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Review

A genetic context for the study of audiogenic seizures

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ABSTRACT

Here, the genetic context for the study of audiogenic seizures is four single-gene, spontaneous mutations that occurred in the Behavior Genetics Laboratory at the University of Chicago from 1959 to 1969. Three of these increased the incidence of audiogenic seizures, and one of these decreased the incidence of audiogenic seizures. The genetics of one of these mutants is described in detail, and the effect of diet on the same mutant is also described in detail. Research on genetic and environmental effects on the cortical EEG of audiogenic seizures is reviewed; this research included two of these mutants. The cortical EEG associated with audiogenic seizures in this study was consistent with audiogenic seizures being a type of brain stem epilepsy as had been proposed by others. Also, I proposed that brain stem pathophysiology is the same regardless of the genetic or environmental pathway to audiogenic seizure susceptibility. Research is also reviewed using these mutants to determine whether or not a strain association between glutamic acid decarboxylase (GAD) activity in whole brain and susceptibility to audiogenic seizures is pleiotropic and whether or not a strain association between nucleoside triphosphatase (NTPase) activity in the granule cell layer of the dentate fascia of the hippocampus and susceptibility to audiogenic seizures is a lineal or collateral pleiotropy. Lastly, pleiotropy as an explanation for strain comorbidities in aggressive behavior and audiogenic seizures is considered.

This article is part of a Special Issue entitled "Genetic and Reflex Epilepsies, Audiogenic Seizures and Strains: From Experimental Models to the Clinic".

1. Genetics and audiogenic seizures

There has long been an interest in genetic aspects of audiogenic seizures. Selective breeding has been used to establish lines of rats [1] or mice [2,3] differing genetically in susceptibility to audiogenic seizures. Crossbreeding of inbred strains of mice differing in susceptibility to audiogenic seizures has been used in the search for the individual genes with effects on this trait. Many of these studies used the susceptible DBA/1 and DBA/2 versus resistant C57BL/6 and C57BL/10 inbred strains [4–8]. Here, I focus on my research with single-gene, spontaneous mutants that occurred in inbred strains of mice. Three of these increased susceptibility to audiogenic seizures, and one of these decreased susceptibility to audiogenic seizures. These mutants are described and are used in genetic analyses of the development and physiology of audiogenic seizures. These mutants provide a genetic context for the study of audiogenic seizures.

Some of the reviewed research with these mutants has never been published in the primary literature. Methods are given for these. Others have been published in the primary literature; the methods can be found in these publications.

2. Single-gene mutants with effects on audiogenic seizures

In the C57BL/6 inbred strain, resistance to audiogenic seizures develops with age. According to Frings et al. [9], mice of the C57BL/6 strain are susceptible to audiogenic seizures from 20 to 25 days *postpartum*. According to Witt and Hall [4] and to Ginsburg and Miller [6], mice of the C57BL/6 strain are highly resistant to audiogenic seizures at 28 to 35 days *postpartum*. However, in the colony of Dr. Dorthea Miller at the University of Chicago, some C57BL/6 mice were susceptible to audiogenic seizures. This colony had been exposed to radiation, and Miller suggested that there had been a radiation-induced mutation that affected audiogenic seizure susceptibility.

To test Miller's hypothesis, I selectively bred lines for high and low audiogenic seizure susceptibility. The selective breeding began with the progeny from four mated pairs in Miller's C57BL/6 colony. After nine generations of selective breeding, eighty to one hundred percent of mice in the four high lines were audiogenic seizure-susceptible whereas 20% of the mice in the low line were audiogenic seizure-susceptible. Susceptible mice had at least one clonic–tonic seizure on four consecutive test days. Tests began at 28 days of age. The rapid response to selection was consistent with the hypothesis that a single-gene radiation-induced mutation with effects on susceptibility to audiogenic seizures had occurred in the C57BL/6 colony of Dr. Dorthea Miller. The hypothesis was critically tested by making Mendelian crosses

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between mice of the selected high line with 100% audiogenic seizure susceptibility (C57BL/6s) and mice of the C57BL/6 strain.

2.1. Methods

The chamber used to test for audiogenic seizures consisted of an isolated closed box of 17″ × 15″ × 17″. The top of the chamber had a large window in it consisting of several layers of glass. An Edwards No 13, Lugen bell was used as the sound stimulus. This produced an average of 94 to 105 dB white noise at the floor level of the chamber. The same stimulus was used in all experiments described in this paper, except that on the cortical EEG. For this, a hand-rung bell was used.

For this genetic analysis, reciprocal F1, F2, BX to C57B/6s, and BX to C57BL/6 were derived from the initial reciprocal crosses of C57BL/6 and C57BL/6s strains. Both male and female mice were tested.

For this study, the mice were tested once a day for four consecutive days beginning at 30 to 32 days of age (N = 1167). They were placed in the testing chamber 1 min before the sound was turned on. They were exposed to the sound for 2 min or until a clonic–tonic convulsion occurred. Only clonic–tonic convulsions were observed. The nomenclature for audiogenic seizures used throughout this paper is that of Frings et al. [10]. All mice that had a clonic–tonic seizure received artificial respiration, since otherwise they died.

Mice were scored as susceptible to audiogenic seizures if they had at least one clonic–tonic seizure over the four test days.

2.2. Results and discussion

There were no sex differences for any population; male and female data were thereby combined for the genetic analyses. There were also no differences between any pair of reciprocal populations; data for a pair of reciprocal populations were combined. Since there were no sex differences, and since there were no differences between any pair of reciprocal population, the mutant gene could not be located in the mitochondrial DNA, be on the Y chromosome or be on the X chromosome, and must be on one of the 22 autosomes. Also, the mutant gene effects would not be mediated by prenatal or postnatal maternal environment.

The data for the C57BL6, C57BL6s, and F1 mice and for the segregating F2, BX to C57BL/6, and BX to C57BL/6s are shown in Fig. 1. The data for the segregating populations are consistent with effects of a single autosomal gene mutant.

The predicted incidence of susceptible mice in the segregating populations was compared with the observed incidence by Chi-square test.

2.3. Comment

Between 1960 and 1969, three more single-gene mutations with effects on audiogenic seizure susceptibility occurred in the mouse colony of the Behavior Genetics Laboratory at the University of Chicago.

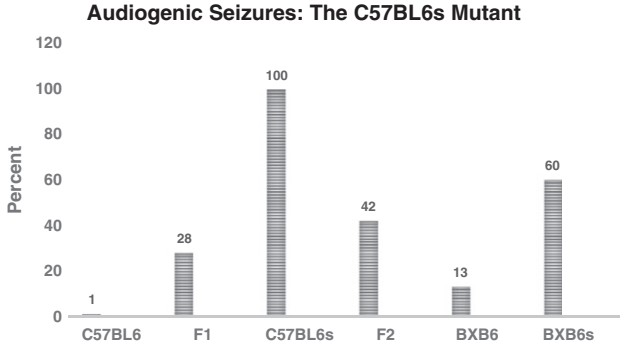

Fig. 1. C57Bl/6s mutant. Percent clonic–tonic seizures in C67BL/6, C57BL/6s, their F1, F2, BX to C57BL6, and BX to C57BL6s.

One of these occurred in C57BL/6 mice that were homozygous for the white-belly allele of the agouti gene. This was designated as C57BL/6A^{w},*s2*. Another occurred in C57BL/10 mice that were homozygous for the black and tan allele of the agouti gene. This was designated as C57BL/10a^{t},*sas*. Genetic analyses similar to these indicated that each mutant was autosomal and that each mutant was not closely linked to the agouti gene on chromosome 2. The genetic analyses for the mutant in the C57BL10 mice carrying black and tan were published by Maxson et al. [11]. This strain also carried a spontaneous seizure mutant (designated as *sps*). The fourth mutant occurred in the DBA/1 strain. This was designated as DBA/1-*asr*. This mutation reduced substantially the audiogenic seizure susceptibility of mice of the DBA/1 inbred strain; this reduction was not due to hearing loss [12–14].

3. C57BL6 inbred strain, diet, and susceptibility to audiogenic seizures

Hoag and Dickie [15] reported that on the Rockland Mouse Breeder Chow, mice of the C57BL/6J and DBA/2J inbred strains have an increased number of young surviving to four weeks of age. On this diet, females of the DBA/2 inbred strain also had heavier progeny at the four-week weaning age. In August 1959, all colonies at the Jackson Laboratory were switched to this diet. Because of the advantage reported by Hoag and Dickie [15], and in order to maintain standard dietary conditions, the mouse colony in the Behavior Genetics Laboratory of the University of Chicago was switched in January 1961 from Purina Laboratory Chow to the Rockland Mouse Breeder Chow. This dietary change occurred while I was selectively breeding for high and low lines of audiogenic seizure-susceptible mice described above. It appeared that mice of the four high lines had much lower levels of audiogenic seizures on the Rockland Breeder Chow than on the Purina Laboratory Chow. This observation was tested in the following experiment.

3.1. Methods

Mice from the C57BL/6s strain were maintained on either Purina Laboratory Chow or Rockland Mouse Breeder Chow. There were 53 males and 53 females for the Purina Laboratory Chow and 67 males and 62 females for the Rockland Mouse Breeder Chow tested for audiogenic seizure susceptibility at 30 and 31 days *postpartum* as described in the genetic study. Mice with a clonic–tonic seizure were scored as susceptible.

The effect of the diet on audiogenic seizure susceptibility was tested with the Npq method for the significance of differences between proportions.

3.2. Results

The data are shown in Fig. 2. On the first test day, males but not females had significantly ($p = 0.005$) lower proportion of audiogenic seizure-susceptible mice on Rockland Mouse Breeder Chow than on the Purina Laboratory Chow. On the second test day, males and females had significantly ($p = 0.0001$) lower proportion of audiogenic seizure-susceptible mice on Rockland Mouse Breeder Chow than on the Purina Laboratory Chow. However, there was no effect of this dietary difference on the proportion of audiogenic seizure-susceptible mice in DBA/1, DBA/2, and Rb1 inbred strains.

3.3. Discussion

There are several implications of these findings. First, genetic effects on audiogenic seizures depend on environmental conditions. Second, diet is now known to be one of the environmental influences on audiogenic seizures. For example, there is evidence that audiogenic seizure susceptibility is greater on soy protein-containing diet than on a casein