enhancer of seizure: A New Genetic Locus in *Drosophila melanogaster* Defined by Interactions With Temperature-Sensitive Paralytic Mutations

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Manuscript received September 26, 1986
Revised copy accepted April 4, 1987

ABSTRACT

Mutations in the *enhancer of seizure* (*e(sei)*) locus have been isolated on the basis of their ability to cause temperature-induced paralysis of alleles at the *seizure* (*sei*) locus at temperatures at which these mutations ordinarily do not paralyze. This enhancer is specific to the *seizure* locus and is without effect on other temperature-sensitive paralytic mutants including *para*, *nap*, *tip-E* and *shi*. This suggests that the enhancer responds specifically to the mechanism of paralysis mediated by the *seizure* mutations. The *e(sei)* is a recessive mutation which maps to 39.0 on the left arm of chromosome *3*. Deficiency mapping has placed it at 69A4-B5 on the salivary gland polytene chromosome map. When a new enhancer allele was isolated following P-M hybrid dysgenesis, there was a concomitant *P*-element insertion at 69B. In the absence of *seizure* mutations, the enhancer mutation causes non-temperature dependent hyperactivity when agitated and interferes with the climbing response. Electrophysiological studies examined the effects of increasing temperature on electrical activity in the adult giant fiber/flight muscle system. Neuronal hyperactivity was seen in both *e(sei)* and *sei* single mutant homozygotes, but not in wild type. The hyperactivity was more severe in the *sei;e(sei)* double mutants. The correlation between the physiological effects and the mutant behavior suggests that both *sei* and *e(sei)* cause membrane excitability defects. Since previous work has shown that *seizure* mutants affect [^{3}H]saxitoxin binding to the voltage-sensitive sodium channel, *e(sei)* may code for a gene product which interacts with this channel.

IN *Drosophila melanogaster* four genetic loci (*para*, *nap*, *sei* and *tip-E*) have been identified which affect the structure and/or production of the voltage-sensitive sodium channel (WU and GANETZKY 1980; JACKSON *et al.* 1984, 1985; JACKSON, WILSON and HALL 1986). One approach to identify additional genes that affect the sodium channel is to screen for second site mutations which enhance or suppress the mutant phenotype of each of these four loci. Such enhancers and suppressors would be expected to be of two types: those which identify gene products which interact directly with the sodium channel protein and those which identify gene products that affect other components of cell excitability besides the sodium channel. An example of the second type of interaction has been presented by GANETZKY and WU (1982) who describe the suppression of mutations affecting potassium channels by *nap*, a mutation which reduces the number of saxitoxin-binding sodium channels (JACKSON *et al.* 1984). Interactions of the first type may be found among the four loci mentioned above that affect sodium channel properties. Indeed, double mutants constructed using these mutations have revealed examples of enhancer and suppressor interactions. For instance, *nap* and *tip-E* each suppress *sei* and there are enhancing interactions between *para* and *nap*, between *para* and *tip-E*, and between *nap* and *tip-E* (WU and GANETZKY 1980; GANETZKY 1984, 1986; JACKSON *et al.* 1985; JACKSON, WILSON and HALL 1986).

In this paper we report the first application of this approach to identify a new genetic locus defined by the ability of mutants in the locus to enhance the temperature-sensitive paralytic phenotype of all *seizure* (*sei*) alleles. Since *sei* alleles affect the parameters of [^{3}H]saxitoxin binding to the voltage-sensitive sodium channels (JACKSON *et al.* 1984, 1985), this enhancer locus (named *enhancer of seizure*, *e(sei)*) may code for a gene product that interacts with this channel. Our electrophysiological studies demonstrate that this locus affects membrane excitability but the molecular nature of the gene product remains to be elucidated. To facilitate cloning and molecular characterization of the *e(sei)* gene using the method of transposon tagging described by BINGHAM, LEVIS and RUBIN (1981), we have isolated a *P*-element induced allele at this locus.

MATERIALS AND METHODS

Stocks and culture conditions: *Drosophila* cultures were grown at 21° on standard cornmeal medium (LEWIS 1960).

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The wild-type Canton-S was provided by J. C. HALL (Brandeis University) and the P-Harwich strain came from A. CHOVNICK (University of Connecticut, Storrs). The *vin* deficiency stocks (AKAM *et al.* 1978) were provided by V. G. FINNERTY (Emory University) and D. B. ROBERTS (Oxford University). The temperature-sensitive paralytic mutants sei^{ts1} and sei^{ts2} (2-106) were provided by B. GANETZKY (University of Wisconsin, Madison) and have been previously described (JACKSON *et al.* 1984, 1985). Double mutants carrying *e(sei)* in combination with either sei^{ts1} or sei^{ts2} were constructed using the multiply inverted, dominantly marked second chromosome *In (2LR)O, dp^{lvl} Cy pr cn^2 bw* abbreviated as *CyO, cn bw* and the third chromosome *In(3LR)TM3, y^+ ri p^p sep Sb bx^{34e} e^s Ser* abbreviated as *TM3, Sb e^s Ser* as balancers. In some cases the benign eye color mutations *brown* (*bw*) and *scarlet* (*st*) were used to follow the segregation of chromosomes carrying the behavioral mutations. The visible markers used have been described by LINDSLEY and GRELL (1968).

Isolation of the original *e(sei)* allele: The original allele of *e(sei)* was isolated serendipitously during a screen for hybrid dysgenesis-induced alleles of *sei.* In the initial experiments we used a *bw* sei^{ts2} stock in which a third chromosome marked with *scarlet* was segregating at an undetermined frequency. This *scarlet*-bearing chromosome apparently carried a spontaneous mutation at the *e(sei)* locus which was uncovered by new dysgenesis-induced enhancer mutations. In the screen dysgenic males (arising as the F_1 offspring of a mating between Canton-S females and P-Harwich males) were mated to virgin females from the *bw* sei^{ts2} stock and their progeny were screened for paralysis at 38°. Subsequent analysis of the temperature-sensitive paralytics showed that the factor causing paralysis of *bw* sei^{ts2} heterozygotes was not a new *sei* allele but rather was a factor segregating with the third chromosome. As documented in this manuscript, this factor was the *e(sei)* locus.

After the *e(sei)* mutation was recognized, the original *bw* sei^{ts2} stock was crossed into a Canton-S background for ten generations so that all third chromosomes were replaced by Canton-S chromosomes. This Cantonized *bw* sei^{ts2} stock lacking the spontaneously induced enhancer allele has been used for all subsequent experiments.

Countercurrent distribution procedure: The countercurrent distribution procedure of BENZER (1967) was adapted to measure the negative geotaxis response of populations of adult flies aged 3–5 days. After adaptation in ambient light at 22°C for 15 min, the flies were allowed to distribute in five cycles each of 15 sec duration. All trials were done at the same time of the day.

Neurophysiology: Neurophysiological studies were done using the giant fiber (GF) system in adult flies. The detailed physiology of the GF pathway and methodology used to study it has been described by TANOUYE and WYMAN (1980). A diagrammatic representation of this pathway is shown in Figure 5. Flies were sucked into a Pipetman micropipet tip sculpted to expose the dorsal head and thorax. The sculpted tip was slipped into a larger micropipet tip (which protected the mounted fly from air currents) and placed on a temperature-controlled Cambion peltier plate. The temperature was measured by a thermistor placed within 1 mm of the fly. In each experiment flies were heated to 42.5° for 2.5 min and returned to 22°. Temperature shifts required less than a minute to stabilize. Tungsten electrodes were used for recording from the wing depresser muscles (DLM) during the heat pulse. In some experiments the giant fiber or the motoneurons that innervate the DLMs were directly stimulated and responses recorded from the DLMs.

Screening for hybrid dysgenesis induced *e(sei)* alleles: To generate new alleles by hybrid dysgenesis, P-Harwich males were mated with homozygous *ebony* (*e*) virgin females of the M cytotype. The F_1 dysgenic hybrid males were then mated *en masse* to *bw* sei^{ts2}; *e(sei) st* virgin females. The F_2 progeny were screened for temperature-sensitive paralytic flies by placing all the F_2 flies (1500–5000 flies per test) onto a shelf in a preheated plexiglass box (WILLIAMSON 1971) at 38° for less than 8 min. Paralyzed flies were trapped on a shelf while mobile flies drowned in a mixture of vinegar and detergent at the bottom of the box. Individual paralyzed flies that recovered were mated to *CyO,cn bw/bw* sei^{ts2}; *TM3, Sb e^s Ser/e(sei) st* flies. All progeny from this cross were tested for paralysis at 38° to determine if *CyO, cn bw/bw* sei^{ts2}; *mutagenized chromosome/e(sei) st* flies paralyzed. Such paralysis confirmed the isolation of a newly induced *e(sei)* allele. Out of a total of 36,260 chromosomes screened, we obtained 4 that carried a newly induced *e(sei)* allele. All of these were isolated in males. In each case the *TM3, Sb e^s Ser/mutagenized chromosome* flies had the ebony phenotype indicating that the new mutation was induced on the *ebony*-bearing M chromosome. It is possible that the 4 isolates do not represent independent mutational events. A stock was established for each by mating males and females that were *CyO, cn bw/+; TM3, Sb e^s Ser/mutagenized chromosome* to each other.

Mapping of *e(sei)*: Mapping experiments were done with respect to the visible markers *veinlet* (*ve* 3-0.2), *hairy* (*h* 3-26.5) and *thread* (*th* 3-43.2). Potentially recombinant third chromosome lines derived from the *ve h th/e(sei) st* females were balanced using the *TM3, Sb e^s Ser* chromosome. To score the *e(sei)* mutation, males from each line were crossed to *bw* sei^{ts2}; *e(sei) st* virgin females and the progeny were tested for paralysis at 38°.

***In situ* hybridization to polytene chromosomes:** *In situ* hybridization to polytene chromosomes used the plasmid pπ 25.1 as probe. The plasmid contains a full length *P* element and was obtained from G. M. RUBIN (University of California, Berkeley). The probe was biotinylated using the BRL nick translation kit (catalog no. 81605B) and biotin-11-dUTP (BRL catalog no. 9507SA). It was hybridized to larval salivary gland polytene chromosomes and detected by the alkaline phosphatase method using the BRL DNA detection kit (catalog no. 8239SA) and the procedures described by ENGELS *et al.* (1985).

RESULTS

***e(sei)* lowers the temperature required to paralyze two different *seizure alleles*:** The first *enhancer of seizure* allele was isolated serendipitously during a screen for hybrid dysgenesis induced alleles of *seizure.* (See MATERIALS AND METHODS for details.) The enhancer mutation was identified on the basis of its ability to induce paralysis of sei^{ts2}/+ flies at 38°. As shown in Figure 1, sei^{ts2} homozygotes paralyze rapidly at 38° but the sei^{ts2}/+ heterozygotes do not normally paralyze at this temperature. To characterize the interaction between the enhancer and the *seizure* locus more fully, we examined the effects of the enhancer on the paralysis behavior of sei^{ts2} homozygotes and heterozygotes at 35°. Figure 1 shows that the behavior of both homozygotes and heterozygotes is completely normal at this temperature. They do not paralyze.

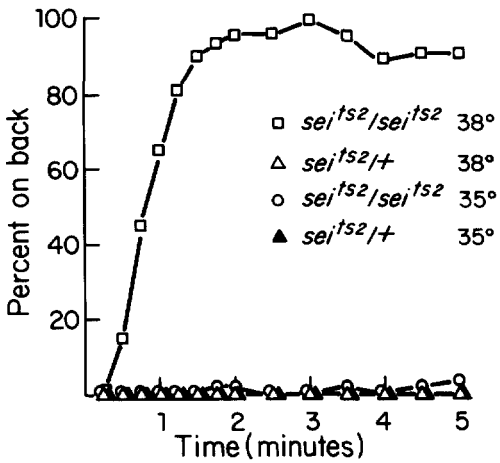

FIGURE 1.—Kinetics of temperature-induced paralysis for sei^{ts2} homozygotes and heterozygotes. About 20 flies grown at 21° and aged 3–6 days after eclosion were transferred to a preheated 25 cm^2 Corning tissue culture flask and immersed in a water bath at the indicated temperature. The percentage of flies on their backs was determined at 15-sec intervals. Flies scored as "on back" included those that were not standing and remained paralyzed until the end of the observation period as well as those that repeatedly fell on their backs but then recovered. Each curve represents 50–60 flies.

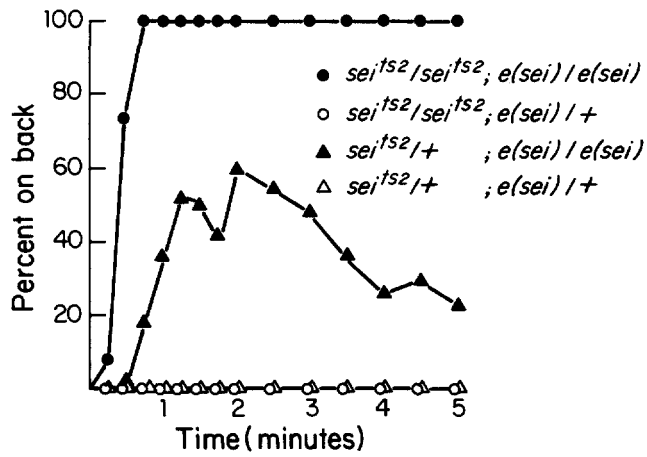

FIGURE 2.—Effects of *e(sei)* on the temperature-induced paralytic behavior of sei^{ts2}. The behavior of the indicated genotypes was tested at 35° as described in Figure 1. Each curve represents 38–108 flies.

As illustrated in Figure 2, sei^{ts2} homozygotes and heterozygotes show completely wild-type behavior at 35° when *e(sei)* is heterozygous demonstrating the recessive nature of the *e(sei)* mutation. In contrast, when the enhancer is homozygous, the sei^{ts2};*e(sei)* double homozygotes paralyze rapidly ($t_{1/2}$ = 24 sec) and remain paralyzed for more than 5 min after transfer back to 22°. Significantly, sei^{ts2}/+; *e(sei)* flies become very hyperactive and frequently fall and recover at 35°. Although these flies do not paralyze, their behavior is dramatically different from that of sei^{ts2}/+ heterozygotes (Figure 1). Previously, JACKSON *et al.* (1985) have shown that sei^{ts2} is codominant with respect to paralytic behavior. Heterozygotes paralyze at 40° with kinetics intermediate between that of homozygous sei^{ts2} and wild type. Thus, the homozygous enhancer affects both sei^{ts2} homozygotes and heterozygotes in the same way by lowering the temperature required for expression of the behavioral defects.

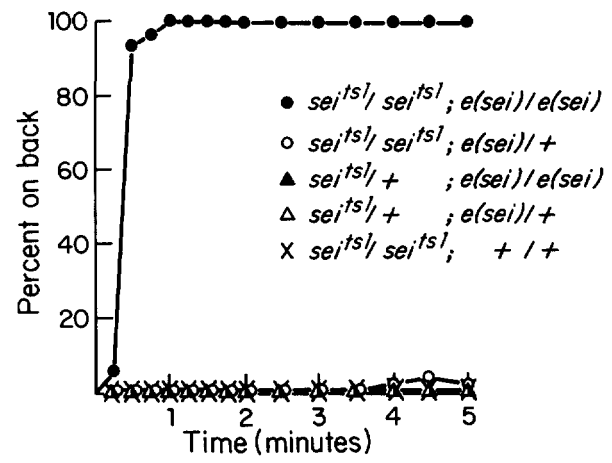

FIGURE 3.—Effects of *e(sei)* on the temperature-induced paralytic behavior of sei^{ts1}. The behavior of the indicated genotypes was tested at 35° as described in Figure 1. Each curve represents 31–50 flies.

The sei^{ts2} allele shows a temperature-dependent change in affinity for [^{3}H]saxitoxin binding and has a partially dominant biochemical and behavioral phenotype (JACKSON *et al.* 1984, 1985). In contrast, the sei^{ts1} allele differs from the sei^{ts2} allele with respect to the biochemical nature of the defect and the genetic dominance of the behavioral and biochemical phenotypes (JACKSON *et al.* 1985). The sei^{ts1} allele shows a reduced number of saxitoxin-binding sodium channels and is completely recessive with respect to both the biochemical and behavioral phenotypes. It was of interest to determine whether the enhancer had different, allele-specific interactions with these two alleles so the interaction between the enhancer and sei^{ts1} was analyzed at 35°. As summarized in Figure 3, sei^{ts1} homozygotes show wild-type behavior which is indistinguishable from that of sei^{ts1};*e(sei)*/+ flies, again demonstrating the recessive nature of the enhancer. The effect of the enhancer mutation is seen in homozygous sei^{ts1} flies in which the *e(sei)* is also homozygous. These double homozygotes paralyze rapidly ($t_{1/2}$ = 21 sec). The sei^{ts1} heterozygotes (triangles) show normal locomotor activity when the enhancer is either homozygous or heterozygous. Although the enhancer does not change the nature of the mutant phenotype from recessive to dominant, it does itself act in a recessive manner to enhance the severity of the behavioral phenotype by lowering the temperature required for paralysis just as it did for the sei^{ts2} allele.

There are two lines of evidence which suggest that the enhancer interacts specifically with the *seizure* locus and not with some other locus on the second chromosome. First, the enhancer interacts with two independently isolated *seizure* alleles and the nature

of this interaction does not change when the alleles are placed in a Canton-S background. Second, the *seizure*-enhancer interaction reflects the dominant or recessive character of the specific *seizure* allele used. This difference in dominance of the paralysis phenotype would not be expected if the enhancer were interacting with another mutation common to the genetic background of the two *seizure* alleles.

Since the enhancer affects two phenotypically distinct *seizure* alleles in exactly the same way (by lowering the temperature required for temperature induced paralysis), the interaction does not appear to be allele specific. Allele-specific interactions are often characteristic of structural interactions between gene products. Since the interaction is not allele specific in this case, the enhancer locus and the *seizure* locus may code for products that interact functionally. That is, *e(sei)* might code for a product that is not directly related to sodium channels but one that affects other aspects of cell excitability. Such functional interactions indicating possible cases of non-direct relationships have been reported for other loci that affect neuronal excitability (GANETZKY and WU 1982).

In order to define further the nature of the enhancer effect on the temperature-induced paralysis phenotype, we constructed double mutants and looked for effects of the enhancer on other paralytic mutants. We found the enhancer has no effect on the temperature-sensitive paralytic phenotype of other mutants including: *para*, *nap*, and *tip-E*. These mutants affect nerve conduction and/or [^{3}H]saxitoxin binding parameters. In addition, the enhancer is without effect on *shibire* which causes a temperature-induced blockade in synaptic transmission (IKEDA, OZAWA and HAGIWARA 1976) and affects synaptic vesicle recycling (POODRY and EDGAR 1979; KOSAKA and IKEDA 1983). Thus, the functional effects of the enhancer appear to be specific for the *seizure* gene product.

The *e(sei)* affects the climbing response of adult flies: After defining the enhancer locus on the basis of its interaction with *seizure* we were interested in determining whether *e(sei)* by itself exhibits a behavioral phenotype. When adult wild-type flies are tapped to the bottom of a container, they rapidly climb the sides of the container and collect around the top. That is, they exhibit a negative geotactic response. We found that when the container holding *e(sei)* flies is lightly tapped, they become hyperactive and this interferes with their climbing response. As summarized in Figure 4, countercurrent distribution analysis (BENZER 1967) was used to quantitate this behavioral defect. Most of the wild-type flies made at least three successful runs out of five attempts whereas most of the *e(sei)* flies made no successful runs. The same results have been obtained in experiments done at 38° (data not shown) suggesting that the abnormal

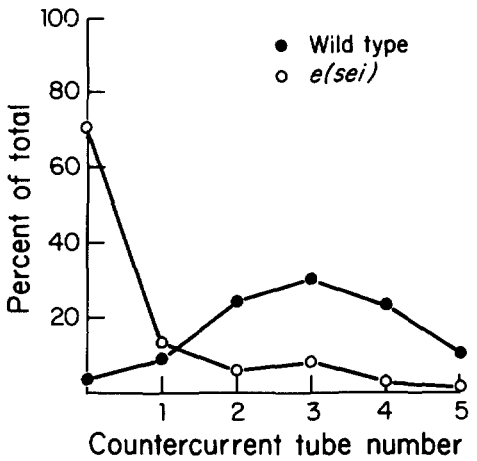

FIGURE 4.—Comparison of the climbing response of *e(sei)* and wild-type Canton-S flies. The curves quantitate the negative geotactic response of flies after five transfers in a countercurrent apparatus as described in MATERIALS AND METHODS. The flies distribute in tubes 0 through 5 according to the number of times (0–5) that they have climbed successfully. Each curve represents the average of five experiments each using 30–70 flies.

climbing response of *e(sei)* flies is not temperature-dependent. The climbing response defect is uncovered by *Df(3L)vin*^{7} isolated by AKAM *et al.* (1978). This deficiency also uncovers the enhancer phenotype of *e(sei)*. The two phenotypes were not separated in recombination mapping studies described in a later section. Therefore, it is likely that this behavioral defect and the enhancer phenotype are due to a single gene mutation.

Electrophysiological correlates of enhancer-seizure interactions: We have examined the effects of increasing temperature on electrical activity in the giant fiber system of mutant and wild-type adults (TANOUYE and WYMAN, 1980). We have used the identified neurons, muscles, and synapses in the flight portion of the system (Figure 5). Briefly, a pair of giant fibers descends from the brain into the thorax where each makes an electrical synapse with an interneuron (PSI) which, in turn, chemically synapses on five motoneurons that innervate the dorsal longitudinal muscles (DLM). Each DLM is innervated by one motoneuron.

During temperature shifts from 22° to 42.5° spontaneous activity was recorded from the DLMs in *e(sei)*, *sei*, and *e(sei)* flies (Figure 6). The appearance of spontaneous activity coincided with the onset of visually observed hyperactivity in *e(sei)*, and paralysis in *sei* or *sei*; *e(sei)* mutants. In contrast, no such activity was recorded from the DLMs of wild-type flies even in temperature shifts to 44° for 4 min. We classified the maximum rate of spontaneous activity in each genotype based on the following arbitrary stages: (1) no activity; (2) intermittent bursts; (3) continuous activity <33 Hz; (4) continuous activity 33–80 Hz and (5) continuous activity >80 Hz. The results for each genotype tested are summarized in Table 1.

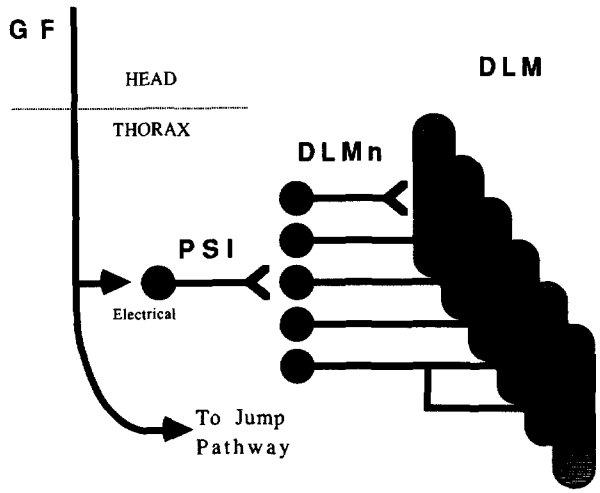

FIGURE 5.—Schematic representation of the portion of the giant fiber pathway involved in flight. The giant fiber (GF) makes an electrical synapse with the peripherally synapsing interneuron (PSI) which in turn makes chemical synapses on five dorsal longitudinal motoneurons (DLMn) that innervate the dorsal longitudinal muscles (DLM).

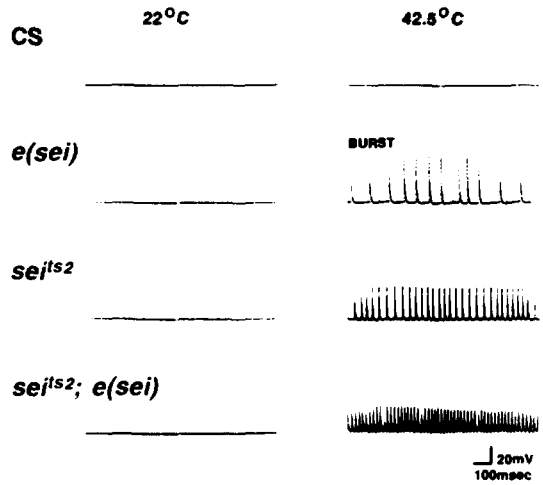

FIGURE 6.—A comparison of the effects of a temperature shift on spontaneous activity in the DLM of mutant and wild-type flies. The wild-type flies were Canton-S (CS). The *e(sei)* mutation showed intermittent bursts of activity followed by periods of quiescence at 42.5°. A representative burst is illustrated. Both sei^{ts2} and sei^{ts2};*e(sei)* showed continuous activity at 42.5°. A representative portion of this activity is illustrated. Note that in general the DLM spike amplitude decreases with increasing frequency of activity.

TABLE 1

Spontaneous activity recorded from the dorsal longitudinal muscles of mutant and wild-type adults

Maximal activity induced by 22° to 42.5° shift	Number of flies					
	Wild type	*e(sei)*	sei^{ts1}	sei^{ts2}	sei^{ts1}; *e(sei)*	sei^{ts2}; *e(sei)*
1. No activity	5	3	0	0	0	0
2. Intermittant bursts	0	12	0	3	0	0
3. Continuous <33 Hz	0	12	1	2	0	0
4. Continuous 33–80 Hz	0	2	6	4	6	6
5. Continuous >80 Hz	0	0	0	0	6	6

The *e(sei)* flies displayed the most variability: The rate of spontaneous activity ranged from stages 1 to 4. Activity started at an average temperature of 36.6° with a large standard deviation of 6.8° (n = 26). The sei^{ts1} allele on average induced higher rates of spontaneous activity than either sei^{ts2} or *e(sei)*. However, there was no difference between sei^{ts1} and sei^{ts2} in the average temperature at which spontaneous activity started. The average onset temperature from pooled data of the two *seizure* alleles was 38.5° (SD = 2.3°, n = 16). Double mutants of *sei*; *e(sei)* displayed much higher rates of activity than any of the single mutants. The temperature at which spontaneous activity started was reduced to 34.7° (SD = 2.1°, n = 24) in the double mutants. This correlated with their lower paralysis temperature. No differences in the rates of spontaneous activity or the temperature at which activity started were seen between sei^{ts1}; *e(sei)* and sei^{ts2}; *e(sei)*.

There are three lines of evidence which suggest that the spontaneous activity seen in mutant flies is neuronally induced. First, others have shown that in wild-type direct stimulation of the muscle by depolarizing current causes muscle spikes that are almost ten times longer in duration than neuronally induced responses (SIDDIQI and BENZER 1976; SALKOFF and WYMAN 1983). In our experiments with mutant flies we found the spontaneous muscle responses induced by the mutants have the duration of neuronally induced responses (see Figure 7). Second, when the motoneurons are directly stimulated, the amplitude of the DLM response decreases with increasing frequency due to reduced transmitter release. This is exactly what we observed in the mutant-induced spontaneous activity shown in Figure 6. Third, cobalt concentrations can be defined which block synaptic transmission without affecting propagation of action potentials or the ability of muscle to respond to direct stimulation. When such a concentration of cobalt was injected into *e(sei)*, *sei*, or *sei*; *e(sei)* flies, the mutant-induced spontaneous activity at high temperatures was blocked in all cases. Thus, DLM spontaneous activity in these mutants is induced via the motoneurons.

There is further evidence that the spontaneous activity does not arise from the PSI or GF. Since a single PSI synapses onto five motoneurons, any spontaneous activity occurring in the PSI or GF should elicit simultaneous responses in all six DLMs (see Figure 5). We found that spontaneous responses were not coincident in two DLMs receiving input from a single PSI. This suggests that the spontaneous activity does not arise from the PSI or GF, but from within the motoneurons themselves or from an unidentified presynaptic input onto the motoneurons.

Although the mutants increased spontaneous activity, they did not block action potentials or alter the

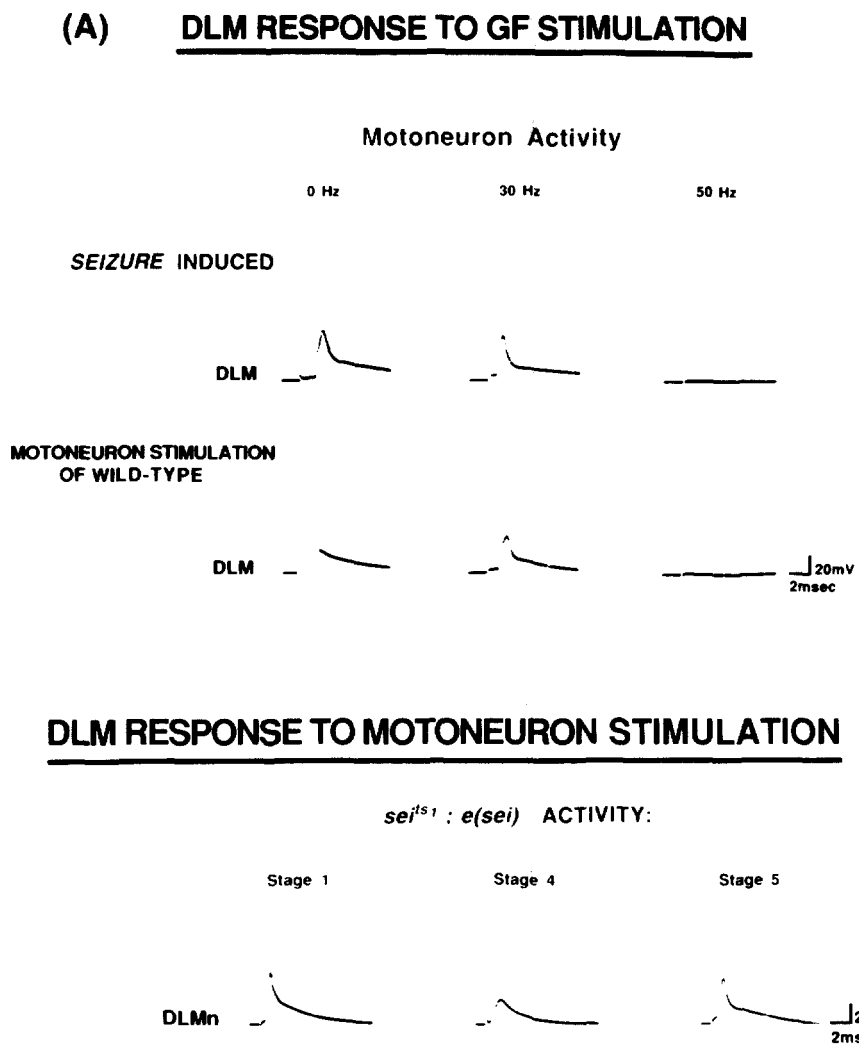

FIGURE 7. Effect of high frequency activity in the motoneuron on the ability of the DLM to respond to: (A) giant fiber stimulation; (B) motoneuron stimulation. The mutant responses were measured at various times during a temperature shift from 22° to 42.5°. The wild-type responses were all collected on Canton-S flies at 40°. Because the time scale has been expanded compared with Figure 6, the background motoneuron-induced activity has been excluded from these traces.

rate of propagation of action potentials. By directly stimulating the giant fiber we obtained normal responses in the DLMs during mutant activity. However, when the mutant-induced spontaneous activity was >33 Hz, direct stimulation of the giant fiber failed to elicit a response in the DLM. This is documented in Figure 7A. This effect could be mimicked in wild-type flies by directly stimulating the motoneurons at >33 Hz (Figure 7A). This indicates the blockade of DLM response is a normal consequence of hyperactivity in the motoneurons and is not an effect associated with the mutants themselves. To see if the neurons function normally when the frequency of spontaneous activity is >33 Hz (*i.e.*, greater than stage 3), motoneurons were directly stimulated and found to produce DLM responses (Figure 7B). However, as shown in Figure 7B the amplitude of the DLM response was reduced when spontaneous activity reached stage 4 rates. In mutant flies that reached rates of >80 Hz (stage 5) the amplitude continued to decrease. An anomaly to this is that all stage 5 mutants had a 30–40-sec quiescent period immediately after reaching their maximum rate of activity. During this quiescent period, if the motoneuron was given a test stimulus, the amplitude appeared to be partially restored. (See the stage 5 example in Figure 7B.) After this quiescent period continuous spontaneous activity resumed at its maximum rate in the mutant and then the rate decreased with time at high temperature. The quiescence was not due to motoneuron fatigue since the DLM responded normally to motoneuron stimulation. The quiescence may be due to fatigue in unknown presynaptic neurons which drive the motoneurons into high rates of spontaneous activity in the double mutants.

The *e(sei)* does not affect [^{3}H]saxitoxin binding parameters: Since *e(sei)* causes neuronal hyperexcitability and interacts with the *sei* mutations which affect [^{3}H]saxitoxin binding parameters (JACKSON *et al.* 1984, 1985), we were interested in determining whether *e(sei)* alone affects saxitoxin binding. As shown in Figure 8 saxitoxin binding to *e(sei)* head membrane extracts is identical to that in wild-type control extracts at 0°. The extracts were assayed only

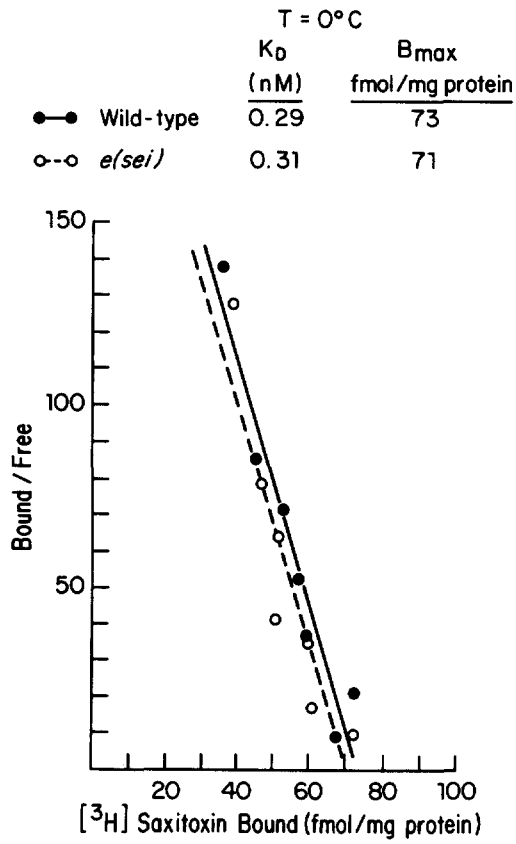

FIGURE 8.—Scatchard analysis of [^{3}H]saxitoxin-binding activity in *e(sei)* and wild-type extracts. Membrane extracts were prepared and assays were performed at 0°C as described by JACKSON *et al.* (1985). Data were plotted after the manner of SCATCHARD (1949). *X*-intercepts indicate the number of saturable saxitoxin-binding sites (B_{max}) in preparations. The slope of lines is the negative reciprocal of the K_D for saxitoxin binding.

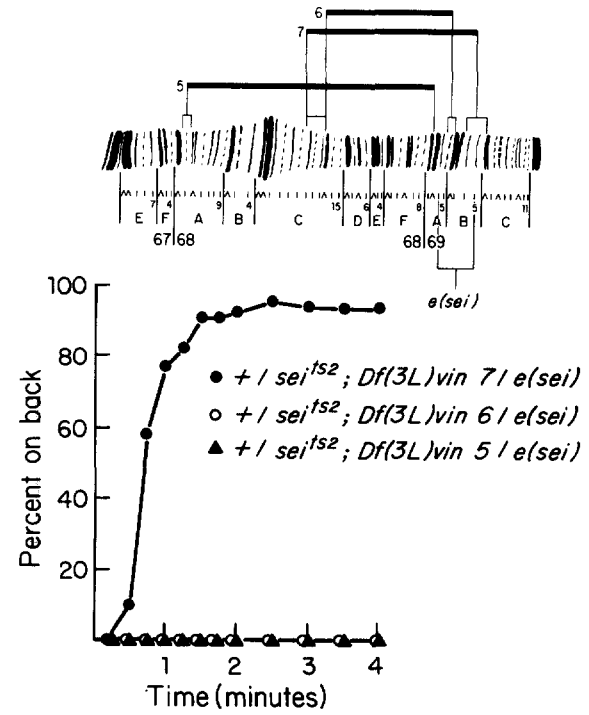

FIGURE 9.—Cytogenetic localization of *e(sei)* to 69A4-B5. *TM3, Sb e^s Ser/Df(3L)vin* males were mated with *bw sei^{ts2}; e(sei) st* females. F_1 progeny that were *bw sei^{ts2}/+; Df(3L)vin/e(sei) st* were tested for paralysis at 38° indicating that the *vin* deficiency uncovered the *e(sei)* mutation. The deficiencies *vin^5* and *vin^6* did not uncover *e(sei)*, whereas *vin^7* did. This places the *e(sei)* locus between the proximal breakpoints of *vin^6* and *vin^7*. The indicated limits for the *vin* deficiencies were taken from AKAM *et al.* (1978). Uncertainty about the exact location of breakpoints is indicated by brackets. The lower graph shows the behavioral data for each deficiency heterozygote. Each curve represents 67–84 flies.

at this temperature because *e(sei)* does not show temperature dependent effects on its own locomotor behavior. (Refer to the RESULTS section on climbing behavior.) The B_{max} values in this experiment are lower than those which we have reported previously. (See for example JACKSON *et al.* 1984, 1985.) This reflects inaccuracies in determining the actual specific activity of the [^{3}H]saxitoxin. To control for this variation the wild-type and mutant extracts are assayed simultaneously and comparisons are made only within single experiments. Within the accuracy of this binding assay, the *e(sei)* does not affect the saxitoxin binding site of the voltage-dependent sodium channel.

The *e(sei)* locus maps to region 69A4-69B5 of the third chromosome: Mapping studies were undertaken to determine if the *seizure*-enhancer interaction, the *e(sei)* climbing response defect, and the *e(sei)* electrophysiological phenotype were all due to a single mutation. As described in MATERIALS AND METHODS, the enhancer was found to be 4.2 map units distal to *th*. Since *th* has been mapped to 43.2, this places *e(sei)* at position 39.0 on the left arm of chromosome 3. The *vin* deficiencies (AKAM *et al.* 1978), which lie in this region were tested for complementation with *e(sei)*. As shown in Figure 9, *Df(3L) vin^7* fails to complement the enhancer mutation whereas *Df(3L) vin^5* and *vin^6* complement it. Thus, *e(sei)* lies in the region 69A4-69B5, which is comprised of six polytene chromosome bands. This localization of *e(sei)* is further strengthened by the finding that a hybrid dysgenesis induced *e(sei)* allele was isolated concomitantly with the insertion of a *P*-factor into the 69B region. Figure 10 shows the localization of the *P* insertion in the region uncovered by the *vin^7* deficiency. The climbing response defect and the electrophysiological phenotypes of *e(sei)* are also uncovered by *vin^7* but not by *vin^5* and *vin^6* suggesting that all three phenotypes result from the same mutation. This conclusion is further supported by the fact that independently isolated hybrid dysgenesis-induced *e(sei)* alleles also display the climbing response defect.

DISCUSSION

The *e(sei)* represents a new class of behavioral mutations that affect membrane excitability. Mutant enhancer flies exhibit brief bouts of hyperactivity when agitated. This behavioral phenotype is more subtle than that of previously isolated membrane excitability

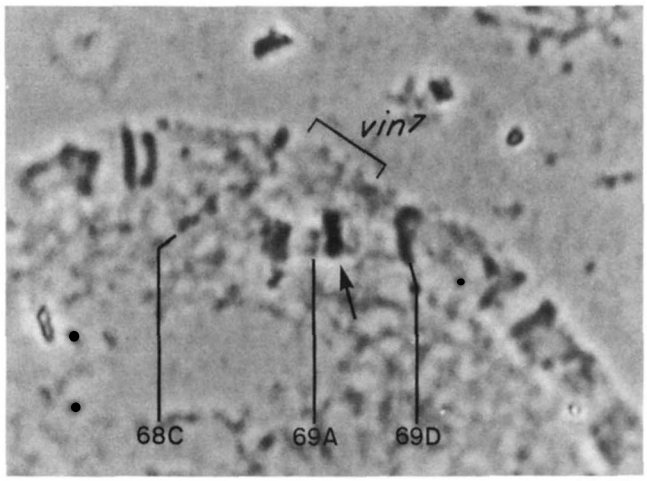

FIGURE 10.—Localization of the *P*-element insertion in a hybrid-dysgenesis induced *e(sei)* allele. Salivary gland polytene chromosome squashes were prepared from larvae carrying the dysgenesis-induced *e(sei)* mutation bearing chromosome over *Df(3L)vin*^{7}. This deficiency, with breakpoints at 68C8-11 and 69B4-5, uncovers the *e(sei)* locus. The plasmid pπ25.1 was biotinylated and used as a hybridization probe as described in MATERIALS AND METHODS. The arrow indicates hybridization to 69B which is within the region uncovered by the deficiency. Proximal is to the right and distal left.

mutants which show phenotypes such as temperature-sensitive paralysis, ether-induced leg shaking and mechanical stress-induced paralysis (GANETZKY and WU 1985; TANOUYE *et al.* 1986). The *e(sei)* defect can be detected by quantitating its interference with the climbing response of adult flies in a countercurrent apparatus. However, it would be difficult to isolate and map mutants, such as the enhancer, on the basis of such subtle effects that can be best measured in populations rather than individual flies. In our experiments the isolation and mapping studies relied instead on the ability of the enhancer to lower the paralysis temperature of *seizure* alleles. Thus, these studies illustrate how gene interactions that modify the mutant phenotypes of known excitability loci can be used to identify additional excitability mutations with novel, unexpected, and subtle behavioral defects.

These studies also provide the first electrophysiological evidence that the *seizure* locus affects cell excitability. Previously, JACKSON *et al.* (1985) observed that at restrictive temperatures both *seizure* alleles induced convulsive seizures which led to rapid paralysis. Additionally, they found that the recovery rate upon return to permissive temperature was dependent on the duration of paralysis. Interestingly, the recovery was accelerated in *nap*^{ts}; *sei* double mutants. That is, *nap*^{ts}, which reduces membrane excitability, appeared to suppress the *seizure* mutant phenotype. On the basis of these results JACKSON *et al.* (1985) proposed that the *seizure* mutations increased membrane excitability. The results of our electrophysiological studies support this proposal. For both *seizure* alleles temperature shifts from 22° to 42.5° induced spontaneous activity in the giant fiber system which increased in frequency with increases in temperature. The onset of spontaneous activity in each mutant occurs at the same temperature as that which causes paralysis. This indicates that the behavioral phenotypes of the mutants result from effects on membrane excitability.

Our experiments indicate that both *seizure* and *enhancer of seizure* cause neuronal hyperexcitability. This suggests two possible mechanisms to explain behavioral paralysis. One is that the high rate of spontaneous firing of mutant neurons at elevated temperature causes a depletion of transmitter in nerve endings. This depletion makes it impossible for the motoneurons to elicit muscle contraction and thus paralysis results. Alternatively, the spontaneous activity may produce tonic contractions in the muscles resulting in paralysis.

The temperature pulse triggered high levels of spontaneous activity (stages 4 and 5) in only a small proportion of the *e(sei)* flies (2 out of 29 total reached stage 4). However, stage 5 activity could be induced in five out of seven *e(sei)* flies at elevated temperature by briefly stimulating the motoneurons at high frequencies (J. C. NELSON, unpublished observations). Thus, it appears that the *e(sei)* defect amplifies existing activity but does not itself initiate high rates of spontaneous activity. The initial activity may be triggered by various means including the *seizure* mutations, mechanical agitation or electrical stimulation. In other words, the *e(sei)*$^{+}$ gene product may function to dampen the effects of background spontaneous electrical activity and thereby control general levels of excitability. This could account for the apparent lack of interaction between *e(sei)* and hypoexcitable mutations such as *nap*^{ts}, *para* and *tip-E* because in the absence of a trigger for spontaneous activity the *e(sei)* defect would remain undetected.

Since both *seizure* alleles affect [^{3}H]saxitoxin binding to sodium channels in Drosophila membrane extracts (JACKSON *et al.* 1984, 1985), it was of interest to determine whether *e(sei)* also affects saxitoxin binding. Our results show that the *e(sei)* mutation does not affect saxitoxin binding at low temperature (0°). However, it will be interesting to use double mutants (*sei*; *e(sei)*) to determine whether *e(sei)* can magnify the effects on saxitoxin binding observed with *sei*^{ts1} and *sei*^{ts2} single mutants.

Ultimately, we are interested in determining the interactions between the *seizure* and *e(sei)* gene products at the molecular level and their relationship to sodium channels. The cytogenetic localization of *e(sei)* to a six band region together with the isolation of a *P*-element induced allele should allow us to clone this locus by the approach of transposon tagging.

We thank ROBERT J. WYMAN for helpful discussions, JANE KEEN

for invaluable assistance with *in situ* hybridizations, and JANET MACLEOD for preliminary mapping studies. This work was supported by The Muscular Dystrophy Association and by National Institutes of Health grant NS16209 to L.M.H. who is an Irma T. Hirschl-Monique Weill Caulier Career Scientist Awardee and a Jacob Javits Neuroscience Investigator. The electrophysiological experiments were supported by National Institutes of Health grant NS07314 and National Science Foundation grant BNS8510678 to ROBERT J. WYMAN.

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Communicating editor: W. M. GELBART