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Differentiation and Central Projections of Peripheral Sensory Cells with Action-Potential Block in *Drosophila* Mosaics

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The ultrastructural differentiation and central projection of identified bristle mechanosensory neurons were examined in *Drosophila* mutants lacking action potentials. Two mutations, $para^{ts1}$ and nap^{ts}, are known to block axonal conduction in centrally located neurons at high temperatures. Their effects on epithelial sensory cells, which are derived from imaginal disks during pupation, have not been determined. Furthermore, the $para^{ts1}$ nap^{ts} double-mutant flies are lethal at all temperatures; thus the synergistic effect of these mutations on neurons has not yet been studied. It is possible to examine the above questions in genetic mosaics. By monitoring a reflex response involving identified bristle sensory cells, we found that the 2 mutations exert similar effects on these epithelial sensory cells as seen in central neurons. This also indicates that the action potential mechanisms in both epithelial sensory cells and central neurons are under similar genetic control.

The $para^{ts1}$ nap^{ts} double-mutant sensory cells in mosaics are nonfunctional at all temperatures, providing an opportunity to examine, at the single cell level, the development of neurons with activity block. Ultrastructural specializations typical of epithelial sensory cells were found in the double-mutant cells. Cobalt backfilling experiments showed that central projections of these nonfunctional sensory cells were not altered, as compared with the active contralateral sensory cells. Therefore, blockage of the action potential mechanism in individual sensory cells has no effect on their pathfinding and arborization.

Sensory axons differentiate and grow from the periphery, forming specific connections in the CNS. The role of nerve activity in the pathfinding and arborization of neurons is not well understood. Connectivity in vertebrate sensory systems has been shown to be maintained and modified by activity (Harris, 1981; Hubel et al., 1977; Sanes and Constantine-Paton, 1983). For example, blockade of Na^+ channel-dependent electrical activity using TTX affects the formation and maintenance of specific sensory connections in retinotectal systems of several vertebrates (Archer et al., 1982; Harris, 1984; Meyer, 1982; Reh and Constantine-Paton, 1985).

Few studies of activity dependence in the development of invertebrate sensory systems have been reported (Palka, 1984). Experience-dependent changes in the CNS of *Drosophila* have recently been detected (Technau, 1984), suggesting that this plasticity may be activity dependent. The experiments described in this paper attempted to examine directly the activity dependence in the formation and maintenance of connectivity within sensory projections in an invertebrate nervous system.

In *Drosophila,* mutations are available that eliminate nerve activity (Hall, 1982). Genetic mosaics can also be produced, in which only a portion of the fly is phenotypically mutant (Hall, 1978; Hotta and Benzer, 1972). Using this mosaic system, it is possible to study at the single cell level the effects of blockade of activity on sensory cell morphology, while surrounding cells remain functionally active. Two mutations, $para^{ts1}$ (paralytic, temperature-sensitive) (Suzuki et al., 1971) and nap^{ts} (no-action-potential, temperature-sensitive) (Wu et al., 1978), are separately located on the first and second chromosomes, respectively. They both result in a temperature-sensitive paralytic phenotype, causing action potential failure in axons at a high temperature (above 29°C for $para^{ts1}$ and 34°C for nap^{ts}). These mutations have been suggested to affect the voltage-sensitive Na^+ channel, on the basis of physiological (Siddiqi and Benzer, 1976; Wu and Ganetzky, 1980; Wu et al., 1978), pharmacological (Suzuki and Wu, 1984; Wu et al., 1983), and toxin binding (Jackson et al., 1984; Kauvar, 1982) studies.

The above results concerning the $para^{ts1}$ and nap^{ts} mutations were obtained using centrally located neurons. It is not known whether these 2 mutations also affect epithelial sensory cells, which represent a developmentally distinct set of excitable cells that differentiate in imaginal disks during pupation. The combined effects of $para^{ts1}$ with nap^{ts} on neurons have not been studied, because they are known to interact synergistically, resulting in lethality of the organism even at 17°C (Ganetzky, 1984; Wu and Ganetzky, 1980).

In this paper, we examine these questions by utilizing a stereotyped reflex behavior of decapitated flies in which stimulation of an identified sensory cell elicits a scratching response from a specific leg (Vandervorst and Ghysen, 1980). We have created small-patch mosaics containing a single mutant mechanosensory cell, identifiable by the associated mutant bristle, which are both derived from a common progenitor cell (Lawrence, 1966). Thus, the function of the mutant sensory cell can be tested by comparing reflex responses from the mutant bristle with responses from normal bristles, which evoke identical responses within each fly. Using this system, we have determined the effects of $para^{ts1}$ and nap^{ts} on the function of these cells. In addition, this mosaic system provides a unique opportunity to characterize and examine the differentiation, pathfinding, and projection formation of inactive $para^{ts1}$ nap^{ts} double-mutant sensory cells, which is impossible to do in a nonviable double-mutant fly. Preliminary accounts of this work have appeared previously (Burg and Wu, 1984, 1985).

Materials and Methods

Generation of mosaics

Genetic mosaics were found in female flies in which 1 of the 2 X-chromosomes was an unstable ring-X chromosome, $In(1)w^{vC}$ (Hinton, 1955).

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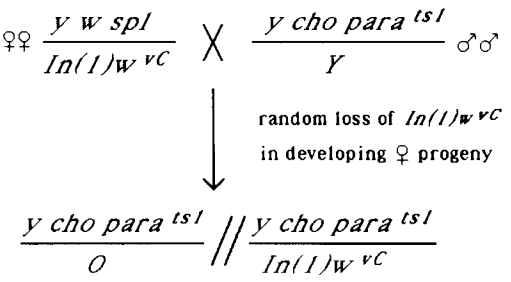

Figure 1. Construction of *para*^{ts1} mosaics. Female flies containing the ring-X chromosome (*In(1)w*^{vC}) were mated with *para*^{ts1} males. Genetic markers on the other X chromosomes used in the maintenance of the ring-X strain were yellow body (*y*), white facets (*w*), and split bristles (*spl*). Loss of the ring-X chromosome in female progeny produces patches of hemizygous tissue (*y cho para*^{ts1}*/O*, detectable by recognizing *y* and *cho* on the cuticle) surrounded by heterozygous tissue (*y cho para*^{ts1}*/ In(1)w*^{vC}). Progeny of other genotypic constructions are not shown.

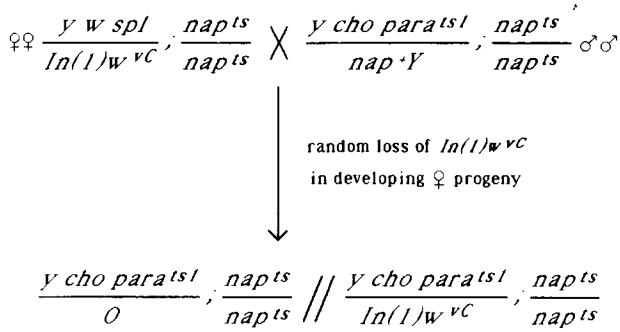

Figure 2. Construction of *para*^{ts1} *nap*^{ts} mosaics. Female flies homozygous for *nap*^{ts} and containing the ring-X chromosome (*In(1)w*^{vC}) were mated to males that carry both *para*^{ts1} on the X chromosome and *nap*^{ts} on the second chromosome. The Y chromosome (*nap*$^{+}$*Y*), an insertional translocation (T(Y;2)L12), carries one normal copy of the *nap* locus, *nap*$^{+}$, which is necessary to overcome the lethal effect of the *para*^{ts1} *nap*^{ts} double-mutant combination (Ganetzky, 1984). Loss of the ring-X chromosome in female progeny produces patches of double-mutant tissue (*y cho para*^{ts1}*/O*; *nap*^{ts}*/nap*^{ts}), surrounded by phenotypically *nap*^{ts} tissue (*y cho para*^{ts1}*/In(1)w*^{vC}; *nap*^{ts}*/nap*^{ts}).

When this ring-X chromosome is lost during development, mosaic flies are produced that contain patches of hemizygous (X/O) male tissue surrounded by normal female tissue. When the remaining X-chromosome carries recessive mutations, they will be expressed in the hemizygous patches of tissue in the phenotypically normal heterozygous tissue. Mutant patches in mosaics can be of varying size: The later during development that the ring-X chromosome is lost, the smaller the mutant patch will be (Garcia-Bellido and Ripoll, 1978; Garcia-Bellido et al., 1979; Zalokar et al., 1980). In these experiments, the X-linked recessive mutations *y* (yellow, 1-0.0) and *cho* (chocolate, 1-13.0) were used (Lindsley and Grell, 1968). These mutations cause yellow body color and chocolate facets, so that the entire cuticle of the fly could be examined and patches of mutant cuticle could be recognized.

Since *para*^{ts1} is X-linked, mosaics containing patches of *para*^{ts1} mutant tissue were produced by recombining *y* and *cho* to *para*^{ts1}. This method of producing mosaic flies containing *y cho para*^{ts1} cuticle is shown in Figure 1. In this particular cross, females were produced containing *In(1)w*^{vC} (ring-X) and *y cho para*^{ts1} chromosomes. In 21% of these 1176 females (*y cho para*^{ts1}*/In(1)w*^{vC}), a loss of the ring-X chromosome occurred that produced visible cuticle mosaicism. In at least 25% of these mosaics, the *para*^{ts1} patches were small, containing only 1–2 bristles (macrochaetae), which result from a late loss of the ring-X chromosome in the imaginal disc.

Since *nap*^{ts} is located on the second chromosome, *nap*^{ts} mutant patches could not readily be produced using the ring-X system. Instead, *para*^{ts1} *nap*^{ts} double-mutant patches in mosaics were examined. As seen in Figure 2, this was done by producing *para*^{ts1} mutant patches in flies that were homozygous for *nap*^{ts}. The resulting tissue in the mutant patch is doubly mutant (*para*^{ts1}*/O*; *nap*^{ts}*/nap*^{ts}), while the rest of the fly would be phenotypically *nap*^{ts} but not *para*^{ts1} (*para*^{ts1}*/In(1)w*^{vC}; *nap*^{ts}*/nap*^{ts}). In this manner, lethality in double-mutant flies can be circumvented by producing small-sized patches of double-mutant tissue in mosaic flies. In this study, cuticle mosaicism occurred in 9% of 2600 flies, with 67% of these mosaics having small patches (see above) of double-mutant tissue containing 1–2 bristles. All flies in this study were raised at 22°C.

Bristle reflex

Mosaic flies that contained a small mutant patch on the dorsal mesothorax were used for the bristle reflex test. These flies behaved normally even at restrictive temperatures (above 29°C for *para*^{ts1} and any temperature for *para*^{ts1} *nap*^{ts}), confirming that the mutant patch did not include a significant amount of internal nerve tissue. They were anesthetized with ether and decapitated, using petroleum jelly to seal the wound. Two hours of recovery time were allowed in a moist environment. A decapitated fly stands still (Fig. 3*A*) and exhibits a stereotypic leg reflex behavior (Vandervorst and Ghysen, 1980). Reflex responses were evoked by moving the bristle back and forth several times with an eyelash, triggering the appropriate leg to scratch the bristle touched. Such flies have routinely responded for up to 3 days, but all tests in this report were conducted within 24 hr of decapitation. The bristles tested were those that normally elicit positive responses with a frequency greater than 70% upon single stimulation (Vandervorst and Ghysen, 1980). Therefore, multiple stimulations will always produce clear-cut results to distinguish between responsive and nonresponsive bristles. In addition to the mutant bristle, surrounding phenotypically normal bristles that would elicit identical responses from the same leg were tested (Fig. 4). Tests on these and other phenotypically normal bristles served as a control to eliminate internal mosaicism that involved the remaining portion of the CNS.

Reflex tests of *para*^{ts1} mosaics were performed on a Peltier stage (Cole-Palmer, model 3832, or Cambion, model 809-3010-01), so that accurate control and rapid changes of temperature could be performed to determine the effects on the reflex behavior at both the permissive (23°C) and restrictive (30°C) temperatures. Reflex tests done with *para*^{ts1} *nap*^{ts} mosaic flies were performed at 17 and 25°C, because the control patches (*para*^{ts1}*/In(1)w*^{vC}; *nap*^{ts}*/nap*^{ts}) do not elicit reliable responses above 25°C.

Electron-microscopic analysis of identified bristle sensory cells

Small mutant patches of homozygous *para*^{ts1} *nap*^{ts} tissue in day old mosaic flies were used for electron-microscopic analysis. The mosaic flies were dissected and fixed in Karnovsky's fixative and postfixed in 1% OsO_4 (in 0.1 M sodium cacodylate buffer). The specimens were then stained *en bloc* using 2% uranyl acetate and embedded in Spurr's resin (Polysciences, Inc.). Serial thin sections were stained with lead citrate and later observed using a Zeiss EM10A EM.

Cobalt backfilling of sensory cells

Mosaic flies containing *para*^{ts1} *nap*^{ts} double-mutant sensory cells were used to investigate the effects of activity blockade on the central projection of the sensory cell. Several considerations make using double-mutant sensory cells more suitable than performing temperature shifts on *para*^{ts1} or *nap*^{ts} mosaics to block action potentials. First, the *para*^{ts1} mutants are known to partially regain movement after prolonged exposure to high temperature, i.e., 30°C (Grigliatti et al., 1972; Suzuki et al., 1971). Thus, a simple temperature shift regiment will not block activity during the entire period of development. Second, the restrictive temperature for *nap*^{ts} is 34°C, and prolonged exposure to this temperature during development is not desirable because it is close to temperatures that induce a heat-shock response. However, the *para*^{ts1} *nap*^{ts} double-mutant sensory cells are nonfunctional even at low temperatures, as shown in Table 1B.

Single mechanosensory cell projections in the thoracic ganglia were visualized by using a modified cobalt backfilling technique. One- to five-day-old small-patch mosaics, containing only one double-mutant bristle, were used for all backfill experiments. A small drop of a 2% $CoCl_2$ solution containing 1.5 μg/ml BSA (Sigma) was placed on the cuticle where a single macrochaeta bristle was pulled or cut. The fly was then placed in a humid chamber for 30–45 min. After incubation, the ganglia and brain were dissected from the fly and immersed in a diluted solution (1:40) of ammonium sulfide for 5 min. Following a saline rinse,

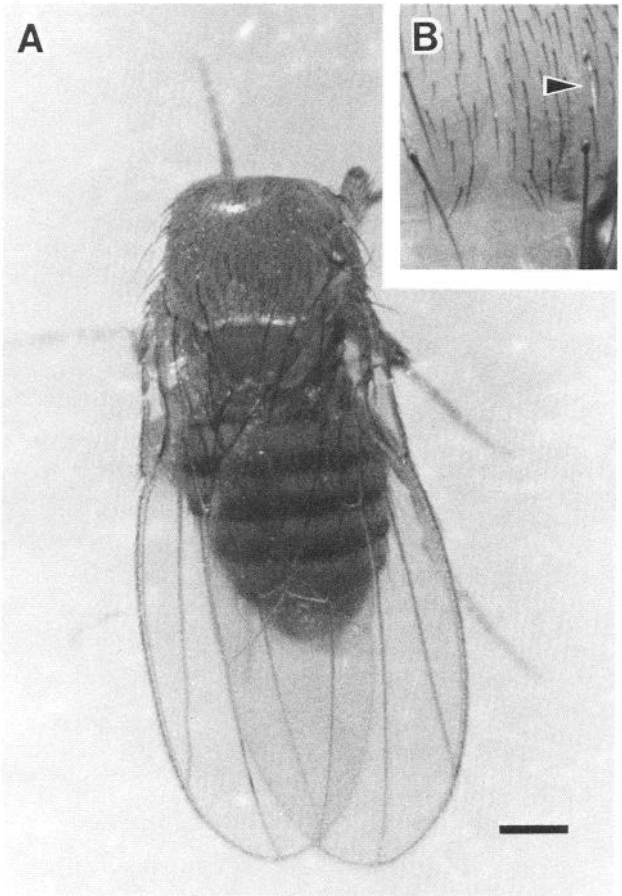

Figure 3. Photomicrograph of a decapitated small-patch mosaic used in the bristle reflex experiment. *A,* Decapitated flies stand upright and are motionless (exposure time, 5 sec). *B,* This particular mosaic fly contains only a single yellow bristle (*arrow*). Bar, 300 μm (*A*), 110 μm (*B*).

specimens were fixed for 1–3 hr using Carnoy's fixative. Afterward, a silver-intensification procedure (Bacon and Altman, 1977) was used to visualize the sensory cell's projection. Specimens were observed as a whole-mount in immersion oil using a compound microscope (Zeiss Photomicroscope I). Backfill of a single sensory cell was indicated by the staining of a single axon in the nerve root, prior to the axon's initial branch point. Photographs in serial planes of focus were taken to obtain detailed morphology of the sensory cell's projection. Camera lucida drawings were made for each stained sensory cell, so that the entire projection could be analyzed.

Results

Blockade of sensory cell function by the para^{ts1} *mutation*

Previous studies of *para^{ts1}* have been performed on neurons derived from centrally located neuroblasts. Small-patch mosaic flies, as shown in Figure 3*B*, were generated in order to test the effect that *para^{ts1}* may have on the function of bristle sensory cells, which arise from imaginal disks during pupation and share a common progenitor cell with bristle-forming cells. These small-patch *para^{ts1}* mosaics were tested for their reflex behavior at low (23°C) and high (30°C) temperatures to determine whether temperature-sensitive blockade of the reflex behavior occurred. Bristles on the dorsal thorax are identifiable and, within certain regions of the mesothorax and humerus, will elicit reflex responses of prothoracic and metathoracic legs, as shown in Figure 4 (also, see Vandervorst and Ghysen, 1980). The phenotypically normal (heterozygous) bristles surrounding the mutant patch

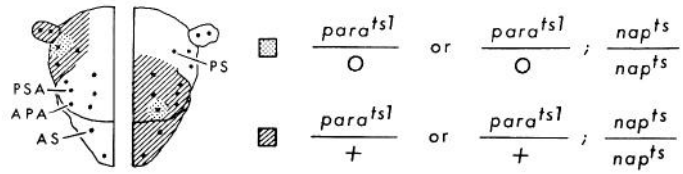

Figure 4. Macrochaeta bristles of the dorsal thorax that elicit reflex responses from specific legs. Shown is a map of the dorsal thorax, with macrochaeta bristles represented by dots. Territories for pro- and metathoracic leg responses are shown on separate halves for clarity. Macrochaetae within the shaded region on the left half, including the humerus and an anterior portion of the notum, elicit responses from the left prothoracic leg. Macrochaetae within the shaded region on the right half, including posterior portions of the notum and the entire scutellum, elicit responses from the right metathoracic leg. Shown for each region described above is an example of a small mosaic patch, identifiable by color, including a single mutant bristle (*dotted region*). In the experiment, when the yellow mutant bristle fails to elicit a reflex response but the surrounding black normal bristles do elicit a response, this indicates that the sensory cell underlying the yellow bristle is mutant, whereas the remaining portions of the reflex circuit are functional. Shown are the locations of the *PS* (presutural), *PSA* (posterior supra-alar), *APA* (anterior post-alar), and *AS* (anterior scutellar) bristles of the dorsal thorax, which were the bristles most frequently studied.

were also tested for the ability to elicit an identical reflex response of the same leg at both temperatures (23° and 30°C). This control was performed to ensure that no other portion of the reflex circuit contained *para^{ts1}* tissue in the mosaics tested.

The exact circuit underlying the reflex behavior in *Drosophila* has not been established. A small group of motorneurons innervating the leg (Ikeda and Kaplan, 1970) is excited by the sensory cells directly or by interneurons indirectly.

In all the small-patch mosaics containing *para^{ts1}* tissue, only the mutant sensory cells failed to elicit a response at the high temperature (Table 1A), while at the low temperature, responses were elicited. In contrast, surrounding normal bristles elicited responses at both temperatures. This result conclusively shows that the bristle mechanosensory cells are affected by *para^{ts1}* much like centrally located neurons are (Siddiqi and Benzer, 1976; Wu and Ganetzky, 1980). This also suggests that the action potential mechanisms used by these 2 sets of cells are under similar genetic control.

Activity blockade in para^{ts1} nap^{ts} *bristle sensory cells*

Since *para^{ts1} nap^{ts}* double-mutant flies are lethal, the combined effects of these mutations on neuronal function have not been studied. To examine these effects, we produced *para^{ts1}* mosaic patches in *nap^{ts}* homozygous flies (see Materials and Methods and Fig. 2). In such mosaics the patch size of doubly mutant tissue was small (Fig. 3*B*), and only occasionally were large patches produced, consistent with a previous report (Ganetzky, 1984). The mosaic flies that contained large patches of *para^{ts1} nap^{ts}* cuticle mostly had reduced movement even at low (17°C) temperatures, presumably because of internal mosaicism involving the CNS.

Results from the reflex behavior experiment performed on mosaics containing small patches of *para^{ts1} nap^{ts}* doubly mutant tissue are shown in Table 1B. At 25°C, which is below the restrictive temperature for either *para^{ts1}* or *nap^{ts}*, no reflex responses were elicited from double-mutant bristles in mosaics ($n = 36$). In comparison, the surrounding bristles that were heterozygous for *para^{ts1}* but homozygous for *nap^{ts}* (see Fig. 4) did elicit homologous responses in 30 of the mosaics tested. Identical results were obtained when the experiment was performed at 17°C, demonstrating that the blockade of sensory cell function by *para^{ts1} nap^{ts}* is not temperature dependent (Table 1B).

Among 36 small-patch mosaics available for testing, 6 did

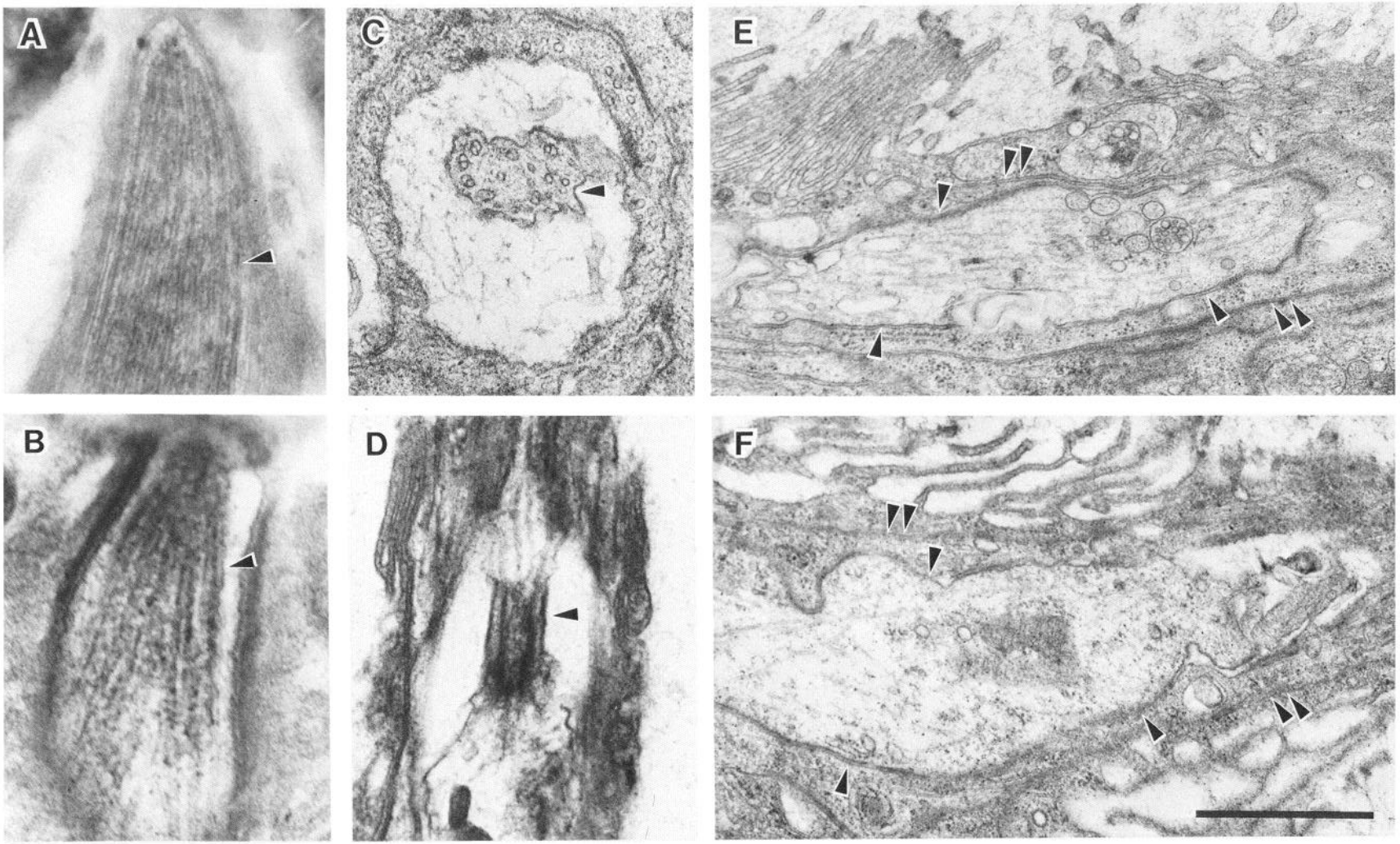

Figure 5. Electron micrographs from functional (*para*^{ts1}/+; *nap*^{ts} in *A, C,* and *E*) and nonfunctional (*para*^{ts1}/*O*; *nap*^{ts} in *B, D,* and *F*) mechanosensory cells of dorsal mesothoracic macrochaetae. *A* and *B,* Apical dendritic attachment of the sensory cell to the bristle shaft, with bundles of microtubules (*arrows*) present in both. *C* (cross section) and *D* (longitudinal section), Ciliary body-like structure common in the epithelial sensory cells (*arrows*). *E* and *F,* Portions of the apical dendrite that are more proximal to the cell body than in *A–D.* Sensory cells (*single arrows*) are surrounded by neurilemma cells, whose outer border is also indicated (*double arrows*). These micrographs indicate that the nonfunctional sensory cells contain typical ultrastructural components of normal sensory cells. Bar: 0.6 μm (*A*), 0.5 μm (*B, C*), 0.7 μm (*D*), 1.0 μm (*E, F*).

Table 1. Bristle reflex of decapitated small-patch mosaic flies

Bristle genotype	Temperature tested (°C)	No. flies tested: Responsive bristles	Nonresponsive bristles	Total
A				
$para^{ts1}/O$	23	50	0	50
	32	0	50	50
$para^{ts1}/+$	23	50	0	50
	32	50	0	50
B				
$para^{ts1}/O;nap^{ts}$	17	0	36	36
	25	0	36	36
$para^{ts1}/+;nap^{ts}$	17	30	6*	36
	25	30	6*	36

Results of bristle reflex behavioral experiments with small-patch mosaics containing 1–2 $para^{ts1}$ (A) or $para^{ts1}$ nap^{ts} (B) bristles. Shown in different rows are the total number of mosaic patches in different flies and the genotypes of mutant ($para^{ts1}/O$ for A and $para^{ts1}/O$; nap^{ts} for B, where nap^{ts} is abbreviated for homozygous nap^{ts}/nap^{ts}) and surrounding phenotypically normal ($para^{ts1}/+$ for A; $para^{ts1}/+$; nap^{ts} for B) sensory cells. Data indicate whether the mutant or surrounding sensory cells elicit responses at the temperatures tested.

Of the 6 $para^{ts1}/+$; nap^{ts} flies indicated by the asterisks, no responses were elicited from any bristle on the fly; see explanation in text. Results show that the sensory cells are affected by $para^{ts1}$ in a temperature-dependent fashion (A) and that $para^{ts1}$ nap^{ts} double-mutant sensory cells are inactive, regardless of the temperature tested (B). Responses elicited by the surrounding sensory cells indicate that the mutant patch involves only the sensory cell, but not the remaining components of the reflex circuit.

not elicit responses from the surrounding bristles (see asterisks in Table 1B). Furthermore, no responses were elicited from any bristle on these 6 flies. The reasons for this result are unknown, although 2 possibilities exist. One is that an internal mosaic patch of $para^{ts1}$ nap^{ts} tissue could be present that prevents the fly from reacting to any stimulation given. A second possibility is that these mosaic flies may not recover as readily as wild-type flies from the decapitation procedure, due to the dominant interaction of $para^{ts1}$ with nap^{ts} (Ganetzky, 1984). Flies heterozygous for $para^{ts1}$ and homozygous for nap^{ts} ($para^{ts1}/In(1)w^{vC}$; nap^{ts}/nap^{ts}) have a reduced viability and become paralyzed at a lower temperature (26°C) than either $para^{ts1}$ (29°C) or nap^{ts} (34°C).

In summary, the combined effect of $para^{ts1}$ nap^{ts} can be conclusively demonstrated in these sensory cells, at all temperatures tested. It is of interest to ask whether the failure of $para^{ts1}$ nap^{ts} sensory cells to initiate a reflex response is due to the prevention of sensory cell differentiation by this genetic interaction.

Differentiation of $para^{ts1}$ nap^{ts} *sensory cells*

To investigate whether any ultrastructural components of the cell were absent due to this double-mutant genotype, EM serial section analysis was performed on 5 phenotypically normal ($para^{ts1}/In(1)w^{vC}$; nap^{ts}/nap^{ts}) sensory cells and 4 double-mutant ($para^{ts1}/O$; nap^{ts}/nap^{ts}) sensory cells from small-patch mosaics.

Figure 5, *A* and *B*, compares the apical portion of the sensory cell in the bristle apparatus from both a normal and a double-mutant bristle apparatus, respectively. The attachment of the apical portion of the sensory cell to the bristle shaft is similar, containing bundles of microtubules in both cases. Minor differences may be attributed to the plane and thickness of sectioning. In all 4 double-mutant sensory cells examined, this region of attachment was similar to that of the control cell.

The modified ciliary body structure within this sensory cell is shown in cross section and longitudinal section in Figure 5, *C* (control) and *D* (double-mutant), showing microtubules within a modified ciliary body located in both sensory cells. This is typical of the arrangement in the apical dendritic portion of the sensory cell (Reed et al., 1975; Thurm, 1968). Figure 5, *E* and *F*, shows that the proximal dendritic region of control and double-mutant sensory cells have similar ultrastructural components. The neurilemma cell can also be seen surrounding the sensory cell in both figures.

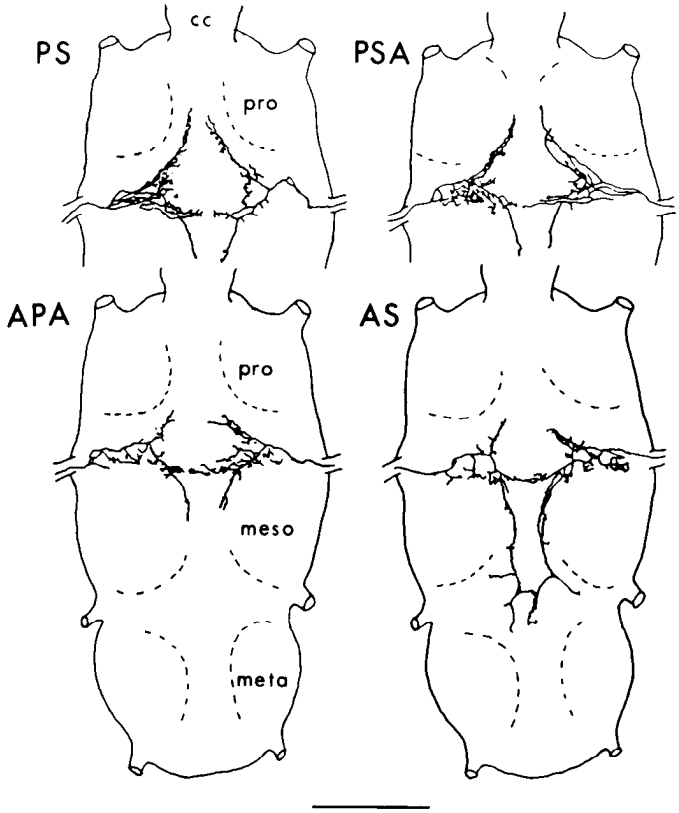

Figure 6. Maps of identified bristle sensory cell central projections revealed by cobalt backfilling. This and the following figures present dorsal views of paired projections in the thoracic ganglia of the sensory cells identified in Figure 4. Paired backfills (a double mutant sensory cell and its contralateral control cell) for the PS, PSA, APA, and AS sensory cells are traced from 4 individual animals. These projections reside on the ventral surface of the ganglia, terminating between the neuromeres. Individual neuromeres, demarcated by *dashed lines*, can be observed and serve as landmarks. The point of entry for each axon is within the posterior dorsal mesothoracic nerve root. Other cut ventral nerve roots for each individual neuromere are shown for clarity. Cervical connective (*cc*) and prothoracic (*pro*), mesothoracic (*meso*), and metathoracic (*meta*) neuromeres are marked. Bar, 100 μm.

Two normal bristle sensory cells in wild-type animals have also been examined and yielded similar results. In micrographs of all sensory cells analyzed, there were no distinct morphological differences between the control and double-mutant sensory cells, even though the double-mutant cells apparently fail to propagate electrical activity. Thus, the compound effect of $para^{ts1}$ and nap^{ts} does not prevent the ultrastructural differentiation of organelles typical of mechanoreceptor cells, although the functioning of these double-mutant organelles requires further physiological examination.

Central projection of $para^{ts1}$ nap^{ts} *sensory cells*

By using mosaic flies containing small patches of $para^{ts1}$ nap^{ts} double-mutant tissue, it was possible to determine, at the single cell level, the effects of the absence of action potential activity

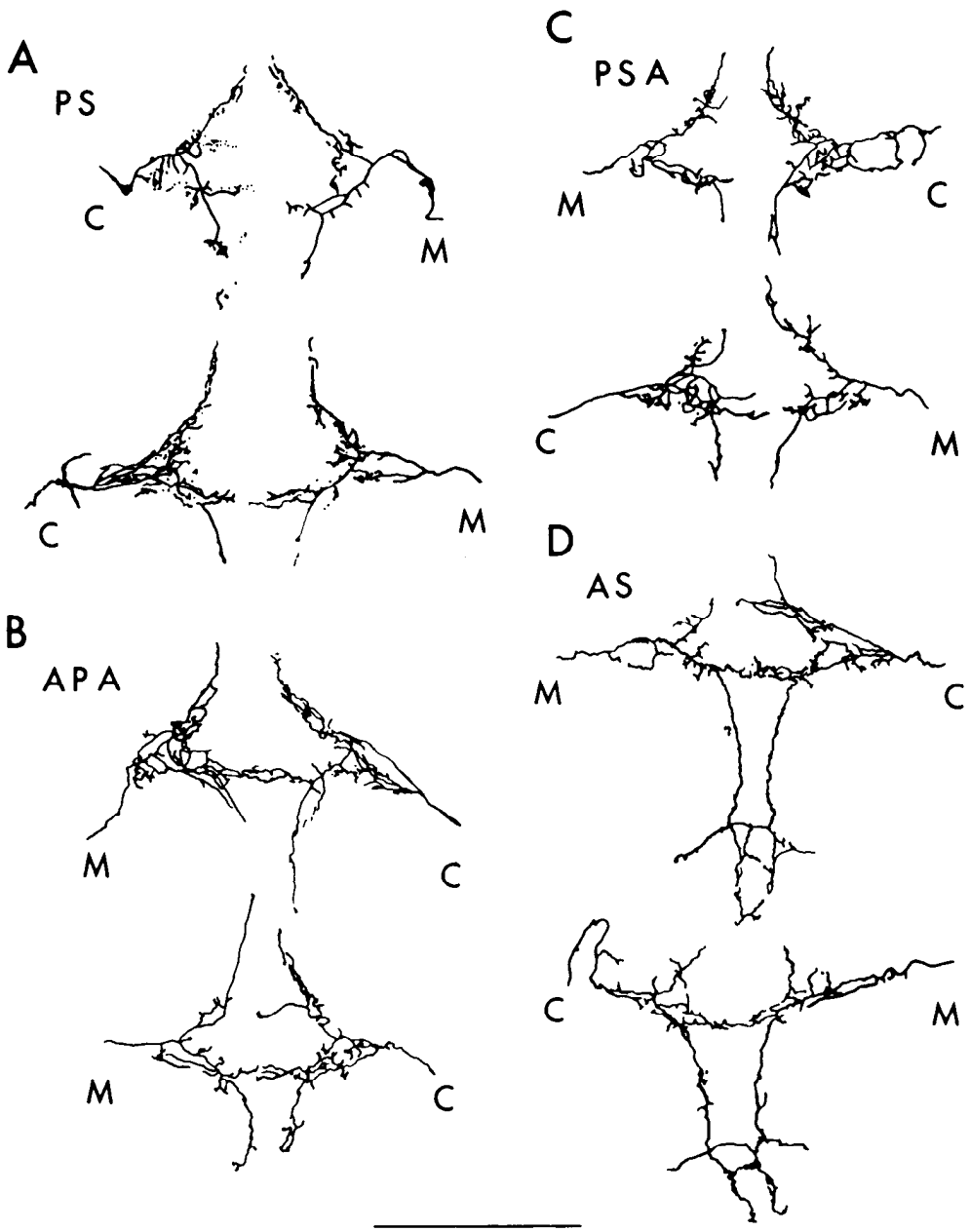

Figure 7. Detailed camera lucida tracings of projections from $para^{ts1}$ nap^{ts} nonfunctional and contralateral control bristle sensory cells in small-patch mosaic flies. Two representative pairs are presented from backfills of the 4 bristles identified in Figure 4, each pair consisting of a double-mutant (*M*) and a contralateral control (*C*) sensory cell projection. *A*, Projections from PS sensory cells; *B*, APA cells; *C*, PSA cells; *D*, AS cells. Note posterior extension of the AS cell's projection in *D*. Bar, 100 μm.

on the final projection and central arborization of these sensory cells. Although the reflex response elicited for each bristle is reproducible from fly to fly (Vandervorst and Ghysen, 1980), the dendritic branching of these cells may be variable in their details. Therefore, for each double-mutant sensory cell that was backfilled, the contralateral phenotypically normal cell was also backfilled to serve as a control.

Figure 4 maps the location of identified bristles whose sensory cell projections (shown in Fig. 6) have been more extensively studied. The sensory cell axon enters the thoracic ganglia via a branch of the posterior dorsal mesothoracic nerve root. This nerve root contains axons from other sensory structures of the mesothoracic segment, such as the wing (Ghysen, 1980; Ghysen and Janson, 1979; Palka and Schubiger, 1979). Upon entering the thoracic ganglia, the axons begin to branch, forming characteristic arborizations for each identified sensory cell. These axons traverse between the pro- and mesothoracic neuromeres and then project anteriorly and posteriorly, close to the ventral surface of the thoracic ganglia (Fig. 6; see also Ghysen, 1978). For the PSA (posterior supra-alar) and APA (anterior post-alar) sensory cells (see Fig. 4), there are dorsally located projections as well, which appear to be more variable in pattern and are stained less frequently than ventrally located projections.

Mosaics produced by ring-X loss contain male tissue within a female fly. As a control for sexual dimorphism of these projections, bilateral cobalt backfilling was also performed on 3 different types of mosaics, in which both the sensory cell in the small male hemizygous patch and the surrounding female sensory cells remained active. The mosaics constructed for this purpose contained hemizygous patches of the following genotypes: (1) $para^{ts1}/O$; $nap^{ts}/+$, (2) $+/O$; $nap^{ts}/+$, or (3) $+/O$. These patches were surrounded by female tissue without ring-X loss (i.e., *O* replaced by $In(1)w^{mC}$). We found no indication of sexual dimorphism between the projections of male and female mechanosensory cells of the dorsal thorax (data not shown).

These experiments also served as a control for the effects that varying the $para^{ts1}$ and nap^{ts} genetic backgrounds could have on sensory cell projection. We have compared the detailed tracings

of AS sensory cell projections having these different genotypes. No distinguishable differences could be found among the projections, demonstrating that our observations of the consequences of activity blockade were not complicated by other unknown factors associated with a specific genetic background.

Figure 7 contains detailed camera lucida drawings of representative paired projections, assembled from different mosaics, of the 4 identified cells presented in Figure 6. There appear to be greater degrees of variability in the arborization of the identified sensory cells between mosaics than within each mosaic backfilled (compare to Figs. 6 and 8). Nevertheless, as shown in Figure 7, the projections of the double-mutant (M) and the control (C) sensory cells have similar major branching patterns. These results are based on successful backfilling of 23 APA (10 M and 13 C), 23 PSA (7 M and 16 C), 5 PS (3 M and 2 C), and 10 AS (3 M and 7 C) sensory cells (for location of cells, see Fig. 4).

Mosaics in which both the double-mutant and contralateral control sensory cells were successfully backfilled have been difficult to obtain (28 out of 113 attempts). Two such successful backfills are shown in Figure 8. Despite a relatively small sample size, the bilateral backfills even more convincingly demonstrate the similarity between the projections of functional and nonfunctional sensory cells.

The variability in this backfill technique may prevent detection of differences in minor details. However, at the present level of resolution, no differences were detected between the central projections of the nonfunctional *para^{ts1} nap^{ts}* sensory cell and the functional sensory cell.

Discussion

Effects of para^{ts1} *and* nap^{ts} *on sensory cell function and differentiation*

The results show that both *para^{ts1}* and *nap^{ts}* mutations affect the function of sensory cells within the mechanosensory apparatus. Signal conduction in these cells is reversibly blocked by *para^{ts1}* and is absent at all temperatures tested in *para^{ts1} nap^{ts}* double-mutant cells, as in central neurons. This suggests that the genetic mechanism responsible for the generation of action potentials is similar in both epithelial mechanosensory cells and central neurons.

Previous studies in nematodes provide examples where some mutations that block function of mechanosensory cells did not prevent normal ultrastructural differentiation (Chalfie and Sulston, 1981). Using genetic mosaics to circumvent the lethality resulting from the *para^{ts1} nap^{ts}* interaction, it was possible to demonstrate that the absence of excitability does not prevent sensory cell differentiation in *Drosophila* as well.

It should be pointed out that simple elimination of action potentials may not be the only mechanism that can account for the observed functional blockade in the double-mutant sensory cells. Variations from this simple condition must be considered and await further investigation. First, defects in synapse formation cannot be ruled out by the cobalt backfilling technique. Although synaptic function in *nap^{ts}* and *para^{ts1}* single mutants has been shown to be physiologically normal (Wu and Ganetzky, 1980; Wu et al., 1978), no information regarding the double-mutant synaptic function is yet available. Second, these mutations may not affect all cell types uniformly. Subthreshold or local electrical activity could still be present in these *para^{ts1} nap^{ts}* sensory cells. Recently, Nelson and Baird (1985) have indirectly shown that in neither *nap^{ts}* nor *para^{ts1}* single mutants is action potential propagation in the cervical giant fiber completely blocked. Presumably, these single-gene mutations reduce but do not completely eliminate the Na^+ current (Jackson et al., 1984; Suzuki and Wu, 1984). The giant fiber, with its larger diameter and hence greater space constant, would have an increased safety margin. Furthermore, intracellular recordings have demonstrated that Ca^{2+} current contributes to the generation of action potentials in the giant fiber (Tanouye et al., 1981). These factors would make the giant fiber less affected than other axons by the *para^{ts1}* or *nap^{ts}* mutations (Ganetzky and Wu, 1985;

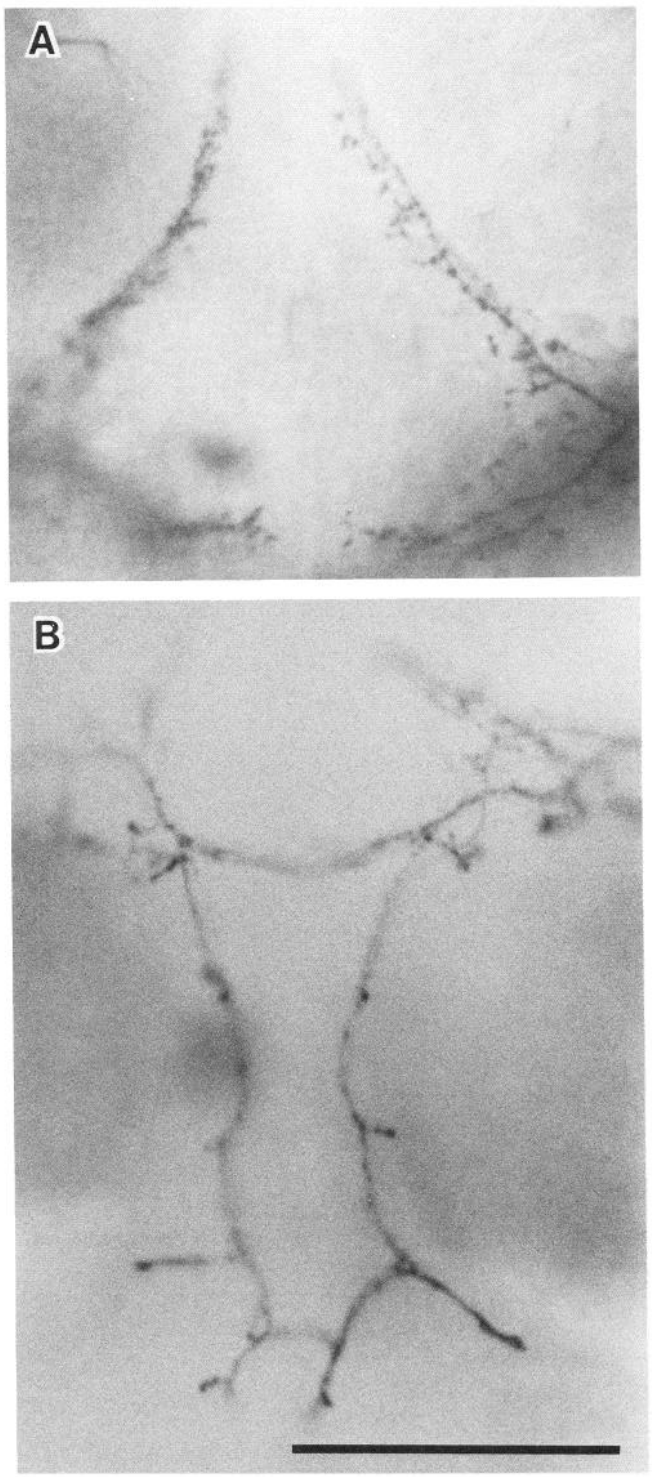

Figure 8. Photomicrographs of 2 paired backfills of *para^{ts1} nap^{ts}* double-mutant and contralateral control bristle sensory cells in small-patch mosaic flies. *A*, PS sensory cell pair, mutant projection on right. *B*, AS sensory cell pair, mutant projection on left. Portions of these projections are not in focus. Figure 6 contains the complete projections of these same 2 preparations. Note the symmetry in the paired projections. Bar, 50 μm.

Siddiqi and Benzer, 1976; Wu et al., 1978). No physiological recordings from the $para^{ts1}$ nap^{ts} double-mutants have been obtained because of the lethal interaction described above. Last, the effects of mutations may depend on developmental stages. Developing sensory cells during pupation may employ a different action potential mechanism, although this is unlikely since developing larval or prepupal neurons in culture have been shown to be affected by $para^{ts1}$ and nap^{ts} (Suzuki and Wu, 1984).

Activity dependence of sensory cell projection

The mosaic system offers certain advantages in studies of the activity dependence of the central projection formation and maintenance of sensory cells. Unlike other experimental systems, the development of a single identified inexcitable cell can be studied in a phenotypically normal environment (for a discussion of mosaics, see Palka, 1979). In previous studies on the dependence of the generation and maintenance of connectivity on activity, TTX was applied to an entire population of cells to eliminate action potentials (Harris, 1984; Meyer, 1982; Reh and Constantine-Paton, 1985). It is difficult to selectively affect a small group of cells in this manner.

Our results indicate that lack of action potentials does not alter the sensory cell central projection. This finding is in contrast to observations on vertebrate sensory systems, in which terminal branching critically depends on activity. For example, in the retinotectal system of the frog, application of TTX increases the branching of the retinal ganglion cell projection in the tectal lamina (Reh and Constantine-Paton, 1985). Meyer (1982) and Harris (1984) have shown that TTX alters the retinotectal projections in other vertebrates. Therefore, the fixed wiring of insect sensory projections may employ cues other than the modulation of nerve activity.

There have been previous demonstrations of the effects of sensory deprivation on projections of sensory cells in invertebrates. For example, unilateral cercal removal in the cricket causes a redistribution of the bilateral central arborization of the remaining cercal sensory cell. The distribution of terminal branching of the remaining cell is shifted towards the sensory-deprived side (Murphy and Lemere, 1984). In our study, without physically removing the sensory cell, differences in branching patterns between functional and nonfunctional sensory cells have not been detected. Therefore, the observed effects of sensory deprivation in the cricket may not be attributable to an absence of activity but rather the physical absence of the contralateral sensory cell.

It should be noted that our results do not necessarily rule out a possible role of nerve activity in the maintenance of arbors as well as in the process of directed growth and final projection of these sensory cells. Different combinations of multiple parallel mechanisms could provide the essentials for normal development. Sensory cells may use other chemical or physical cues in the environment. For example, chemical signals from developing or degenerating cells, electrical fields set up by other neurons (Patel and Poo, 1984), or the mere physical presence of neighboring cells (Silver and Sidman, 1980) could provide important extrinsic influences.

This study can be extended to investigate the effect of other mutations in *Drosophila* that alter specific cellular properties (Hall, 1982) important to the development of the nervous system. For example, it will be possible to investigate the effect of blocking different ionic conductances during development. Using the mosaic system, these types of problems can be investigated at a single cell level in a normally developing system *in vivo*.

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