus J, Juzysch W, Shaham Y. Effects of naltrexone and fluoxetine on alcohol self-administration and reinstatement of alcohol seeking induced by priming injections of alcohol and exposure to stress. *Neuropsychopharmacology* 1999;21:435–444.10.1016/S0893-133X(99)00024-X [PubMed: 10457541]
- Liu X, Weiss F. Additive effect of stress and drug cues on reinstatement of ethanol seeking: exacerbation by history of dependence and role of concurrent activation of corticotropin-releasing factor and opioid mechanisms. *J Neurosci* 2002;15:7856–7861. [PubMed: 12223538]
- Martin-Fardon R, Ciccocioppo R, Massi M, Weiss F. Nociceptin prevents stress-induced ethanol-but not cocaine-seeking behavior in rats. *Neuroreport* 2000;11:1939–1943. [PubMed: 10884047]
- Matthews DB, Morrow AL, O'Buckley T, Berry RB, Mittleman G, Goldowitz D, et al. The effect of acute mild footshock on ethanol self-administration and plasma corticosterone levels in three commonly used mouse strains. *Alcohol*. in press
- Mori T, Makino J. Response types to shock and avoidance learning in inbred strains of mice. *Shinrigaku Kenkyu* 1994;4:285–302.
- Reeves RH, Crowley MR, Lorenzon N, Pavan WJ, Smeyne RJ, Goldowitz D. The mouse neurological mutant weaver maps within the region of chromosome 16 that is homologous to human chromosome 21. *Genomics* 1989;5:522–526.10.1016/0888-7543(89)90018-9 [PubMed: 2575584]
- Roberts LE. Central, peripheral and artifactual determinants of skin resistance in the mouse. *J Comp Physiol Psychol* 1967;64:318–328.10.1037/h0024805 [PubMed: 6050579]
- Santos J, Montagutelli X, Acevedo A, Lopez P, Vaquero C, Fernandez M, et al. A new locus for resistance to gamma-radiation-induced thymic lymphoma identified using inter-specific consomic and inter-specific recombinant congenic strains of mice. *Oncogene* 2002;21:6680–6683.10.1038/sj.onc.1205846 [PubMed: 12242666]
- Singer JB, Hill AE, Burrage LC, Olszens KR, Song J, Justice M, et al. Genetic dissection of complex traits with chromosome substitution strains of mice. *Science* 2004;304:445–448.10.1126/science.1093139 [PubMed: 15031436]
- Swedberg MD. The mouse grid-shock analgesia test: pharmacological characterization of latency to vocalization threshold as an index of antinociception. *J Pharmacol Exp Ther* 1994;269:1021–1028. [PubMed: 8014847]
- Vengeliene V, Siegmund S, Singer MV, Sinclair JD, Li TK, Spanagel R. A comparative study on alcohol-preferring rat lines: effects of deprivation and stress phases on voluntary alcohol intake. *Alcohol Clin Exp Res* 2003;27:1048–1054.10.1097/01.ALC.0000075829.81211.0C [PubMed: 12878910]
- Volpicelli JR. Uncontrollable events and alcohol drinking. *Br J Addict* 1987;82:381–392.10.1111/j.1360-0443.1987.tb01494.x [PubMed: 3555574]
- Wahlsten D. Phenotypic and genetic relations between initial response to electric shock and rate of avoidance learning in mice. *Behav Genet* 1972;2:211–240.10.1007/BF01065691 [PubMed: 4664210]

- Weller CP, Sulman FG. Drug action on tail shock-induced vocalization in mice and its relevance to analgesia. *Eur J Pharmacol* 1970;9:227–234.10.1016/0014-2999(70)90304-3 [PubMed: 5437157]
- Whitney GD. Vocalization of mice: a single genetic unit effect. *J Hered* 1969;60:337–340. [PubMed: 5399132]
- Whitney GD. Vocalization of mice influenced by a single gene in a heterogeneous population. *Behav Genet* 1973;3:57–64.10.1007/BF01067689 [PubMed: 4715984]
- Yingling J, Toyo-Oka K, Wynshaw-Boris A. Miller-Dieker syndrome: analysis of a human contiguous gene syndrome in the mouse. *Am J Hum Genet* 2003;73:475–488.10.1086/378096 [PubMed: 12905154]
- Zerbollo DJ. Differences between three inbred mouse strains on a wheel-turn avoidance task. *Psychon Sci* 1967;7:201–202.

Fig. 1.

Vocalization score as a function strain. Insertion of A Chr 1 into B6 background increases vocalization threshold to a mild footshock in mice. Bars are mean vocalization $\pm$ SE. * $P < 0.05$

Fig. 2.

(a) Interval mapping of F2 animals to sensitivity of acute footshock. The solid line corresponds to $P = 0.05$, dashed $P = 0.01$, dotted $P = 0.005$ based on 1,000 permutations. The Dominance effects of the two QTL are in opposite directions. (b, c) The mean phenotypic value and the individual observations are plotted against allelic classes at markers near the two QTL locations. The QTLs each have dominance deviations in opposite directions, which together would account for the intermediate F1 phenotype

Fig. 3. Vocalization score as a function of D2.B6 strains. Bars are mean vocalization $\pm$ SE. * $P < 0.05$