Genetic Epilepsy Model Derived From Common Inbred Mouse Strains

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Manuscript received March 30, 1994
Accepted for publication June 18, 1994

ABSTRACT

The recombinant inbred mouse strain, SWXL-4, exhibits tonic-clonic and generalized seizures similar to the commonest epilepsies in humans. In SWXL-4 animals, seizures are observed following routine handling at about 80 days of age and may be induced as early as 55 days by rhythmic gentle tossing. Seizures are accompanied by rapid, bilateral high frequency spike cortical discharges and followed by a quiescent post-ictal phase. Immunohistochemistry of the immediate early gene products c-Fos and c-Jun revealed abnormal activation within cortical and limbic structures. The seizure phenotype of SWXL-4 can be explained and replicated fully by the inheritance of susceptibility alleles from its progenitor strains, SWR/J and C57L/J. Outcrosses of SWXL-4 with most other common inbred strains result in F_1 hybrids that have seizures at least as frequently as SWXL-4 itself. Quantitative trait locus mapping reveals a seizure frequency determinant, *Szf1*, near the pink-eyed dilution locus on chromosome 7, accounting for up to 32% of the genetic variance in an F_2 intercross between SWXL-4 and the linkage testing strain ABP/Le. These studies demonstrate that common strains of mice such as SWR and C57L contain latent epilepsy susceptibility alleles. Although the inheritance of susceptibility may be complex, these results imply that a number of potentially important and practical, noninvasive models for this disorder can be constructed and studied in crosses between common mouse strains.

"IDIOPATHIC" epilepsies have no specifically identified causative factors, and have long been suspected to have a genetic basis (ANDERMAN 1982). Only a few human primary epilepsy genes have been mapped to date (DELGADO-ESCUETA *et al.* 1994; LEHESJOKI *et al.* 1991; LEPPERT *et al.* 1989) and none of these genes have been cloned. Further progress in identifying epilepsy genes is generally hampered by problems commonly encountered in human genetics, that is, small family size, heterogeneous phenotypes, overlapping clinical classifications, variable expressivity, and multifactorial inheritance (ANDERMAN 1982; ANDERSON *et al.* 1986; WHITEHOUSE *et al.* 1993).

The study of genetic models of epilepsy in animal systems overcomes many of the obstacles encountered in humans. Both rat and mouse genetic epilepsy models have been identified (BUCHHALTER 1993; NOEBELS 1986; SEYFRIED *et al.* 1986). Genetically simple models of primary generalized epilepsy in mice are represented by several single gene neurological mutations including *stargazer* (NOEBELS *et al.* 1990), *tottering* (NOEBELS and SIDMAN 1979) and *lethargic* (HOSFORD *et al.* 1992). Genetically complex mouse models for epilepsy include the audiogenic-induced seizures of the DBA and C57BL/6J × DBA/2J (BXD) recombinant inbred (RI) strains (COLLINS 1972; NEUMANN and COLLINS 1991; SEYFRIED 1983), and the handling-associated seizures of the EL inbred mouse strain (IMAIZUMI *et al.* 1959). EL mice[1] exhibit a phenotype that has been compared to partial complex seizures with secondary generalization in humans (SEYFRIED *et al.* 1992). Seizures occur with routine handling at about 80–100 days of age, and are inducible earlier by rhythmic stimulation, that is, repeated gentle tossing. EL seizures reportedly initiate in the parietal cortex, then spread to the hippocampus from which they generalize to other brain areas (ISHIDA *et al.* 1993). A number of biochemical studies have revealed elevated brain levels of acetylcholine and γ-aminobutyric acid (MURASHIMA and SUZUKI 1989) and decreased ammonia, glutamate, glutamine, and aspartate in EL mice (FLAVIN *et al.* 1991; HIRAMATSU *et al.* 1989; SEYFRIED *et al.* 1992). There is also tissue damage in older EL mice, as evidenced by hippocampal gliosis without accompanying neuronal loss (BRIGANDE *et al.* 1989).However, it is unclear whether a causal relationship exists between any of these findings and seizure susceptibility. The genetic basis of epilepsy in EL mice is multigenic, as at least two dominant genes, *El1*, chromosome 9, and *El2*, chromosome 2, contribute significantly to seizure frequency in crosses between EL and ABP or DBA/2 strains (RISE *et al.* 1991).

Here we report that the SWXL-4 strain, one of six extant RI strains derived in the 1970s from an F_2 cross between the inbred strains SWR/J and C57L/J (TAYLOR 1976, 1989) display seizures very similar in phenotype and mode of induction to those of EL mice. Because SWXL-4 is an RI strain and thus contains a generally random assortment of alleles from both progenitor strains, we examined whether the heritability was due to combinations of susceptibility alleles from progenitor

[1] We note that EL/Suz is the same strain as "El" or "E1" described previously in the literature. However, as with all mouse strains the designation EL/Suz is consistent with the rules of the International Committee on Laboratory Animal Nomenclature, with the strain symbol in caps and substrain designation attached.

strains SWR and C57L or a new mutation in the SWXL-4 stock itself.We also determined whether this strain could be used to identify quantitative trait loci (QTL) influencing seizure frequency.

MATERIALS AND METHODS

Mice: Mice used in this study, SWXL-4/Ty (SWXL-4), SWR/J (SWR), C57L/J (C57L), and ABP/LeJ (ABP), were maintained as inbred strains at The Jackson Laboratory by brother-sister mating. (SWXL-4 × ABP)F_1 or (ABP × SWXL-4)F_1 males or females (SxAF1) were backcrossed with ABP females or males, respectively, to obtain SxAxA backcross mice. (ABP × SWXL-4)F_1 males and females were intercrossed to produce the AxSF2 generation. (C57L × SWR)F_1 (LxSWF1) males and females were intercrossed to produce the LxSWF2 generation. Mice were raised on either Wayne or 96W/Old Guilford grain, depending on breeding success. The Jackson Laboratory is fully accredited by AAALAC (American Association for the Accreditation of Laboratory Animals Care). All procedures conformed to guidelines for humane treatment of animals, and were approved by the ACUC (Animal Care and Use Committee) according to NIH procedures.

Seizure tests: Mice were weaned at 21 days, with males and females housed separately. At 30 days of age, mice were subjected to rhythmic gentle tossing, as described previously for the EL inbred strain with minor modifications (Flavin *et al.* 1991; Rise *et al.* 1991). This procedure was standardized to consist of ~1.0-cm vertical displacement at a rate of 256 cycles/minute on a quiet, mechanically driven plastic mouse box. For convenience, up to six animals were tested simultaneously. Tests lasted for no more than 30 sec. Mice were scored as either plus or minus for the full seizure phenotype described in RESULTS and were removed from the box when they developed seizures. Testing was done once every 3 days for 60 days. This seizure testing regimen was chosen empirically. Tests were begun at 30 days of age for comparison with previous studies involving the EL strain. A "window" of seizure threshold assessed by the 20 tests was chosen for statistical rigor–to assay seizure frequency as a reliable trait, rather than one seizure episode. The seizure frequency of animals tested for longer periods overlaps with that of mouse strains that do not seize spontaneously, such as ABP or C57L.

Genetic typing: Genomic DNA was prepared from mouse tail tips using a modified salt-out procedure as described by Taylor *et al.* (1993). Backcross and intercross progeny were typed by the polymerase chain reaction (PCR) using SSLP (simple sequence length polymorphism) markers purchased from Research Genetics, Inc., according to Dietrich *et al.* (1992). Markers were radiolabeled with ^{32}P, either by oligonucleotide 5′ end-labeling or direct incorporation into the PCR reaction, and PCR products were electrophoresed on denaturing polyacrylamide gels. Gels were dried and exposed to X-ray film for 4–24 hr.

QTL analysis: Genotype and trait data were entered and stored using Excel (Microsoft, Inc). MAPMAKER/QTL was used as previously described for interval mapping (Lander *et al.* 1987; Paterson *et al.* 1988). The lod score corresponding to a $P < 0.05$ level of significance for each cross (generally assumed to be lod 3.0) was determined by simulation (Efron and Tibshirani 1993): 200 mock seizure frequency datasets were made using the random number generator of Excel, with the same mean and standard deviation as the actual datasets, MAPMAKER/QTL was run once for each "phenotype" throughout the entire genome in the SxAxA cross or the mapped intervals in the AxSF2 cross, and the frequency of lod scores recorded. A lod score of 3.0 or higher was observed 10 times in each cross, indicating it as the $P < 0.05$ level for these crosses. Student's t tests and nonparametric χ^2 tests were carried out using Excel; for the latter, we analyzed all SxAxA animals that had a seizure frequency or onset at least as extreme as either SWXL-4 or SxAF1. To adjust seizure frequency differences between males (0.36) and females (0.29) in the SxAxA cross prior to MAPMAKER/QTL analysis, the mean difference (0.07) was added to females values (see Figure 5A). A similar result was obtained when subtracting the difference from the male values, or with the proportional difference (1.22) used as a factor to adjust the female values (data not shown).

Electroencephalography (EEG): Chronic electrocorticographic (EEG) recordings in freely moving mice were obtained using subdural silver wire electrodes implanted bilaterally through small cranial burr holes under tribromoethanol (Avertin) (0.02 ml/g) i.p. anesthesia (Noebels and Sidman 1979). Electrodes were soldered to a microminiature plug that could be subsequently connected to the preamplifiers of a Grass model 6 encephalograph. Daily monitoring was performed in the home cage with visual correlation of EEG activity.

Immunohistochemistry: Mice were anesthetized with tribromoethanol and perfused transcardially with 0.1 M phosphate buffer (PB) (pH 7.4) and 4% paraformaldehyde in 0.1 M PB, respectively. Brains were removed, fixed for 24 hr, and immersed in 30% sucrose in 0.1 M PB for 5 days. Horizontal sections were cut at 40 μm on a freezing microtome. The tissue slices were pretreated in 0.03% H_2O_2 in absolute methanol followed by 2% bovine serum albumin (BSA) in 0.1 M phosphate-buffered saline (PBS) for 3 hr. Free-floating slices were incubated at 4° with c-Fos polyclonal (sheep) antibody (Cambridge) 1:400 in 1% BSA in PBS or c-Jun polyclonal (rabbit) antibody (Oncogene Science) 1:400 in 1% BSA in PBS. Sections were then incubated for 60 min with secondary biotinylated (rabbit anti-sheep) antibody (Jackson ImmunoResearch, 1:100; for c-Fos, goat anti-rabbit antibody, Vector Laboratories, 1:100) followed by an avidin-biotin-horseradish peroxidase (HRP) complex. Specific staining was accomplished with the addition of 3,3′-diaminobenzidine (DAB) and hydrogen peroxide to form the DAB substrate (Vectastain kit, Vector Laboratories).

RESULTS

SWXL is a set of recombinant inbred (RI) strains produced by brother-sister-inbreeding lines from the F_2 generation of the progenitor strains SWR/J and C57L/J (Taylor 1976). Of seven SWXL strains that survived the initial RI set construction, SWXL-4 was the only strain observed to undergo seizures spontaneously or after routine handling (moving animals from cage to another). In addition, of several RI strains tested, SWXL-4 was the most sensitive to the seizure-inducing compound, isonicotinic acid hydroxide (Taylor 1976).

Seizure description: The behavioral phenotype of SWXL-4 is similar to that of the epileptic EL mouse, proposed as a model for temporal lobe epilepsy or partial complex seizures with secondary generalizations (Brigande *et al.* 1989; Imaizumi *et al.* 1959; Seyfried *et al.* 1992). The SWXL-4 seizure begins with an arrest of normal behavior, accompanied by twitching and lurching forward. The twitching is rapidly followed by a total loss of postural control, with tonic movements starting in the

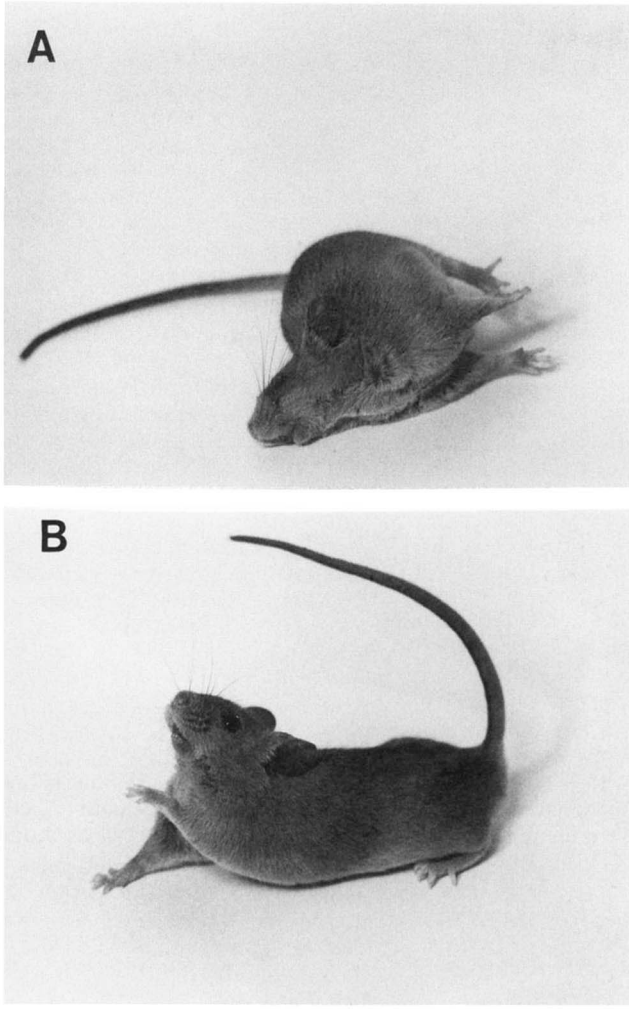

FIGURE 1.—SWXL-4 mouse in seizure. (A) SWXL-4 mouse a few seconds after the start of the seizure, on its right side. Note the loss of postural control, tonic extension of forelimbs and hindlimb, and the dorsoflexion of the neck. (B) SWXL-4 mouse approximately 5 sec later. Note the regain of postural control, tonic extension of the tail, clonic movement of the left forelimb, and salivation.

hindlimbs then progressing to tonic-clonic movements in both limb sets, and mild dorsoflexion of the neck. Postural control is regained, the tail becomes erect and there is flexion of the neck (Figure 1). The mouse then usually exhibits increased clonus in alternating forelimbs, hindlimb clonus and salivation, followed by chewing/grooming automatisms and general hyperactivity. SWXL-4 mice do not respond readily to physical stimuli during the active phase of seizure, which lasts for about 30 sec. An EEG during this period is shown for an (ABP × SWXL-4)F_1 hybrid in Figure 2A (the results for SWXL-4 itself are virtually identical). Except for recurrent seizures, the behavior, breeding habits and lifespan of SWXL-4 appear to be normal.

Seizures are observed during routine handling of SWXL-4 animals at 80–90 days of age. Because it is impractical to measure seizure susceptibility by constant surveillance, seizures in SWXL-4 were induced by rhythmic gentle tossing of younger animals, as done previously for EL mice (FLAVIN *et al.* 1991; RISE *et al.* 1991). The qualitative features of the seizure appear identical, whether they occur spontaneously or following stimulation. The two seizure parameters we examined were number of test stimuli until the first seizure, *i.e.*, *seizure onset*, and *seizure frequency per 20 tests*. There is also a developmental component to seizure induction by gentle tossing: SWXL mice stimulated at 21 days do not seize any sooner than those begun at 30 days. Moreover, as in the case of EL mice and analogous to the audiogenic seizure models, there is a "priming" effect of rhythmic gentle tossing on seizure onset–at least in young mice. In older mice it would be difficult to distinguish priming from the cumulative effects of routine handling. Using our testing procedure, of all common inbred strains and hybrids between common strains, only SWXL-4 (or its progenitors), EL or hybrids involving these strains led to a seizure frequency higher than 0.2, or an onset earlier than 15 tests (our unpublished results).

Localization of seizure activity by EEG and immediate early gene expression: We examined one SWXL-4 and four (ABP × SWXL-4)F_1 animals using cortical EEG recording to determine whether the behavioral seizures were associated with epileptiform discharges. F_1 hybrid mice showed generalized, high frequency synchronous cortical discharges throughout the active phase of the seizure (Figure 2A). Similar discharges were observed during seizures of SWXL-4 mice (data not shown). In both cases, the baseline interictal EEG pattern was normal compared to unaffected mice. In brain sections from these same F1 hybrids, we examined the activity of immediate-early proteins c-Fos and c-Jun (Figure 2B). These nuclear proteins are markers of synchronous neuronal activation in several experimental models of epilepsy (DRAGUNOW and ROBERTSON 1987; LE GAL LA SALLE and NAQUET 1990; MORGAN *et al.* 1987). Regional staining patterns of antibodies to the immediate early gene proteins c-Fos and c-Jun were examined in horizontal brain sections cut from F_1 hybrids approximately 6 hr following repeated seizure episodes. Seizures in F_1 hybrids were specifically associated with increased levels of c-Jun staining of neurons in superficial layers of the neocortex extending into the entorhinal cortex (Figure 2B), when compared with the same genotype in the absence of a prior seizure. Numbers of c-Jun staining neurons in the hippocampus were slightly elevated, particularly in the CA1 region of the pyramidal cell layer. Abnormal patterns of increased c-Fos staining were most prominent in dentate granule cells (Figure 2B). No clear increases were identified within other synaptically linked structures of the hippocampal formation, and no other consistent abnormality in either staining pattern was observed in the remainder of the forebrain.

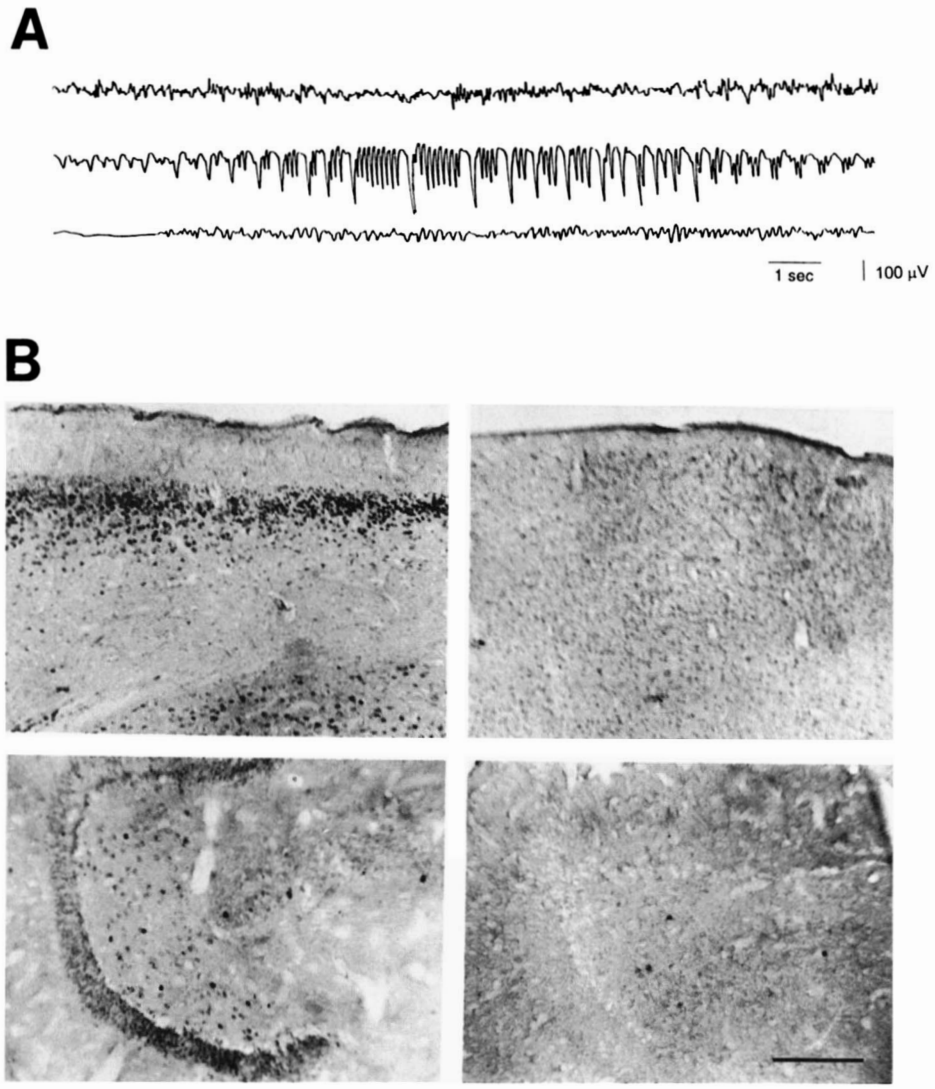

FIGURE 2.—EEG and immunohistochemistry studies of brain sections. (A) Continuous tracing (left-to-right) of monopolar EEG recording showing generalized cortical discharge activity during rhythmic gentle tossing-induced seizure in a single (ABP × SWXL-4)F_1 hybrid mouse. Three other F_1 mice and SWXL-4 gave similar results, but are not shown here. The seizure episodes typically lasted 20–30 sec and were associated with behavioral arrest followed by a complex pattern of ictal behaviors described in the text. (B) Immunohistochemical staining of immediate early gene proteins c-Jun and c-Fos reveal seizure-induced involvement of cortical and limbic structures in (ABP × SWXL-4)F_1 hybrid mice, compared with nonseizure control. *Upper*, dark neuronal nuclei stained by c-Jun antibody are prominent in the superficial pyramidal layers of entorhinal cortex 6 hr after a seizure (left), and absent from unstimulated control (F_1 hybrid, same genotype) brain stained before a seizure (right). *Lower*, a similar pattern of increased c-Fos staining 6 hr after seizure activity (left) reveals activation of granule cells in the dentate gyrus that are unstained in the control mouse brain (right). Calibration bar: 150 μm.

Multigenic origin of epilepsy alleles: Because SWXL-4 is an RI strain and neither other SWXL lines nor SWR and C57L progenitors have frequent, spontaneous or induced seizures, we hypothesized the trait resulted from either a specific combination of progenitor alleles, or a new mutation in SWXL-4 itself. Therefore, we tested various F_1 hybrids of SWXL-4, SWR and C57L, "combining" putative susceptibility alleles from progenitors. SWXL-4 animals had a mean seizure frequency of 0.45 and mean onset of 9.7 tests (Figure 3). In contrast, progenitors SWR and C57L alone had significantly lower mean seizure frequencies and onsets. Although the mean seizure frequency of F_1 hybrids between progenitors was only modestly greater than that of the parental strains, the mean onset of the F_1 hybrid was like that of SWXL-4 itself (Figure 3). These results suggest that dominant or additive alleles from each progenitor are sufficient to achieve the early seizure onset of SWXL-4, but these alleles alone do not fully account for recurrent seizures.

However, both the high seizure frequency and early onset of SWXL-4 were recovered in a significant fraction of LxSWF2 intercross progeny (Figure 3). About one-sixth (14/92) of all F_2 progeny had a seizure frequency the same or higher than the SWXL-4, and about one-third (27/92) of the F_2 progeny had an onset earlier than that of SWXL-4. That the high seizure frequency of SWXL-4 was replicated in F_2 but not F_1 progeny suggests either one or more recessive gene(s) is necessary for recurrent seizures, or dominant SWXL-4 alleles are influenced by complex nonallelic interactions with modifier genes. Also, because fewer than 9/16 of the F_2 progeny had an early onset, it appears that at least two dominant alleles influence this trait. (SWXL-4 × C57L)F_1 or (SWR × SWXL-4)F_1 hybrids exhibited mean seizure frequencies and onsets at least as high as SWXL-4 itself (Figure 3), implying that either progenitor might have additional seizure alleles that were not fixed in SWXL-4.

Mapping epilepsy genes using SWXL-4: We investigated whether SWXL-4 could be used to identify QTL for seizure alleles that vary between common inbred mouse strains. ABP was chosen as a partner strain because (a) it contains six recessive visible mutations as

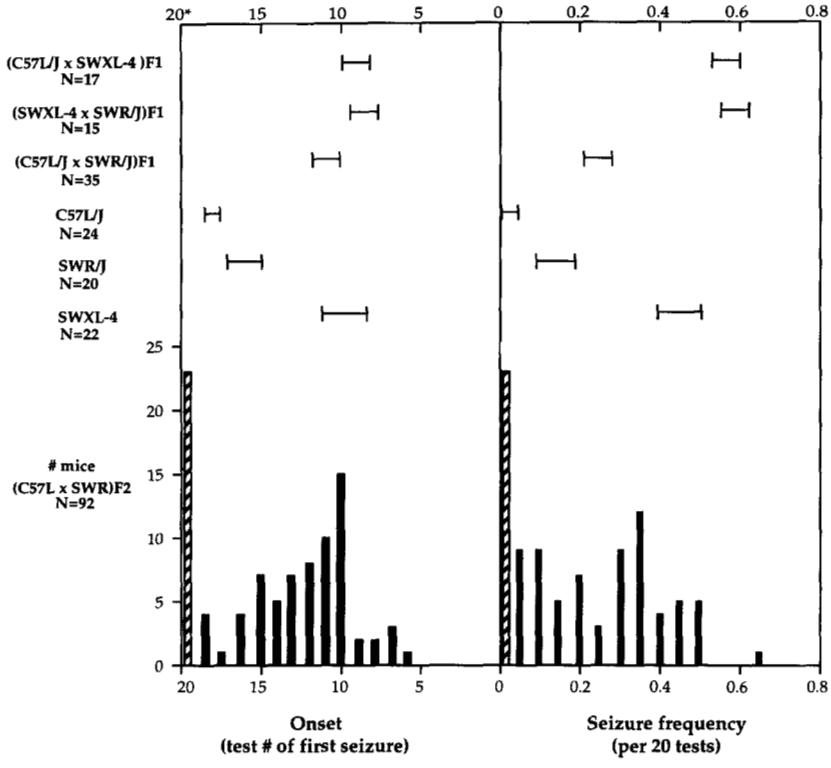

FIGURE 3.—Distribution of seizure traits in crosses between SWR and C57L. (**Top**) Graph comparing onset (left) and seizure frequency (right) ± 2 SE (horizontal I bars) in SWXL-4, progenitor strains SWR/J and C57L/J, and various F_1 hybrids, as described in MATERIALS AND METHODS. The mean values for seizure frequency were SWXL-4 0.45 ± 0.05; SWR/J 0.14 ± 0.04; C57L/J 0.02 ± 0.02; (C57L × SWR)F_1 0.24 ± 0.03; (SWXL-4 × SWR)F1 0.59 ± 0.03; (C57L × SWXL-4)F_1 0.57 ± 0.03. The mean values for onset were SWXL-4 9.7 ± 1.3; SWR/J 16.0 ± 0.9; C57L/J 18.0 ± 0.3; (C57L × SWR)F_1 11.0 ± 0.6; (SWXL-4 × SWR)F_1 8.6 ± 0.7; (C57L × SWXL-4)F_1 9.0 ± 0.7. (**Bottom**) Frequency distribution of the traits onset (left) and seizure frequency (right) in 92 LXSWF2 intercross progeny. The mean seizure frequency in the F_2 cross was 0.20 ± 0.16 SD; the mean onset was 14.0 ± 4.4 SD. The hatched column represents 23 individuals that did not seize at all. The correlation between traits in this cross was 0.87.

linkage markers (*a*, nonagouti; *b*, brown; *p*, pink-eyed dilution; *wa1*, waved; *bt*, belted; and *se*, short ear), (b) it has a small inherent phenotypic variance in seizure frequency and (c) it was used as a partner strain in crosses with EL to detect several seizure QTLs (RISE *et al.* 1991) (our unpublished results). ABP was relatively insensitive to rhythmic gentle tossing, but SxAF1 hybrids had an onset like SWXL-4 and a seizure frequency slightly higher (Figure 4)–suggesting that both traits are generally dominant. To follow the segregation of putative dominant seizure alleles from SWXL-4, SxAF1 hybrids were backcrossed to ABP and 110 progeny seizure-tested as described in MATERIALS AND METHODS. We also analyzed 107 F_2 progeny of an intercross between hybrids–AxSF2. In both crosses the correlation between the seizure frequency and onset was high, but imperfect ($r = 0.86$, SxAxA; $r = 0.70$ AxSF2); we thus analyzed the traits separately. The frequency distributions for seizure frequency (Figure 4A), and onset (Figure 4B) did not appear to reflect the segregation of a simple genetic trait; although both were slightly irregular, they were nevertheless continuous. To determine how much of each segregating trait could be explained by additive genetic factors, the $V_{(E)}$ ("environmental" variance) as measured from F_1 mice was subtracted from the $V_{(T)}$ (total variance from the respective F_2 or backcross) to arrive at a $V_{(G)}$ (genetic variance) for seizure frequency or onset. The heritability of both traits in all crosses was high (Table 1). Because we were concerned about susceptibility alleles inherited from "resistant" parental strains, we did not estimate the number of genes involved in the trait based on Wright's formula (WRIGHT 1968; Lander and BOTSTEIN 1989) which includes the assumption that "high" alleles come from one parent and "low" alleles from the other.

QTL mapping: To identify dominant SWXL-4-derived seizure QTLs in the SxAxA backcross, we employed SSLP that were, based on known maps, about 15 cM apart so that a putative QTL was on average no more than 7.5 cM away from a marker. Over 300 primer pairs were tested for SSLPs between SWXL-4 and ABP, and 117 were chosen for linkage analysis. Because of the high heritability, we reasoned that selecting progeny from the high and low 20% tails of each trait distribution would allow us to map alleles whose presence was associated with "high" trait values and whose absence was associated with "low" trait values–*i.e.*, genes of major effect (LANDER and BOTSTEIN 1989). Of 118 genetic intervals covering all chromosomes, no significant seizure frequency or onset QTLs were found. Several regions had weak associations, but none had a lod score of 3.0 or higher (data not shown). These generally negative results were also obtained using conventional tests including the parametric Student's *t*-test on trait values, or nonparametric χ^2 tests assuming various trait thresholds (data not shown).

We looked for evidence of trait differences in backcross males *vs.* females which might have reduced the power to detect associations. Backcross males had a seizure frequency of 0.36, higher than that of females, 0.29 ($P < 0.05$, two-tailed Student's *t*-test). Because the parental strains did not show sex differences, we first tested

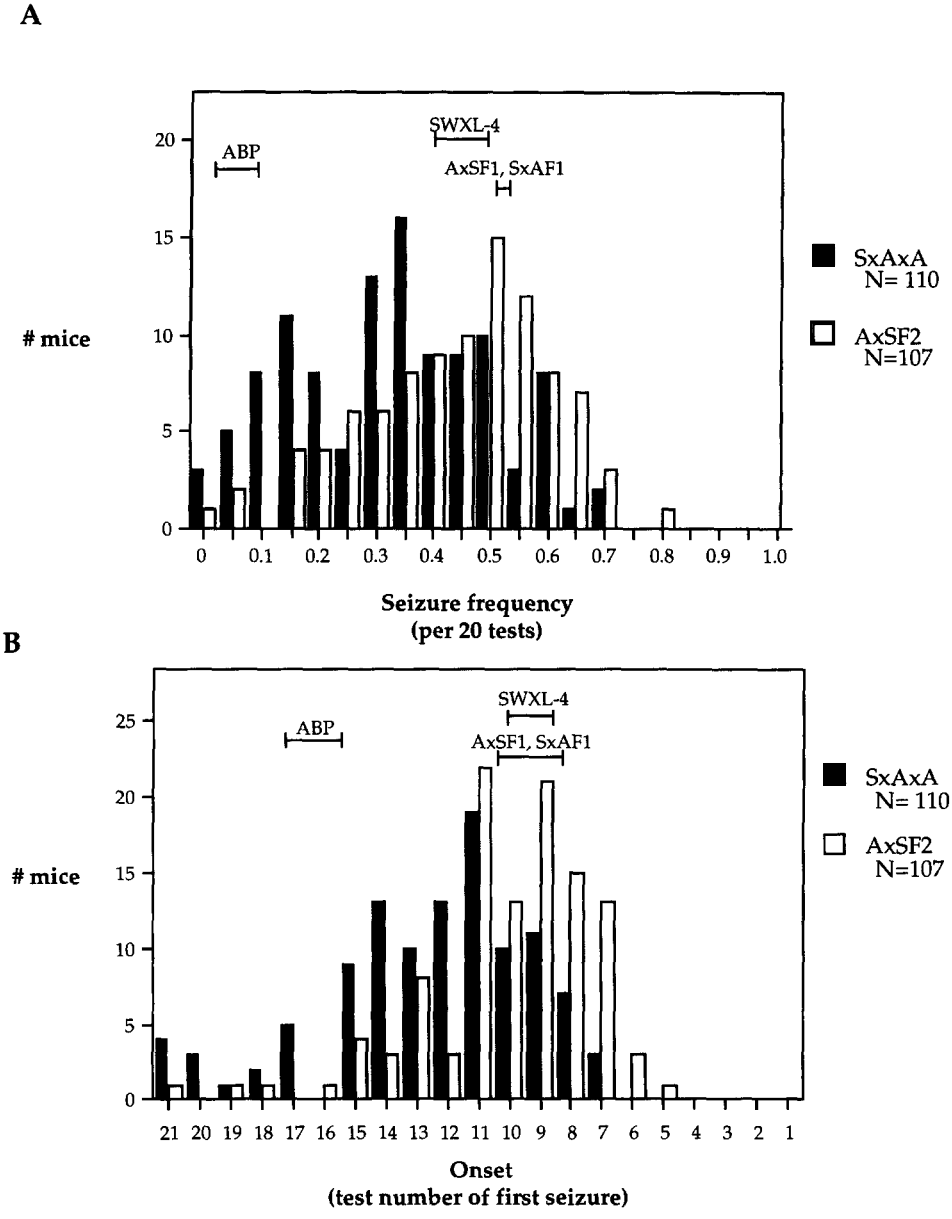

FIGURE 4.—Seizure trait distributions in crosses between SWXL-4 and ABP. Histograms showing the distribution of seizure frequency (A) and onset (B), as described in MATERIALS AND METHODS, for the SxAxA backcross (black columns) and the AxSF2 intercross (white columns). The mean values for seizure frequency ± 2SE of parents and F_1 hybrids were SWXL-4 0.45 ± 0.05 (n = 22); ABP 0.06 ± 0.03 (n = 28); AxSF1, SxAF1 0.53 ± 0.03 (n = 26, n = 8, respectively). The mean values for onset ± 2 SE of parental strains and F_1 hybrids were SWXL-4 9.7 ± 1.3 (n = 22); ABP 16.50 ± 1.10 (n = 28); AxSF1, SxAF1 9.62 ± 1.26.

whether the apparent difference in the backcross was perhaps the result of segregating autosomal loci with sex-limited effects, using MAPMAKER/QTL analysis of male and female datasets separately. No significant QTLs were found (data not shown). Based on the average difference between sexes, we adjusted the seizure frequency (MATERIALS AND METHODS) and again performed MAPMAKER/QTL analysis across the genome. This trait adjustment gave a single significant lod score of 3.1, between *p* and *D7Mit38* on chromosome *7*, explaining about 14% of the seizure frequency variance in the backcross (Figure 5A).

Given the effort of covering the genome in the SxAxA cross and the deficit of significant QTLs, we examined AxSF2 progeny mainly for regions that were marginally associated with seizures in the SxAxA cross. We also typed about 20 novel, polymorphic endogenous IAP proviruses that segregated normally but were not detectably linked to one another or to other markers–presumably identifying additional regions (data not shown). In all, regions were screened on chromosomes *1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 14, 15* and *16*, including 36 intervals spanning over 620 cM, plus an additional portion flanking each linkage group, plus several unlinked markers. No sex differences were found in the AxSF2 cross. Only one region, on proximal chromosome *7*, was significantly associated with seizure frequency, (lod 4.3, Figure 5B), explaining 22% of the total variance, and over 30% of the genetic variance (Figure 5B). We will refer to this QTL as *Szf1* (for *s*eizure *f*requency *1*, quantitative trait locus). Most of the effect on the phenotype comes from the homozygous SWXL-4 *Szf1* genotype, with a smaller effect of heterozygotes at the likelihood peak (Figure 5 legend). It is possible that

TABLE 1

Heritability of seizure frequency and onset in outcrosses of SWXL-4 or progenitor strains

Factors	LxSWF1	SxAF1	LxSWF2	SxAxA	SxAF2
Mean onset					
$V_{(T)}$	3.47	2.40	21.60	11.24	7.56
$V_{(E)}$	3.47	2.40	3.47	2.40	2.40
$V_{(G)}$	(0)	(0)	18.19	8.84	5.16
$V_{(G)}$ as % of $V_{(T)}$	(0%)	(0%)	84%	79%	68%
Mean seizure frequency					
$V_{(T)}$	0.007	0.008	0.030	0.030	0.026
$V_{(E)}$	0.007	0.008	0.007	0.008	0.008
$V_{(G)}$	(0)	(0)	0.023	0.022	0.018
$V_{(G)}$ as % of $V_{(T)}$	(0%)	(0%)	76%	73%	68%

the chromosome 7 association in the SxAxA cross is identical to *Szf1*; although the likelihood curve peaks are 15 cM apart, the confidence interval in the F_2 cross is quite broad. However, included in the interval there is a second peak where seizure frequency and onset are both significantly affected. Without further analysis it is not clear whether this broad extension of the *Szf1* likelihood curve results from the effects of a second locus. The use of the "simultaneous search" feature of MAPMAKER/QTL to scan additional genomic regions accounting for the additive effects of *Szf1*, did not resolve the issue, nor did it allow detection of any additional significant QTLs on other chromosomes.

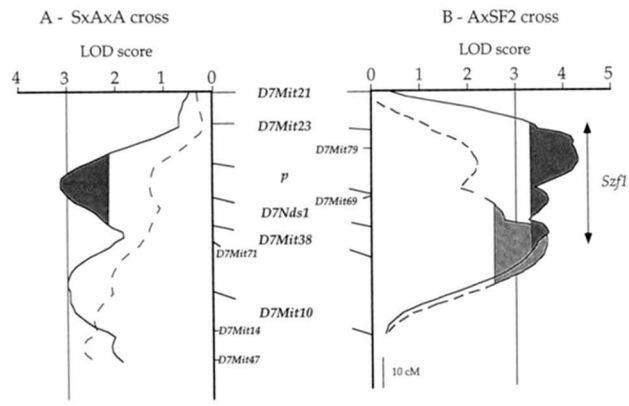

FIGURE 5.—Associations between chromosome 7 markers and seizure traits in SxAxA and AxSF2 mice. Lod score plots for seizure frequency (solid line) and onset (stippled line) for chromosome 7 in SxAxA (A) and AxSF2 (B) crosses. Markers shown in large font were typed in both crosses. The peaks of significant associations (lod > 3.0; see MATERIALS AND METHODS for rationale) are shown in the AxSF2 cross for seizure frequency, lod = 4.3, accounting for 22% of the total variance (32% of the additive genetic variance, relative effect of S/S to S/A genotypes at the peak was 0.210 *vs.* 0.082 seizure frequency, respectively) and for mean onset, lod = 3.6, accounting for 15% of the phenotypic variance (22% of the additive genetic variance, relative effect of S/S to S/A genotypes at the peak was 2.4 *vs.* 1.2 tests, respectively). In the SxAxA cross, for the sex-adjusted seizure frequency phenotype (see MATERIALS AND METHODS), the lod score was 3.11, explaining 13.5% of the total variance (18% of the additive genetic variance). The shaded portions represent 1-lod (approximately 95%) confidence intervals.

DISCUSSION

In this report we describe a new polygenic model for tonic-clonic and generalized epileptic seizures, the SWXL-4 RI strain. The behavioral and electrocorticographic phenotypes as well as the mode of seizure induction (*i.e.*, rhythmic tossing) are generally similar to those of the EL inbred mouse, a model studied extensively in the past by neurobiologists (FUKAHORI and ITOH 1990; IMAIZUMI *et al.* 1959; SEYFRIED *et al.* 1992; SUZUKI and NAKAMOTO 1977) and recently by geneticists (FUETA *et al.* 1986; RISE *et al.* 1991). In addition, as a result of seizure there is increased expression in the cerebral cortex and hippocampus of c-Jun and c-Fos, similar to that found in other experimental models of limbic seizures.

Among inbred mouse strains, the EL mouse is considered to be a valuable resource as an idiopathic epilepsy model, presumably bearing several rare mutant alleles that are not present in other strains. Here we demonstrate that even common mouse strains, such as SWR and C57L contain epilepsy susceptibility alleles that, when inherited in combination, are capable of transmitting very similar epileptogenic disorders. Other inbred strain combinations, such as RI strains between NZB and RF, are also capable of producing mice with handling-sensitive seizures (WIMER *et al.* 1990). As epilepsy is a genetically heterogenous disorder in humans, (ANDERMAN 1982; ANDERSON *et al.* 1986; GREENBERG and DELGADO-ESCUETA 1993), the availability of such genetically diverse mouse models for epilepsy provides an important experimental resource to complement human genetic studies.

Although it has long been known that determinants for susceptibility to audiogenically stimulated seizures also vary among common mouse strains (COLLINS 1972; NEUMANN and COLLINS 1991; SEYFRIED 1983), the rhythmic gentle tossing models represented by SWXL-4 and EL have several practical advantages over certain audiogenic seizure and other induced seizure models. First, we have never observed mortality during or resulting from seizures, and husbandry is not adversely affected; for example, even chronically epileptic females nurse their young. Second, rhythmic gentle tossing of younger mice is easily performed, is noninvasive, and although the model technically results in an induced seizure, the procedure may simply accelerate the cumulative effects of routine handling and day-to-day activity that results in the spontaneous seizures of older animals. Last, in both models the seizure phenotype is operationally dominant; that is, most F_1 hybrids between SWXL-4 or EL and other inbred strains exhibit seizures. Thus, without multiple backcrosses it should be possible to test, for example, whether a specific dominant allele (*e.g.*, trans-

genic insertion) has a significant role or effect in the development or expression of seizures in mice.

The overall inheritance of epilepsy in the SWXL-4 model is clear: alleles inherited from both progenitor strains are necessary and sufficient to explain the behavioral phenotype of the RI strain. Moreover, because F_1 hybrids between SWXL-4 and SWR or C57L have a more severe phenotype than SWXL-4 itself, either progenitor may have additional seizure alleles that were not fixed in the SWXL-4 RI strain. In this respect it is possible that a model with a more severe seizure phenotype could be constructed by selection from crosses with one of the progenitors. Finally, in crosses between SWXL-4 and ABP QTL analysis of seizure frequency or onset can be used to identify significant alleles with influences on greater than 20% of the phenotype, namely, *Szf1* on proximal chromosome 7.

Specifically, however, we do not fully understand the genetic determinants that define the seizure susceptibility of SWXL-4 as inherited from its progenitors. The only mapped QTL, *Szf1*, though SWXL-4 derived, was not detectable in the LxSWF2 cross (our unpublished results), and therefore may have been common to SWR and C57L. Given that the heritability of seizures is high and apparently dominant, that the ABP strain itself does not experience seizures frequently, and that the phenotypic frequency distributions were not grossly abnormal, it was confounding that we could find no major seizure QTLs in the SxAxA backcross despite a thorough genome-sweep. One possibility is that the seizure phenotypes chosen for analysis did not optimally reflect underlying genetic factors. Seizure frequency and onset are semi-quantitative traits, and rely on the occurrence of a qualitative event, the seizure itself. It is possible that this qualitative event is influenced by unknown, epigenetic phenomena, or perhaps by the testing procedure itself. However, given the apparent high heritability of the traits, we think it is unlikely that complications of this type presented the major obstacle to map significant QTLs in the SxAxA cross.

An alternative explanation for the inability to find definitive seizure QTLs in the SxAxA backcross invokes the segregation of multiple seizure alleles that overlap in function, *i.e.*, heterogeneity. Just as heterogeneity can be an obstacle to genetic analysis of complex traits in human populations (ANDERSON *et al.* 1986; WHITEHOUSE *et al.* 1993), it is possible that heterogeneity within the SxAxA cross lessened the detectability of any one QTL–especially in our small sample of 110 mice. Considering that the seizure frequency of SxAF1 hybrids is slightly higher than that of SWXL-4 (Figure 4), perhaps even ABP contributes susceptibility alleles that masked the SWXL-4 alleles in the backcross. Finally, it is possible that the critical SWXL-4 seizure alleles act epistatically, that is, they rely on the presence of other segregating alleles. The approach we chose to search for seizure genes, using MAPMAKER/QTL, would have detected segregating QTLs that contribute additively to a quantitative trait, but not those that act only epistatically. Given the complexities of the linkage genetics so far, we will now turned to the LxSWF2 cross between progenitor strains to map seizure trait determinants, in the hope that at least limiting the segregation to SWR and C57L alleles might minimize the number of seizure determinants involved and thus allow us to more readily detect their effects.

The *Szf1* QTL was not found previously in the EL strain. However, *Szf1* is in the same general vicinity as the audiogenic seizure prone gene from the DBA/2J strain, *Asp3* (NEUMANN and COLLINS 1991, 1992), and the neurological mouse mutation, quivering, *qv* (YOON and LES 1957). The likelihood peak of *Szf1* maps in a region of proximal chromosome 7 that is highly homologous with human chromosome *19q13*. Several possible candidate genes reside in this homology segment, including calmodulin 3 (BERCHTOLD *et al.* 1993), a sodium-potassium ATPase (YANG-FENG *et al.* 1988), neurotrophin 4, and neurotrophin 5 (BERKEMEIER *et al.* 1992; IP *et al.* 1992). In addition, the 1-lod confidence interval which extends more distally, necessitates the inclusion of several GABA receptor subunit genes located near the *p* locus (CULIAT *et al.* 1993) as candidates for *Szf1*.

This work was supported by the National Institutes of Health grants NS31348 (to W.N.F.) and NS29709 (to J.L.N.). We are grateful to MARTHA BUZZELL for observant animal care, to THOMAS N. SEYFRIED for advice on seizure testing, to ROSS CHAFETZ for excellent technical assistance, to JOE NADEAU and ED LEITER for review of this manuscript, to an anonymous reviewer for attentive constructive criticism, and to NICHOLAS SCHORK for advice on simulation. The Jackson Laboratory is fully accredited by AAALAC (American Association for the Accreditation of Laboratory Animals Care). All procedures conformed to guidelines for humane treatment of animals, and were approved by the ACUC (Animal Care and Use Committee) according to NIH procedures.

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Communicating editor: N. A. JENKINS