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Selective Breeding for Acoustic Priming

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Data on eight generations of selective breeding for acoustic priming efficacy are reported. The realized heritability of this trait is approximately 0.2–0.3, indicating that the trait is determined in part by genetic factors. Animals selectively bred for acoustic priming do not differ in terms of first-trial (i.e., non-priming-induced) audiogenic seizures. These data suggest that acoustic priming and first-trial audiogenic seizures are controlled by different genetic mechanisms.

KEY WORDS: audiogenic seizures; mice; acoustic priming; genetics.

INTRODUCTION

Audiogenic seizures are a series of psychomotor reactions exhibited by some animals in response to an intense acoustic stimulus. Susceptibility to audiogenic seizures in mice (Hall, 1947; Ginsburg and Miller, 1963; Schlesinger *et al.*, 1966), in rats (Maier, 1942), and in rabbits (Nellhaus, 1963) has long been known to depend on the genotype of the animal being tested. Behavior genetic analyses of audiogenic seizure susceptibility have employed strain comparison studies, selective breeding experiments, and single-gene studies. Despite many years of such research, there still exists a good deal of uncertainty concerning the precise mechanisms of inheritance of this behavior. Witt and Hall (1949) and Lehman and Boesiger (1964) have suggested a single-gene model to explain the inheritance of sound-induced convulsions. Fuller *et al.* (1950) and Schlesinger *et al.* (1966) have

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interpreted their data to favor a multiple-factor theory of inheritance. Several investigators have identified single major genes which contribute to the expression of this trait; examples of such findings are those reported by Ginsburg *et al.* (1967) and by Collins and Fuller (1968). Some of these differences in results and interpretation, as suggested by Schlesinger *et al.* (1966) and by Fuller (1975), are probably due to the following factors: the precise age of the animals at the time of testing; the strains of animals used in the experiments; the precise testing procedure used, most especially whether the animals are tested only once or are repeatedly exposed to the acoustic stimulus.

The importance of this last point, i.e., whether animals are given only a single exposure to the seizure-inducing stimulus or are tested repeatedly, was dramatically underscored when Henry (1967) discovered that animals from seizure-resistant strains could be rendered susceptible to audiogenic seizures by previous exposure to an intense acoustic stimulus. Henry (1967) performed this experiment with C57BL/6J mice and termed the phenomenon *acoustic priming*. Since that time, acoustic priming has been demonstrated in CF1 mice (Itturian and Fink, 1967), SJL mice (Fuller and Collins, 1968), and heterogeneous stock animals (HS mice) maintained by the Laboratory for Behavioral Genetics, Boulder, Colorado (Boggan *et al.*, 1971).

It soon became an important question to determine whether acoustic priming efficacy *per se* is determined by genetic factors, and, if this is the case, whether the mechanisms which underlie first-trial susceptibility to audiogenic seizures are independent of those which might account for acoustic priming efficacy. With respect to this question, the following data have been reported: Collins and Fuller (1968) have reported data which suggest that whereas the *asp* locus could account for susceptibility to audiogenic seizures the mechanisms which determine acoustic priming efficacy appear to be more complex, i.e., polygenic. Henry and Bowman (1970) have reported on strain differences in acoustic priming. Fuller (1975) has tested for both audiogenic seizure susceptibility and acoustic priming in animals derived from the thirteenth generation of a randomly bred population of mice (Binghamton HET mice). In this experiment, the familial distribution of spontaneous audiogenic seizures and priming-induced audiogenic seizures was such as to suggest that the two variables were inherited independently; a strong genetic influence on priming-induced audiogenic seizures was evident.

In this report, we wish to present data derived from a selective breeding program designed to produce lines of animals which differ in susceptibility to priming-induced audiogenic seizures. In addition, animals selectively bred for acoustic priming efficacy were tested for spontaneous audiogenic

seizure incidence to determine whether or not these two processes were mediated by a common genetic mechanism.

METHODS

Subjects

Twenty mating pairs of heterogeneous stock (HS) mice were obtained from the Institute for Behavioral Genetics, University of Colorado, Boulder, Colorado. The origin of this population of animals has been described by McClearn *et al.* (1970). The offspring of these mating pairs constituted the base population from which successive selected generations were obtained. Animals were individually selected for mating on the basis of their different seizure severity scores. Approximately equal numbers of males and females were used in all seizure tests, and no animal was used more than once in any experiment.

During the course of this experiment, the animals were maintained under standard conditions of temperature (74°F ± 3°) and controlled lighting (12-hr light cycle, 7 a.m. to 7 p.m.), with *ad libitum* access to Purina Mouse Breeder Chow and tapwater.

Testing Procedures

Three experiments were performed: (1) Animals were primed at 19 days of age and retested at 22 days of age. (2) Selectively bred animals were primed at 19 days of age and retested at 27 days of age. (3) Nonprimed selectively bred animals were tested for audiogenic seizures at 21 days of age.

Priming Procedure

At 19 days of age, the animals were removed from their home cages one at a time and placed into a large chromatography jar, 45.5 cm in height and 29.5 cm in diameter. After a 10-sec adaptation period, an electric bell mounted over the chromatography jar was sounded for 60 sec. This 5-inch electric bell delivered approximately 116 + 3 db of noise, as measured at the level of the mouse. During this period, the animals were observed and records were made of the incidence of wild running, clonic, tonic, and lethal seizures. After being primed, the animals were returned to their home cages.

Retest Procedure

At age 22 or 27 days, animals were again removed from their home cages one at a time and replaced into the chromatography jar. The mice

were allowed to adapt for 10 sec and the bell was then sounded for 60 sec. During this period, the animals were observed and records were made of the incidence of wild running, clonic, tonic, and lethal seizures. For the purposes of statistical analyses, each mouse was given a seizure severity score based on its response: no response, 0; wild running, 1; clonic seizure, 2; tonic seizure, 3; lethal seizure, 4.

Since the animals with seizure severity scores of 4 had to be used for breeding purposes, lethal seizures were not in fact allowed to develop. In order to save these animals, they were removed from the chromatography jar after the tonic phase of the seizure and immediately upon relaxation of the pinnae. This behavior signals the onset of the respiratory failure characteristic of the lethal phase of an audiogenic seizure. When removed from the jar, these animals were revived by artificial respiration; nevertheless, they were assigned a seizure severity score of 4. We were successful in saving approximately 90% of these animals.

Upon completion of the retesting procedure, the animals were weaned and placed into separate cages depending on the litter from which they came, their sex, and their seizure severity score.

Mating Procedure

Only those mice primed at 19 days of age and retested when 22 days of age were used for matings. Three lines were selected: a *high line* selected for maximum seizure severity scores, a *low line* for minimum seizure severity scores, and an *unselected line* as a control group for the two selected lines. High-line matings were set up using only the offspring of the previous generation's high line which had obtained seizure severity scores of 4. Low-line matings were set up in the same way; only animals with scores of 0 were used. The control line was randomly mated from the offspring of the previous generation's unselected line.

RESULTS

The data obtained for eight generations of selective breeding for acoustic priming efficacy are summarized in Fig. 1 and Table I. Figure 1 presents the average seizure severity scores obtained in 22-day-old mice which had been primed at 19 days of age for unselected control animals, animals selectively bred for low seizure severity scores, and animals selectively bred for high seizure severity scores. Table I summarizes the same data, and includes the standard deviations associated with each of these averages and the number of animals in each line tested in each generation.

Inspection of Fig. 1 indicates that the greatest change in the means of

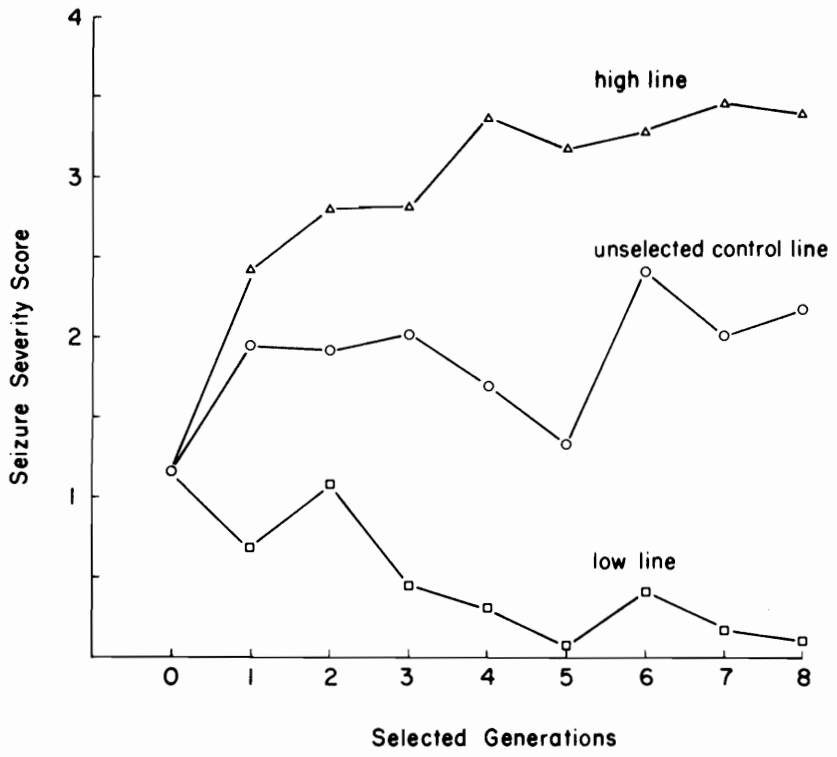

Fig. 1. Acoustic-priming-induced seizure severity scores as a function of selectively bred generations.

the seizure severity scores in both selected lines occurred in the first generation of selective breeding. In the line bred for high seizure severity scores, the average phenotypic scores did not change significantly after four generations; the average phenotypic scores in the low line did not change after five generations of selective breeding.

The data obtained in these experiments were used to calculate the heritabilities (h^2) associated with this phenotype in these animals. Heritabilities were calculated separately for the high and low lines, and for the divergence between the two lines, after five and eight generations of

Table I. Seizure Severity Scores During Eight Generations of Selective Breeding

Generation	Average seizure severity scores + 1 SD[a]		
	Unselected line	High line	Low line
Base population	1.42 + 1.56 (86)	—	—
First selected generation	1.96 + 1.62 (76)	2.42 + 1.58 (47)	0.74 + 1.07 (42)
Second selected generation	1.94 + 1.68 (52)	2.80 + 1.47 (35)	1.16 + 1.46 (36)
Third selected generation	2.01 + 1.72 (51)	2.83 + 1.62 (48)	0.43 + 0.92 (44)
Fourth selected generation	1.73 + 1.80 (48)	3.40 + 1.30 (27)	0.32 + 0.69 (55)
Fifth selected generation	1.31 + 1.68 (53)	3.22 + 1.22 (46)	0.09 + 0.29 (43)
Sixth selected generation	2.49 + 1.57 (53)	3.30 + 1.31 (49)	0.35 + 0.87 (54)
Seventh selected generation	2.03 + 1.61 (57)	3.48 + 1.13 (62)	0.17 + 0.52 (47)
Eighth selected generation	2.20 + 1.60 (44)	3.30 + 1.31 (51)	0.15 + 0.58 (40)

[a] Numbers in parentheses indicate the number of mice tested per generation.

Table II. Seizure Severity Scores at Two Priming–Retest Intervals

Group	Priming–retest ages (days)	*N*	Seizure severity scores ± 1 SD
High line	19–22	62	3.48 ± 1.13
High line	19–27	70	3.40 ± 0.99
Low line	19–22	47	0.17 ± 0.52
Low line	19–27	10	0.00 ± 0
Unselected line	19–22	57	2.03 ± 1.61
Unselected line	19–27	11	1.64 ± 1.12

selective breeding. For the high line, these estimates of h^2 were 0.28 ± 0.042 and 0.22 ± 0.029 after five and eight generations, respectively; in the low lines, these estimates were 0.32 ± 0.062 and 0.29 ± 0.037 after five and eight generations, respectively. Heritabilities calculated from the divergence scores yielded estimates of 0.25 ± 0.044 and 0.19 ± 0.029 after five and eight generations, respectively.

Animals derived from the seventh and eighth generations were also tested for audiogenic seizures after a longer priming–retest interval (priming at 19 days of age and retest at 27 days of age). These data, in comparison to those obtained with the shorter priming–retest interval, are summarized in Table II. The scores of the mice primed at 19 days and retested at 22 days were not significantly different from those primed at 19 days and retested at 27 days (high line: $t = 0.29$, df = 80, not significant; low line: $t = 1.03$, df = 55, not significant; unselected line: $t = 0.76$, df = 66, not significant).

Animals derived from the seventh and eighth selected generations were also tested for audiogenic seizures at 22 days of age without having previously been exposed to a priming stimulus. Average seizure severity scores obtained on mice of the high line were 0.04 ± 0.002 ($N = 70$), low line 0.00 ± 0 ($N = 25$), unselected line 0.00 ± 0 ($N = 31$). These data indicate quite clearly that these animals are not susceptible to audiogenic seizures without first having been exposed to a priming stimulus.

DISCUSSION

Acoustic priming is a procedure which renders mice from seizure-resistant genotypes susceptible to sound-induced seizures by exposing them to a loud acoustic stimulus during some critical period of neural development. The convulsions observed in animals which are genetically susceptible to audiogenic seizures and those observed in animals which have been acoustically primed are phenotypically identical, at least insofar as their overt behavior is concerned. For this reason, it is possible to think of

acoustic priming as a method which produces a phenocopy of susceptibility to audiogenic seizures in animals which are normally resistant to this method of seizure induction. Acoustic priming *per se* is of considerable interest for several reasons, not the least important of which is the fact that it is possible to conceptualize acoustic priming as an instance of neural plasticity. Understanding some of the mechanisms which mediate acoustic priming might therefore provide important insights into variables which underlie other instances of neural plasticity.

Several experiments mentioned briefly in the introduction to this report have suggested that acoustic priming efficacy is under genetic control. Until recently, this interpretation was based on the observations made in several laboratories that animals from some strains can be primed more readily than mice of other genotypes. The familial distribution of acoustic priming efficacy observed in animals of a heterogeneous stock has led to the same conclusion. Finally, Fuller (1975) has reported that after two generations of selective breeding for acoustic priming, animals selected for a high incidence of priming-induced convulsions showed a seizure incidence of 92.5%, whereas animals selected for a low incidence of priming-induced convulsions showed only a 3% incidence of audiogenic seizures.

Herein we have reported on the results of a bidirectional selective breeding experiments for acoustic priming efficacy carried out for eight generations. Simultaneously, an unselected control line was maintained and tested. The phenotype, on the basis of which animals were selected for mating, was the severity of the priming-induced response, which varies between 0 (no response) and 4 (a lethal response); animals which showed signs of a lethal seizure were given artificial respiration, saved, and used as breeders. Brother by sister matings were not used.

After eight generations of selective breeding, two lines have been established which differ significantly in terms of acoustic priming efficacy. It seems unlikely that environmental fluctuations could account for these differences in acoustic priming efficacy since both the high line and the low line differ from the unselected control animals which were tested simultaneously. It also seems unlikely that the differences in response of the two lines could be due to inbreeding since brother by sister matings were not used. Further, the number of offspring per litter was counted in both of the selected lines and in the control line in every generation. No differences in the number of pups per litter were observed either among the three lines or in any of the lines as a function of the generation. Since this measure shows inbreeding depression in both rabbits and mice (e.g., Chai, 1969; Roberts, 1960), it seems fair to conclude that inbreeding was not a significant problem in this experiment.

These data can be interpreted as additional evidence to indicate that

acoustic priming efficacy is, in part, controlled by genetic factors. Our estimates of heritability suggest that between 20 and 30% of the variability in response to acoustic priming in these animals can be accounted for by genetic factors. In addition, it is important to point out that the response to acoustic priming in both lines did not differ regardless of whether the priming–retest interval was 3 or 7 days. These data suggest that this variable is not important in determining the differences in the two selected lines.

On the other hand, our data indicate that the genetic mechanisms which underlie priming-induced susceptibility to sound-induced convulsions and genetic susceptibility to audiogenic seizures are mediated by different genetic mechanisms. In our tests, the lines selected for high and low priming-induced convulsions did not differ significantly in audiogenic seizure incidence. These data are in good agreement with those reported by Fuller (1975), who found familial distributions of priming-induced and spontaneous audiogenic seizure incidence sufficiently different to lead him to the conclusion that different genes mediate these two types of behavior.

We are continuing these breeding experiments; however, we have sufficient material available so that we would be happy to send mating pairs of our high and low lines to laboratories interested in acoustic priming.

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