Audiogenic Seizure Prone (asp): A Gene Affecting Behavior in Linkage Group VIII of the Mouse

Abstract. *The incidence of initial audiogenic seizures in mice from crosses between a susceptible and a resistant inbred strain is attributable to the effects of a single pair of alleles. The locus responsible for this behavioral variation is located in Linkage Group VIII of the mouse. Sensitization-dependent convulsions are influenced in whole or in part by alleles at an independent locus.*

Mice from certain inbred strains, such as DBA/2J, commonly convulse during the presentation of an intense auditory stimulus. Mice of other strains, such as C57BL/6J, appear to be unaffected by the same treatment. High seizure risk has been attributed to the effects of a single dominant gene (*1*), alleles at two loci (*2*), multiple genetic factors (*3, 4*), and alleles residing at or near the dilute-locus (*5*). Recent studies have indicated that audiogenic seizures may be induced in mice of a supposed seizure-resistant strain by exposing subjects to an auditory stimulus at a critical age and retesting them after an appropriate interval of time (*6*). The diversity in genetic explanations may have resulted in part from an entanglement of two potentially independent phenomena—initial susceptibility and sensitization-dependent susceptibility. Accordingly, a new study was conducted to evaluate the genetic basis of seizure susceptibility. The results show that the incidence of initial seizures in crosses between C57BL/6J and DBA/2J mice is accounted for by the effects of a single gene pair. We name the allele responsible for high seizure risk *asp* (audiogenic seizure prone). The *asp*-locus is loosely linked to the *b*-locus in Linkage Group VIII, and is independent of the *d*-locus in Linkage Group II. Sensitization-dependent seizures appeared to be influenced by alleles at an independent locus.

C57BL/6J (B6) and DBA/2J (D2) highly inbred mice were obtained from the Production Branch of the Jackson Laboratory. Nine generations were derived. These comprised P_1 (B6), P_2 (D2), and F_1; the traditional B_1, B_2, and F_2 segregating generations; and additional B_1F_1, B_2F_1 and B_1B_2 segregating generations. These latter generations are required according to recently developed nonparametric methods for genetic analysis (*7*). The number of mating pairs for the neoclassical crosses were as follows: B_1F_1, 8; B_2F_1, 7; and B_1B_2, 15. Experiments were conducted in first and second litter replications in which mice from all crosses were tested during identical time periods. The experimental chamber was a sound-deadened box with dimensions 27.5 by 27.5 by 24 cm. An electric bell, 15 cm in diameter, which produced a sound level reading of 102 to 104 db above 0.0002 dyne/cm^2 was used as the stimulus. Mice aged 21 days ($\pm$ 3) were placed singly in the chamber and the bell was rung for a maximum of 1 minute, or until the onset of clonic convulsion. Elapsed time from the onset of bell ringing to wild running, onset of clonic convulsion, onset of tonic extension (if it occurred), and termination of convulsion were recorded. Mice which did not convulse on the initial test were retained and retested 48 hours later.

Table 1 summarizes the numbers and proportions of clonic seizures observed for each experimental group. These data are summed across sex, reciprocal cross, and replication. The proportion of seizures expected according to an allelic pair model for a given segregating generation was calculated by deriving the generation's expected genotypic constitution, and multiplying these frequencies by the risk of seizure determined from the seizure incidence of the genetically uniform populations. For example, under a single allelic pair model, the expected genotypic constitution of the F_2 generation is ¼ (P_1) + ½ (F_1) + ¼ (P_2), and the expected proportion of seizures for F_2 is then ¼ (0.000) + ½ (0.011) + ¼ (0.983), or 0.251. Chi-square tests were computed for goodness of fit for five of the six segregating generations. The test for B_1 data was omitted because of the low number of convulsions expected. In no case was the allelic pair expectation rejected statistically. For the total experiment including B_1, χ^2 was 0.44, $P \simeq .50$, d.f. = 1. Since these data did not lead to a rejection of the allelic pair model, they provide no support for a hypothesis that the initial seizure risk is attributable to multiple genetic factors. Accordingly, we name the autosomal allele which in double dose is associated with high seizure risk *asp* (audiogenic seizure prone) (*8*). The alternative allele may be designated *asp*$^+$, or more simply, +.

The generations were also segregating for black (*B*) and brown (*b*) alleles at one locus and dense (*D*) and dilute (*d*) at another locus. Table 1 presents

Table 1. Summary of data and genetic analyses for the incidence of inital audiogenic seizures for all generations.

Generation	Number of subjects	Proportion of seizures observed	Proportion of seizures expected, 1-unit model	χ^2	Seizures according to coat color				Percent recombination $\hat{p} \pm s_{\hat{p}}$	
					Black *B/- D/-*	Dilute black *B/- d/d*	Brown *b/b D/-*	Dilute brown *b/b d/d*	*b*-locus	*d*-locus
P_1 C57BL/6J	45	0.000			0/45					
P_2 DBA/2J	58	.983						57/58		
F_1	89	.011			1/89					
B_1	115	.035	0.006		4/115					
B_2	119	.513	.497	0.12	15/37	12/27	18/27	16/28	40.3 ± 4.5	50.4 ± 4.6
F_2	105	.247	.251	.01	11/51	5/25	6/15	4/14	40.7 ± 6.5	52.0 ± 7.5
B_1F_1	128	.070	.128	3.81	9/111	0/3	0/12	0/2		
B_2F_1	96	.344	.374	0.37	10/41	8/22	9/21	6/12	38.7 ± 6.1	42.7 ± 6.5
B_1B_2	185	.168	.191	.66	15/131	1/16	15/36	0/2	27.9 ± 5.6	47.8 ± 7.6
Total	940			0.44*					40.1 ± 3.2†	49.1 ± 3.3†

* χ^2 for the total experiment ($P \simeq .50$, d.f. = 1). † Joint maximum likelihood estimate of the recombination proportion for generations B_2, F_2, and B_2F_1.

Table 2. Summary of data and genetic analyses for the incidence of sensitization-dependent audiogenic seizures for all generations.

Generation	Number of subjects	Proportion of seizures observed	Proportion expected *asp*/+; +/+	Proportion expected, 1-unit model
P_1 C57BL/6J	44	0.5909		
P_2 DBA/2J	1	.0		
F_1	84	.8095		
B_1	109	.5229	0.7002	0.7002
B_2	58	.9138	.8095	.9048
F_2	79	.7595	.7366	.8025
B_1F_1	117	.7778	.7158	.7513
B_2F_1	63	.8730	.7658	.8536
B_1B_2	153	.7712	.7591	.8042
Total	708		$\chi^2 = 6.403$*	$\chi^2 = 0.179$

* $P < .025$, d.f. = 1. B_1 data not included in model comparisons.

the number of seizures observed for each of the four coat color phenotypes. To determine whether the *asp*-locus was associated with either *b*- or *d*-loci, maximum likelihood estimates of the recombination proportion in coupling were computed for each segregating generation in which a meaningful estimate could be obtained. For linkage of the *asp*- and *b*-loci for the entire experiment, omitting B_1 data, χ^2_L was 15.9 ($P < .0001$, d.f. = 1). Estimates of the recombination proportion, $\hat{y}$, for each generation are listed in Table 1. Since recombination estimate for B_1B_2 could be smaller owing to larger sampling variance of parents, a joint maximum likelihood estimate of $\hat{y}$ was computed for B_2, F_2, and B_2F_1 data, by using an iterative procedure. The conservative estimate of the recombination between *asp*- and *b*-loci was 40.1 percent (S.E. = 3.2 percent). There was no differential viability associated with the *asp*-locus ($\chi^2 = 1.23$, $P > .25$) or with the *b*-locus ($\chi^2 = .09$, $P > .90$).

Estimates of the recombination proportion for *asp*- and *d*-loci computed for the same generations did not indicate an association. The comparable joint estimate of the recombination proportion for *asp*- and *d*-loci was 49.1 percent (S.E. = 3.3 percent). This negative evidence is of importance, since considerable attention has been given to the hypothesis that audiogenic seizures result from certain deficiencies in phenylalanine metabolism associated with the dilute-locus (*5*).

Data corroborating an association between initial audiogenic convulsion and *b*-locus phenotypes, but not *d*-locus phenotypes, may be found in a report by Schlesinger *et al.* (*4*). Their table 7 presents frequencies of seizure according to coat color for the B_2 generation derived from the same strains. Reanalysis of their data indicated that the recombination proportion between initial convulsion and *b*-locus phenotypes was 38.3 percent (S.E. = 6.3 percent), while that between initial convulsion and *d*-locus phenotypes was 51.7 percent (S.E. = 6.5). It thus appears fairly certain that an allele influencing initial seizure risk is loosely linked to the *b*-locus and is therefore located in Linkage Group VIII of the mouse. Further studies are needed to ascertain on which side of the *b*-locus the *asp*-locus resides.

Mice which did not convulse on the initial test were retested 48 hours later. Table 2 summarizes these data. A higher proportion of F_1's convulsed relative to B6. The single remaining D2 subject did not convulse. The proportion of seizures was 9 percent higher in brown (*b*/*b*) than in black (*B*/-) mice ($\chi^2 = 3.65$, $P \simeq .06$, d.f. = 1). There was no association of induced seizure risk and *d*-locus phenotypes ($\chi^2 = 0.54$, $P \simeq .40$, d.f. = 1).

Two genetic hypotheses were examined for these data. First, the *asp*-locus may control sensitization-induced seizures. In this case, since few, if any, *asp*/*asp* homozygotes remained for second testing, the genotypic composition of the segregating generations would consist of *asp*/+ and +/+. Expected genotypic compositions for each generation were derived and multiplied by the risk of seizure as inferred from that of P_1 and F_1. The expected proportions according to this hypothesis are listed in Table 2. For goodness of fit χ^2 was 6.40, $P < .025$, d.f. = 1; B_1 data were omitted. Thus, the *asp*/+ hypothesis did not fully account for the incidence of sensitization-induced audiogenic seizures.

A second genetic hypothesis was examined. Sensitization-induced seizures may depend upon alleles at a locus independent of *asp*. In this case, the retest data may be considered to be a new experiment and the allelic pair model applied in the usual way. The probability that the D2 genotype would produce a convulsion on the second test was considered to be 1.00, despite the failure of the single remaining D2 to convulse during retesting. Expected proportions according to this hypothesis are listed in Table 2. For five segregating generations, with B_1 deleted, χ^2 was 0.179, $P > .50$, d.f. = 1. The data more nearly fit the independent locus hypothesis.

The B_1 data clearly do not conform to either single locus hypothesis. This may well indicate that more than one locus influences sensitization-induced seizures. In view of the almost complete inviability of P_2, further genetic analysis seems unwarranted. The data clearly indicate that the degree to which an acute prior experience transforms an initially resistant mouse into a convulsible one is dependent upon his genotype. Generations B_2 and B_2F_1, which contain a larger proportion of D2 allelomorphs, were most responsive to the effects of initial exposure, while generations B_1 and B_1F_1, which contain a larger proportion of B6 allelomorphs, were least responsive. Frequencies of induced seizures in generations F_2 and B_1B_2 were somewhat intermediate, as expected from a consideration of their genotypic constitutions.

These results support a view that seizure risk in mice derived from B6 and D2 strains is accounted for by at least two independent mechanisms. Initial seizure risk is attributable to the effects of a single pair of alleles at the *asp*-locus. Initial risk for the F_1 hybrid resembles that of the B6 parent. Subsequent risk is influenced in whole or in part by genetic mechanisms independent of the *asp*-locus. Induced risk for the F_1 is high, resembling that presumed for the D2 parent. These findings help to clarify the discrepant incidences of seizure previously reported for B6, D2, and their F_1. If a single test is administered, the required genetic explanation is simple, and the mode of inheritance of seizure susceptibility is recessive. If multiple tests are given, and a convulsible mouse is defined as one which convulses on at least one trial, the genetic influences appear to be complex, and the mode of inheritance shows average dominance.

These experiments demonstrate the detection and location of a new locus from the study of elicited behavioral

variation. Such systems offer substantial advantages for the study of biological processes intervening between a gene substitution and a behavioral difference. The familiar neurological mutants are aberrant in so many ways that it is difficult to establish causal relationships between structure and behavior. Genes such as *asp* may facilitate research on the genetic basis of variation in phenotypes chosen for their intrinsic behavioral significance.

ROBERT L. COLLINS
JOHN L. FULLER

Jackson Laboratory,
Bar Harbor, Maine 04609

References and Notes

1. G. Witt and C. S. Hall, *J. Comp. Physiol. Psychol.* **42**, 58 (1949).
2. B. E. Ginsburg and D. S. Miller, *Colloq. Int. Centre Nat. Rech. Sci. Paris* **112**, 217 (1963).
3. K. Schlesinger, R. C. Elston, W. Boggan, *Genetics* **54**, 95 (1966).
4. J. L. Fuller, C. Easler, M. E. Smith, *ibid.* **35**, 622 (1950).
5. D. L. Coleman, *Arch. Biochem. Biophys.* **91**, 300 (1960); S. D. Huff and J. L. Fuller, *Science* **144**, 304 (1964).
6. K. R. Henry, *ibid.* **158**, 938 (1967); J. L. Fuller and R. L. Collins, *Develop. Psychobiol.*, in press.
7. R. L. Collins, *Genetics* **56**, 551 (1967).
8. Committee on Standardized Genetic Nomenclature for Mice, *J. Hered.* **54**, 159 (1963).
9. Supported in part by PHS research grant MH 11327 from the National Institutes of Mental Health and in part by an allocation from the American Cancer Society Institutional grant IN-19 to the Jackson Laboratory. We thank Dr. E. L. Green for providing the algebraic expressions for maximum likelihood estimates of the recombination proportion for generations B_2F_1 and B_1B_2. We acknowledge the able technical assistance of Geddes Simpson and Frank Clark. The principles of laboratory animal care as advocated by the National Society for Medical Research are observed in this Laboratory.

30 September 1968

Mutants at the Bobbed Locus in Drosophila melanogaster: Relation to Ribosomal RNA Synthesis?

Abstract. *An allele of the bobbed locus (bobbed bristle) behaved in crosses with other bobbed alleles as a weak isoallele to which extreme bobbed alleles were dominant. The results are consistent with the hypothesis that the bobbed locus contains multiple cistrons, some threshold number of which are needed to produce ribosomal RNA and the normal phenotype.*

In 1963, I was using a w^{m4} (white-mottled 4) stock of *Drosophila melanogaster* which was homozygous for the X chromosome inversion In(*1*)w^{m4} and was phenotypically wild with respect to the *bb* (bobbed bristle) locus. In crosses between this and another inversion stock, In(*1*)w^{mJ}, which lacks the nucleolus-organizing region (*1*), the resulting F_1 females were bobbed. When w^{m4} females were mated with *car bb* (carnation bobbed, a stock with a typical *bb* allele), with bb^l (deficiency for the *bb* locus, lethal when homozygous) and with $Y^S \cdot XY^L/O$ (attached XY, no free Y) males, the results were: w^{m4}/*car bb* females, phenotype wild; w^{m4}/bb^l females, phenotype bobbed; w^{m4}/O males, phenotype bobbed.

These results seem worth considering in terms of the hypothesis of Ritossa *et al.* (*2*). These authors showed that the site of ribosomal DNA (rDNA), which is complementary to ribosomal RNA (rRNA), is in the nucleolus-organizing region and that there is a multiplicity of rDNA cistrons for each of the two rRNA's. A consideration of the properties of the *bb* locus led to the conclusion that it and the rDNA locus are one and the same. According to the hypothesis (i) the array of cistrons present at the *bb* locus in a single X chromosome of a wild-type strain (about 130 cistrons for each of the two rRNA's) is sufficient to produce a normal, wild phenotype; (ii) mutant *bb* "alleles" result from deletion of some of the rDNA cistrons; and (iii) mutant *bb* "alleles" are additive in effect, as pointed out by Stern (although this generalization has been questioned) (*3*).

Let us suppose that x represents the number of cistrons (for each of the rRNA's) required to produce the wild phenotype, and that *any* reduction below x cistrons will result in a fly with a bobbed phenotype. It may be noted in passing that x must be less than 130, but we do not know how much less.

Now an "allele" a with $(x/2) + y$ cistrons (where y may be zero or any positive integer) will produce a wild phenotype when homozygous, because there will be a total of at least x cistrons present. We may therefore consider a a wild-type allele. If another "allele" b has fewer than $(x/2) - y$ cistrons, its homozygote will be bobbed. The heterozygote a/b will also be phenotypically bobbed, because the total number of cistrons will be less than x. Therefore b behaves like a dominant *bb* allele, or, to put it another way, a is an isoallele of *bb*+ with weakened dominance.

The results of the w^{m4} crosses can be explained if we assume that the w^{m4} stock had an isoallele at the *bb* locus which had more than half of the cistrons required for a wild phenotype [like allele a, with $(x/2) + y$ cistrons], while the *bb* allele of *car bb* had at least $(x/2) - y$ cistrons. With a total of at least x cistrons, the heterozygote w^{m4}/*car bb* would be phenotypically wild. The heterozygotes with w^{mJ} and bb^l [in which very little rDNA is present (*4*)] would be like the a/b heterozygote, in which the extreme *bb* allele has a dominant effect. The bobbed phenotype of the w^{m4}/O males would be expected on the assumption that the w^{m4} allele had fewer than x cistrons.

Ritossa *et al.* say, "One would expect the *bb* phenotype to appear only when the deletion of the rRNA cistrons is greater than one-half of the wild locus." As shown above, however, it is consistent with their hypothesis that (i) a bobbed phenotype can result from a genotype in which one of the "alleles" still has more than half of the cistrons of the wild locus; and (ii) *bb* "alleles" may act as dominants in combination with wild-type "alleles." These points are conceded in a later paper by Ritossa *et al.* in a discussion of a dominant bobbed mutant of *Drosophila funebris* (*4*), which, however, appears to be atypical.

Departures can be expected from the strict quantitative relations suggested above between rDNA and bobbed phenotype, for apparently there is not a simple proportionality between the amounts of rDNA and rRNA present, either in the range above the assumed threshold x (that is, in wild phenotypes) or in the range below, in bobbed phenotypes (*5*).

The w^{m4} stock used in 1963 is no longer extant. Even if it had been maintained, it might well have been impossible to repeat the results obtained previously, owing to the lability of the *bb* locus. Similar stocks should be obtainable, however (if the lability is due to unequal crossing-over in the multicistronic locus) by selection of alleles that show a slight bobbed effect in combination with bb^l and subsequent selection for wild phenotype in combination with *car bb*.

EILEEN S. GERSH
School of Veterinary Medicine,
University of Pennsylvania,
Philadelphia 19104

References and Notes

1. G. Lefevre, Jr., and M. D. Wilkins, *Genetics* **53**, 175 (1966).
2. F. M. Ritossa, K. C. Atwood, D. L. Lindsley, S. Spiegelman, *Nat. Cancer Inst. Monogr.* No. 23, (1966), p. 449.
3. C. Stern, *Biol. Zentralbl.* **49**, 261 (1929); W. P. Spencer, *Amer. Nat.* **79**, 85 (1945).
4. F. M. Ritossa, K. C. Atwood, S. Spiegelman, *Genetics* **54**, 819 (1966); H. Lüers, *Z. Induct. Abstamm. Vererbungs.* **74**, 70 (1937).
5. B. I. Keifer, *Proc. Nat. Acad. Sci. U.S.* **61**, 85 (1968); F. M. Ritossa, *ibid.* **59**, 1124 (1968).
6. Supported by PHS grant GM-11379.

12 September 1968