INDIRECT SUPPRESSION INVOLVING BEHAVIORAL MUTANTS WITH ALTERED NERVE EXCITABILITY IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

Two classes of *X*-linked behavioral mutants of *Drosophila melanogaster*, leg-shaking mutants and bang-sensitive mutants, are suppressed by nap^{ts} (no action potential, temperature-sensitive), an autosomal temperature-sensitive paralytic mutation. So far, nap^{ts} is found to suppress thirteen mutations at seven loci, two of which produce leg shaking and five bang-sensitivity. Suppression is recessive, occurs at temperatures permissive for nap^{ts}, and is indirect and function-specific rather than allele-specific. At restrictive temperatures, nap^{ts} is known to completely block all nerve activity. Several of the mutants suppressed by nap^{ts} are shown by neurophysiological experiments to have increased nerve excitability. The physiological defect of these mutants as well as their behavioral defect is suppressed by nap^{ts}. Thus, suppression occurs within individual neurons at the level of excitable membranes and apparently depends on the reduction in membrane excitability caused by nap^{ts} even under permissive conditions. We suggest that all mutants suppressed by nap^{ts} may have related defects leading to enhanced nerve excitability. Genetic interactions of this type help reveal functional relationships between different behavioral mutants and suggest ways of isolating new mutants with altered excitable membranes.

BEHAVIOR of a complex organism such as Drosophila is a composite of sensory input, integration, and motor output. These functions are mediated by the basic units of the nervous system, the receptor cells, the individual neurons and muscle fibers. These are characterized by their electrically excitable outer membrane which enable them to receive, process, and transmit information in the form of electrical impulses called action potentials. These brief electrical impulses involve sudden fluxes of sodium and potassium ions across the cell membrane due to transient increases of membrane permeabilities to these ions (HODGKIN and HUXLEY 1952). Information is transferred between neurons or between a neuron and a muscle fiber at synapses which are specialized regions of contact. Release of a chemical neurotransmitter at the presynaptic terminal is evoked upon arrival of action potentials. The transmitter interacts with the

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postsynaptic cell causing it to fire an action potential in turn (reviewed by KUFFLER and NICHOLLS 1976). The special properties of electrically excitable cell membranes are determined by a set of membrane proteins that establish and maintain the ionic gradients across the membranes and control the voltage-dependent and ion-selective permeabilities involved in the generation of action potentials (STEVENS 1979). Thus, one approach to the genetic dissection of behavior is to focus on genes that encode the protein components controlling excitable membranes. Isolation of appropriate mutants will identify the genes that specify these macromolecular components and characterization of these mutants will reveal the normal role of particular gene products in the function of the nervous system. For these reasons recent work in a number of laboratories has concentrated on the neurophysiological analysis of behavioral mutants of *Drosophila melanogaster* with altered excitable membranes (reviewed by HALL and GREENSPAN 1979).

A further extension of this approach is to inquire about the functional relationships that exist among the various gene products involved in membrane excitability. One way to obtain information on this question is through evidence of indirect suppression between two mutants. Indirect suppression, in contrast to informational suppression, does not restore the function of a mutant protein by altering its amino acid sequence. Instead, the mutant protein is still made in altered form but the functional consequences of this are compensated for, or modified by, mutations at other loci. Opportunities for indirect suppression are particularly plentiful in complex biological structures or processes that involve a number of interdependent steps. For example, indirect suppression has been useful in the analysis of various biochemical pathways, ribosome assembly, and phage morphogenesis (reviewed by HARTMAN and ROTH 1973).

Excitable membranes should present opportunities for indirect suppression. Receptors that bind neurotransmitter are known to be protein complexes (WEILL, MCNAMEE and KARLIN 1974) and the ion-selective, voltage-dependent gates or channels that regulate membrane permeability may also be comprised of several different polypeptide subunits (HARTSHORNE and CATTERALL 1981). Moreover, as described, there are numerous interdependent steps within a cell and between cells involved in the relay of electrical impulses from one part of the nervous system to another. Mutants that affect related or sequential steps in this pathway might also interact in special ways. To explore these possibilities, we have examined various behavioral mutants in Drosophila for evidence of indirect suppression.

We report here that even under permissive temperatures, *nap*^{ts} (no action potential, temperature-sensitive), a temperature-sensitive paralytic mutation that blocks action potentials at 37° (WU *et al.* 1978) suppresses the behavioral defect caused by mutations at seven other loci. In several instances, we are able to demonstrate directly by neurophysiological experiments that suppression occurs within individual neurons at the level of the excitable membrane and apparently depends on the reduction in membrane excitability caused by *nap*^{ts}. We infer the existence of analogous suppression mechanisms in the other cases.

These results suggest that all mutants suppressible by *nap*^{ts} may share related neurophysiological defects. Information of this type is useful in elucidating the primary defect in a collection of behavioral mutants and in developing screens for the isolation of new mutants with particular defects.

MATERIALS AND METHODS

Mutants: The isolation and characterization of *nap*^{ts} (2–56.2) has been previously described (WU *et al.* 1978; WU and GANETZKY 1980). Isolation and characterization of *Sh*^{5} (Shaker) and *Hk* (Hyperkinetic) were described by KAPLAN and TROUT (1969). Other *Sh* mutants used in these studies are *Sh*^{KS133}, *Sh*^{rKO120}, *Sh*^{E62}, and *Sh*^{M}. The first three were induced with ethyl methanesulfonate in the laboratory of S. BENZER and *Sh*^{M} was of spontaneous origin in the same laboratory. The *Sh* mutants are either dominant or incompletely recessive so complementation tests are difficult. However, they all map to the same genetic region (see Table 1). Map positions for *Sh*^{5}, *Sh*^{KS133} and *Sh*^{rKO120} have been reported previously (KAPLAN and TROUT 1969; JAN, JAN and DENNIS 1977). *Sh*^{E62} and *Sh*^{M} were mapped to the right of *f* in this study, close to the position of other *Sh* mutants. Although considered to be alleles in these studies, recent evidence (TANOUYE, FERRUS and FUJITA 1981) suggests that the *Sh* region may be functionally complex.

bas (bang-sensitive) was isolated by GRIGLIATTI *et al.* (1973) and provided to us by L. HALL.

bas^{MW1} was isolated in the laboratory of S. BENZER (JAN and JAN 1978). Originally thought to be allelic to *bas*, we show here that they are separate loci (see RESULTS). For this reason the mutant has been renamed *bss*^{MW1} (bang-senseless).

tko^{25t} (technical knockout) was isolated and described by JUDD, SHEN and KAUFMAN (1972). Mutant stocks were provided to us by B. JUDD.

PC80, RH11, PC64 and *PC75* are mutant lines isolated in the Benzer laboratory because of their "easily-shocked" phenotype (BENZER 1971). We show here (see RESULTS) that *PC80* and *RH11* are alleles and name the locus they define *eas* (easily-shocked). *PC64* defines another locus which we named *kdn* (knockdown). *PC75* is allelic to *bss*^{MW1} (see RESULTS) and is now designated *bss*^{2}.

TABLE 1

List of mutants found to be suppressed by nap^{ts}

Mutant	Map position	References
Leg-shaking mutants		
Sh^{5}	1–58.2	KAPLAN and TROUT 1969
Sh^{rKO120}	1–57.4	JAN *et al.* 1977
Sh^{KS133}	1–57.9	JAN *et al.* 1977
Sh^{M}	1–58.7	This paper
Sh^{E62}	1–57.7	This paper
Hk	1–30.9	KAPLAN and TROUT 1969
Bang-sensitive mutants		
bas	1–49.5	GRIGLIATTI *et al.* 1973
bss^{MW1}	1–54.0	JAN and JAN 1978; This paper
bss^{2}	1–54.0	This paper
eas	1–53.6	This paper
eas^{2}	1–53.2	This paper
kdn	†	This paper
tko^{25t}	1– 0.99	JUDD *et al.* 1972

† *kdn* was located in the region between *cv* and *v*. Further mapping has not been done.

The *X*-linked behavioral mutants used in these studies are listed in Table 1. See LINDSLEY and GRELL (1968) for description of other mutants used in these studies. Wild-type controls were from the Canton-S strain.

Cytological mapping: The following duplications and deficiencies were used in the mapping of several of the mutants described in this report:

$Dp(1;4)r^+f^+$: carries 14A–16A2 appended to chromosome *4*.

$T(1;2)r^{+75c}$: carries 14B13–15A9 inserted into chromosome *2* in salivary region 35D-E.

$T(1;3)f^{+71b}$: carries 15A4–16C2,3 inserted into the heterochromatin of chromosome *3*.

$Df(1)r^{D1}$: deleted for 14D–15D.

All the above chromosomes were provided by G. LEFEVRE.

$Df(1)sd^{72b26}$: deleted for 13F1–14B1, provided by C. POODRY.

We also used *T(Y;2)L12*, an insertional translocation of salivary region 41–43A into B^SYy^+ (SANDLER and CARPENTER 1972). This duplicated fragment carries the nap^+ locus (see WU *et al.* 1978) and will be abbreviated in this report as the nap^+Y chromosome.

Construction of double mutant stocks: Double mutant males carrying one of the *X*-linked mutants listed in Table 1 and homozygous for nap^{ts} were constructed by two successive backcrosses to $\hat{X}X/Y;\ nap^{ts} = C(1)FMA4,\ In(1)w^{m4} + AB/In(1)FM7,\ y^2bb^-/Y;\ nap^{ts}$ females. Control males carrying the nap^+Y chromosome were constructed in similar fashion by crosses to $\hat{X}X/nap^+Y;\ nap^{ts}$ females.

Behavioral tests: Leg-shaking behavior in *Sh* and *Hk* flies was monitored after exposing flies to ether for 15–30 seconds in a standard etherizer. Under these conditions wild-type flies show occasional twitching of tarsal segments while *Sh* and *Hk* display vigorous shaking of the entire leg.

To measure the duration of paralysis of bang-sensitive mutants, a standard "bang" was delivered to flies. This consisted of vibrating flies in culture vials (one to five flies per vial) on a vortex mixer (Scientific Products, S8223) at top speed for 10 seconds. Length of paralysis was measured as the time required after "banging" until flies regained the ability to stand upright. Varying the duration of the "bang" between 5 and 15 seconds had little or no effect on the length of paralysis. Wild-type flies are unaffected by this treatment.

Flies were grown and tested at 21–23° for all experiments described here.

Neurophysiology: Intracellular recordings from muscles and extracellular recordings from nerves were carried out on the larval neuromuscular preparation according to procedures described previously (JAN and JAN 1976a,b; WU *et al.* 1978).

Photography: Photographs were taken with a Wild dissecting microscope equipped with dark-field illumination. Long exposure times (5–20s) were used to record motion.

RESULTS

Behavior of leg-shaking mutants: Mutants of several distinct loci have been described that display abnormal, vigorous leg-shaking behavior when etherized (KAPLAN and TROUT 1969). Two of these mutants previously studied in some detail are *Hk* (KAPLAN and TROUT 1969; IKEDA and KAPLAN 1970) and *Sh* (KAPLAN and TROUT 1969; JAN, JAN and DENNIS 1977; JAN and JAN 1979; TANOUYE, FERRUS and FUJITA 1981; SALKOFF and WYMAN 1981; GANETZKY and WU 1982). Etherized *Sh* flies display rapid leg-shaking, and with certain alleles wing-scissoring as well (Figure 1). It has been reported (KAPLAN and TROUT 1969) that anesthetization with chloroform or ethyl acetate does not elicit this behavior in mutant flies and we have found a similar lack of effect using carbon dioxide. However, leg-shaking behavior is displayed by mutant flies when anesthetized by nitrogen or triethylamine indicating that the behavioral response is not strictly dependent on ether. Moreover, abnormal loco-

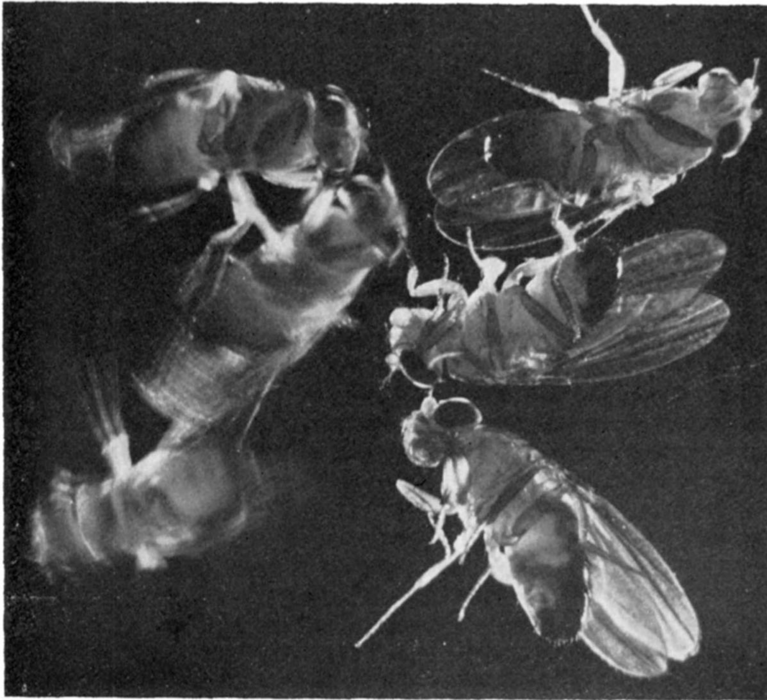

FIGURE 1.—Sh^{KS133} mutant flies (left) display rapid and vigorous leg shaking and wing scissoring after ether anesthesia. The *Sh* phenotype is suppressed by nap^{ts} since Sh^{KS133}; nap^{ts} double-mutant flies (right) appear like normal flies and are completely immobilized after anesthetization. Exposure time: ten seconds.

motor activity is apparent in mutant individuals even when they are not anesthetized. *Sh* flies, particularly those older than one week, walk in jerky uncoordinated steps. Often they stand quivering on the bottom of the culture bottle. The behavior of *Hk* flies when etherized is similar to that of *Sh*.

Suppression of shaking behavior by nap^{ts}: Homozygous nap^{ts} flies have normal locomotor activity at permissive temperatures (less than 34°) and do not differ from wild type in their response to ether (WU *et al.* 1978). Double mutant males hemizygous for Sh^{KS133} and homozygous for nap^{ts} were constructed and found not to shake following anesthetization for ether, nitrogen or triethylamine (Figure 1) even at permissive temperatures for nap^{ts}. This effect of nap^{ts} on *Sh* can also be seen in double mutant flies that are not anesthetized since $Sh;nap^{ts}$ double mutants are noticeably more coordinated than *Sh* individuals. Thus, nap^{ts} acts as a suppressor of the *Sh* behavioral defect and, moreover, does so at temperatures where nap^{ts} is itself behaviorally normal.

The ability of nap^{ts} to suppress the *Sh* phenotype is not shared by other temperature-sensitive paralytic mutants. For example, $para^{ts}$ (SUZUKI, GRIGLIATTI and WILLIAMSON 1971) which is similar to nap^{ts} both in behavior and in some neurophysiological aspects (WU and GANETZKY 1980), does not suppress the

Sh defect at permissive temperatures. Another temperature-sensitive paralytic mutant, shi^{ts}, (GRIGLIATTI *et al.* 1973) also is unable to suppress *Sh*.

By what mechanism does nap^{ts} suppress *Sh*? There are two general mechanisms of intergenic suppression—informational or direct suppression and indirect suppression. Informational suppressors operate via the protein-synthesizing system to restore a functional amino acid sequence in the mutant protein. Indirect suppressors revert a mutational defect to a normal or nearly normal phenotype without modifying the production of the mutant gene product (cf. HARTMAN and ROTH 1973; RIDDLE and BRENNER 1978). The following observations indicate that the suppression by nap^{ts} is indirect rather than informational.

A number of mutations at the *Sh* locus have been isolated in several laboratories (KAPLAN and TROUT 1969; JAN, JAN and DENNIS 1977). Although each of these mutants has the same general defect they are distinguishable behaviorally and neurophysiologically (JAN, JAN and DENNIS 1977; TANOUYE, FERRUS and FUJITA 1981; SALKOFF and WYMAN 1981; GANETZKY and WU 1982) indicating that they are probably distinct alleles. The following *Sh* alleles were tested for suppression by nap^{ts} (see Table 1): Sh^{5}, Sh^{KS133}, Sh^{rKO120}, Sh^{E62} and Sh^{M}. In each case, suppression of leg-shaking behavior by nap^{ts} was complete. Thus suppression by nap^{ts} is not allele specific.

In Sh/Y; $nap^{ts}/+$ flies shaking behavior was not suppressed. By use of an insertional translocation that moves nap^{+} into the *Y* chromosome, we constructed flies carrying two doses of nap^{ts} and a single dose of nap^{+} *i.e.* $Sh/nap^{+}Y$; nap^{ts}/nap^{ts}. These flies also displayed the typical *Sh* phenotype indicating that suppression by nap^{ts} is completely recessive and results from loss of a function on which manifestation of the *Sh* phenotype depends rather than from a compensatory gain of function. Dominant suppressors of the latter type have been reported for several uncoordinated mutants in nematodes (RIDDLE and BRENNER 1978).

To examine the range of suppression by nap^{ts}, we constructed Hk/Y; nap^{ts}/nap^{ts} double mutants and found that leg-shaking behavior was suppressed in these flies too. Typical *Hk* behavior is again restored by the presence of a single dose of nap^{+} in $Hk/nap^{+}Y$; nap^{ts}/nap^{ts} flies.

Physiological mechanism of suppression of Shaker mutants: It has been observed that legs from *Sh* or *Hk* flies, continue to shake after being severed from the thorax at the coxa (Figure 2). However, legs similarly severed from *Sh*; nap^{ts}, or *Hk*;nap^{ts} or wild-type individuals shake very little or not at all (Figure 2).

Synaptic inputs to motor neurons in Drosophila are confined to the thoracic ganglion. In the cut leg the motor axons that innervate the muscles do not receive any synaptic inputs. Therefore, shaking behavior and its suppression by nap^{ts} do not require neural connections in the central nervous system. Altered properties of either the motor axon or muscle are sufficient to produce leg-shaking in *Sh* and *Hk*. Apparently nap^{ts} exerts its suppressive effect at the same level.

As reported previously, (JAN and JAN 1976a,b; WU *et al.* 1978) the Drosophila larva is well-suited for studies of the physiological properties of motor

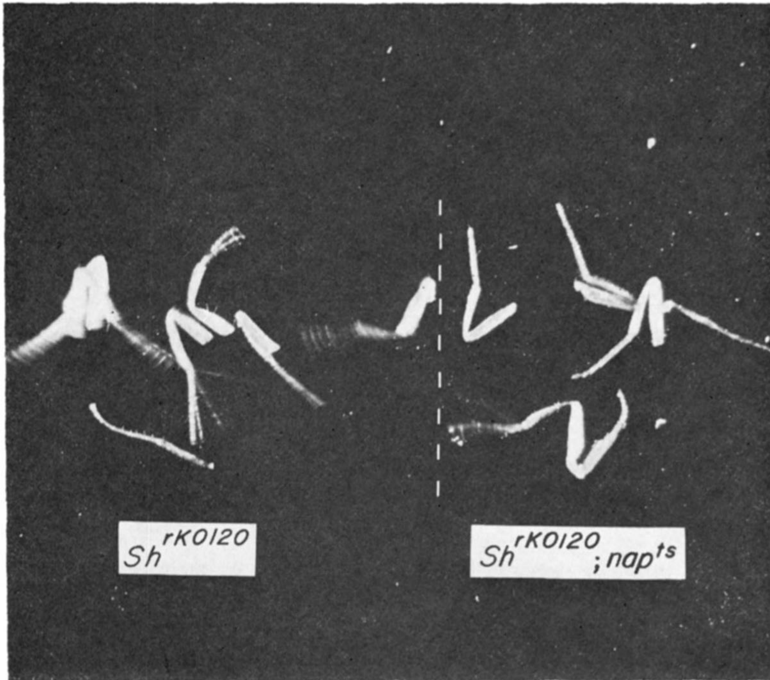

FIGURE 2.—Legs severed from anesthetized Sh^{rKO120} flies (left) continue to twitch while those from Sh^{rKO120}; nap^{ts} flies (right) remain relatively immobile. 23°. Exposure time 12 seconds.

axons and synaptic transmission at the neuromuscular junction. This preparation has been used in detailed neurophysiological studies of *Sh* (JAN, JAN and DENNIS 1977; JAN and JAN 1979), nap^{ts} (WU *et al.* 1978; WU and GANETZKY 1980) and the interactions between them (GANETZKY and WU 1982).

In *Sh* larvae, the membrane of the motor neuron is hyperexcitable due to a defect in potassium conductance and repetitive action potentials are generated in response to a single stimulus applied to the nerve (Fgure 3). As previously reported (JAN and JAN 1979; GANETZKY and WU 1982), the repetitive firing of the motor axons is associated with a prolonged release of neurotransmitter at the neuromuscular junction which produces a postsynaptic response (excitatory junctional potential or ejp) of long duration and large amplitude, strikingly different from the wild-type response (Figure 3). In *Sh/Y*; nap^{ts} double mutants, repetitive firing is absent and the time course and amplitude of the ejp are similar to that observed in wild-type and nap^{ts} individuals.

A detailed description of the physiological basis of repetitive firing in *Sh* and its suppression by nap^{ts} is presented elsewhere (GANETZKY and WU 1982). The results of those experiments can be summarized here by saying that suppression of *Sh* by nap^{ts} is consistent with our previous demonstration that excitability of axonal membranes is reduced in nap^{ts} individuals even under permissive conditions (WU and GANETZKY 1980). This effect apparently counter-

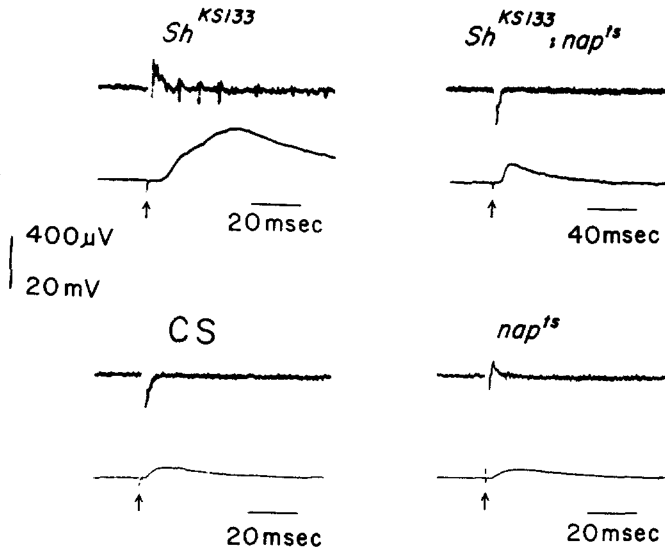

FIGURE 3.—Simultaneous intracellular recordings of muscle response (lower trace of each panel) and extracellular recordings of action potentials from nerve (upper traces) that innervates the muscle in mutant and wild-type (CS) larvae. Composition of bathing medium and details of neurophysiological techniques were previously described (WU *et al.* 1978). Ca^{2+} concentration for these experiments was 0.2 mM. In CS the nerve response to a stimulation (arrow) consists of a single compound action potential and the muscle response (ejp) has a small amplitude at this Ca^{2+} concentration. In Sh^{KS133} a single stimulus applied to the nerve elicits multiple nerve action potentials (seen here as regularly spaced downward deflections) and an ejp of greater amplitude and duration than wild type. Note the stepwise increase in amplitude of the ejp correlated with the occurrence of extra nerve action potentials. In the Sh^{KS133}; nap^{ts} double mutant the repetitive firing and prolonged ejp are suppressed. The nerve and muscle responses of nap^{ts} are similar to those of wild-type under these conditions. Voltage calibration: 400 µV for nerve traces; 20 mV for muscle traces. Note different time scale for Sh^{KS133}; nap^{ts} traces. 23°.

balances the increased membrane excitability due to the defective potassium permeability in *Sh*. Thus, suppression of *Sh* by nap^{ts} is demonstrated at both the behavioral and the cellular level.

No abnormality in neuromuscular transmission has been detected in similar experiments with *Hk* larvae (GANETZKY and WU unpublished observations). However, intracellular recordings from the thoracic ganglion of *Hk* adults have demonstrated that some neurons produce rhythmic bursts of repetitive action potentials not found in wild type (IKEDA and KAPLAN 1970). Although no attempts have been made to repeat these experiments on *Hk*; nap^{ts} adults, since we know that nap^{ts} also affects adult flies (WU *et al.* 1978) we would predict that abnormal nerve activity in *Hk* adults is suppressed by nap^{ts}.

Genetic and cytological mapping of bang-sensitive mutants: Another group of behavioral mutants that we have studied are bang-sensitive paralytics which become paralyzed for several minutes following a sudden jolt or vibration of the culture vial. A number of mutants with this phenotype have been inde-

pendently isolated (BENZER 1971; JUDD, SHEN and KAUFMAN 1972; GRIGLIATTI *et al.* 1973; HOMYK and SHEPPARD 1977; HOMYK, SZIDONYA and SUZUKI 1980). Complementation tests and recombinational and cytological mapping described below revealed that the bang-sensitive mutants we studied comprise five distinct loci. Each of these mutants was tested in combination with nap^{ts} and in every case the bang-sensitive phenotype was suppressed. Before detailing the double-mutant interactions, a brief description of each bang-sensitive mutant is given below.

GRIGLIATTI *et al.* (1973) isolated a bang-sensitive paralytic mutant named *bas* (bang-sensitive) which mapped between *v* (1–33.0) and *f* (1–56.7) at 47.2 on the *X* chromosome. We mapped *bas* using the markers *g* (1–44.4) and *sd* (1–51.5). The map position of *bas* based on 290 recombinants between *g* and *sd* is 49.5, in reasonable agreement with previous results (GRIGLIATTI *et al.* 1973). The *bas* locus is not uncovered by $Df(1)sd^{72b26}$ nor is it covered by $Dp(1;4)r^+f^+$ placing it to the left of 13F (Figure 4).

JAN and JAN (1978) described another bang-sensitive mutant (bas^{MW1}) isolated in BENZER's laboratory which they considered to be an allele of *bas*. However, we have found that the two mutants are not alleles. The semidominant behavior of bas^{MW1} (Figure 6A) may have confused previous complementation tests. However, heterozygotes for bas^{MW1} are distinguishable from homozygotes or hemizygotes by the length of time they remain paralyzed. The phenotypes of bas/bas^{MW1} heterozygotes and $+/bas^{MW1}$ heterozygotes are the same indicating that *bas* and bas^{MW1} do complement.

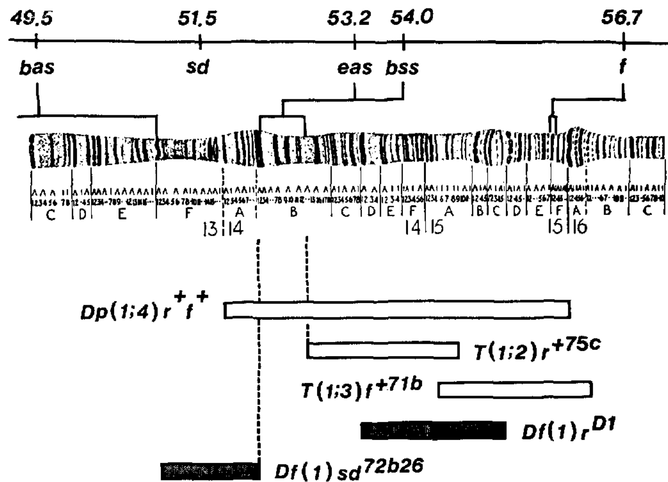

FIGURE 4.—Summary of recombination and cytological mapping of mutant loci in the proximal region of the *X* chromosome that cause paralysis when flies are subjected to mechanical shock. Top line: Recombination map. Below: Diagram of salivary chromosome banding pattern. The breakpoints of various duplications and deletions used to determine the cytological positions of the mutants are shown. Open bars are duplications, the stippled bars represent deletions.

To confirm this, we mapped bas^{MW1} relative to *g*, *sd*, and *f* and located it at 1–54.0 based on 107 recombinants between *sd* and *f*. Cytological localization of bas^{MW1} agrees with this recombinational mapping (Figure 4). $Dp(1;4)r^+f^+$ covers bas^{MW1} but $T(1;2)r^{+75c}$ and $T(1;3)f^{+71b}$ did not cover bas^{MW1} nor did $Df(1)sd^{72b26}$ uncover it. This places bas^{MW1} in 14B quite separate from *bas*, establishing that they are distinct loci. To indicate that it represents a new locus, we propose that bas^{MW1} be renamed bss^{MW1} (bang-senseless) and we will use this nomenclature hereafter.

PC75 is allelic to bss^{MW1} as the following results demonstrate. Flies of the genotype *PC75*/+ become paralyzed when vibrated but they can be distinguished from hemizygotes or homozygotes by their shorter duration of paralysis, similar to the semidominant nature of bss^{MW1}. Cytological mapping using the previously described deletions and duplications placed *PC75* in 14B. The duration of paralysis in $PC75/bss^{MW1}$ flies corresponded to that of *PC75* or bss^{MW1} homozygotes. In addition, *PC75* and bss^{MW1} appear to be genetically inseparable because no wild type recombinant sons were found among 3100 male offspring produced by $PC75/bss^{MW1}$ females. Finally, the neurophysiological abnormality which is characteristic of bss^{MW1} (see below) was found to be present in *PC75* also (GANETZKY, unpublished results). *PC75* is now designated as bss^2.

Three other bang-sensitive mutants which we examined are *PC80*, *RH11*, and *PC64*. Complementation tests among these recessive mutants revealed that *PC80* and *RH11* are alleles and distinct from *bas* and bss^{MW1}. *PC64* is not allelic to *bas*, bss^{MW1}, or *PC80*. Thus, these three mutants define two more bang-sensitive loci. *RH11* was mapped and found to lie between *sd* and *f* very close to the bss^{MW1} locus. To be certain that these mutants defined separate loci, *PC80* was mapped directly against bss^{MW1}. Male offspring from $sd\ bss^{MW1}\ f/PC80$ females were tested for wild-type behavior and scored for *sd* and *f*. Among 2123 progeny tested, only nine were found that were not bang-sensitive. These nine males were all $sd\ f^+$ in phenotype. This places *PC80* about 0.8 map units left of bss^{MW1} at 53.2. Cytologically, *PC80* and *RH11* are located in 14B1–13, confirming the proximity of this locus to bss^{MW1} (Figure 4). We propose the name *easily-shocked* (*eas*) for the locus defined by *RH11* and *PC80*.

PC64 mapped to the interval between *cv* and *v* and represents a locus distinct from those previously discussed. This mutant, which we named knockdown (*kdn*) has not yet been further localized.

The remaining bang-sensitive paralytic mutant is technical knockout (tko^{25t}, 1–0.99) isolated and mapped by JUDD, SHEN and KAUFMAN (1972).

At present, it is unknown whether any of these mutants are allelic to the behaviorally similar stress-sensitive mutants that map to seven sites on the *X* chromosome (HOMYK and SHEPPARD 1977; HOMYK, SZIDONYA and SUZUKI 1980.

Behavioral characterization of bang-sensitive mutants: All the bang-sensitive mutants described above shared a common behavioral defect. When they are subjected to a sudden jolt, such as vibration from a vortex mixer, they become paralyzed (Figure 5). Paralysis lasts for 50 to several hundred seconds depend-

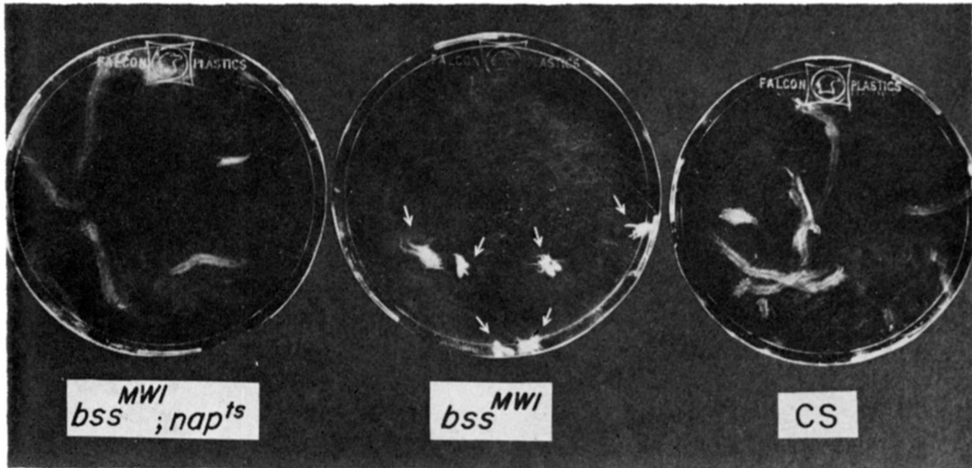

FIGURE 5.—Bang-sensitive behavior of bss^{MW1} and its suppression by nap^{ts}. Movement of flies immediately following stimulation was recorded as lighter tracks in this long (four seconds) exposure photograph. After being subjected to five second vibration on a vortex mixer bss^{MW1} flies were paralyzed (arrows), while CS flies showed increased activity in response to the agitation. The phenotype of bss^{MW1} was suppressed by nap^{ts} since the bss^{MW1}; nap^{ts} flies remain unparalyzed after the treatment. 23°.

ing on the mutant (see Table 2). After recovery from paralysis, the mutant flies again display normal locomotor ability.

The behavior of bss^{MW1} can be considered representative of this class of mutants. The length of paralysis following a standard "bang" (see MATERIALS AND METHODS) as a function of age is shown in Figure 6A. Hemizygous bss^{MW1} males are paralyzed for 100–400 sec, the length of paralysis increasing with age. The bang-sensitive phenotype is semidominant, heterozygous $bss^{MW1}/+$ females are paralyzed for about 50 seconds after banging. A single dose of bss^{MW1} is partially dominant over two doses of the wild-type allele since $bss^{MW1}/+/Dp(1;4)r^+f^+$

TABLE 2

Suppression of bang sensitive paralytic mutants by nap^ts

	$\frac{m}{Y}\ \frac{+}{+}$	$\frac{m}{Y}\ \frac{nap^{ts}}{nap^{ts}}$	$\frac{m}{nap^{+}Y}\ \frac{nap^{ts}}{nap^{ts}}$
bas	96.5 ± 14.9 (10)	0 (32)	149.4 ± 40.1 (9)
bss^{mW1}	216.0 ± 52 (11)	0 (27)	263.0 ± 65.4 (11)
eas^1	99.3 ± 11.5 (8)	0 (15)	109.5 ± 32.2 (10)
eas^2	56.5 ± 10.8 (10)	0 (10)	46.1 ± 4.8 (9)
kdn	237.9 ± 117.7 (12)	13.4 ± 15.4 (19)	182.8 ± 81.0 (7)
tko^{25t}	91.8 ± 26.3 (12)	0 (12)	89.0 ± 21.3 (15)

Duration of paralysis for flies of the indicated genotypes. For each row, *m* represents the bang-sensitive mutant indicated on the left. Flies were tested as described in MATERIALS AND METHODS. Duration of paralysis is given as the mean value ± standard deviation. Number of flies tested is indicated in parentheses. Flies were 5–7 days old when tested.

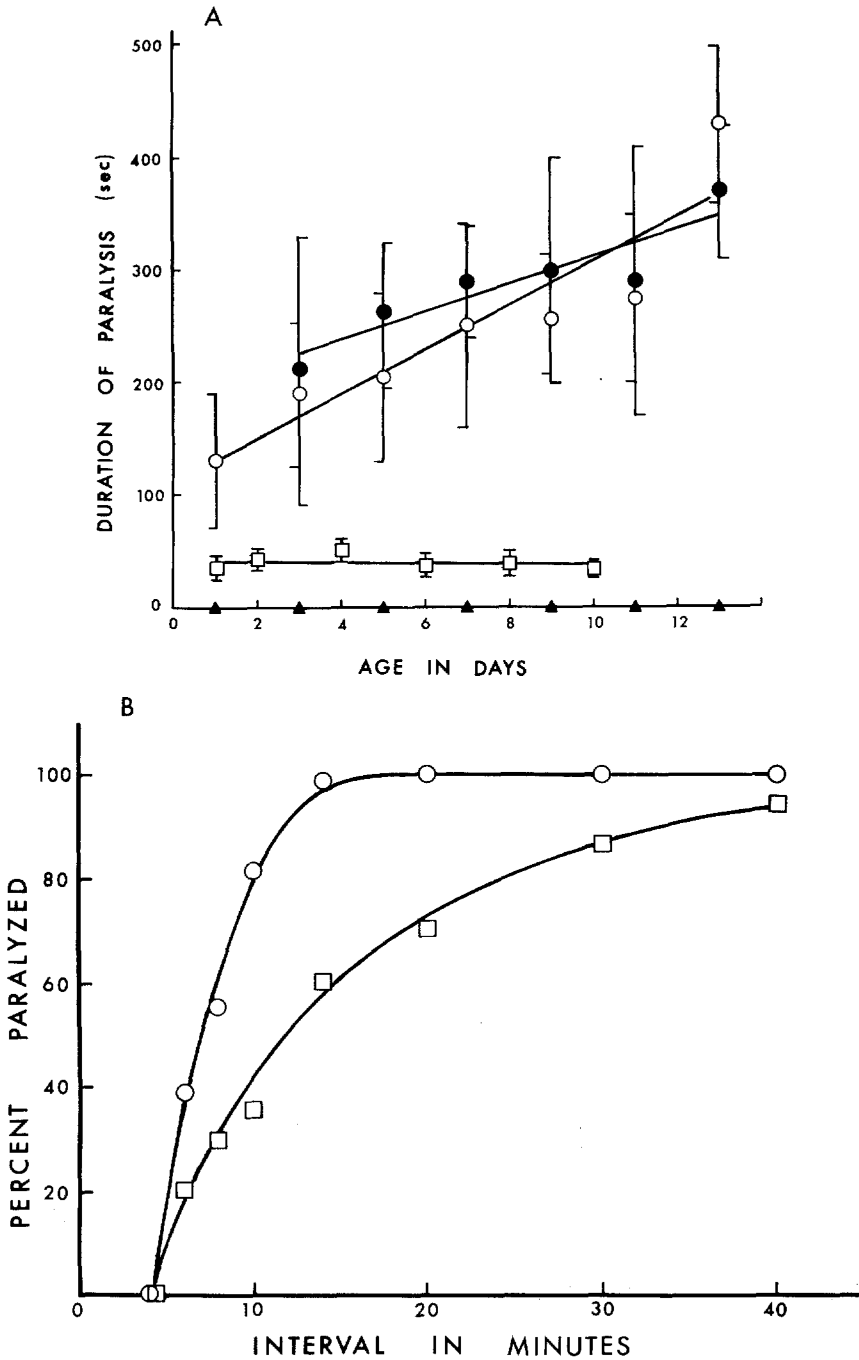

FIGURE 6.—A. Duration of paralysis as a function of age following a standard vibrational shock (see MATERIALS AND METHODS). Duration of paralysis increased with age in bss^{MW1}/Y males (○). Double mutant bss^{MW1}/Y; nap^{ts} *males* (▲) were not paralyzed by the same

flies are paralyzed for 40–50 sec following banging. The behavioral defect caused by bss^{MW1} is thus not due to loss of a wild-type function but to a mutant gene product that alters the normal process.

Immediately after recovering from bang-induced paralysis bss^{MW1} flies are insensitive to further vibration. This refractory period persists for several minutes. About 7 min of recovery following paralysis are required before 50 percent of a population of bss^{MW1} flies can be reparalyzed (Figure 6B). A similar refractory period was also found in $bss^{MW1}/+$ heterozygotes. However, the heterozygotes are apparently more resistant to reparalysis than homozygotes since a longer recovery period was required for half the population to become reparalyzed (Figure 6B). The refractoriness found in bss^{MW1} is characteristic of all the mutants in this class and has been previously noted for *bas* and tko^{25t} (GRIGLIATTI *et al.* 1973; JUDD, SHEN and KAUFMAN 1972).

One additional characteristic of *bas* not previously reported is that it also has a temperature-sensitive paralytic phenotype. At 38° *bas* flies become paralyzed (without banging) within 10–20 sec whereas wild-type flies are not affected by this temperature for 30–40 min. Following a five min exposure to 38° *bas* flies require two to three min at 21° before normal locomotor ability is regained. In the mapping experiments described above, the temperature-sensitive phenotype and the bang-sensitive phenotype of *bas* were inseparable and probably result from a single mutation. None of the other bang-sensitive mutants exhibit temperature-sensitive paralysis.

Suppression of bang-sensitive behavior by nap^{ts}: The behavior of bss^{MW1}/Y males compared to that of wild-type and $bss^{MW1}/Y;nap^{ts}$ males is shown in Figure 5 and 6A. The bang-sensitive phenotype of bss^{MW1} is suppressed by nap^{ts}. Double mutants do not pass out even when they are vibrated on the vortex mixer twice the usual length of time. Suppression occurs at temperatures permissive for nap^{ts} and is independent of the age of flies tested. The behavior of $bss^{MW1}/nap^{+}Y;\ nap^{ts}/nap^{ts}$ males is indistinguishable from that of bss^{MW1}/Y males (Figure 6A) demonstrating that suppression again is due to a loss of the nap^{+} function.

Results demonstrating suppression of each of the other bang-sensitive mutants by nap^{ts} are summarized in Table 2. Except for *kdn*, suppression by nap^{ts} was complete. Of 19 *kdn*;nap^{ts} double mutants tested, six did not pass out at all, 12 were stunned for periods ranging from 5–30 sec and one passed out for 60 sec. Nonetheless, it is evident that nap^{ts} reduces the severity of the behavioral defect even for *kdn*.

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treatment. Adding a single dose of nap^{+} in $bss^{MW1}/nap^{+}Y;\ nap^{ts}$ males (●) restores bang-sensitive phenotype. Heterozygous bss^{MW1} females (□) are paralyzed for shorter duration demonstrating semidominant nature of bss^{MW1}. Each point is based on the behavior of 10–20 individuals. Error bars indicate standard deviation. Linear regression lines are fitted to data points. The difference between bss^{MW1}/Y and $bss^{MW1}/nap^{+}Y;\ nap^{ts}$ is not statistically significant showing that the nap^{ts} suppressive effect is recessive.

B. Refractory period of bang-sensitive behavior for bss^{MW1}/Y males (○) and $bss^{MW1}/+$ females (□). Individual flies were tested for paralysis by subjecting them to the standard stimulus at intervals following recovery from previous vibration-induced paralysis.

Suppression of bss^{MW1} *physiological defect by* nap^{ts}: Of the various bang-sensitive mutants, bss^{MW1} is best characterized neurophysiologically (JAN and JAN 1978). Previous analysis of the larval neuromuscular junction in this mutant indicated that bss^{MW1} has abnormal long-term facilitation, *i.e.* under conditions where a single nerve stimulus evokes only a small ejp, repetitive stimulation leads to the production of a large, prolonged ejp in bss^{MW1} but not in wild-type (JAN and JAN 1978).

We have confirmed this result and found in addition that bss^{MW1} nerves are hyperexcitable, generating multiple action potentials together with the occurrence of the large prolonged ejp (Figure 7).

As shown in Figure 7, the physiological abnormality of bss^{MW1} is suppressed in bss^{MW1}/Y; nap^{ts} larvae. The nerve response and the accompanying ejp are like that of wild-type larvae under comparable conditions. The neurophysiological phenotype of $bss^{MW1}/nap^{+}Y$; nap^{ts} larvae is indistinguishable from that of bas^{MW1}/Y larvae. As with *Sh*, suppression of the bss^{MW1} behavioral defect is paralleled by suppression of the neurophysiological defect at the cellular level.

We have also examined *bas*, eas^{1}, eas^{2}, tko^{25t} and *kdn* for neurophysiological abnormalities at the larval neuromuscular junction but have detected no apparent difference from wild type. This does not eliminate the possibility of a

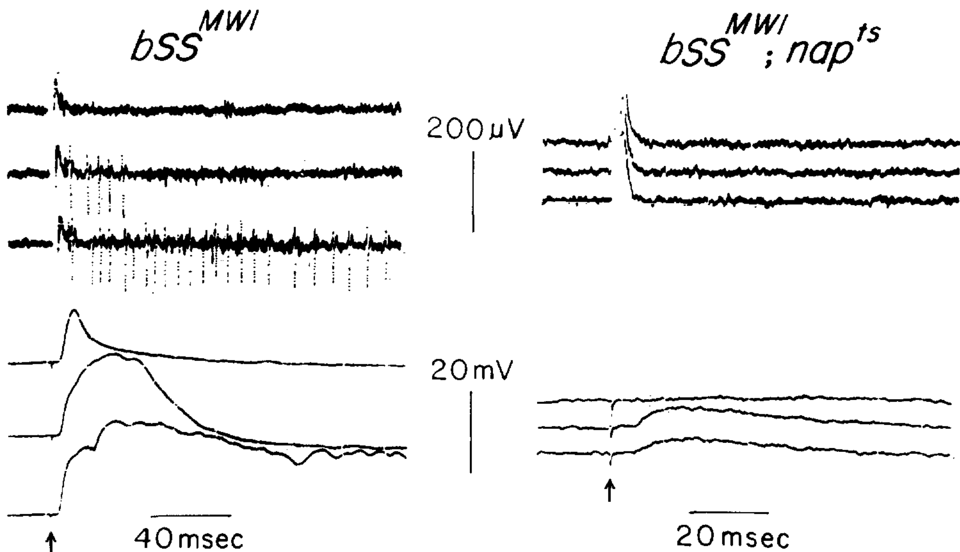

FIGURE 7.—Simultaneous recordings of nerve (upper traces) and muscle (lower) traces in bss^{MW1}/Y males and bss^{MW1}/Y; nap^{ts} double mutants. The first nerve record is associated with the first muscle record, the second with the second etc. for both genotypes. Three successive stimuli applied to bss^{MW1}/Y at a frequency of ten Hz. elicits a long train of repetitive nerve action potentials (downward deflections in nerve records) correlated with a prolonged ejp. In the double mutant, both the repetitive nerve firing and the prolonged ejp were not observed under the same experimental conditions. The response of wild-type is like that of the double mutant (see Figure 3). External Ca^{2+} concentration was 0.15 mM. 23°.

defect in another part of the nervous system such as the ganglion or a defect expressed only in the adult nervous system.

DISCUSSION

Genetic suppression has been demonstrated in many different biological systems and can involve a variety of different mechanisms (reviewed by HARTMAN and ROTH 1973). In many of these cases, analysis of suppressors has provided key insights in the genetic dissection of biochemical, regulatory and morphogenetic pathways.

We are interested in the genetic dissection of excitable membranes (WU *et al.* 1978; WU and GANETZKY 1980; GANETZKY and WU 1982) whose function in nervous systems underlies behavior. Detailed mutational analyses of excitable membranes have been carried out in *Paramecium* (KUNG *et al.* 1975), but no extensive genetic studies have been carried out in higher organisms. In analogy with other biological pathways involving a series of interdependent steps, it seemed possible that some mutants that disrupt membrane excitability could suppress the defect of other such mutants. These interactions could provide evidence for physical or functional relationships between the products of the genes involved.

Here, we have demonstrated the validity of this idea. The mutant, nap^{ts}, isolated as a temperature-sensitive paralytic mutant suppresses an extensive collection of behavioral mutants at seven other loci. This wide range and the lack of allele specificity indicates that suppression by nap^{ts} is indirect rather than informational. One mechanism of indirect suppression involves physical interaction between altered gene products producing a conformational change that restores normal function (JARVIK and BOTSTEIN 1975). In the present case, it is unlikely that the nap^{ts} gene product physically interacts with the products of seven other mutant loci to cause an appropriate conformational change that restores normal function to each. Instead, the behavioral and neurophysiological phenotypes of the single mutants and the double mutant combinations suggests that suppression is most likely due to opposing effects on membrane excitability.

The neuronal excitation process is determined by the relative membrane permeability to sodium and potassium ions. The regenerative sodium ion influx initiates an action potential. This excitatory effect is rectified or even terminated by the counter-balancing of the potassium efflux. Previous neurophysiological experiments have led to the suggestion that *Sh* interferes with the potassium permeability (JAN, JAN and DENNIS 1977; JAN and JAN 1979; TANOUYE, FERRUS and FUJITA 1981; SALKOFF and WYMAN 1981) and nap^{ts} with the sodium permeability mechanism (WU *et al.* 1977; WU and GANETZKY 1980; GANETZKY and WU 1982). These opposite effects on membrane excitability are in agreement with, and provide an explanation for, the suppression of *Sh* by nap^{ts}. The neurophysiological phenotype of the *Sh;*nap^{ts} double mutant provides clear evidence that both mutations exert their effect within individual neurons and alter the properties of excitable membranes in opposing manner. It has been suggested that

bss^{MW1} is defective in a sodium pump leading to excess accumulation of sodium ions within neurons (JAN and JAN 1978). Such a defect might also be expected to be opposed by *nap*^{ts} if it causes a reduction in sodium permeability. To our knowledge, this work is the first demonstration of genetic suppression by a mechanism that involves excitability of neuronal membranes.

One of the surprising outcomes of this investigation was the number of behavioral mutants whose phenotype is suppressible by *nap*^{ts}. It is simplest to assume that *nap*^{ts} suppresses all of these mutants in a similar way. If as suggested above, that mechanism involves a reduction in membrane excitability, the results presented here may indicate that all mutants suppressible by *nap*^{ts} have a related underlying defect causing increased nerve activity.

Besides *Sh* and *bss*^{MW1}, at least *Hk* has also been shown to display enhanced excitability in some part of the nervous system. Although no such physiological abnormality has yet been found at the larval neuromuscular junction for the remaining mutants, we believe it is likely that these also have related defects in nerve activity. This defect may be expressed only in a particular part of the nervous system or only at a particular developmental stage and thus not be detectable by our present methods. One prediction based on the interpretation suggested above is that other mutants that are identified as having increased membrane excitability should be suppressed by *nap*^{ts}. We are attempting to verify this prediction experimentally.

Several additional conclusions illustrating the value of double mutant interactions in the analysis of membrane excitability seem warranted. First, the double mutant interactions can be very sensitive to subtle defects which might not otherwise be noticed. For example, one of the remarkable results of this study is that *nap*^{ts} is a very effective suppressor at permissive temperatures. This was not anticipated because the behavior of *nap*^{ts} at these temperatures appears normal and initially no defect in axonal conduction was detected except at restrictive temperatures (WU *et al.* 1978). The suppression of *Sh* by *nap*^{ts} at permissive temperatures provided a clue that axonal conduction in *nap*^{ts} might not be completely normal even at this temperature. Subsequent neurophysiological analysis of membrane excitability in *nap*^{ts} at permissive temperatures confirmed that this was the case (WU and GANETZKY 1980).

Second, this kind of approach can also reveal unsuspected functional relationships among gene products in the nervous system. The suppression of a collection of mutants by *nap*^{ts} suggests a related defect may underly their behavioral phenotypes as illustrated by the presence of abnormal, repetitive nerve firing both in *Sh* and *bss*^{MW1}. Interactions of the kind described can thus be very useful to organize available behavioral mutants into distinct categories and to provide some insight into possible neurophysiological perturbations.

Finally, these results immediately suggest a means for isolating additional mutants that affect the primary events of membrane excitability. Identification of a family of interdependent genes that control the nerve functions underlying behavior should provide interesting material for future analysis by neurophysiological and molecular techniques.

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