Dosage Effects of a Drosophila Sodium Channel Gene on Behavior and Axonal Excitability

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ABSTRACT

The effects of *para* mutations on behavior and axonal excitability in Drosophila suggested that *para* specifically affects sodium channels. This hypothesis was confirmed by molecular analysis of the *para* locus, which demonstrates that the encoded *para* product is a sodium channel polypeptide. Here we characterize the effects of altered $para^+$ dosage on behavior and axonal excitability, both in an otherwise wild-type background and in combination with two other mutations: nap^{ts}, which also affects sodium channels, and Sh^{KS133}, which specifically affects potassium channels. Whereas it was previously shown that decreased dosage of $para^+$ is unconditionally lethal in a nap^{ts} background, we find that increased dosage of $para^+$ suppresses nap^{ts}. Similarly, we find that para hypomorphs or decreased dosage of $para^+$ suppresses Sh^{KS133}, whereas increased dosage of $para^+$ enhances Sh^{KS133}. The electrophysiological basis for these effects is investigated. Other genes in Drosophila that have sequence homology to sodium channels do not show such dosage effects, which suggests that the $para^+$ product has a function distinct from that of other putative Drosophila sodium channel genes. We conclude that the number of sodium channels present in at least some Drosophila neurons can be affected by changes in $para^+$ gene dosage, and that the level of $para^+$ expression can strongly influence neuronal excitability.

ELECTRICAL signaling within the nervous system of multicellular organisms such as Drosophila requires the propagation of action potentials along axons. During an action potential, sodium channels in axonal membranes open permitting an influx of sodium ions, which depolarizes the axon. Subsequently, the opening of potassium channels results in an efflux of potassium ions, which contributes to the repolarization of the axon.

In Drosophila, electrophysiological assays of behavioral mutants have identified those in which either sodium channels or potassium channels are perturbed (reviewed recently by GANETZKY and WU 1986; TANOUYE *et al.* 1986). For example, mutations in the *nap* (*no action potential*) or *para* (*paralytic*) genes cause larval and adult paralysis (WU *et al.* 1978; SUZUKI, GRIGLIATTI and WILLIAMSON 1971) and eliminate action potential propagation in certain axons at elevated temperature (SIDDIQI and BENZER 1976; WU *et al.* 1978; WU and GANETZKY 1980). These observations led to the suggestion that *nap* and *para* mutations specifically affect sodium channels (WU and GANETZKY 1980). The recent cloning and sequence analysis of the *para* gene has shown that it encodes a sodium channel protein (LOUGHNEY, KREBER and GANETZKY 1989). Mutations in the *Sh* (*Shaker*) and *eag* (*ether a go-go*) genes cause flies to shake their legs under ether anesthesia (KAPLAN and TROUT 1969). In addition, *Sh* mutations confer increased transmitter release at the larval neuromuscular junction (JAN, JAN and DENNIS 1977) and can eliminate the rapidly inactivating potassium current I_A from larval (WU and HAUGLAND 1985) and pupal (SALKOFF and WYMAN 1981) muscle. *Sh* has recently been shown to encode a family of potassium channels (KAMB, IVERSON and TANOUYE 1987; PAPAZIAN *et al.* 1987; TEMPEL *et al.* 1987; BAUMANN *et al.* 1987; TIMPE *et al.* 1988). Mutants defective in *eag* display spontaneous action potentials in larval axons (GANETZKY and WU 1983) and show a reduction in a second potassium current, the delayed rectifier I_K in larval muscles (WU *et al.* 1983).

Mutations in these four genes interact in nonadditive fashion. For example, *para* null mutations, which are recessive lethal mutations in a nap^+ background, become dominant lethal mutations in a nap^{ts} background (GANETZKY 1984). It was proposed that this phenotypic enhancement reflects the related functions of *nap* and *para* as genes affecting sodium channels. Mutations in *Sh* and *eag* also display phenotypic enhancement: transmitter release at the larval neuromuscular junction is increased by at least one order of magnitude in the double mutant compared with either single mutant (GANETZKY and WU 1983). In addition, *eag Sh* adults display downturned wings and an indentation of the dorsal thorax, properties not

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exhibited by either single mutant (R. KREBER and B. GANETZKY, unpublished data). This synergism between *eag* and *Sh* presumably reflects the simultaneous reduction of two potassium currents, both of which function to repolarize neuronal membranes. Finally, the nap^{ts} mutation suppresses the leg-shaking behavior of *Sh* (GANETZKY and WU 1982b) and *eag* mutations, as well as certain of their electrophysiological defects (GANETZKY and WU 1982a). These interactions presumably reflect the opposing functions of the *nap* product and the *Sh* and *eag* products in membrane excitability.

In this paper we characterize the effects on behavior and axonal excitability of altering $para^+$ dosage. We have found that certain *para* hypomorphs as well as decreased dosage of $para^+$ suppress the leg-shaking behavior and some electrophysiological defects of *Sh* mutants. We have also found that increased dosage of $para^+$ confers behavioral phenotypes opposite to those conferred by decreased dosage of $para^+$. In particular, whereas decreased dosage of $para^+$ is lethal in a nap^{ts} background, increased dosage of $para^+$ suppresses the temperature sensitivity of nap^{ts} mutants. Similarly, whereas decreased dosage of $para^+$ suppresses the Sh^{KS133} mutation, increased dosage of $para^+$ enhances Sh^{KS133}, resulting in a downturned wing and indented thorax phenotype similar to that observed in *eag Sh* double mutants. In addition to effects on behavior, increased dosage of $para^+$ has effects at the electrophysiological level consistent with an increase in membrane excitability at least in certain neurons. These results, combined with previous studies of *para* mutant phenotypes (WU and GANETZKY 1980; SIDDIQI and BENZER 1976; O'DOWD, GERMERAAD and ALDRICH 1989) and the recent molecular analysis of the *para* locus (LOUGHNEY, KREBER and GANETZKY 1989), demonstrate that $para^+$ encodes sodium channels whose level of expression can significantly alter neuronal membrane excitability. These dosage effects are not exhibited by other Drosophila genes that were identified on the basis of sequence homology to vertebrate sodium channels (SALKOFF *et al.* 1987; OKAMOTO *et al.* 1987; RAMASWAMI and TANOUYE 1989), which suggests that $para^+$ has a function distinct from other putative sodium channel genes in Drosophila. Because the phenotypes conferred by modifying $para^+$ gene dosage most likely reflect corresponding changes in the number of sodium channels in axonal membranes, we conclude that $para^+$ dosage is a rate-limiting factor in the synthesis of these sodium channels. In addition, we conclude that approximately twofold changes in sodium channel number is sufficient to produce striking behavioral and electrophysiological effects.

MATERIALS AND METHODS

Mutants and chromosomes: Sh^{KS133} (1–57.7) is a dominant *Sh* allele described by JAN, JAN and DENNIS (1977) that causes rapid leg-shaking behavior under ether anesthesia.

Hk^1 (1–30.5) is a recessive mutation that causes rapid leg-shaking behavior under ether anesthesia (KAPLAN and TROUT 1969).

$para^{lk5}$ (GANETZKY 1984) is a recessive lethal *para* mutation that has no detectable chromosome rearrangements and behaves similarly to deficiencies for *para*.

$l(1)l^{D23}$ is a recessive lethal mutation that lies close to *para* (GANETZKY 1984).

$Df(1)r\text{-}D^{17}$, kindly supplied by J. RAWLS, is deleted for region 14F6 to 15A6 and does not uncover $para^+$.

nap^{ts} (2–56.2) is a recessive temperature-sensitive paralytic mutation (WU *et al.* 1978).

$Df(2R)M\text{-}c^{33a}$ is deleted for 60E2 to 60E11, which includes the *DSC1* locus, a putative sodium channel gene (SALKOFF *et al.* 1987; OKAMOTO *et al.* 1987).

Df(2L)E71 is deleted for 36F2–6 to 37C6-D1.

Df(2L)TW158 is deleted for 37B2–8 to 37E2-F4.

$Dp(1:4)r^+f^+$ carries region 14A–16A2 appended to chromosome 4. This duplication contains the entire *para* gene and also covers $l(1)l^{D23}$ and $Df(1)r\text{-}D^{17}$. The presence of this duplication was scored by the f^+ marker. This duplication will be referred to as $Dp\ para^+$.

$Dp(2;Y)bw^+$ is referred to as $bw^+\ Y$. This duplication arose by transposition of region 58F-60F, which includes *DSC1* (SALKOFF *et al.* 1987), into the *Y* chromosome.

Dp(2;Y)G, contains region 36B5-C1 to 40F inserted into the *Y* chromosome.

$Tp(1;2)r^{+75c}$, referred to as $Tp(1;2)para^+$, contains region 14B13 to 15A9, which includes *para*, inserted into 35DE of the second chromosome.

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

C(1)DX ywf is a compound *X* chromosome that will be represented here as $\overline{XX}$ *ywf*.

FM7 is an *X* chromosome balancer marked with *B*.

CyO is a second chromosome balancer marked with *Cy*.

Descriptions of other genetic markers used in these experiments can be found in LINDSLEY and GRELL (1968).

Behavioral tests: *Leg-shaking*: Ether-induced leg-shaking was monitored after exposing flies to ether for 15–30 sec. Under these conditions, wild-type flies are immobile except for an occasional tarsal twitch, whereas *Sh* and *Hk* flies display vigorous shaking of all six legs.

Temperature sensitivity: Flies were tested for temperature-sensitive paralysis by placing flies in an empty vial submerged in a water bath at 34 or 37.5°. Under these conditions, wild-type flies remain active for up to 30 min, whereas nap^{ts} and certain *para* mutants become paralyzed within 10 sec of exposure to elevated temperature.

Other phenotypes: Flies exhibiting down-turned wings or an indentation of the dorsal thorax between the dorsocentral bristles were scored by visual inspection.

Construction of special strains and chromosomes: For data shown in Figure 1, double mutants carrying either $l(1)l^{D23}\ f\ car$ or $para^{lk5}\ f\ car$ on the *X* chromosome, and nap^{ts} on the second chromosome were constructed by two successive backcrosses to $\overline{XX}\ y^2\ bb^-;nap^{ts}\ cn$ females.

For data shown in Table 2, the $para^{lk5}\ Sh^{KS133}$ chromosome was constructed by crossing $para^{lk5}\ f\ car$ (the chromosome on which $para^{lk5}$ was isolated) to $B\ Sh^{KS133}$ and selecting recombinants between *f* and *B*. The control $para^+\ Sh^{KS133}$ chromosome was constructed by selecting *f B* recombinants from $g\ sd\ f/B\ Sh^{KS133}$ heterozygotes to produce a $g\ sd\ f\ B\ Sh^{KS133}$ chromosome. This chromosome was made heterozygous with $para^{lk5}\ f\ B\ Sh^{KS133}$ and $g^+\ sd$ recombinants were selected, to produce a $g^+\ sd\ f\ B\ Sh^{KS133}$ derivative. This chromosome is expected to carry the same genetic material

as $para^{lk5}$ *f B* Sh^{KS133} for the region distal to *g* (1–44.0) and proximal to *Sh* (1–57.7). These crosses were designed to minimize genetic differences at loci other than at *para*.

For the data shown in Figure 3, the *g sd* $para^{60}$ Sh^{KS133} chromosome was constructed by crossing *g sd f* to $para^{60}$ Sh^{KS133} and selecting for *sd* f^+ recombinants that were temperature sensitive. The control chromosome used was *g sd* $para^+$ Sh^{KS133}. To obtain Sh^{KS133} larvae carrying *Dp* $para^+$, a *v* $Df(1)r\text{-}D^{17}$ *f* Sh^{KS133} chromosome was constructed by crossing *v* $Df(1)r\text{-}D^{17}$ *f*; *Dp* $para^+$ to *B* Sh^{KS133} and selecting recombinants between *B* and *Sh*. Because $Df(1)r\text{-}D^{17}$ is lethal when hemizygous, only *Dp* $para^+$ bearing *v* $Df(1)r\text{-}D^{17}$ Sh^{KS133} males will be viable.

For the data shown in Figure 4, the *g* $Df(1)r\text{-}D^{17}$ *f* chromosome was constructed by crossing *v* $Df(1)r\text{-}D^{17}$ *f* to *g sd f* and selecting *g* sd^+ recombinants. The *g* $para^{lk5}$ *f car* chromosome was constructed by crossing *g* $Df(1)r\text{-}D^{17}$ *f* to $para^{lk5}$ *f car* and selecting *g car* recombinants. Chromosomes carrying $para^{lk5}$ were distinguished from those carrying $Df(1)r\text{-}D^{17}$ by complementation with $para^{103}$. $para^{103}/para^{lk5}$ flies exhibit temperature-sensitive paralysis, whereas $Df(1)r\text{-}D^{17}/para^{103}$ flies do not.

Two other genes have been identified in Drosophila that have homology to vertebrate sodium channels, *DSC1*, located at 60E2–3; 60E11–12, and *DIC37*, located at 37B-D (SALKOFF *et al.* 1987; OKAMOTO *et al.* 1987; RAMASWAMI and TANOUYE 1989). Changes in dosage of these genes were accomplished with the following duplications and deficiencies: deficiency $Df(2L)M\text{-}c^{33a}$, which removes *DSC1*; deficiencies *Df(2L)E71* and *Df(2L)TW158*, which together span the region containing *DIC37*; duplication *Dp* bw^+ *Y*, which contains *DSC1*; and duplication *Dp(2;Y)G*, which contains *DIC37*.

Isolation of suppressors of Sh^{KS133}: Sh^{KS133} males were mutagenized with ethylmethane sulfonate (EMS) according to LEWIS and BACHER (1968) except that 0.25% EMS was used. Following mutagenesis, the males (150–600) were mated to an equivalent number of $\overline{XX}$ *ywf* females with 25 pairs per bottle. Males were allowed to mate for 4–5 days at 25° and were then discarded. EMS was inactivated following use with 10% sodium thiosulfate as described by OSHIMA and TAKANO (1980).

Mapping: The map positions of the five *para* suppressor mutations were determined as follows. Sh^{KS133} males carrying one of the suppressor mutations were mated to females carrying either *y cho cv v f* (for $para^{60}$, $para^{63}$, $para^{74}$, and $para^{141}$) or *g sd f* Sh^{KS133} (for $para^{103}$). F_2 sons were scored for visible markers and ether-induced leg-shaking. In addition, temperature-sensitive paralysis was scored in crosses mapping $para^{60}$ and $para^{103}$. In all cases, temperature-sensitive paralysis was inseparable from the suppressor phenotype.

Electrophysiology: Larval dissections and nerve and muscle recordings were accomplished as described previously (JAN and JAN 1976; GANETZKY and WU 1982b; STERN and GANETZKY 1989). Nerves innervating the body wall muscles were cut near the ventral ganglion and were stimulated with a suction electrode. For intracellular muscle recordings, microelectrodes were pulled on a Flaming Brown micropipette puller to tip resistances of 30–60 MΩ and were filled with 3 M KCl. For extracellular nerve recordings a small loop of nerve near the nerve terminal was drawn into a second suction electrode. All dissections and recordings were performed at room temperature in standard saline containing 0.1 mM Ca^{2+}. Nerves were stimulated for 0.1 msec at a voltage of 1.5 to 2 times threshold voltage (which was about 2 V). Duration of the excitatory junctional potential (ejp) was measured from onset of response to peak response. All experiments were performed at 21–22°.

RESULTS

Mutant isolation: It was found previously that nap^{ts}, originally isolated on the basis of temperature-sensitive paralysis, suppresses the leg-shaking behavior of *Sh* mutations (GANETZKY and WU 1982b). To identify mutations in additional genes that suppress *Sh*, males carrying Sh^{KS133} were mutagenized, crossed to attached *X* females, and their sons scored for suppression of leg-shaking. Nine sons of 35,000 tested failed to exhibit ether-induced leg-shaking. The segregation pattern of the nine suppressor mutations suggested that each was located on the *X* chromosome.

Mapping and complementation tests: There were two reasons for suspecting that some of these new mutations might be defective at the *para* locus. First, nap^{ts} shares many properties with certain *para* mutations (GANETZKY and WU 1985), and nap^{ts} suppresses *Sh*. Second, two of the nine suppressor mutants exhibited rapid and reversible temperature-sensitive paralysis, which is a phenotype also exhibited by other *para* mutants (SUZUKI, GRIGLIATTI AND WILLIAMSON 1971). To determine which, if any, suppressor mutant was defective at *para*, complementation tests were performed between each suppressor mutation and $para^{lk5}$, a recessive lethal mutation that acts as a null point mutation (GANETZKY 1984). As shown in Table 1, five of the nine suppressor mutations failed to complement $para^{lk5}$. Four of the mutants demonstrated temperature-sensitive paralysis and somewhat reduced viability in combination with $para^{lk5}$, and the fifth was unconditionally inviable in combination with $para^{lk5}$. The other four mutations complemented $para^{lk5}$ and will not be described here further.

To confirm that these five suppressor mutations were alleles of *para*, the map position of each was determined. Table 1 shows that the map position of the five mutations is very close to that of $para^{ts1}$ (1–54.1). Therefore we conclude that these five suppressor mutants are alleles of *para*.

The suppression of leg-shaking by at least one of these mutations, $para^{103}$, is not gene specific because $para^{103}$ also suppresses the leg-shaking defect of the *Hyperkinetic*^{1} (Hk^1) mutation. This result is consistent with an earlier report that $para^{ts1}$ partially suppresses the leg-shaking defect of Hk^2 (WILLIAMSON and KAPLAN 1976).

Decreased and increased dosage of $para^+$: In certain genetic backgrounds, *para* null mutations and some *para* hypomorphs confer dominant phenotypic defects. For example, the null mutation $para^{lk5}$, which is a recessive lethal mutation in a nap^+ background, becomes a dominant lethal mutation in a nap^{ts} background (GANETZKY 1984). Similarly, $para^{lk5}$ is a dom-

TABLE 1

Complementation tests and map positions of new *para* alleles

Genotype	Complementation with *para*[a]		Map position[d]
	Temp. paralyzed[b]	Relative viability[c]	
para^{+} *Sh*^{KS133}		1.0 (57/103)	
para^{60} *Sh*^{KS133}	ND	0.0 (0/61)	1-53.7 (4/16)
para^{63} *Sh*^{KS133}	34	0.6 (19/57)	1-53.5 (6/25)
para^{74} *Sh*^{KS133}	37	0.7 (35/88)	1-53.0 (4/19)
para^{103} *Sh*^{KS133}	34	0.7 (43/112)	1-54.1 (14/28)
para^{141} *Sh*^{KS133}	37	0.4 (16/79)	1-54.5 (6/19)

ND, not determined.

[a] Complementation tests of the five suppressors of *Sh*^{KS133} with *para*^{lk5} were performed by crossing *suppressor*^{x} *Sh*^{KS133} males to *para*^{lk5} *f car*/FM7 females and the *suppressor*^{x} *Sh*^{KS133}/*para*^{lk5} *f car* daughters were tested for complementation.

[b] Four of the five *para* mutations, when heterozygous with *para*^{lk5}, became paralyzed within 10 seconds at temperatures between 34 and 37°. At least ten flies of each genotype were tested. The fifth mutation, *para*^{60}, could not be tested because it was unconditionally lethal when heterozygous with *para*^{lk5}. Flies carrying the parental *para*$^{+}$ chromosome heterozygous with *para*^{lk5} did not become paralyzed after at least 5 min at 37°.

[c] The ratio of viable *para*^{x} *Sh*^{KS133}/FM7 daughters to *para*^{x} *Sh*^{KS133}/*para*^{lk5} *f car* daughters (listed in parentheses) was determined. The viability for the different *para* alleles was normalized to that for *para*$^{+}$, which was assigned a value of 1.0.

[d] The new *para*^{x} were mapped relative to *y cho cv v f* (for *para*^{60}, *para*^{63}, and *para*^{74}), relative to *g sd f* (for *para*^{141}) and relative to *g sd f Sh*^{KS133} (for *para*^{103}). For *para*^{60}, *para*^{63}, *para*^{74}, and *para*^{141}, the map position was determined by comparing the number of shaking flies that were *f* to the total number of shaking flies (these numbers listed in parentheses). With this method, the map distance between *para*^{x} and *f* is expressed relative to the map distance between *f* and Sh^{KS133}. For *para*^{103}, the map position was determined by selecting the *sd f* recombinants (which numbered 28), of which 14 exhibited leg-shaking and 14 did not.

inant suppressor of the leg-shaking behavior of the four additional *Sh* alleles tested (*Sh*^{5}, *Sh*^{M}, *Sh*^{rKO120}, *Sh*^{E62}). These dominant effects of *para*^{lk5} are likely to result from a reduction of *para*$^{+}$ function rather than an antimorphic effect of this allele, because deficiencies of *para* are also dominant lethal mutations in a *nap*^{ts} background and dominant suppressors of *Sh*^{KS133}.

The results described above demonstrate that a 50% reduction in *para*$^{+}$ dosage can alter behavioral phenotypes, suggesting that axonal excitability at least in certain neurons is sensitive to reductions in *para*$^{+}$ dosage. Therefore it was of interest to determine if increasing *para*$^{+}$ dosage, by introduction of a duplication for *para*$^{+}$ (*Dp para*$^{+}$), would also cause phenotypic effects but opposite in direction from decreased *para*$^{+}$ dosage.

In agreement with previous observations of WILLIAMSON and KAPLAN (1973) we found that flies bearing a duplication for the region that included *para*$^{+}$ (*Dp para*$^{+}$), showed leg-shaking behavior under ether anesthesia. This phenotype was specifically the result of the increased dosage of *para*$^{+}$ because *para*^{lk5}; *Dp para*$^{+}$ males, which have the wild-type dose of *para*$^{+}$ but an extra dose of all other genes on *Dp para*$^{+}$, do not shake. Because the phenotypic defects caused by decreased *para*$^{+}$ dosage are most apparent in *nap*^{ts} and *Sh*^{KS133} backgrounds, the effect of *Dp para*$^{+}$ was further examined in these backgrounds.

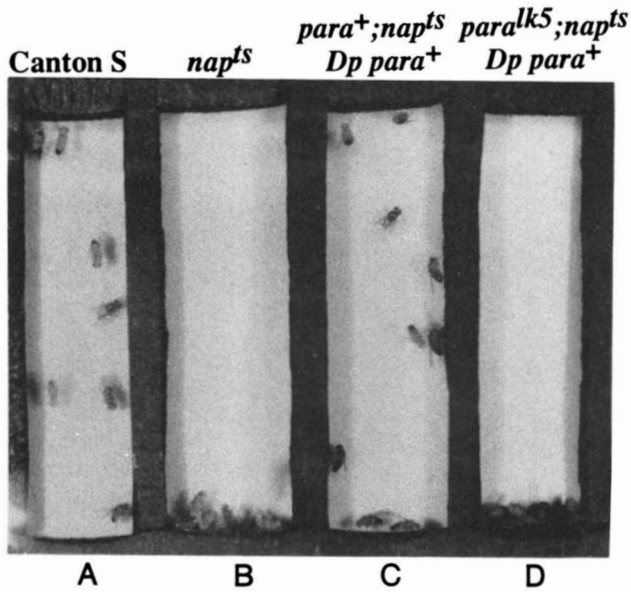

FIGURE 1.—Suppression of *nap*^{ts} by *Dp para*$^{+}$. Male flies of the indicated genotypes were placed between glass sheets in a water bath held at 37.5°. The Canton S flies remain standing and moving on the vial walls for 30 min or more. In contrast, the *nap*^{ts} flies become paralyzed quickly upon exposure to 37.5° and fall to the bottom of the vial. The *nap*^{ts}; *Dp para*$^{+}$ flies can remain standing and moving at 37.5°, indicating that *Dp para*$^{+}$ suppresses *nap*^{ts}. The *para*^{lk5}; *nap*^{ts}; *Dp para*$^{+}$ flies become paralyzed upon exposure to 37.5° and fall to the bottom of the vial. Therefore, suppression of *nap*^{ts} by *Dp para*$^{+}$ specifically requires an extra dose of *para*$^{+}$. The *X* chromosomes in the flies shown in the two vials at the right are *l(1)l*^{D23} f car and *para*^{lk5} *f car*, respectively.

Suppression of *nap*^{ts} by *Dp para*$^{+}$: Although *nap*^{ts} flies are fully active at permissive temperatures (18–25°), they become rapidly paralyzed at the restrictive temperature of 37.5° (WU *et al.* 1978; shown in Figure 1B). In contrast, wild-type flies (Canton S) remain fully active at this temperature for 30 min or more (Figure 1A). Males homozygous for *nap*^{ts} and carrying *Dp para*$^{+}$ remain almost as active at 37.5° as Canton S (Figure 1C), demonstrating that *Dp para*$^{+}$ suppresses the temperature-sensitive paralytic phenotype of *nap*^{ts}.

To determine if the suppression of *nap*^{ts} resulted from an extra dose of *para*$^{+}$ or an extra dose of another gene on the duplication, the behavior of *para*^{lk5}; *nap*^{ts} flies carrying *Dp para*$^{+}$ was analyzed for temperature-sensitive paralysis. These flies are paralyzed at 37.5° (Figure 1D), demonstrating that suppression of *nap*^{ts} results specifically from doubling of *para*$^{+}$ dosage.

Enhancement of *Sh*^{KS133} by *Dp para*$^{+}$: Increased *para*$^{+}$ dosage also exerts phenotypic effects in a *Sh*^{KS133} background. In contrast to Canton S flies, *Sh*^{KS133}; *Dp para*$^{+}$ flies exhibit downturned wings and an indentation of the dorsal thorax (Figure 2A), a phenotype

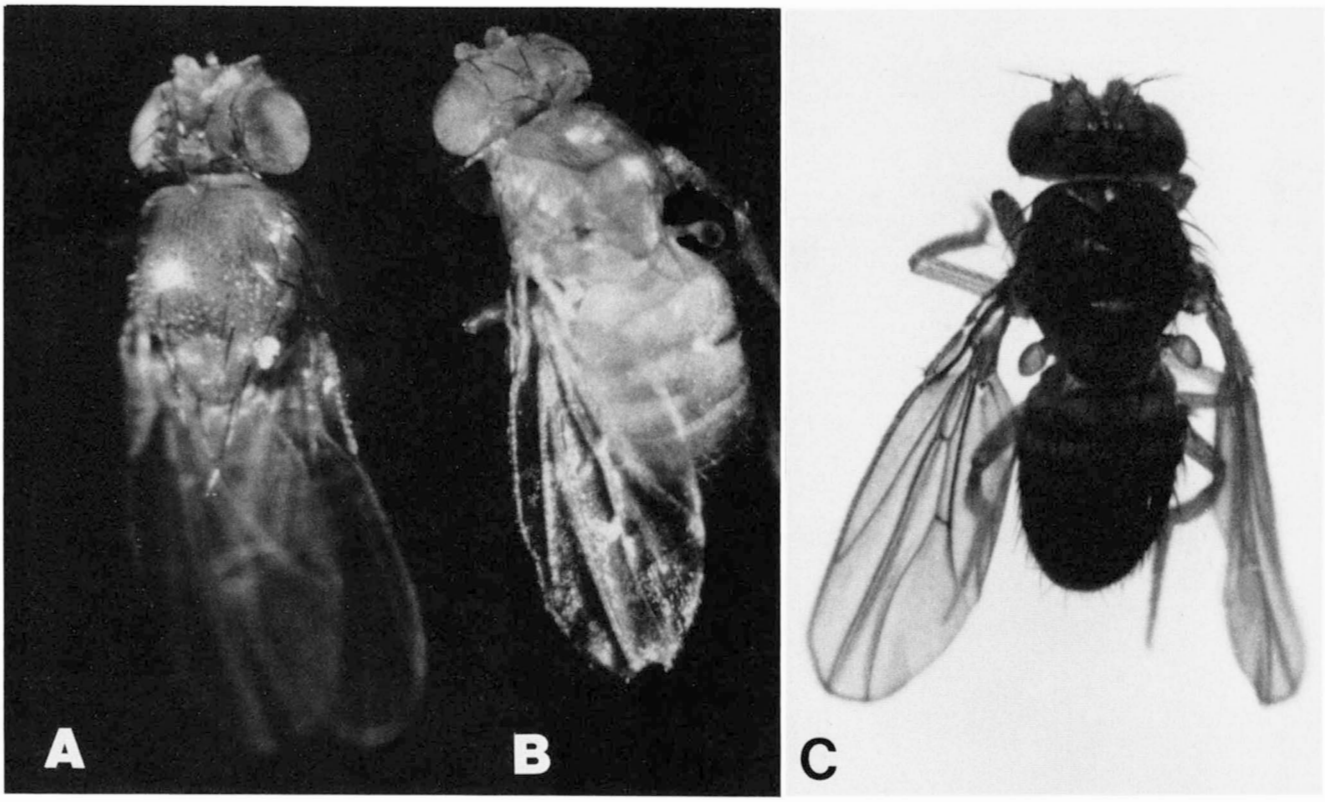

FIGURE 2.—The Canton S fly on the left (A) displays straight wings and a normal thorax. The Sh^{KS133}; *Dp para*$^{+}$ fly on the right (B) displays downturned wings and an indentation of the dorsal thorax; C, photographed separately, shows an *eag* Sh^{KS133} fly. Note the similarity in phenotype between this fly and the Sh^{KS133}; *Dp para*$^{+}$ fly shown in B.

TABLE 2

Enhancement of Sh^{KS133} by increased dosage of *para*$^{+}$

Genotype[a]	Wings and thorax normal	Wings down, normal thorax	Wings down, indented thorax
$para^{lk5}$ Sh^{KS133}; *Dp para*$^{+}$ ♂	96	2	0
para^{+} Sh^{KS133}; *Dp para*$^{+}$ ♂	4	14	20
Sh^{KS133}; *Tp(1; 2) para*$^{+}$ ♀	38	20	34
Sh^{KS133}; *Tp(1; 2) para*$^{+}$ ♂	27	31	39

[a] For the first cross listed, $para^{lk5}$ *f* Sh^{KS133}; *Dp para*$^{+}$ males were crossed to *XX ywf* females and male progeny were counted and scored. For the second cross, *para*$^{+}$ *f* Sh^{KS133} males were crossed to *XX ywf*; *Dp para*$^{+}$ females and f^{+} male progeny (those that carried *Dp para*$^{+}$) were counted and scored. Construction of these *X* chromosomes and additional markers present are listed in MATERIALS AND METHODS. For the third and fourth crosses, Sh^{KS133}; *Tp(1; 2) para*$^{+}$/*CyO* males were crossed to Sh^{KS133} females and Sh^{KS133}; *Tp(1; 2) para*$^{+}$/+ males and females were counted and scored.

not observed in either Sh^{KS133} flies or *Dp para*$^{+}$ flies alone. This effect was caused by the extra dosage of *para*$^{+}$ rather than another gene on the duplication because in $para^{lk5}$ Sh^{KS133}; *Dp para*$^{+}$ flies, in which *para*$^{+}$ dosage is restored to normal, the wing posture and thoracic cuticle phenotypes are also returned to normal (Table 2).

This enhancement of Sh^{KS133} occurs even when an extra copy of *para*$^{+}$ is introduced into females, which should cause only a 1.5-fold, rather than a 2-fold, increase in *para*$^{+}$ dosage. In addition, this enhancement occurs when a different *para*$^{+}$ duplication, *Tp(1;2)para*$^{+}$, is used to increase *para*$^{+}$ dosage (Table 2). The penetrance of this phenotype was only slightly lower in females than in males. We conclude that these effects of *para*$^{+}$ dosage are not specific to the particular duplication used, and furthermore, that increasing the normal dosage of *para*$^{+}$ by 50% is sufficient to confer observable phenotypes.

It is of interest that Sh^{KS133}; *DP para*$^{+}$ flies closely resemble eag^{1} Sh^{KS133} flies in appearance (R. KREBER and B. GANETZKY, unpublished data, shown in Figure 2C). Because *eag* mutations produce their phenotypic effects through an increase in membrane excitability, it is likely that the similar phenotype caused by increased dosage of *para*$^{+}$ also involves increased membrane excitability.

Electrophysiological consequences: The results described above demonstrate that altering *para*$^{+}$ dosage has significant behavioral effects. To determine if these effects are correlated with electrophysiological abnormalities, synaptic transmission at the larval neuromuscular junction was assayed. Motor axons innervating the larval body wall muscles are stimulated with an electrode, thus evoking an action potential, which is propagated to the nerve terminal, causing transmitter release at the neuromuscular junction. The result-

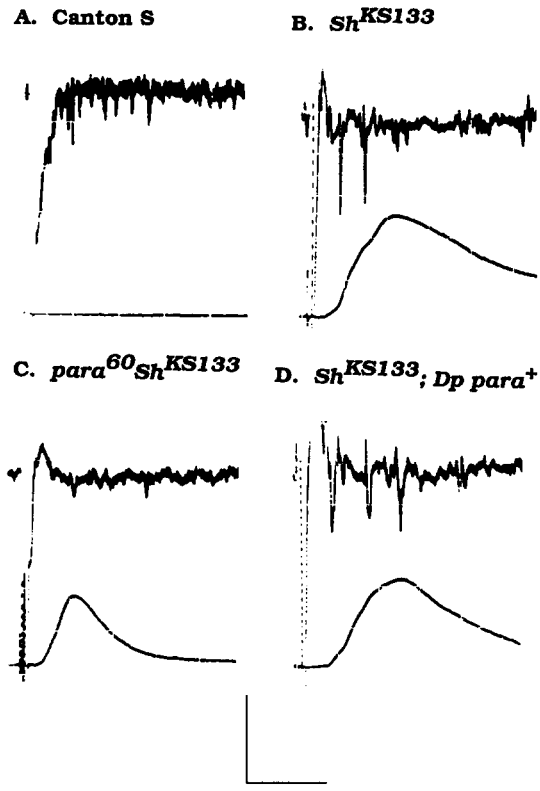

FIGURE 3.—Simultaneous intracellular recordings from muscle (lower traces) and extracellular recordings from the nerve (upper traces) that innervates them. Representative traces are shown. (A) Response of a Canton S larva. (B) Response of a Sh^{KS133} larva. (C) Response of a $para^{60}$ Sh^{KS133} larvae. The ejp, although larger than the ejp of the Canton S larva, is smaller than the ejp of the Sh^{KS133} larva, and the extra action potentials are absent. (D) Response of a Sh^{KS133}; *Dp* $para^{+}$ larva. This response is similar to the response of the Sh^{KS133} larva shown in B). External [Ca^{2+}] was 0.1 mM. Calibration bars: sweep speed 40 msec. Lower voltage trace 20 mV. Upper voltage trace 0.2 mV for (A), 0.4 mV for (B), (C) and (D).

ing depolarization of the muscle membrane, called the excitatory junctional potential (ejp), can be monitored with an intracellular electrode. Because transmitter release requires Ca^{2+} influx, at low external [Ca^{2+}], such as 0.1 mM, very little transmitter is released following nerve stimulation at a wild-type neuromuscular junction and only a very low amplitude ejp is evoked (JAN and JAN 1976 and Figure 3A). Sh^{KS133} mutants, however, are defective in the potassium current I_A (SALKOFF and WYMAN 1981; WU and HAUGLAND 1985). Because I_A is required for nerve terminal repolarization, Sh^{KS133} mutants display prolonged depolarization of the nerve terminal, which leads to increased release of transmitter and a prolonged and large amplitude ejp (JAN, JAN and DENNIS 1977, and Figure 3B) even at 0.1 mM [Ca^{2+}]. This increased ejp is associated with the generation of extra action potentials in motor neurons (JAN and JAN 1979, and Figure 3B), most likely as a result of the prolonged depolarization of the nerve terminal.

It was shown previously that in addition to suppression of leg-shaking, nap^{ts} also suppresses some of the

TABLE 3

Summary of ejp properties at the neuromuscular junction in larvae of the indicated genotypes

Genotype	Resting potential (mv)	ejp Amplitude (mv)	ejp Duration (ms)	
Wild type	−38 ± 5.6	≤1[a]	≤10[a]	(9)
Sh^{KS133}	−39 ± 4.2	16.5 ± 5.2	27 ± 6	(7)
$para^{60}$ Sh^{KS133}	−38 ± 2.7	11.2 ± 3.3	15 ± 4	(7)
Sh^{KS133}; *Dp* $para^{+}$	−34 ± 3	15.2 ± 5.8	24 ± 10	(5)

The number of larvae of each genotype from which these recordings were made is shown in parentheses above.

[a] At the [Ca^{2+}] used in these experiments (0.1 mM) most nerve stimulations in wild type failed to evoke any ejp at all (JAN and JAN 1976). The values shown are upper limits for amplitude and duration of the occasional successful responses observed under these conditions.

electrophysiological defects of *Sh* (GANETZKY and WU 1982a, b). In particular, the time course and amplitude of ejps in Sh^{KS133}; nap^{ts} double mutants are reduced compared to Sh^{KS133}; nap^{+}, and the extra neuronal action potentials are eliminated. To determine if any of the five *para* mutations isolated in this study could similarly suppress the electrophysiological defects of Sh^{KS133}, recordings from the $para^{60}$ Sh^{KS133} double mutant were performed. We chose the $para^{60}$ mutation for this analysis because, of the five, it displays the most extreme phenotypes at room temperature, including unconditional lethality when heterozygous with $para^{lk5}$ (Table 1) and unconditional lethality in double mutant combination with nap^{ts} (not shown). The $para^{60}$ allele partially suppresses the increase in membrane excitability caused by Sh^{KS133}. Representative recordings are presented in Figure 3 and data from all the larvae examined are summarized in Table 3. In the double mutant the ejp amplitude and duration were reduced compared to Sh^{KS133} alone although both were still greater than wild type. In addition, no extra action potentials were observed in the double mutant, in contrast to the 2–3 extra action potentials that were typically seen in Sh^{KS133}.

These results indicate that a decrease in expression or activity of the $para^{+}$ product results in a decrease in membrane excitability. To determine if increased dosage of $para^{+}$ caused a corresponding increase in membrane excitability that further enhanced transmitter release in Sh^{KS133}, electrophysiological recordings were performed on *Dp* $para^{+}$; Sh^{KS133} larvae. Synaptic transmission in *Dp* $para^{+}$; Sh^{KS133} larvae did not differ noticeably from Sh^{KS133} larvae either in ejp amplitude or duration or in the number of extra action potentials following nerve stimulation (Table 3 and Figure 3D). Therefore, no further enhancement of nerve terminal depolarization or transmitter release by *Dp* $para^{+}$ beyond that caused by Sh^{KS133} is apparent under these conditions.

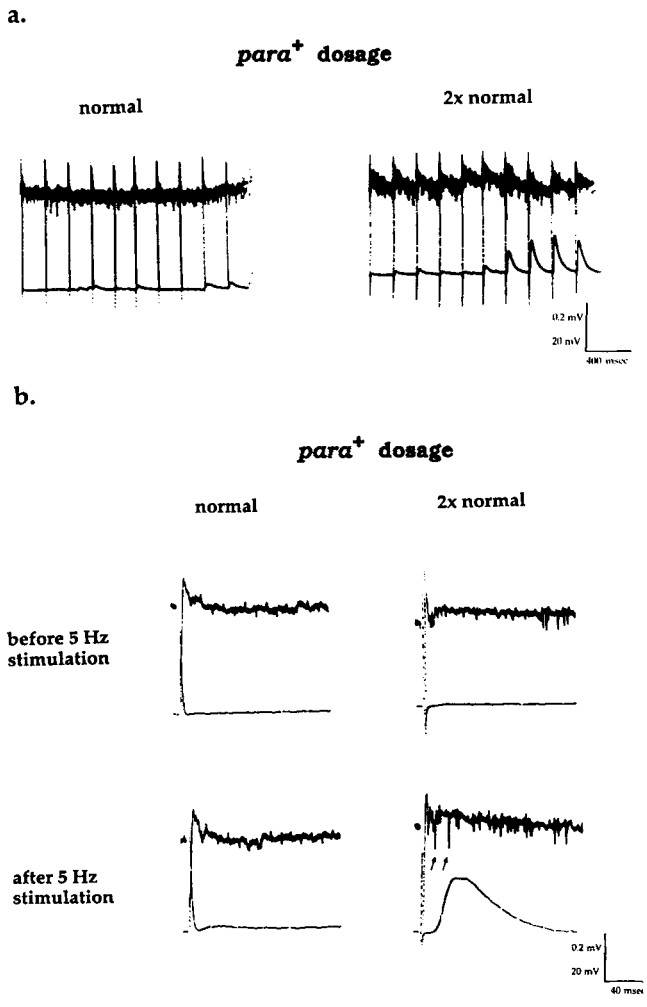

FIGURE 4.—Simultaneous intracellular recordings from muscle (lower traces) and extracellular recordings from the nerve (upper traces) that innervates them. Representative traces are shown. (a) Comparison of the response of a $para^{lk5}$; *Dp* $para^{+}$ male larva (left trace) with a $para^{+}$; *Dp* $para^{+}$ male larva (right trace) to 2 sec of 5 Hz nerve stimulation. Calibration bars: sweep speed 400 msec, upper voltage trace 0.2 mV, lower voltage trace 20 mV. (b) Responses of larvae of the same genotype as in (a), shown at a faster sweep speed. The top traces show responses upon initial stimulation, the lower traces show responses after 2 sec of 5 Hz nerve stimulation. Whereas the ejps of the larva carrying normal dosage of $para^{+}$ closely resembled wild type (JAN and JAN 1977; STERN and GANETZKY 1989) and showed no increased response to repetitive stimuli, the ejps of the larva carrying an extra dose of $para^{+}$ showed a striking increase in amplitude and duration following prior 5 Hz nerve stimulation, accompanied by the appearance of two extra neuronal action potentials (marked by arrows). Calibration bars: sweep speed 40 msec. Upper voltage trace 0.2 mV. Lower voltage trace 20 mV. External $[Ca^{2+}]$ was 0.1 mM.

However, an increase in dosage of $para^{+}$ did have effects on synaptic transmission in an otherwise wild-type background. Figure 4a shows recordings from nerve and muscle in response to 2 sec of 5 Hz nerve stimulation at 0.1 mM $[Ca^{2+}]$. The traces on the right are from a male larva carrying *Dp* $para^{+}$. The first several responses from this larva resembled those of wild type: the ejps were low in amplitude and no extra neuronal action potentials were observed. However, in contrast to wildtype larvae, subsequent stimulation at 5 Hz produced a facilitated response resulting in ejps of considerably increased amplitude and duration (Table 4), associated with two extra action potentials (marked by arrows in Figure 4b, lower right). These observations suggest that the increased ejps produced result from prolonged depolarization of the nerve terminal and the consequent increased release of transmitter. These effects on synaptic transmission specifically require duplication of $para^{+}$, rather than other genes present on *Dp* $para^{+}$, because in $para^{lk5}$; *Dp* $para^{+}$ larvae, $para^{+}$ dosage is reduced to normal and synaptic transmission resembles wild type (JAN and JAN 1977; STERN and GANETZKY 1989): ejps remain low in amplitude throughout the stimulus train (Table 4) and no extra action potentials are observed in response to 5 Hz stimulation (Figure 4, left traces).

Dosage effects of other putative sodium channel genes: Because $para^{+}$ is a sodium channel structural gene (LOUGHNEY, KREBER and GANETZKY 1989), we interpret these dosage effects as resulting from corresponding changes in the number of sodium channels present in neuronal membranes. On the basis of homology to previously cloned sodium channel genes from other organisms, two other loci in Drosophila have been identified as potential sodium channel genes (SALKOFF *et al.* 1987; OKAMOTO *et al.* 1987; RAMASWAMI and TANOUYE 1989). However, because no mutations have been isolated in these genes, their function remains uncertain. To determine if either of these genes can exert phenotypic effects similar to $para^{+}$, we tested whether they also show dose-dependent interactions with nap^{ts} and Sh^{KS133}.

We found that changes in *DSC1* dosage had no effect on the behavior or viability of Sh^{KS133} or nap^{ts} mutants. Flies heterozygous for $Df(2L)M\text{-}c^{33a}$, which deletes *DSC1*, show no suppression of the Sh^{KS133} behavioral defect. Furthermore, in a nap^{ts} background, heterozygosity for $Df(2L)M\text{-}c^{33a}$ had no additional effect on viability or temperature-sensitive paralysis. Similarly, the presence of *Dp* bw^{+} *Y*, which carries an extra dose of *DSC1*, did not suppress the nap^{ts} phenotype nor enhance the Sh^{KS133} phenotype.

The putative sodium channel gene at 37B-D, *DIC37*, is located in a region deleted by *Df(2L)E71* or *Df(2L)TW158* (or both) and is contained within *Dp(2;Y)G*. As was the case for *DSC1*, we found that changes in dosage of *DIC37* had no observable phenotypic effects: neither deficiency when heterozygous suppressed Sh^{KS133}, and *Dp(2;Y)G* did not enhance Sh^{KS133} or suppress nap^{ts} or $para^{ts}$. These results indicate that the gene dosage effects we have observed for $para^{+}$ are specific for this locus and not shared with other putative sodium channel genes in Drosophila.

TABLE 4

Effect of increased dosage of *para*$^+$ on response to repetitive nerve stimulation at the larval neuromuscular junction

Genotype	Resting potential (mv)	Time until facilitation (s)	Ejp amplitude (mv)		Ejp duration (msec)	
			Before	After	Before	After
para^{ik5}; *Dp para*$^+$ (4)	−40 ± 6.5	>60	≤1		≤10	
para$^+$; *Dp para*$^+$ (5)	−46 ± 3.0	2.3 ± 1.8	≤1	21.8 ± 6.4	≤10	25 ± 3

The muscle responses to repetitive nerve stimulation at 5 Hz in male larvae of the indicated genotype are shown ([Ca^{2+}] = 0.1 mM). The number of larvae of each genotype that were examined is given in parentheses. The columns labelled "before" and "after" refer to the onset of facilitation in larvae with increased dosage of *para*$^+$. In the control larvae no comparable facilitation was observed during the 60-sec interval during which 5 Hz stimulation was maintained (Figure 4).

DISCUSSION

Here we show that significant perturbations of behavior and membrane excitability in Drosophila can be caused just by altering the dosage of the *para*$^+$ locus. Our results indicate that extra doses of *para*$^+$ lead to an increase in neuronal membrane excitability, presumably resulting from overexpression of sodium channels and a corresponding enhancement of sodium currents. In contrast, decreased *para*$^+$ dosage leads to a reduction in membrane excitability, presumably because of underproduction of sodium channels and the consequent reduction of sodium currents. These results lead us to conclude that a rate-limiting step for the incorporation of functional sodium channels in neuronal membranes in Drosophila is the degree of expression of the *para* locus. Furthermore, we conclude that approximately twofold increases or reductions in the level of sodium channels can cause observable changes in behavior and neuronal membrane excitability, raising the possibility that differences in membrane excitability among various neurons might normally involve regulating the expression or functional activity of *para*$^+$.

The behavioral and electrophysiological experiments reported here assay the properties of only a small subset of neurons in the Drosophila nervous system. Other neurons might not be similarly affected by variations in *para*$^+$ dosage. Putative sodium channel genes at loci other than *para* have been identified (discussed below) on the basis of sequence similarity with vertebrate sodium channels. It is not known if these genes encode functional sodium channels, but if so, then the properties of certain neurons could depend primarily on sodium channels encoded by these genes rather than by *para*. The discussion that follows focuses primarily on those neurons whose properties could be examined in these studies.

Deletions of *para*$^+$ reduce membrane excitability: The phenotypic effects on behavior and synaptic transmission associated with altered dosage of *para*$^+$ are most readily revealed in combination with other mutations that alter membrane excitability. For example, *para* null mutations, which are recessive lethal mutations in a *nap*$^+$ background, become dominant lethal mutations in a *nap*^{ts} background (GANETZKY 1984). This lethality can be explained if *nap*^{ts} causes a reduction in expression or function of the *para*$^+$ gene product (LOUGHNEY *et al.* 1989). According to this view, in a *nap*^{ts} background the level of functional sodium channels produced by a single dose of *para*$^+$ is below a threshold required for viability. This interpretation is consistent with the previous observation that *nap*^{ts} mutants exhibit a 40% reduction in sodium channel number (KAUVAR 1982; JACKSON *et al.* 1984). A prediction of this model is that it should be possible to restore a normal phenotype to *nap*^{ts} homozygotes by increasing the level of *para*$^+$ expression. In accord with this prediction, we have found that addition of a single extra dose of *para*$^+$ via a chromosome duplication can rescue the *nap*^{ts} paralytic phenotype.

Phenotypic interactions of *para*$^+$ deletions with *Sh* mutations lend further support to the notion that neuronal membrane excitability is dependent upon the dosage of the *para* locus. We found that heterozygous *para* null mutations suppress the leg-shaking phenotype associated with *Sh*^{KS133}. Similarly, in mutational screens for suppressors of *Sh*^{KS133}, we isolated five new *para* mutations. These new mutations appear to be hypomorphic alleles because, unlike *para* null mutations, they are recessive suppressors of *Sh*^{KS133} and are viable when homozygous. In addition to suppression of the *Sh*^{KS133} behavioral phenotype, the *para*^{60} allele also blocks the repetitive firing of action potentials in motor axons and partially suppresses the increase in transmitter release associated with *Sh*^{KS133}.

The suppression of *Sh* phenotypes by *para* mutations closely resembles the suppression of *Sh* by *nap*^{ts} (GANETZKY and WU 1982a), which has been attributed to a decrease in sodium currents that counteracts the increase in membrane excitability caused by *Sh* mutations (GANETZKY and WU 1982a, b). Suppression of *Sh*^{KS133} by hypomorphic *para* mutations can be

similarly attributed to a reduction in sodium currents resulting from either a decrease in number of sodium channels (LOUGHNEY, KREBER and GANETZKY 1989), or from the production of functionally defective sodium channels. The dominant suppression caused by *para* null alleles suggest that even a 50% reduction of sodium channel levels is sufficient to block the manifestation of *Sh* behavioral defects.

Duplications of *para*$^+$ increase membrane excitability: Whereas heterozygous deletions of the *para* locus suppress Sh^{KS133}, the addition of extra doses of *para*$^+$ have the opposite effect leading to a marked enhancement of the *Sh* phenotype. This enhancement is visibly manifested by a phenotype of downturned wings, often associated with an indentation of the cuticle of the dorsal thorax near its anterior margin. This particular phenotype is strikingly similar to that observed in *eag* Sh^{KS133} double mutants, suggesting the possibility of a common underlying mechanism. Like *Sh*, *eag* mutations cause an abnormal increase in membrane excitability, apparently as a result of a defect in a class of potassium channels different from those affected by *Sh* (GANETZKY and WU 1983; WU *et al.* 1983). Furthermore, in the double mutant there is a striking enhancement of transmitter release at the larval neuromuscular junction. We believe a similar type of abnormal hyperexcitability leads, in the adult, to the downturned wing phenotype. For example, excessive activity of the motor axons that innervate the dorsal longitudinal flight muscles (DLM) of the adult could cause these wing depressor muscles to be in a perpetually hypercontracted state, in turn causing both the downturned wings and indented dorsal thorax phenotype. For the case of Sh^{KS133}; *Dp para*$^+$ flies, the enhanced membrane excitability would be produced as a consequence of an increase in sodium current caused by the *para*$^+$ duplication concomitant with a decrease in potassium currents caused by Sh^{KS133}. Unlike *eag* Sh^{KS133} double mutants, Sh^{KS133}; *Dp para*$^+$ larvae display no obvious enhancement of the Sh^{KS133} electrophysiological defect. However, this result does not rule out the possibility that extra doses of *para*$^+$ enhance the *Sh* defect in other parts of the nervous system or at other developmental stages that are more directly related to the manifestation of the downturned wing phenotype.

Although in the examples described, the presence of other membrane excitability mutations enabled the phenotypes associated with altered dosage of *para*$^+$ to be clearly revealed, phenotypic effects caused by increased dosage of *para*$^+$ can also be observed in an otherwise wild-type background. For example, increased dosage of *para*$^+$ causes leg-shaking in adults, a phenotype associated with enhanced nerve activity in other mutations such as *Sh*, *eag* and *Hk* (KAPLAN and TROUT 1969). In response to repetitive nerve stimulation, ejps in larvae carrying *Dp para*$^+$ display an aberrant abrupt increase in amplitude and duration associated with the generation of extra action potentials in the motor axon. These results provide further support for the view that sodium channels are being overexpressed in at least some neurons in these larvae and that this increase in sodium channel number causes an increase in membrane excitability. The exact mechanism of this increased response in *Dp para*$^+$ larvae and why it is elicited only in response to repeated nerve stimulation is not known.

Similar effects are not observed for other putative sodium channel genes: Two other loci sharing amino acid similarity with the a subunit of vertebrate sodium channels have been identified in Drosophila, *DSC1*, at 60D-E, and *DIC37*, at 37B-D (SALKOFF *et al.* 1987; OKAMOTO *et al.* 1987). Because no mutation of either of these genes has been identified their function is still unknown. To assess whether they are functionally similar to *para*$^+$, we examined the phenotypic consequences of altered dosage of these loci. In contrast to our results with *para*$^+$, altered dosage of these other two loci do not cause any significant phenotypic effects. We conclude that if these loci do encode sodium channel polypeptides they are distinct from the *para*$^+$ product in the nature of their regulation, their subcellular localization, their functional activities or the time or place of their action.

Relationship of *para*$^+$ dosage to the control of sodium channel numbers and the regulation of membrane excitability: As suggested in our arguments above, the most straightforward interpretation of our data is that the number of functional sodium channels incorporated into the membranes of at least some classes of neurons is affected by the gene dosage of *para*$^+$. Therefore, we conclude that transcriptional activity of *para*$^+$ is a rate-limiting step for expression of functional sodium channels at the neuronal membrane. Although previous segmental aneuploid studies for enzyme-encoding genes in Drosophila have shown that generally a proportionality exists between the level of enzymatic activity and dosage of the corresponding structural gene (HODGETTS 1975; HALL and KANKEL 1976; GREENSPAN 1980), our results were unexpected in light of studies on sodium channel biosynthesis in rat brain neurons (SCHMIDT, ROSSIE and CATTERALL 1985). These studies indicated that in embryonic rat brain neurons developing *in vitro*, over 50% of the newly synthesized sodium channel subunits are present as a stable intracellular pool that may act as a precursor to the cell surface sodium channels. Furthermore, it was suggested that post-translational assembly is a rate-limiting step in the biogenesis of functional sodium channels. If Drosophila neurons contained a similar large reservoir of intracellular sodium channels available for incorpo-

ration into the cell membrane, altered dosage of *para*$^+$ should not cause the marked phenotypic effects described here. However, our behavioral and electrophysiological assays have been carried out on mature third instar larvae or adults, when neurons are already well developed. In rat, the intracellular pool described for embryonic neurons was absent in the neurons of adult brain. Therefore, the apparent difference in sodium channel regulation between rat and Drosophila neurons may depend on the stage of development. In this regard, it is of interest to note that doubling the dose of *Sh*$^+$, the structural gene for A-type potassium channels, results in an increase in amplitude of this current in muscles of mature larvae (F. N. HAUGLAND and C.-F. WU, cited in WU and GANETZKY 1988) but not in developing pupal muscle (SALKOFF 1983; TIMPE and JAN 1987). On the other hand, heterozygous deletions of *Sh*$^+$ resulted in reductions in A current amplitude in both larval and pupal muscles (F. N. HAUGLAND and C.-F. WU, personal communication; TIMPE and JAN 1987).

Our results demonstrate that changes in the level of sodium channel expression associated with altered dosage of *para*$^+$ significantly alter neuronal excitability. Electrophysiological studies of identified neurons in other organisms reveal that particular neurons have differing characteristic patterns of electrical activity. Thus, the different levels of neuronal excitability achieved in our experiments by modifying the dosage of *para*$^+$ may correspond to variations in excitability normally present in certain types of neurons. One way that neurons could normally acquire different patterns of activity is by regulating the expression or functional activity of sodium channels. Such normal regulatory mechanisms could alter the magnitude of sodium currents with consequences comparable to those produced in our experiments by altered dosage of *para*$^+$.

The availability both of genetic and molecular tools to study *para* offer unique opportunities for analysis of sodium channels. Further investigations utilizing these tools will enable the elucidation of the molecular mechanisms that regulate sodium channel biosynthesis and function *in vivo* as well as the phenotypic consequences of altering these mechanisms.

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LITERATURE CITED

BAUMANN, A., I. KRAH-JENTGENS, F. MULLER, F. MULLER-HOLTKAMP, R. SEIDEL, N. KECSKEMETHY, J. CASAL, A. FERRUS and O. PONGS, 1987 Molecular organization of the maternal effect region of the *Shaker* complex of Drosophila: characterization of an I_A channel transcript with homology to vertebrate Na^+ channel. EMBO J. **6:** 3419–3429.

GANETZKY, B., 1984 Genetics of membrane excitability in Drosophila: lethal interactions between two temperature-sensitive paralytic mutations. Genetics **108:** 897–911.

GANETZKY, B., and C.-F. WU, 1982a Drosophila mutants with opposing effects on nerve excitability: genetic and spatial interactions in repetitive firing. J. Neurophys. **47:** 501–514.

GANETZKY, B., and C.-F. WU, 1982b Indirect suppression involving behavioral mutants with altered nerve excitability in Drosophila. Genetics **100:** 597–614.

GANETZKY, B., and C.-F. WU, 1983 Neurogenetic analysis of potassium currents in Drosophila: synergistic effects on neuromuscular transmission in double mutants. J. Neurogenet. **1:** 17–28.

GANETZKY, B., and C.-F. WU, 1985 Genes and membrane excitability in Drosophila. Trends Neurosci. **8:** 322–326.

GANETZKY, B., and C.-F.WU, 1986 Neurogenetics of membrane excitability in Drosophila. Annu. Rev. Genet. **20:** 13–44.

GREENSPAN, R. J., 1980 Mutations of choline acetyltransferase and associated neural defects in *Drosophila melanogaster*. J. Comp. Physiol. **137:** 83–92.

HALL, J. C., and D. R.KANKEL, 1976 Genetics of acetylcholinesterase in *Drosophila melanogaster*. Genetics **83:** 517–535.

HODGETTS, R. B., 1975 The response of dopa decarboxylase activity to variations in gene dosage in Drosophila: a possible location of the structural gene. Genetics **79:** 45–54.

JACKSON, F. R., S. D. WILSON, G. R. STRICHARTZ and L. M. HALL, 1984 Two types of mutants affecting voltage-sensitive sodium channels in *Drosophila melanogaster*. Nature **308:** 189–191.

JAN, L. Y., and Y. N. JAN, 1976 Properties of the larval neuromuscular junction in *Drosophila melanogaster*. J. Physiol. **262:** 189–214.

JAN, Y. N., L. Y. JAN and M. J. DENNIS, 1977 Two mutations of synaptic transmission in *Drosophila*. Proc. R. Soc. Lond. B **198:** 87–108.

JAN, Y. N., and L. Y. JAN, 1979 Genetic dissection of synaptic transmission in *Drosophila melanogaster*, pp. 161–168 in *Insect Neurobiology and Pesticide Actions*, edited by F. E. RICKETT. Society of Chemical Industry, London.

KAMB, A., L. E. IVERSON and M. A. TANOUYE, 1987 Molecular characterization of *Shaker*, a Drosophila gene that encodes a potassium channel. Cell **50:** 405–413.

KAPLAN, W. D., and W. E. TROUT III, 1969 The behavior of four neurological mutants of Drosophila. Genetics **61:** 399–409.

KAUVAR, L. M., 1982 Reduced ^{3}H-tetrodotoxin binding in the *nap*^{ts} paralytic mutant of Drosophila. Mol. Gen. Genet. **187:** 172–173.

LEWIS, E. B., and F. BACHER, 1968 Method of feeding ethyl methane sulfonate (EMS) to Drosophila males. Drosophila Inform. Serv. **43:** 193.

LINDSLEY, D. L., and E. H. GRELL, 1968 *Genetic Variations of Drosophila melanogaster*. Carnegie Inst. Wash. Publ. 627.

LOUGHNEY, K., R. KREBER and B. GANETZKY, 1989 Cloning and sequence analysis of the *para* locus, a sodium channel gene in Drosophila. Cell **58:** 1143–1154.

LOUGHNEY, K., M. STERN, R. KREBER and B. GANETZKY, 1989 Genetic and molecular analysis of a gene encoding sodium channels in Drosophila, 201–215 in *Molecular Biology of Neuroreceptors and Ion Channels*, edited by A. MAELICKE. Springer Verlag, Dortmund.

O'DOWD, D. K., S. E. GERMERAAD and R. A. ALDRICH, 1989 Alterations in the expression and gating of Drosophila sodium channels by mutations in the *para* gene. Neuron **2:** 1301–1311.

OKAMOTO, H., K. SAKAI, S. GOTO, E. TAKASU-ISHIKAWA and Y. HOTTA, 1987 Isolation of Drosophila genomic clones homologous to the eel sodium channel gene. Proc. Jpn. Acad. **63B:** 284–288.

OSHIMA, T., and I. TAKANO, 1980 Mutants showing heterothallism from a homothallic strain of *Saccharomyces cerevisiae*. Genetics **94:** 841–857.

PAPAZIAN, D. M., T. L. SCHWARZ, B. L. TEMPEL, Y. N. JAN and L. Y. JAN, 1987 Cloning of genomic and complementary DNA from *Shaker*, a putative potassium channel gene from Drosophila. Science, **237:** 749–753.

RAMASWAMI, M., and M. A. TANOUYE, 1989 Two sodium-channel genes in Drosophila: implications for channel diversity. Proc. Natl. Acad. Sci. USA **86:** 2079–2082.

SALKOFF, L., 1983 Genetic and voltage-clamp analysis of a Drosophila potassium channel. Cold Spring Harbor Symp. Quant. Biol. **48:** 221–231.

SALKOFF, L., and R. WYMAN, 1981 Genetic modification of potassium channels in Drosophila *Shaker* mutants. Nature **293:** 228–230.

SALKOFF, L., A. BUTLER, A. WEI, N. SCAVARDA, K. GRIFFEN, C. IFUNE, R. GOODMAN and G. MANDEL, 1987 Genomic organization and deduced amino acid sequence of a putative sodium channel gene in Drosophila. Science **237:** 744–749.

SCHMIDT, J. W., S. ROSSIE and W. A. CATTERALL, 1985 A large intracellular pool of inactive Na channel subunit in developing rat brain. Proc. Natl. Acad. Sci. USA **82:** 4847–4851.

SIDDIQI, O., and S. BENZER, 1976 Neurophysiological defects in temperature-sensitive paralytic mutants of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **73:** 3253–3257.

STERN, M., and B. GANETZKY, 1989 Altered synaptic transmission in Drosophila *Hyperkinetic* mutants. J. Neurogenet. **5:** 215–228.

SUZUKI, D. T., T. GRIGLIATTI and R. WILLIAMSON, 1971 Temperature-sensitive mutants in *Drosophila melanogaster*. VII. A mutation (*para*^{ts1}) causing reversible adult paralysis. Proc. Natl. Acad. Sci. USA **68:** 890–893.

TANOUYE, M. A., C. A. KAMB, L. E. IVERSON and L. SALKOFF, 1986 Genetics and molecular biology of ionic channels in Drosophila. Annu. Rev. Neurosci. **9:** 255–276.

TEMPEL, B. L., D. M. PAPAZIAN, T. L. SCHWARZ, Y. N. JAN and L. Y. JAN, 1987 Sequence of a probable potassium channel component encoded at the *Shaker* locus of Drosophila. Science **237:** 770–775.

TIMPE, L. C., and L. Y. JAN, 1987 Gene dosage and complementation analysis of the *Shaker* locus in Drosophila. J. Neurosci. **7:** 1307–1317.

TIMPE, L. C., T. L. SCHWARZ, B. L. TEMPEL, D. M. PAPAZIAN, Y. N. JAN and L. Y. JAN, 1988 Expression of functional potassium channels from *Shaker* cDNA in *Xenopus* oocytes. Nature **331:** 143–145.

WILLIAMSON, R. L., and W. D. KAPLAN, 1973 A duplication that causes leg shaking. Drosophila Inform. Serv. **50:** 134.

WILLIAMSON, R. L., and W. D. KAPLAN, 1976 The developmental focus of the suppression of *Hk*^{2} leg shaking by *para*$^{ts-1}$ in *Drosophila melanogaster*. Genetics **83**(Suppl.): s82-s83.

WU, C.-F., and B. GANETZKY, 1980 Genetic alteration of nerve membrane excitability in temperature-sensitive paralytic mutants of *Drosophila melanogaster*. Nature **286:** 814–816.

WU, C.-F., and B. GANETZKY, 1988 Genetic and pharmacological analyses of potassium channels in Drosophila, pp. 311-323 in *Neurotox '88*, edited by G. G. LUNT. Elsevier, New York.

WU, C.-F., and F. N. HAUGLAND, 1985 Voltage clamp analysis of membrane currents in larval muscle fibers of Drosophila: alterations of potassium currents in *Sh* mutants. J. Neurosci. **5:** 2626–2640.

WU, C.-F., B. GANETZKY, L. Y. JAN, Y. N. JAN and S. BENZER, 1978 A Drosophila mutant with a temperature-sensitive block in nerve conduction. Proc. Natl. Acad. Sci. USA **75:** 4047–4051.

WU, C.-F., B. GANETZKY, F. N. HAUGLAND and A.-X. LIU, 1983 Potassium currents in Drosophila: different components affected by mutations of two genes. Science **220:** 1076–1078.

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