GENETIC STUDIES OF MEMBRANE EXCITABILITY IN DROSOPHILA: LETHAL INTERACTION BETWEEN TWO TEMPERATURE-SENSITIVE PARALYTIC MUTATIONS

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ABSTRACT

Two mutants of *Drosophila melanogaster*, *para*^{ts1} (*1*-53.9) and *nap*^{ts} (2-56.2) both display similar temperature-sensitive paralysis associated with blockage in the conduction of nerve action potentials, suggesting that the two gene products have a similar function. This idea is supported by the observation that the double mutant is unconditionally lethal. Genetic analysis of this synergistic interaction has revealed the following: 1) it specifically involves the *para* and *nap* loci; (2) all *para* alleles interact with *nap*^{ts}, but the strength of the interaction varies in an allele-dependent fashion; (3) lethality of the double mutant occurs during the first larval instar with *para*^{ts1} but differs with other *para* alleles; (4) hypodosage of *para*$^{+}$ causes lethality in a *nap*^{ts} background. These results together with previous electrophysiological, behavioral and pharmacological studies of these mutants suggest that both *para* and *nap* affect sodium channels and possibly encode different subunits.

ELECTRICAL signals in the nervous system propagate within neurons along axons and between neurons at synapses and involve brief, regulated ionic fluxes across the membrane of excitable cells. The electrical excitability of these cells is controlled by a set of proteins that mediate the conductance of specific ions across the membrane. Although the function of these proteins, known as channels, has been fairly well defined in physiological terms, only recently has it been possible to begin to elucidate the details of their molecular structure and properties (CATTERALL 1982). Using Drosophila, several laboratories have taken a mutational approach to perturb membrane excitability in the nervous system as a means of dissecting the mechanisms involved and to identify the genes and their products that regulate these mechanisms (see HALL 1982 for review). Among a collection of such mutants should be those that directly affect the structure and function of channel proteins.

Because of the likely molecular complexity of channels and the interdependent physiological functions of different channels involved in propagating action potentials, the analysis of double mutants can provide information about functional relationships between gene products that affect the same or sequential steps in the mechanisms of excitability. We have previously demonstrated that specific nonadditive interactions do occur in certain double mutant combinations in which one mutation that alters membrane excitability either suppresses or markedly enhances the defect caused by a second such mutation (GANETZKY and WU 1982a,b, 1983).

One interaction of this type involves the temperature-sensitive (ts) paralytic mutants *para*^{ts} (paralytic, *1*-53.9) and *nap*^{ts} (no action potential, *2*-56.2). At 37.5°, *nap*^{ts} flies become completely paralyzed in less than 5 sec. The paralysis is instantly reversible when the flies are returned to 25° (WU *et al.* 1978). At this temperature (25°) the flies are fully viable and fertile and behaviorally normal. Many cycles of paralysis and recovery do not appear to have an adverse effect on the flies. Larvae are also paralyzed at the restrictive temperature. Electrophysiological experiments demonstrated that the propagation of action potentials is blocked at the restrictive temperature in *nap*^{ts} adults and larvae. Although action potentials are propagated at permissive temperatures, *nap*^{ts} apparently alters membrane excitability even under these conditions. This is demonstrated both by the enhanced sensitivity of the mutant to the drug tetrodotoxin (TTX), which blocks sodium channels, and by the suppressive effect of *nap*^{ts} on other mutations that cause increased membrane excitability (WU and GANETZKY 1980; GANETZKY and WU 1982a,b).

The properties of *para*^{ts} are similar to those of *nap*^{ts} (SUZUKI, GRIGLIATTI and WILLIAMSON 1971). The *para*^{ts1} allele, which is the best characterized, has a restrictive temperature of 29° for adults and about 37° for larvae. Kinetics of paralysis and recovery are very rapid for *para*^{ts1}, the transition occurring in a matter of seconds. Failure of action potentials at the restrictive temperature has been demonstrated in *para*^{ts1} larvae and adults (SIDDIQI 1975; SIDDIQI and BENZER 1976; WU and GANETZKY 1980). No physiological or behavioral abnormalities have been detected at permissive temperatures for any of the temperature-sensitive *para* alleles.

The similar paralytic behavior of *para*^{ts} and *nap*^{ts} and their common defect in blocking the propagation of action potentials at restrictive temperatures suggested a functional relationship between the two mutants. This idea was further strengthened by the discovery that the double mutant was lethal even at permissive temperatures (WU and GANETZKY 1980). In this report, the synergistic interaction between *para*^{ts} and *nap*^{ts} is further analyzed. It is suggested that the synthetic lethality of these two mutations results from their combined effects on the structure, function or stability of sodium channels.

MATERIALS AND METHODS

Mutants: The isolation and initial characterization of *para*^{ts1} and *nap*^{ts} have been described elsewhere (SUZUKI, GRIGLIATTI and WILLIAMSON 1971; WU *et al.* 1978). The *para* alleles, *para*^{ST76}, *para*^{ST109} and *para*^{ts115}, were isolated in the laboratory of S. BENZER (SIDDIQI and Benzer 1976; B. GANETZKY, unpublished data). The allele, *para*^{lk5}, is an unconditional recessive lethal *para* allele isolated by R. KREBER that is free of any detectable chromosome aberration.

Two additional *X*-linked temperature-sensitive paralytic mutations, *shi*^{ts1} (shibire, *1*-52.2; GRIGLIATTI *et al.* 1973) and *com*^{ST53} (comatose, *1*-40.0; SIDDIQI and BENZER 1976), that differ from *nap*^{ts} and *para*^{ts} in behavioral and physiological properties were tested in double mutant combinations with *nap*^{ts} as controls for the locus specificity of the interaction.

Description of other genetic markers used in this study can be found in LINDSLEY and GRELL (1968).

Special chromosomes: The following chromosome rearrangements were employed in these studies.

Df(1)r^{D1}: Deleted from 14C2-4 to 15B2-C1 and deficient for *para$^+$*.

Df(1)l^{D34}: An apparent small deletion that uncovers several lethal complementation groups (M. M. GREEN, personal communication) including the *para* locus but looks cytologically normal.

l(1)l^{D23} and *l(1)l^{D17}* are lethal mutations uncovered by *Df(1)l^{D34}*. These mutations complement each other and *para*. Neither has any cytologically visible abnormality.

Df(1)l^{D7}: Deleted from 14C7-D1 to 14E3-F1 and deficient for *para$^+$*.

In(1)l^{D30}: Inversion with breakpoints at 14C6-D1 and 15E-F. The distal break is associated with a recessive lethal phenotype that is uncovered by *Df(1)l^{D34}* and *Df(1)l^{D7}*. These chromosomes were provided by M. M. GREEN. In this report, they will be abbreviated as *l^{D34}*, *l^{D23}*, *l^{D17}*, *l^{D7}* and *l^{D30}*.

Dp(1;4)r$^+$f$^+$: Carries 14A-16A2 appended to chromosome *4*. This duplication will be referred to as *Dp(1;4)para$^+$* in this report.

FM7: An *X* chromosome balancer marked with *B* (Bar eyes).

In(2LR)O: A second chromosome balancer marked with *Cy* (Curly wings).

R(1)w^{vC}: An unstable ring chromosome used to generate gynandromorphs.

C(1)FMA4, In(1)w^{m4} + AB/In(1)FM7, y^{2}bb$^-$: A compound *X* chromosome that will be represented here as $\widehat{XX}$.

T(Y;2)L12: An insertional translocation of 41–43A, which contains *nap$^+$* (WU *et al.* 1978), inserted into the long arm of *B^{S}Yy$^+$*. This chromosome will be abbreviated as *nap$^+$Y*.

Crosses: Flies were grown on standard cornmeal molasses medium. Crosses were performed in shell vials at 25° except where otherwise indicated. Most matings were set up with one or two pairs of parents per vial with ten to 20 replicate vials. Parents were transferred once before being discarded, and progeny were counted from both the original and the transfer vial. Crosses set up at 18° generally had low fecundity, and the protocol was modified slightly for these crosses. Five pairs of parents were used in each vial, and parents were allowed to mate for 1–2 days at 25° before the vial was transferred to 18°.

Lethality: The convention adopted in this work is to consider as lethal any genotype whose survival compared with appropriate controls is less than 5% of the expected value.

RESULTS

Lethal interaction in para^{ts1}; nap^{ts} *double mutant*: Because of the marked similarity in behavior and physiology between *nap^{ts}* and *para^{ts1}*, it was of interest to examine the phenotype of the double mutant. However, attempts to recover double mutant individuals were unsuccessful because of their complete lethality (WU *et al.* 1978; WU and GANETZKY 1980). Since *para^{ts1}/nap$^+$Y; nap^{ts}* males survive, a convenient way to demonstrate and quantify the lethal interaction is by crossing these males to $\widehat{XX}$*/Y; nap^{ts}* females (Table 1A). In this cross, all male offspring are double mutants and their survival can be compared with that of female offspring which serve as controls. As shown, with *para^{ts1}* there were no surviving double mutant males among several thousand progeny scored, whereas a similar cross with *para$^+$* yielded 52% males. The lethality of the *para^{ts1}; nap^{ts}* double mutant was observed even when the temperature at which the flies were grown was decreased to 18° (Table 1). No *para^{ts1}; nap^{ts}* males were observed in pupal cases or stuck in the medium at either 18° or 25°. Thus, the lethality occurred at some earlier developmental stage. Any reduction in viability caused by *para^{ts1}* alone under these conditions can be measured in crosses of *para^{ts1}/nap$^+$Y; nap^{ts}* males to $\widehat{XX}$*/nap$^+$Y; nap^{ts}* females (Table 1B). The results demonstrate no marked difference from *para$^+$* controls. Similarly, the results of control crosses using *para$^+$* males (Table 1A) indicate that, when homozygous, *nap^{ts}* does not by itself cause major reduction in viability. To show that the synthetic lethality involves a specific interaction

TABLE 1

Viability of different para *alleles in double mutant combinations with* nap^{ts}

para^x	% ♂ offspring (25°)	% ♂ offspring (18°)
A.		
para$^+$	52.0 (2965)	52.0 (732)
para^{ts1}	0.0 (2023)	0.0 (1589)
para^{ST76}	21.5 (3573)	30.0 (1405)
para^{ST109}	37.6 (2272)	9.5 (858)
para^{ts115}	11.2 (3132)	4.0 (579)
B.		
para$^+$	51.0 (3485)	51.6 (1296)
para^{ts1}	49.4 (1170)	56.7 (1596)
para^{ST76}	43.4 (2054)	50.0 (2350)
para^{ST109}	47.7 (2195)	39.5 (508)
para^{ts115}	47.1 (1532)	47.3 (302)

Data shown are results of crossing *para*^{x}/*nap*$^+$*Y*; *nap*^{ts}/*nap*^{ts} by (A) $\widehat{XX}/Y$; *nap*^{ts}/*nap*^{ts} or (B) $\widehat{XX}$/*nap*$^+$*Y*; *nap*^{ts}/*nap*^{ts} at the temperatures indicated. In each case, the number shown represents the percent of male offspring, *para*^{x}/*Y*; *nap*^{ts}/*nap*^{ts} (A) and *para*$^+$/*nap*$^+$*Y*; *nap*^{ts}/*nap*^{ts} (B) among the total surviving progeny. The numbers in parentheses are the numbers of progeny counted.

between the *nap* and *para* loci, we examined two other *X*-linked temperature-sensitive paralytic mutants, shibire (GRIGLIATTI *et al.*, 1973) and comatose (SIDDIQI and BENZER 1976), in combination with *nap*^{ts} at 25° and found no significant reduction in survival compared with controls (46.7% surviving double mutant males for shibire and 46.3% for comatose).

Interaction of nap^{ts} *with other* para *alleles*: The allele specificity of the interaction was examined by repeating the crosses with additional *para* alleles (Table 1). Several points about these results are worth noting. First, the other *para* alleles tested are able to survive to varying degrees in combination with *nap*^{ts}. Second, despite the survival of some double mutant adults, these *para* alleles also clearly display a strong synergism with *nap*^{ts}. This is evidenced by significantly reduced viability of double mutants compared with controls and much enhanced temperature sensitivity and general weakness of the survivors. Only with *para*^{ST109}; *nap*^{ts} males was it possible to maintain a double mutant strain. Third, comparison of the data in Table 1 indicates that the other *para* alleles are similar to *para*^{ts1}, in that they do not by themselves cause an appreciable decrease in survival under these conditions. Fourth, raising the double mutants at a lower temperatures does not increase their viability. For both *para*^{ST109} and *para*^{ts115}, survival of the double mutants at 18° was less than at 25°. With *para*^{ST76}, the survival of double mutant males at 18° is somewhat elevated compared with 25°, which can be attributed to the greater viability of *para*^{ST76} itself at 18° (*cf.* Table 1B).

With *para*^{ST76}, *para*^{ST109} and *para*^{ts115}, unlike *para*^{ts1}, many of the double mutant males at 25° appeared to die at or near eclosion and were observed stuck in the medium or in their pupal cases. These males were not counted

TABLE 2

Survival of heteroallelic combinations of para *alleles in a* nap^{ts} *background*

$para^x$	Cy ♂♂	Cy♂♂$^+$	Cy ♀♀	Cy$^+$♀♀	% Cy$^+$ ♀♀
A.					
$para^+$	602	570	823	502	20.1
$para^{ts1}$	870	714	1072	0	0.0
$para^{ST76}$	399	404	585	2	0.14
$para^{ST109}$	226	192	259	10	1.4
$para^{ts115}$	163	142	190	1	0.2
B.					
25°	596	537	887	255	11.2
18°	1405	1176	2661	694	11.7

Data shown are results of crossing (A) $para^x/nap^+Y$; nap^{ts}/nap^{ts} × $para^{ts1}/para^{ts1}$; CyO/nap^{ts} at 25° or (B) $para^{ST76}/nap^+Y$; nap^{ts}/nap^{ts} × $para^{ST109}/para^{ST109}$; CyO/nap^{ts} at the indicated temperature.

and are not included in the data tabulated in Table 1. However, it was noted in the 18° crosses with $para^{ST109}$ and $para^{ts115}$ that very few males were stuck in the medium or pupal cases, suggesting that lethality occurred earlier and was more complete with these alleles at 18° than at 25°. With $para^{ST76}$, no significant decrease in the number of males dying near eclosion was observed when the crosses were done at 18° instead of 25°.

The conclusion from these experiments is that most if not all *para* alleles appear to interact synergistically with nap^{ts}, supporting the idea of a functional relationship between the corresponding gene products. However, the strength of the interaction varies in an allele-dependent manner. These differences indicate that the various *para* alleles modify the structure and function of the $para^+$ gene product in distinct ways.

The lethal interaction between $para^{ts1}$ and nap^{ts} does not occur only in males. As shown in Table 2, $para^{ts1}/para^{ts1}$; nap^{ts} females also fail to survive. A single dose of $para^+$ in $para^+/para^{ts1}$; nap^{ts} females rescues these flies, although their viability is still reduced by about 40% when compared with their $para^+/para^{ts1}$; nap^+/nap^{ts} sisters (502 *vs.* 823). The basis of this partial dominance will be addressed later. Females heterozygous for $para^{ts1}$ and other mutant *para* alleles have viabilities that are intermediate to those associated with either *para* allele alone in a nap^{ts} background (Table 2, *cf.* Table 1A). Similar results were found for $para^{ST76}/para^{ST109}$; nap^{ts} females at both 25° and 18°. Thus, in these heterozygous combinations, there does not appear to be any special type of interaction between different *para* alleles.

Time of lethality: At what developmental stage do $para^{ts1}$; nap^{ts} double mutants die? Assaying for embryonic lethality by egg hatch studies proved unsuccessful because of a high background of unhatched eggs in all of the relevant crosses. Instead, first instar larvae were scored to determine whether double mutant animals hatched at the expected frequency. Males of genotype *y cho* $para^{ts1}/nap^+Yy^+$; nap^{ts}, were crossed to $\widehat{XX}$, y^2bb^-/Y; nap^{ts} females. After a brief period of egg-laying, the embryos produced in this cross were collected and allowed to hatch into first instar larvae. These were mounted on slides, and

TABLE 3

Determination of lethal phase for para^{ts1}; nap^{ts} *double mutant*

Mouthhook phenotype	Larval instar		
	First	Second	Third
y	42	10	1
y^{+a}	62	50	122

Data shown are numbers of larvae at different instars resulting from the cross: *y cho para^{ts1}/nap^{+}Y y^{+}; nap^{ts}/nap^{ts}* × *$\widehat{XX}$,y^{2}bb^{-}/Y; nap^{ts}/nap^{ts}* at 25°.

[a] Includes surviving $\widehat{XX}$,*y^{2}/y* metafemales. See text.

their mouthhooks were scored for *y* or *y*$^{+}$. Regular male offspring from the cross are doubly mutant for *para^{ts1}* and *nap^{ts}* and have light brown mouthhooks indicative of *y*. Regular, *y*$^{+}$, female offspring ($\widehat{XX}$, *y^{2}bb^{-}/nap^{+}Yy^{+}*) have black mouthhooks. Metafemales ($\widehat{XX}$, *y^{2}bb^{-}/y cho para^{ts1}*) produced in this cross have dark brown mouthhooks, which are readily distinguishable from *y* but less reliably from *y*$^{+}$. Since the main question was whether or not double mutant larvae survived at each stage, no attempt was made to distinguish the two female classes. The presence of *cho* provided another marker for male larvae but could be reliably scored only after the first larval instar. Of 104 first instar larvae scored, 42 were *y* (Table 3). This indicates that double mutants can complete embryogenesis and hatch at nearly the expected frequency. A second timed collection of embryos was allowed to develop into second instar larvae which were scored as before. Of 60 larvae scored, only ten were *y*. A similar experiment for third instar larvae revealed just one *y* larva among 123 scored. As in the cross shown in Table 1, no male adults eclosed nor were any pharate males observed. Thus, it appears that most of the double mutants die between the first and second larval instars, although the possibility cannot be ruled out that lethality occurs to some extent throughout development from embryogenesis until pupation.

The time of lethality is different for other *para* alleles. As described earlier, certain *para* alleles had a high frequency of mortality near eclosion in a *nap^{ts}* background. These results may indicate that these *para* alleles affect adults more severely than larvae, consistent with previous behavioral data indicating that the temperature required for paralysis is greater in larvae than adults (SIDDIQI and BENZER 1976).

Dominant interaction between nap *and* para: In the crosses presented in Table 2 it was observed that *para^{+}/para^{ts1}; nap^{ts}* females have reduced viability relative to *para^{+}/para^{ts1}; nap^{+}/nap^{ts}* females. These latter females appear phenotypically normal in all respects. To verify the reduced viability of *para^{ts1}* heterozygotes in a *nap^{ts}* background, *para^{ts1}/nap^{+}Y; nap^{ts}* males were crossed to *para^{+}; nap^{ts}* females. All of the female offspring are genotypically *para^{+}/para^{ts1}; nap^{ts}*, and their viability can be compared directly with their male sibs (*para^{+}/nap^{+}Y; nap^{ts}*) which serve as controls. At 25° the viability of *para^{+}/para^{ts1}; nap^{ts}* females is clearly reduced (Table 4). The surviving females have

TABLE 4

Dominant effects on viability of para *alleles in a* nap^{ts} *background*

$para^x$	Temperature	♂♂	♀♀	% ♀♀
$para^+$	25°	1922	2209	53.4
	18°	1161	1160	49.9
$para^{ts1}$	25°	1449	831	36.4
	18°	1939	248	11.3
$para^{ST76}$	25°	1565	1261	44.6
	18°	1445	529	26.8
$para^{ST109}$	25°	510	436	46.1
	18°	688	489	41.5
$para^{ts115}$	25°	847	761	47.3
	18°	839	568	40.4

Data shown are results of crossing $para^x/nap^+Y$; nap^{ts}/nap^{ts} × $para^+/para^+$; nap^{ts}/nap^{ts} at the indicated temperature.

enhanced temperature sensitivity and become paralyzed at 27° (an observation originally made by D. S. LEONARD). Flies homozygous for nap^{ts} do not paralyze below 35°, $para^{ts1}$ flies do not paralyze below 29° and $para^+/para^{ts1}$ heterozygotes (like $para^+$) exhibit no paralysis at temperatures up to 38°–39°. Despite the lowered temperature for paralysis in these females, their viability is reduced even further when the cross is done at 18°. Comparable results, but less extreme, are observed with other *para* alleles (Table 4). Apparently, when nap^{ts} is homozygous, an otherwise recessive mutant *para* allele begins to manifest dominant phenotypic effects.

Two possibilities that could account for this dominant interaction were considered. In nap^{ts} homozygotes, the mutant *para* product could be modified in such a way that it interferes with the function of the normal $para^+$ product. Alternatively, there may be a requirement for a critical level of functional activity affected by both the *para* and *nap* gene products and below which a fly cannot survive. Thus, when the overall level of *para* function is reduced below a certain threshold (*e.g.*, by being heterozygous for a mutant allele), further reduction by homozygosity for nap^{ts} could render the fly inviable.

One test to distinguish these alternatives was to examine the viability and paralytic behavior of $para^{ts1}/Y/Dp(1;4)para^+$; nap^{ts} males. These males had normal viability and their restrictive temperature was that expected for nap^{ts} homozygotes (36–37°). Thus, no dominance of $para^{ts1}$ was evident in these males, although the opportunity was present (as in $para^{ts1}/para^+$ females) for the "poisoning" of a $para^+$ subunit by a mutant subunit. This result, therefore, argues against such a model. However, because of dosage compensation, the level of *para* function in $para^{ts1}/Dp(1;4)para^+$ males should be twice that of $para^{ts1}/para^+$ females. Hence, in this case, any reduction caused by homozygosity for nap^{ts} will be less effective in the males than females. The failure to

TABLE 5

Viability of different para *alleles when heterozygous with aberrations that delete or inactivate* para$^+$ *in a* nap$^+$ *background*

$para^x$	l^{D7}	l^{D30}	l^{D34}
$para^+$	572/577	786/771	695/637
$para^{ts1}$	1/635	9/335	3/1825
$para^{ST76}$	151/211	56/83	27/80
$para^{ST109}$	4/245	1/104	1/169
$para^{ts115}$	106/234	42/118	48/205

Data are presented as the ratio of $l^x/para^+$ ♀♀ to $FM7/para^+$ ♀♀ in crosses of $para^+/Y \times l^x/FM7$ at 25°.

detect dominance in the duplication-bearing males can, therefore, be accommodated by the model proposing that a certain level of *para* function is essential in a nap^{ts} background.

Additional evidence favors the interpretation that there is some essential function controlled by the products of the *para* and *nap* loci and that there is a critical level of activity below which flies cannot survive. Functionally, $para^{ts1}$ appears to be hypomorphic because it is essentially lethal when heterozygous with deletions that uncover it, including $Df(1)r^{D1}$,l^{D34} and l^{D7} (Table 5). Similarly, l^{D30}, an inversion that apparently has a breakpoint at or near the *para* locus, behaves as an amorphic *para* allele (Table 5). The few eclosing adults heterozygous for $para^{ts1}$ and one of the aberrant chromosomes are weak, small, paralyzed at temperatures of approximately 27° and die within a week after eclosion. Thus, the *para* locus encodes an essential function and $para^{ts1}$ can be lethal even in a nap^+ background when the overall level of *para* function is sufficiently low. In the terminology of NASH and JANCA (1983), $para^{ts1}$ is a haplo-specific lethal mutation.

Females heterozygous for $para^+$ and l^{D34}, l^{D7} or l^{D30} suffer no major decrease in viability in a nap^+ background (Table 5). Remarkably, however, the same heterozygotes are lethal in a homozygous nap^{ts} background (Table 6). The heterozygous lethal effect associated with these chromosome aberrations depends specifically on their deletion or inactivation of $para^+$. This is demonstrated by the identical results obtained with $para^{lk5}$, a null *para* allele not associated with any detectable chromosome abnormality (Table 6). In effect, a recessive lethal *para* allele becomes a dominant lethal in a nap^{ts} background. In contrast, two other recessive lethal (presumptive) point mutations, l^{D17} and l^{D23}, in genes that either are adjacent to $para^+$ or extremely close, are quite

TABLE 6

Dominant lethality associated with deleting or inactivating para$^+$ *in a* nap^{ts} *background*

	CyO/nap^{ts}		*nap^{ts}/nap^{ts}*	
l^x	l^x/*FM7*	*FM7*/*FM7*	l^x/*FM7*	*FM7*/*FM7*
l^{D7}	318	174	2	60
	1976[a]	NC	3	NC
l^{D30}	701	403	0	148
	1376[a]	NC	0	NC
l^{D34}	621	408	0	145
$para^{lk5}$	943	665	0	296
l^{D17}	982	723	401	222
l^{D23}	1403	740	489	223

Data shown are the female offspring of *FM7/Y; CyO/nap^{ts}* × *l^xFM7; CyO/nap^{ts}* at 25°. NC, not counted.

[a] These crosses are independent replicates in which only the two relevant classes of female offspring were counted.

viable when heterozygous in a *nap^{ts}* background (Table 6). The results presented in Table 6 consequently lend strong support to the idea that the interaction of *para* mutations with *nap^{ts}* is a result of reduced function of the *para* locus rather than production of an altered *para* gene product. In a *nap^{ts}* background, there is a more stringent requirement for the amount of *para* activity necessary to survive such that even a wild-type allele becomes haplolethal.

Although $para^{ts1}$ is lethal when heterozygous with l^{D7}, l^{D30} or l^{D34}, the other conditional *para* alleles vary in viability when similarly tested (Table 5). If the reduction in viability under these conditions is taken as a measure of the amount of residual function of a particular *para* allele, then comparison of these results with the data in Table 1 indicates that these differences in activity are not the sole basis of the allelic differences in the interaction with *nap^{ts}*. In particular, $para^{ST109}$ which is virtually lethal when heterozygous with l^{D7}, l^{D30} or l^{D34} survives better in combination with *nap^{ts}* than other alleles (*e.g.*, $para^{ts115}$) that have greater viability when heterozygous with lethal *para* alleles. One possibility to explain the allelic variation of the interaction is that the products of the *para* and *nap* loci physically interact. In this case, mutant alleles that affect the association of the products can have effects in the double mutant that do not necessarily correlate with their individual phenotypes.

DISCUSSION

The behavioral phenotypes of $para^{ts1}$ and *nap^{ts}* are similar in several respects that distinguish them from other ts paralytic mutants. Both mutations cause the instantaneous paralysis of larvae and adults at the restrictive temperature with equally instantaneous reversal of paralysis at permissive temperatures (SUZUKI, GRIGLIATTI and WILLIAMSON 1971; SIDDIQI and BENZER 1976; WU *et al.* 1978). For both mutants, there is a sharp cut-off for the restrictive temperature below which flies will not become paralyzed, regardless of the length of exposure to that temperature. The time required to recover from paralysis for both mutants does not increase with longer exposures to the restrictive temperature. The neurophysiological defects in these mutants also are similar. Both mutations produce a temperature-dependent block in the propagation of action potentials in the nerves of larvae and adults (SIDDIQI 1975; SIDDIQI and BENZER 1976; WU *et al.* 1978; WU and GANETZKY 1980). This apparent similarity between $para^{ts1}$ and nap^{ts} is supported by the observation that the two mutations interact synergistically to cause lethality of the double mutant. The usual interpretation of such genetic interactions is that there is a functional overlap between the gene products involved (*cf.* SIMPSON 1983).

Although nap^{ts} and $para^{ts1}$ are conditional mutants with respect to paralysis, lethality of the double mutant is unconditional, suggesting that these mutations cause functional defects even at permissive temperatures. In the case of nap^{ts}, this is consistent with our previous electrophysiological and genetic data demonstrating that conduction of nerve impulses is abnormal even at low temperatures. Under permissive conditions, nerve conduction in nap^{ts} is abnormally sensitive to blockage by TTX, a drug that specifically blocks sodium channels (WU and GANETZKY 1980). In addition, nap^{ts} acts as an unconditional suppressor of a number of behavioral mutants with hyperexcitable nerve membranes by counterbalancing their physiological defects (GANETZKY and WU 1982a,b). Finally, neurons cultured *in vitro* from nap^{ts} are more resistant than wild type at both permissive and restrictive temperatures to the toxic effects of veratridine (WU, SUZUKI and POO 1983), a drug that causes persistent activation of sodium channels (*cf.* WEST and CATTERALL 1979).

At permissive temperatures, $para^{ts1}$ differs from nap^{ts} in that it is not more sensitive to TTX than wild type, nor does it act as a suppressor of other behavioral mutants. However, $para^{ts1}$ neurons cultured *in vitro* are more resistant to veratridine than wild type at permissive temperatures but less so than nap^{ts}. At restrictive temperatures the resistance of $para^{ts1}$ neurons is equal to that of nap^{ts} (SUZUKI and WU 1984). Moreover, when heterozygous with a deletion that uncovers it, $para^{ts1}$ is unconditionally lethal.

The simplest interpretation of our results is that, whereas nap^{ts} and $para^{ts1}$ each block nerve conduction in a temperature-dependent way, they interact in double mutants such that propagation of action potentials is markedly diminished or entirely abolished under all conditions. This would be expected to have lethal consequences if more than a small portion of the ventral nervous system is so affected. Observations made on genetic mosaics are consistent with this interpretation (B. GANETZKY, unpublished results; M. BURG and C.-F. WU, personal communication). Gynandromorphs with patches of male tissue doubly mutant for $para^{ts1}$ and nap^{ts} and marked by *y* were produced by loss of the unstable ring-*X* chromosome in *y cho* $para^{ts1}/R(1)w^{vC}$; nap^{ts}/nap^{ts} embryos. Control mosaics were produced by ring loss in *y cho* $para^{ts1}/R(1)w^{vC}$; CyO/nap^{ts}

embryos. The distribution of mosaic boundaries was significantly different in experimental and control mosaics, anterior and ventral regions being most sensitive to the lethal effects of the double mutant. The average probability of inclusion in a patch of male tissue was 0.545 in control *vs.* 0.015 in experimental mosaics for head landmarks, 0.452 *vs.* 0.103 for ventral thorax, 0.485 *vs.* 0.199 for dorsal thorax and 0.365 *vs.* 0.381 for dorsal abdomen. Among the experimental class of gynandromorphs, there were several cases in which a doubly mutant clone encompassed an entire leg. In these cases, the leg was structurally normal but completely immobile even at low temperature, as though motor control of the leg were totally lost. Examination of a specific behavioral reflex in mosaic flies further supports this observation. In normal flies, stimulation of specific thoracic mechanoreceptor bristles evokes a reflex cleaning response by an appropriate leg (VANDERVORST and GHYSEN 1980). At 21° $para^{ts1}$ and nap^{ts} respond the same as wild type. However, in small mosaic clones in which a single bristle (and its associated sensory neuron) is doubly mutant, the cleaning reflex could not be evoked by stimulating the bristle (M. BURG and C.-F. WU, personal communication). Stimulation of neighboring bristles on the same fly that are not doubly mutant still evoked the reflex. These results suggest that the function of the doubly mutant sensory neuron is unconditionally blocked.

The lethal interaction does not require the presence of an altered product from the *para* locus. This is demonstrated by the lethality of individuals carrying $para^{+}$ heterozygous with an amorphic *para* allele in a nap^{ts} background. Conversely, $para^{ts1}$ can have lethal effects in a nap^{+} background as revealed by the unconditional lethality of flies carrying a deletion of $para^{+}$ heterozygous with $para^{ts1}$. These results can be explained readily by an extension of the model proposed by NASH and JANCA (1983) for haplo-specific lethal mutations. In this view, the effect of homozygosity for nap^{ts} can be interpreted as reducing the activity of mutant and normal *para* alleles relative to some threshold level of wild-type activity (T) necessary for survival. When nap^{ts} is homozygous, the activity in $para^{ts1}/para^{ts1}$ or $para^{+}/Df\ para$ flies presumably falls below T, resulting in lethality.

This model also accounts fairly readily for the reduced viability of $para^{ts1}/para^{+}$ heterozygotes in a nap^{ts} background and of the $para^{ST76}$, $para^{ST109}$ and $para^{ts115}$ alleles when heterozygous with a *para* deletion in a nap^{+} background (*cf.* Table 5). The relative viabilities of the latter group of flies imply a ranking of the *para* alleles with decreasing activities, namely, $para^{+} > para^{ST76} > para^{ts115} > para^{ST109} > para^{ts1}$. A ranking of *para* alleles based on their viability in a nap^{ts} background (*cf.* Table 1) yields the order $para^{+} > para^{ST109} > para^{ST76} > para^{ts115} > para^{ts1}$. Thus, nap^{ts} appears to show allele-specific interactions that depend on something other than residual wild-type activity associated with each *para* allele.

One explanation for these allele-specific interactions is that the products of *para* and *nap* are physically associated. Different *para* alleles could affect the nature of the physical interaction in ways that do not strictly correlate with their activity in a nap^{+} background. A physical interaction between gene products could also account for the effect of *nap*^{ts} on the activity of mutant and normal *para* alleles, in the threshold model proposed. The decrease in viability exhibited by certain *para* alleles in combination with *nap*^{ts} at 18° *vs.* 25° (*cf.* Table 1) is also consistent with the notion of a physical association, since many cold-sensitive mutations are believed to disrupt processes of assembly (*e.g.*, GUTHRIE, NASHIMOTO and NOMURA 1969; WRIGHT 1973).

Can the effects of *para*^{ts1} and *nap*^{ts} and the nature of their interaction be accounted for at a molecular level? The electrophysiological and pharmacological data argue that *nap*^{ts} alters the function and/or number of sodium channels in the nerve membrane (WU *et al.* 1978; WU and GANETZKY 1980; GANETZKY and WU 1982a; WU, SUZUKI and POO 1983). In addition, ligand-binding studies demonstrated a reduction in the number of pharmacologically defined sodium channels in *nap*^{ts} brain membrane preparations (HALL *et al.* 1982; KAUVAR 1982; JACKSON *et al.* 1984). In these studies, the maximum specific binding of TTX or saxitoxin (STX) was reduced by 40% but the K_d was unaltered. These results were interpreted as indicating that *nap*^{ts} reduces the number but "appears not to alter the receptor structure" (JACKSON *et al.* 1984) and that the 40% reduction in channel number was alone sufficient to explain all of the phenotypes of *nap*^{ts} (HALL *et al.* 1982). However, it is known that sodium channels have at least three distinct binding sites for different classes of toxins and that the binding site for one toxin can be altered by mutation without affecting the binding properties of the other sites (WEST and CATTERALL 1979). Moreover, a large body of neurophysiological data indicates that active nerve conduction can be maintained by only a relatively small fraction of the total population of sodium channels. This fraction can be estimated from experiments in which sodium inward current is reduced until conduction fails, either by blocking channels with TTX or by lowering the external sodium concentration. From such experiments estimates are available for a variety of physiological preparations including rabbit vagus nerve (KEYNES, RITCHIE and ROJAS 1971; COLQUHOUN and RITCHIE 1972), leg nerve of crab (KEYNES, RITCHIE and ROJAS 1971) and giant axons of squid (HODGKIN and KATZ 1949; CUERVO and ADELMAN 1970; KEYNES, RITCHIE and ROJAS 1971) and lobster (NARAHASHI, MOORE and SCOTT 1964; TAKATA *et al.*, 1966). In giant axons of lobster and squid the fraction is about 15%; in the other preparations it is 10% or less. Furthermore, this fraction appears to increase only slightly with temperature (COLQUHOUN and RITCHIE 1972). In Drosophila, as in other organisms, the minimum dose of TTX required to block completely the compound action potential recorded from peripheral nerves in wild-type individuals (WU and GANETZKY 1980) is many times higher than the K_d (KAUVAR 1982). Thus, it seems unlikely that a 40% reduction in sodium channel number is sufficient to account for the behavioral, electrophysiological and pharmacological properties of *nap*^{ts} at permissive and restrictive temperatures if the remaining 60% still function normally (WU and GANETZKY 1980; WU, SUZUKI and POO 1983). Instead, it is possible that *nap*^{ts} alters a site on the sodium channel that affects both the function and stability of individual channels but does not affect the binding affinity of STX or TTX. In fact, altered sodium channels with exactly these properties were produced by mutation in a mouse neuroblastoma cell line (WEST and CATTERALL 1979).

Attempts to purify the sodium channel from rat suggest that it contains one large subunit of greater than 250,000 daltons and two smaller subunits (HARTSHORNE and CATTERALL 1981; BARCHI 1983; CATTERALL 1984). The large subunit is a glycoprotein that contains about 30% carbohydrate by weight (MILLER, AGNEW and LEVINSON 1983). Furthermore, electrophysiological and pharmacological studies of sodium channels during differentiation of chick skeletal muscle in culture showed that sodium channels are first incorporated in cell membranes in an immature and nonfunctional form (BAUMGOLD, PARENT and SPECTOR 1983a,b). Subsequently, they undergo a structural change, presumably as a result of posttranslational modification, to become mature functional channels. Thus, it is reasonable to expect that mutations in genes encoding the various channel subunits or the enzymes involved in their posttranslational modification could display nonadditive interactions in double mutants.

As a working hypothesis, it is suggested that products of the *nap* and *para* loci directly interact, either as sodium channel subunits or as enzyme and substrate in the posttranslational modification of sodium channel polypeptides. For example, if nap^+ and $para^+$ encode different subunits of sodium channels, nap^{ts} could modify one subunit such that it still interacts with the $para^+$ product, but the ensemble is unstable even at low temperatures (resulting in a decrease in apparent channel number). At high temperature, the channel function could be blocked completely. The effect of $para^{ts}$ in a nap^+ background could be similarly interpreted. However, when both subunits are mutated, the multimeric structure could fail to assemble, be completely unstable or lack function.

Although this interpretation still awaits definitive evidence, it can account in a fairly straightforward way for most of the results reported here. Whatever the *nap* and *para* gene products are, it is clear from our previous studies and those reported here that these products play essential and functionally related roles in the electrical excitability of Drosophila neurons. Identification of these gene products using recombinant DNA techniques will now be extremely valuable in clarifying the nature of their interaction and in elucidating further the molecular basis of membrane excitability.

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