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Office of Naval Research

Final Report

Report Prepared By: Benson E. Ginsburg
Dorothea Starbuck Miller

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PRINCIPAL INVESTIGATORS: Benson E. Ginsburg, Dorothea S. Miller
Assistants: Mildred Zamis Potas, Dahrl Fohrman

TITLE OF PROJECT: Metabolic Changes Associated with Nerve Conduction
Objectives: Analysis of (a) the several modes of inheritance of susceptibility to sound induced seizures in four strains of inbred mice; (b) metabolic concomitants of seizure susceptibility as related to genotype; and (c) the basis for the observed sex difference in seizure susceptibility and in reaction to protective agents.

SUMMARY OF RESULTS

- a. See semiannual progress reports of July, 1952, February, 1953, July, 1953 and January, 1954.
- b. The following publications will summarize most of our results:
 - J. L. Fuller and B. E. Ginsburg. Effect of adrenalectomy on the anti-convulsant action of glutamic acid in mice. Am. J. Physiol. 176, March, 1954. (Reprints enclosed.)
 - B. E. Ginsburg and J. L. Fuller. A comparison of chemical and mechanical alterations of seizure patterns in mice. J. Comp. and Physiol. Psych. August, 1954.
 - B. E. Ginsburg. Genetics and the physiology of the nervous system. Symposium of Association for Research in Nervous and Mental Diseases. (In press).
 - Miller, D. S., B. E. Ginsburg, and M. Z. Potas. Mode of inheritance of susceptibility to audiogenic seizures in mice. (In preparation.)

Reprints of these papers will be furnished when available.

- c. Because of the termination of the grant, we have not completed the experiments dealing with the sex difference in seizure susceptibility. These experiments are continuing under our present grant from U.S.P.H.S.

GENETIC ANALYSIS.

Our breeding results are now complete, involving crosses of the two seizure-susceptible lines (DBA/1 and DBA/2) with the two seizure-resistant sublines (C57BL/6 and C57BL/10). Each of these lines and sublines is highly inbred and genetically as uniform as has been possible to achieve with mammalian materials.

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All four strains were crossed, and reciprocal matings were made to test the possible effects of sex linkage and maternal influences. Mice resulting from these crosses were tested in each generation, and backcrosses of the F₁ generations to each parental type were made.

I. INCIDENCE OF SEIZURES

Table I presents a summary of total convulsive incidence in the four crosses. Males and females in reciprocal crosses have been pooled.

Table I: Total convulsive incidence in F₁, F₂ and backcross generations in crosses of DBA/1 and DBA/2 with C57BL/10 and C57BL/6 mice.

Mating	F ₁		F ₂		Bx to C57		Bx to DBA	
	N	%	N	%	N	%	N	%
DBA/1 x C57BL/10	207 363	57.02	221 563	39.25	156 876	17.81	673 1038	64.84
*(Expected)				(40)		(15)		(69)
DBA/1 x C57BL/6	158 328	48.17	182 413	44.07	232 512	25.44	576 832	69.23
(Expected)				(45)		(24)		(64)
DBA/2 x C57BL/10	312 351	88.89	312 501	62.27	191 901	21.20	865 944	91.63
(Expected)				(58)		(22)		(94)
DBA/2 x C57BL/6	466 517	90.14	319 448	71.21	384 929	41.33	874 940	92.98
(Expected)				(68)		(41)		(94)

* Expected under the interpretation presented in the text.

Among the critical features observed in these crosses are the following:

1. In F₁ - there is a high degree of dominance of susceptibility in crosses to DBA/2. In similar crosses to DBA/1, dominance is incomplete or lacking.
2. In F₂ - three of the four crosses show a significant drop in incidence from the F₁ figure. The drop is greater in crosses involving C57BL/10 than in the corresponding crosses involving C57BL/6.
3. In the backcross to C57BL (resistant lines), the incidence is extremely low. The frequency of seizures is greater in crosses involving C57BL/6 than in the corresponding C57BL/10 crosses.
4. In the backcross to DBA, the incidence is intermediate between that of the F₁ generation and that of the particular DBA line.

Supplementary results have been obtained by breeding F_3 generations from F_2 individuals which failed to convulse on from 4 to 14 trials. Generations bred from such negative F_2 mice have been obtained in three of the four crosses listed in Table 1: DBA/1 x C57BL/10 (4 matings), DBA/2 x C57BL/10 (2 matings), and DBA/2 x C57BL/6 (1 mating). The incidence of seizures in the progeny resulting from these crosses is shown in Table 2.

Table 2: Convulsive incidence in F_3 mice bred from parents selected as negative in the F_2 generation.

Cross	Mating	Incidence		Possible genotypes		Expected incid.
		N	%	♀	♂	
DBA/1 x C57BL/10	#1	$\frac{11}{36}$	30.56	aaBb	AAbb	29
	#2	$\frac{10}{21}$	47.62	aaBB	AAbb	57
	#3	$\frac{0}{16}$	00	aabb, AAbb	AAbb	00
	#4	$\frac{20}{55}$	36.36	aaBb	AAbb	29
DBA/2 x C57BL/10	#5,6	$\frac{27}{64}$	42.19	AAbb	aaBb	44
DBA/2 x C57BL/6	#10	$\frac{20}{44}$	45.45	AAbb	aaBb	45

In only one of these matings (#3) was the expectation of litters as resistant as their parents realized. It is apparent that the negative (non-convulsing) parents can carry and transmit "convulsing" genes, which recombine in their offspring to result in a generation with a moderately high convulsive incidence.

The following genetic interpretation is proposed, as consistent with the breeding data herein cited:

Two loci, each on a separate chromosome, are involved. Since the two DBA lines were derived from a common stock, as were the two C57BL sublines, the simplest assumption is that the two loci involved are the same, that the susceptible strains are homozygous AABB (audiogenic seizure susceptible) at both loci, and that the resistant strains are aabb. On this assumption, two possibilities remain to account for the differences in incidence between the two susceptible lines, and for the differences obtained in crosses between each of these and the two resistant sublines. These are:

(a) That each of the strains has different alleles at one or both of these loci. Thus, if the genotype of DBA/1 is represented as AABB, that of DBA/2 would be represented as A'A'B'B', A'A'BB, or AAB'B'. A similar situation would obtain for the C57BL sublines.

(b) That the same genes are involved (AABB for DBA/1 and DBA/2; aabb for C57BL/6 and C57BL/10) but that they come to different expression on different genetic backgrounds. Since all four strains differ from each other in many genes, alternative (b) affords the simplest assumption, though (a) is possible, and both may be true. (b) is given some additional support by the fact that no major modifying factors have thus far been detected as segregants in our crosses.

A. In DBA/1 crosses, each gene contributes to the convulsive incidence after a certain threshold is reached.

1. In crosses with C57BL/10, the normal alleles from the C57BL parent compensate for the presence of A alone, but not for B in the homozygous condition. Therefore individuals of genotype AAbb, AaBb, or aaBB do not convulse, but aaBB individuals may. If one of each pair is present (AaBb) the incidence is 57% (F₁ figure). Above this level, each gene increases the seizure risk.

AABB = 81%
 AABb = 69%
 AaBB = 69%
 AaBb = 57%
 aaBB = 57%
 AAbb = 0
 Aabb = 0
 aaBb = 0
 aabb = 0

On this basis, the expected incidence in F₂ would be 40%, which accords well with the observed frequency of 39%. The low incidence in the backcross to C57 would also be explained, since no "one dose" individuals convulse.

In the F₂ generation, those mice selected as negative could have a variety of negative genotypes (AAbb, Aabb, aaBb, aabb), resulting in the different frequencies observed in the four matings of the F₃ generation bred from non-convulsing F₂ parents. For example, if the F₂ male has the genotype AAbb, a cross with a female of genotype aaBb would result in a convulsive incidence of 29%. The frequency obtained in F₃ matings #1 and #4 is close to this figure. The female of mating #3, which had all non-convulsing offspring could have the genotype aabb, Aabb, or AAbb.

2. In crosses of DBA/1 with C57BL/6, the C57BL/6 genes do not compensate for the presence of either A or B. Each "convulsing gene" appears to add to the convulsive risk, in a cumulative manner.

AABB = 81
 AABb = 64
 AaBB = 64
 AaBb = 48 (F₁ figure)
 AAbb = 48
 aaBB = 48
 Aabb = 24
 aaBb = 24
 aabb = 0

This scheme would explain the negligible drop in incidence from F_1 to F_2 (found only in this cross), with only the aabb group showing no tendency to convulse. In the backcross to C57BL, "one dose" individuals show a seizure risk of 24% rather than 0, resulting in a higher frequency than in the corresponding C57BL/10 backcross group.

B. In DBA/2, there is a high degree of dominance of the convulsing genes. One gene pair (BB) appears to be necessary for seizure-susceptibility. The other gene pair (AA or Aa) alone does not result in seizures, but if added to B, increases the incidence.

1. In crosses of DBA/2 by C57BL/10, the C57 alleles compensate for the presence of gene A alone or of Bb alone, but not for the homozygous condition BB. If one of each pair is present, the incidence is 89%.

AABB	= 100
AABb	= 94
AaBB	= 94
AaBb	= 89
aaBB	= 89
Aabb	= 0
Aabb	= 0
aaBb	= 0
aabb	= 0

This formulation is in agreement with the observed drop in incidence in the F_2 generation, with the low frequency in the backcross to C57, and with the incidence in the backcross to DBA, which is higher than that of the F_1 generation.

The F_3 generation bred from negative F_2 parents also shows a convulsive incidence in close agreement with expectation. If the non-convulsing F_2 females have the genotype AAbb, and the male aaBb, their offspring should show a convulsive index of 45%; the actual figure obtained was 42.2%.

2. In crosses of DBA/2 by C57BL/6, the situation is similar, except that (as in the crosses with DBA/1) the C57BL/6 genes compensate less well for the presence of the "convulsing genes." Gene AA or Aa in the absence of B does not result in seizures, but when added to gene B, increases the incidence. However, it appears that the heterozygous condition Bb results in a seizure risk of about 70%, rather than 0 as in the corresponding C57BL/10 cross.

AABB	= 100
AABb	= 95
AaBB	= 95
AaBb	= 90 (F_1 figure)
aaBB	= 90
aaBb	= 70
Aabb	= 0
Aabb	= 0
aabb	= 0

In contrast to the corresponding C57BL/10 cross, the higher incidence observed in the F_2 generation and in the backcross to C57 support this formulation.

The above tentative explanation is in conformity with our extensive breeding results. Although some of the details may be revised, there is no doubt that the four crosses (two susceptible lines by two resistant sublines) give different results, and therefore the total genetic basis for the same phenotype appears to be different in the several lines.

II. LATENCY OF FATAL CONVULSIONS

In addition to the incidence of convulsions, we have found that the latency, i.e., the time intervening between the onset of sound stimulation and the onset of the convulsion, gives an interesting corroborative indication of the degree of susceptibility.

In the less susceptible DBA/1 line, the preponderance of fatal convulsions occur from 41-45 seconds after the onset of stimulation. In the DBA/2 line, this later peak occurs at 35-40 seconds. In DBA/2 mice, more than half of the fatal convulsions show a very short latency - from 6 to 20 seconds. Such "premature" convulsions are rare in the DBA/1 line and in the DBA/1 by C57BL crosses.

In the pure lines, the latency of fatal convulsions is rarely longer than 60 seconds (2% in DBA/1, none in DBA/2). However, in the crosses with the resistant line, in which the susceptibility is reduced, such "delayed" convulsions are more frequent.

The distribution of premature and delayed convulsions and the most frequent latency period in the various groups are summarized in Table 3. The greatest frequency of premature convulsions, coupled with the lowest frequency of delayed seizures, occurs in the backcrosses to DBA, in which the convulsive incidence is highest. These groups also show the earliest peak period. Conversely, in the backcross to C57, the lowest incidence group, the frequency of premature seizures is low and the number of delayed convulsions is highest.

Table 3: Percentage of premature and of delayed fatal convulsions and the most frequent latency period.

	% premature (25 sec.)	% delayed (60 sec.)	Peak period (secs.)
DBA/1	2.2	2.2	41-45
<u>DBA/1 crosses</u>			
F ₁	0.2	16.94	41-50
F ₂	00	19.28	46-55
Ex to DBA/1	1.0	7.85	41-45
Ex to C57	0.6	32.87	46-60
DBA/2	53.2	00	6-10 36-40
<u>DBA/2 crosses</u>			
F ₁	0.9	16.94	41-50
F ₂	10.02	13.35	41-50
Bx to DBA/2	14.23	4.41	36-40
Bx to C57	4.11	25.19	41-50

The distribution of latencies of fatal convulsions is shown graphically in figure 1, which presents the percentage of animals which died at each time interval in the F_1 , F_2 and backcross generations of the cross of DBA/2 by C57BL/6. The F_1 curve resembles a normal curve, with a peak at 41-45 seconds. Since all the F_1 animals are of the same genotype (AaBb), this is according to expectation. In F_2 there is evidence of segregation of high-, medium-, and low-susceptibility animals, with one large group following the F_1 curve, and, in addition, an early and a delayed group. In the backcross to DBA/2 there is a higher frequency of premature convulsions, and the peak of the distribution is at 36-40 seconds. In the backcross to the resistant line the premature group is lacking, and the peak is reached at 46-50 seconds.

The curves of latency in the other crosses show the same trends, except that in the DBA/1 crosses there are no premature seizures.

III. METABOLIC CONCOMITANTS.

Many of these have been reported and most are summarized in the A.R.N.M.D. report. In general, the most significant findings have been that some substrates related to the tricarboxylic acid cycle have marked effects upon the seizures when administered parenterally in neutral solution, while others have no effect whatever. Of those having effects, some enhance, while others depress the incidence of seizures. The effects are, in some cases, either ameliorated or obliterated by metabolic competitors (usually structural analogues) of the particular substrates. In most cases the actions are strain specific. Substances enhancing seizure susceptibility in DBA/1 mice, for example, have no effect on C57BL mice, although the seizure incidence of the latter may be increased by other means. Mono-sodium glutamate, which exerts a strong protective effect against seizures in DBA/1 mice and protects weaning age males more effectively than females, has a lesser effect on DBA/2 animals. Moreover, whereas in DBA/1 the effect of glutamate decreases with repeated doses, the converse is true in DBA/2, where the maximal effect is obtained only after a repeated series of administrations of the agent. Not only is the response to this (and other) substances different in the two susceptible strains, but the mechanism of adaptation which counteracts a cumulative effect of glutamate in the DBA/1 line, does not exist at all in the DBA/2's.

Whether these differences argue for differences in the major alleles correlated with seizure susceptibility, or merely for differences in the total genotype, is not decided by these experiments.

Fig. 1: Latency of fatal convulsions in F₁, F₂, and backcross generations of DBA/2 by C57BL/6 crosses.

30

25

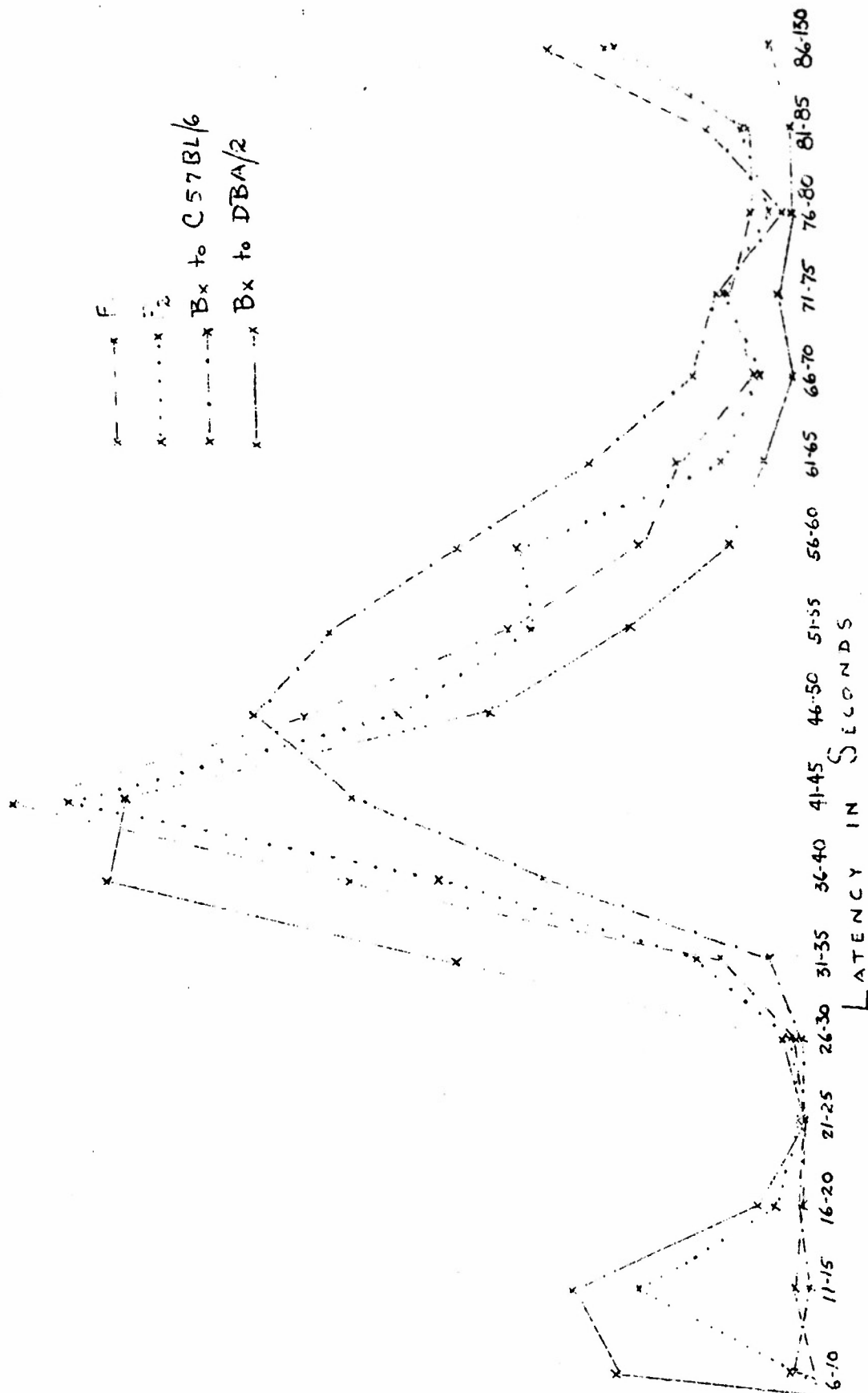
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PERCENT OF ANIMALS WHICH DIED



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