Single Neuron Mosaics of the *Drosophila gigas* Mutant Project beyond Normal Targets and Modify Behavior

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gigas is a lethal mutant that differentiates enlarged cells, including the nucleus. This trait manifests only after the completion of the mitotic program. We have taken advantage of this phenotype to test *in vivo* the capacity of normal target cells to arrest the growth of mutant sensory axons. Single neuron connectivity changes have been analyzed in mosaics after horseradish peroxidase retrograde tracings. A mutant mechanoreceptor neuron, growing over a genetically normal substrate, contacts its normal target, and in addition projects to novel areas of the CNS. The mutant axon does terminate its growth eventually, and the new additional targets that are reached correspond to mechanoreceptor domains in other ganglia, indicating that this territorial constraint is operational in the mutant. *gigas* neurons maintain their stereotyped profile and represent an expanded version of the normal branching pattern. The ultrastructure of

the invading projections does not reveal gliotic or necrotic reactions from the new cell contacts. The functional consequences of the connectivity changes produced by the mutant mechanoreceptors have been studied in grooming behavior. Mosaic flies carrying a single *gigas* mechanoreceptor show modified, albeit context-coherent, grooming responses after stimulation of the mutant bristle, whereas the response from neighboring normal sensory neurons remains unchanged. All of these experiments indicate that target recognition and growth arrest are two dissectible processes of neural development, and they highlight the autonomous features of the growth cone during pathfinding.

Key words: mechanoreceptor; target recognition; growth cone; neural branching; pathfinding; grooming reflex

Work in different organisms indicates that neural pathfinding is mediated by the qualitative and quantitative expression of a combination of molecules, such as fasciclin, semaphorins, and netrins. These molecular clues can act bifunctionally as attractants to some neurites and repellents to others (Culotti, 1994; Keynes and Cook, 1995; Goodman, 1996) or as guidance and stop signals (Chiba et al., 1995; Fan and Raper, 1995). However, the basic question of what makes an axon stop when it reaches its proper target remains largely unanswered. Current views propose the existence of target-specific signals, implying that growth arrest and target recognition are a functionally related set of mechanisms (Luo et al., 1993; Garrity and Zipursky, 1995). In this context, the growing axon is viewed as a relatively passive responder to the signals encountered, and its path becomes the result of the match between substrate signals and the particular subset of receptors expressed in the growth cone. This scheme fits well the data from some systems. For example, in the vertebrate retinotectal projections, graded distributions of tyrosine kinase receptors and their ligands are thought to mediate pathway specification (Nakamoto et al., 1996; Drescher et al., 1997).

Target recognition is interpreted as a response to a target-derived growth cone collapsing signal, as deduced from ectopic

and *in vitro* expression of semaphorins, connectin, and agrin (Nose et al., 1994; Matthes et al., 1995; Campagna et al., 1995; Puschel, 1996). In the retinotectal example, it is proposed that ganglion axons stop growing in response to a threshold of repulsive activity (Baier and Bonhoeffer, 1992; Holland et al., 1996). Candidate molecules for guidance and stop signals have been identified through either *in vitro* (Stahl et al., 1990; Ullrich et al., 1995) or *in vivo* (White et al., 1992; Phillis et al., 1993; Seeger et al., 1993; Martin et al., 1995; Callahan et al., 1996) approaches. When the biological significance of these molecules is tested in the corresponding null mutants, however, the resulting phenotypes in general are surprisingly mild and variable (Whitlock, 1993). This fact has forced to invoke synergistic relationships among structural motives of the known proteins (Engel, 1991), lending support to the combinatorial aspects of the chemoaffinity theory (Tessier-Lavigne and Goodman, 1996).

We followed a nonbiased procedure (Ferrús and García Belido, 1976) and isolated the *gigas* (*gig*) mutant on the basis of its enlarged cell phenotype. The connectivity of *gig* photoreceptors was studied in eye mosaics and found to be normal, although the number of synapses was increased threefold (Canal et al., 1994). In contrast to the visual centers, the proprioceptive system is not overtly structured in units limited by glial cells (Cantera, 1993; Giangrande et al., 1993). Each macrobristle of the thorax is innervated by one sensory neuron (Hartenstein and Posakony, 1989), and its projections can be individually traced by retrograde labeling (Ghysen, 1980, 1992). Also, single neurons can be stimulated (Vandervorst and Ghysen, 1980), allowing a direct correlation between axon branching and behavior at the cellular level (Corfas and Dudai, 1991). We have generated small patches of mutant cells and studied the structural and functional conse-

Received Sept. 29, 1997; revised Nov. 12, 1997; accepted Nov. 14, 1997.

This research has been funded by grants from the Spanish (DGICYT 93-0149, 96-0006) and the European Science Foundation (ENP 16). The critical comments of Drs. P. Bovolenta, J. M. Devaud, A. Nieto, and F. de Pablo, as well as members of the Ferrús laboratory, are appreciated. Dr. R. Rodríguez provided key guidance and help on the electron microscopy. Dr. B. Hämmerle provided the material for Figure 1.

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quences of a *gig* mechanoreceptor neuron projecting to a genetically normal CNS. The data show that in this sensory system, target recognition and axon growth arrest are two independent features of pathfinding.

MATERIALS AND METHODS

Fly strains and mosaics. Mosaics were obtained from *mwh jv gig¹⁰⁹/+* flies that had been x-ray-treated at a dose of 500 rad (Philips MG 151 Be, 150 rad/min, 100 kV, 15 mA, and 2 mm Al filter) between 72 and 96 hr of development. Other alleles (*gig²⁵*, *gig^{8C5}*) yield the same phenotype in mosaics, as we have shown previously (Canal et al., 1994). Morphological studies were performed in 3- to 4-d-old adults. We tested the age effect on neuronal branching on six 15- to 20-d-old mosaics. The results reported in this study are based on a total of 86 mutant and 106 normal mechanoreceptor neurons (for nomenclature of bristle sensillae, see Ferris, 1950). The mutant group corresponds to 51 horse radish peroxidase (HRP) tracings and 58 grooming reflexes, and it includes 23 cases from which both types of data were obtained. As controls, we used nonmutant mechanoreceptors from the same mosaic fly or from flies of the same genotype. The control behavioral tests ($n = 58$) were obtained from the bristle contralateral to the mutant side.

Neuronal tracings. Mutant bristles were identified by the enlarged *gig* phenotype. Mutant spots embraced one to two bristles, and they appeared at a 10% frequency. These cells carry the additional cuticular markers *mwh* and *jv*, which do not affect the axon profile or the grooming reflex (our unpublished data). HRP retrograde fillings of single thoracic mechanoreceptors were performed in flies immobilized in modeling clay. The bristle was removed with forceps, leaving a circular open socket. A capillary filled with the HRP solution (29 mg/ml in PBS, type VI Sigma; Sigma, St. Louis, MO) was applied on the socket and maintained firmly pressed during 5 hr at room temperature in a humid chamber. The heads, wings, and abdomens were removed, and thoraces were fixed overnight in 2.5% glutaraldehyde in Sørensen buffer (10 mM sodium phosphate, 23 mM potassium dihydrogen phosphate, 100 mM sucrose, 3 mM magnesium chloride, pH 7.2). After dissection, thoracic ganglions were washed in PBS (10 mM sodium phosphate buffer, 150 mM sodium chloride, pH 7.5) and preincubated for 20 min in DAB (0.5 mg/ml) in PBS (Sigma). H_2O_2 was added to a final concentration of 0.003%, and after 10 min the ganglions were washed in PBS, dehydrated in graded ethanol series, clarified with methyl salicylate (Sigma), whole-mounted on DPX, and examined under a Zeiss Axiophot microscope. Neural arborizations were drawn using a camera lucida and displayed as ventral views of the thoraco–abdominal ganglion.

Electron microscopy of HRP tracings. Thoraces from flies treated as above were fixed overnight at 4°C (4% paraformaldehyde, 1% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2). After dissection, thoracic ganglions were washed several times in 0.1 M phosphate buffer and preincubated for 5–10 min in DAB (0.5 mg/ml) in PBS (Sigma), followed by H_2O_2 catalysis. Specimens were washed several times in 0.1 M phosphate buffer and post-fixed in 2% OsO_4 in 0.1 M phosphate buffer for 45 min at 4°C in the dark. After dehydration in graded ethanol series, the thoracic ganglions were included in Araldite resin. Blocks were sectioned parasagittally to the thoracic ganglion. Silver sections (60–70 nm) were cut in a Reichert Ultracut E ultramicrotome, collected on Formvar-coated, single-slot grids, and stained with uranyl acetate (10 min) and lead citrate (10 min). Observations were performed in a JEOL 1200 EX electron microscope.

Grooming behavior. We followed essentially the procedure of Vanderwort and Ghysen (1980). Mosaic flies are cold-anesthetized, decapitated with a razor blade, and left to recover for 30 min at room temperature in a humidified chamber. Single macrobristles were stimulated by manual tickling with a fine hair, and the reaction of the free flies was monitored visually. Before the stimulation of the mutant bristle, other nonmutant bristles including its contralateral homolog were tested at 1 min intervals. If a significant number of bristles, in particular the contralateral homolog, did not show responses, the fly was discarded for behavioral tests. No case was found in which the mutant bristle had a detectable reflex response and the contralateral homolog did not. The evaluation of the response was based on five stimulations at 1 min intervals. After the behavioral test was completed, mosaics were prepared for HRP tracing as described above. The age of mosaics used in behavioral tests ranged from 2 to 10 d.

RESULTS

We have examined the issue of growth limits in neural projection by making utilitarian use of the mutant *gigas* (*gig*). This mutation causes cells to grow beyond the normal size after the mitotic program has finished. Thus, the phenotype is expressed at the time of differentiation and not during the proliferative phase of development (Canal et al., 1994). The increment of cell size parallels that of the nucleus and its contents (Fig. 1). On average, the diameter of mutant nuclei is double that of normal nuclei. This seems to be an upper limit to nuclear enlargement, because aged mosaics (see below) do not exceed this increment. In turn, this indicates that the presumed additional rounds of DNA synthesis in the mutant eventually cease.

The *gig* mechanoreceptors modify their projection

We studied 33 somatic spots embracing only one or two mutant thoracic bristles [anterior scutellar (ASC), anterior notopleural (ANP), posterior notopleural (PNP), and humeral (HU)] in heterozygous flies. We chose three types of neurons because they represent three different types of branching patterns. (1) The ASC exhibits a clear distinction between a major (ipsilateral) and a minor (contralateral) branch, (2) the ANPs and PNPs show two ipsilateral branches of equivalent lengths, and (3) the HU presents only one branch. Figure 2 shows a case of an ASC neuron and a control. The axon of the *gig* neuron is two to three times thicker than wild type, generates more collaterals and boutons, and projects into areas that the wild type never reaches. The distinction between the major and minor branches is maintained in the mutant. It appears that the *gig* branching pattern is an expanded version of the normal counterpart. Figure 3 shows the various profiles obtained among five controls and 10 mutant ASC neurons that could be grouped into four morphological classes. The wild type always shows a characteristic terminal bend in the metathoracic neuromere. The *gigas* B and C phenotypes are the most frequent classes and show this bend either in the fused abdominal ganglion (class B, $n = 4$) or duplicated in the normal site and in the abdominal ganglion (class C, $n = 3$). Occasionally, the abnormal projection results in more profuse branching at the normal site (class D, $n = 2$) or in a long extension toward the brain (class E, $n = 1$).

The invasion of foreign territories by the mutant growth cone provides an ideal experimental condition to test the specificity of position-specific clues. The duplication of the characteristic bend of ASC neuron (class C in Fig. 3) suggests that the *gig* growth cone interprets properly the homologous features in each metamere despite their differential genetic identity, at least with respect to the expression of the *bithorax* gene complex (Duncan, 1996). The *gig* branching pattern is generally characterized by changes in the extent of main branches, but not in their number or direction. This suggests strongly that mutant axons follow the main pathways normally followed by other thoracic mechanosensory neurons.

To explore the ultrastructural effects of an invading projection into the abdominal ganglion, in particular the possibility of a gliotic reaction, we performed an electron micrograph analysis of HRP-traced mutant class B ASC neurons (Fig. 4). Sections taken at either of the three levels marked in Figure 4A do not reveal any abnormal glial envelopes or necrotic reactions in the vicinity. Also, glial cell nuclei stained with anti-REPO and viewed under confocal microscopy in these mosaics did not show significant changes in their number and size (not shown). Although synaptic figures could not be resolved because of the HRP precipitate, the behavioral tests indicate that the mutant neurons establish functional contacts (see below).

Figure 1. The *gigas* nuclear phenotype. Nuclei stained with an antibody recognizing the glia-specific transcription factor REPO. *A*, Optic stalk of a wild-type third instar larva. *B*, Equivalent view of a homozygous *gigas* stalk. *C*, Ventral mesothoracic nerve of a wild-type larval CNS. *D*, The homologous nerve on a homozygous mutant larva. Note the substantial increment in the size of mutant nuclei. Scale bar (shown in *A*): *A*, *B*, 12 μ m; *C*, *D*, 8 μ m.

The *gig* mechanoreceptors maintain their gestalt

The ANT and PNP bristles have almost identical patterns of projection, and their normal pattern consists of two very similar branches on the ipsilateral side, ending in the pro- and mesothoracic neuromeres, respectively (Fig. 5). We studied 15 wild-type and 15 mutant cases. The most frequent phenotype (class B, $n = 8$) is an extended projection along the anterior branch toward the brain. When the extension takes place along the posterior branch (class C, $n = 4$), the anterior one has the normal length. As in the previous ASC neuron, this mutant trait manifests in only one of the branches but not in both. Class D ($n = 3$) phenotype appeared less frequently, and it is not clear whether it corresponds to more profuse branching at the normal site (equivalent to class D of the ASC neuron in Fig. 3) or to incomplete HRP tracings. The absence of fine collaterals suggests the latter. The normal

HU neuron has a single ipsilateral branch that never extends beyond the prothoracic neuromere ($n = 15$) (Fig. 6). All mutant cases ($n = 8$) that were studied show an extended projection in the same direction, although they reach very posterior areas of the mesothoracic neuromere. No contralateral or cephalic extension was found in the HU *gig* neurons.

To summarize, in the three types of neurons studied the mutant condition maintains the general shape of the projection, and the only structural feature that can be recognized as abnormal is the cell size and the additional targets reached.

The additional target reached by *gig* neurons still belongs to the mechanoreceptor domain

It is important to point out that the mutant neurons project and extend fine collaterals with boutons in the sensory areas normally

Figure 2. Projection from normal and *gigas* anterior scutellar (ASC) bristles. Photographs of HRP-filled ASC neurons in an irradiated same sibling control (*A*) and one *gig* mosaic (*B*). The mutant neuron corresponds to class B phenotypes represented in Figure 3. Note the relative position of the terminal bend (arrowhead) in each case. Although both mutant and control neurons contact the CNS at the same entry point, between the prothoracic (*pr*) and mesothoracic (*ms*) neuromeres, the *gigas* neuron terminates in the abdominal (*ab*) ganglion. Anterior is to the top in all figures. Scale bar, 50 μ m.

Figure 3. Projections patterns from the anterior scutellar (ASC) bristle. These camera lucida drawings of a ventral view of the fused thoracico-abdominal adult ganglion show representative cases of the wild type (*A*) and the four morphological classes (*B–E*) of mutant projections found among 10 mosaics. Class *A* is the normal profile found in nonirradiated CS flies as well as in irradiated same sibling controls ($n = 5$). Classes *B* and *C* are the most frequent cases ($n = 4$ and 3, respectively). Note the reproduction of the normal branching pattern, albeit into a larger dimension (also see Fig. 2). In class *C*, note the double bend (arrowheads) repeated in the normal site, the metathoracic neuromere, and the abdominal ganglion. Class *D* projection was observed in two instances; class *E* was found only once. Note the projection toward the head (asterisk).

Figure 4. Ultrastructure of a mutant ASC neuron. *A*, Camera lucida drawing of a *gigas* HRP tracing corresponding to class *B* in Figure 3. *B–D*, EM sections processed for HRP taken at levels 1–3, respectively, as shown in *A*. Axons containing HRP show the characteristic black precipitate (arrows in *C*). In *D*, note the absence of gliosis or necrosis around the neural projection into the abnormally invaded abdominal ganglion (level 3). *m*, Mitochondria. Scale bar (shown in *D*): *B*, 1 μ m; *C*, 1.5 μ m; *D*, 0.6 μ m.

innervated by wild-type mechanosensory axons that are located in the ventral side of the insect thoracic ganglion (Merritt and Murphey, 1992). The most aberrant projection target found among the mutant neurons was a cephalic extension in ASC (class E) (Fig. 3) and ANP/PNP (class B) (Fig. 5). We traced three individuals of the latter type and found that the final target was in the brain mechanosensory center (BMC), in which normal head and antennal mechanoreceptors project. To confirm the apparent restriction of the mutant projections to mechanosensory domains, we traced mutant vertical (V) neurons. In the controls ($n = 13$), this neuron projects to the BMC (Fig. 7). In all mutant cases ($n = 12$), however, the V neurons branched at the BMC site and continued toward the thorax until the approximate location of the HU target. Finally, the normal mechanosensory neurons of the antenna exhibit an occasional projection toward the thoracic ganglion (Fig. 8). In the mutant antennal mosaics, this feature was encountered more frequently (27 vs 13%) and showed extended and more profuse branching. Taken together, all of these morphological observations suggest that the *gigas* mutation does not interfere with the normal mechanisms of pathfinding; however, the signals to arrest growth at the normal targets seem to be ignored.

The *gig* neurons finalize their projections

The normal mechanosensory neurons extend their axons during the second half of metamorphosis, and functional contacts are present at eclosion (Palka et al., 1986; Hartenstein and Posakony, 1989; Whitlock and Palka, 1995). Most neural tracings were performed in 3- to 4-d-old flies. We traced six mutant neurons aged 15–20 d to test for extreme time effects. This length of time represents about one third of the average life span of this insect under laboratory conditions. None of these aged mosaics deviated from the regular observations and were included in Figure 3 (class B), Figure 5 (classes B and C), and Figure 6 (class B). It can be concluded that the *gig* effect consists in an extended period of axonal growth along compatible pathways that nevertheless runs off at coherent targets. Alternatively, the *gig* neurons might have

grown faster than wild type during the normal time period of axonal growth. This alternative appears unlikely, because the homozygous mutant develops during the same time schedule as the wild type (not shown).

The *gig* mechanoreceptors elicit modified behavioral responses

The functional consequences of the *gig* condition were assayed using the grooming reflex in a group of 58 *gig* mosaics with one to two mutant macrobristles in the thorax. In the normal reflex and on gentle touch of a single bristle, the fly extends a leg to brush off the bristle area. Each bristle elicits a response from a specific leg, with characteristic probability (Vandervorst and Ghysen, 1980). For the purpose of this study, the response in the wild-type bristles can be classified as (1) high responders (~100% probability), (2) medium responders (~50%), and (3) low responders (0–10%). Very often, we find that the probability of response and the leg used in the case of *gig* bristles differ from the response obtained from the contralateral homolog that is used here as an internal control (Table 1). Of the 58 cases tested, 24 of them yielded a normal response, whereas 34 exhibited some type of abnormality. The deviations included an enhanced ($n = 8$) or reduced ($n = 10$) cleaning activity in terms of either probability of response or brushing vigor. In seven cases the mutant bristle gave no response after repeated stimulation. An interesting group ($n = 9$) of behaviors was classified as qualitatively “different.” It included the use of the contralateral leg, in addition to the ipsilateral one, for the cleaning reflex (three cases), the normal use of the ipsilateral leg but with an unusual tic movement in the leg and bending of the abdomen (one case shown in Fig. 5*B*), and the scissoring of wings as an additional movement during grooming. It is important to realize that all abnormal movements triggered by the mechanical activation of a *gig* bristle can be considered as coherent with the stimulus modality. For example, jump, flight, or courtship wing vibration were never elicited. In all cases, the abnormal responses were observed in addition to, rather than instead of, the normal responses. The obvious excep-

Figure 5. Projection patterns from the anterior and posterior notopleural (ANP, PNP) bristles. The data from these two neurons were found to be very similar in wild-type as well as in mutant mosaics; consequently they were pooled. The camera lucida drawings show the normal class (*A*) ($n = 15$) and the three mutant classes (*B–D*) found among 15 mosaics. Class *B* is the most frequent case ($n = 8$). The anterior projection terminates always in the brain mechanosensory center (*arrow*). The brain is shown in a frontal view. Class *C* ($n = 4$) shows an extended projection in the opposite direction (toward posterior) than in the previous class. Class *D* ($n = 3$) might represent incomplete tracings (see Discussion).

tion is the class of “absent” responses. Also, the normal grooming activity from the nonmutant bristles was not modified. All of these data demonstrate the specificity of individual mechanosensory neurons and suggest an equivalent degree of precision in the information processing at the postsynaptic integrative centers.

In a fraction of cases ($n = 23$), tracing and behavior could be obtained from the same neuron. Among the neurons eliciting “normal” responses (first column in Table 1), two HU neurons had an extended projection to the mesothoracic neuromere, three PNP neurons reached the metathoracic ganglion (class C in Fig. 5), and one ASC extended into the abdominal ganglion. Among the neurons eliciting “enhanced” responses (second column in Table 1), one ANP extended to the metathoracic ganglion. The class of “reduced” responses (third column in Table 1) included two HU neurons that reached the mesothoracic neuromere; one PNP projected to the brain (class B in Fig. 5) and another PNP did it in the metathoracic neuromere. The class of “absent” responses (fourth column in Table 1) included one HU that extended to the mesothoracic neuromere and two ASCs terminating in the abdominal ganglion. Finally, among the cases of “different” responses (fifth

column in Table 1), one ANP and two PNP correspond to class B in Figure 5 and extended their projection to the brain. The remaining six cases correspond to neurons (PSC, PPA, ADC, PSA, PDC), the branching patterns of which were not traced enough times to allow a confident characterization of their abnormality. It should be noted that all mutant projections gave rise to new collateral branches at the normal site of projection, which presumably made new additional synaptic contacts. However, largely coincident patterns of projection (e.g., HU neuron) yield normal as well as all types of abnormal responses. Perhaps it is safe to conclude that the connectivity changes elicited by *gigas* often translate into behavioral changes, although the levels of resolution of the grooming reflex and the HRP tracings do not allow a correlation that could serve as a predictor.

DISCUSSION

In all neural systems, target recognition and growth arrest of the projecting neurons are two synchronous events thought to be causally related; however, the *gigas* phenotype in the tactile neurons proves that these are two separable mechanisms, at least

Figure 6. Projection pattern from the humeral bristle (HU). Drawings are of normal (*A*) and mutant neurons (*B*). This neuron showed only one class of abnormal projection ($n = 8$) in which the extension proceeds farther caudal into the mesothoracic neuromere and branches profusely along the way.

Figure 7. Projection pattern from the vertical (V) bristle of the head capsule. Drawings correspond to wild-type individuals (*A*) and mutant mosaics (*B*). In all mutants examined ($n = 12$), the neuron projects to the normal mechanosensory center in the brain (*arrow*) where it branches, and in addition it goes beyond, toward the thoracic neuromere, and terminates in a mechanosensory area most similar to the target of the HU neuron (see Fig. 6). Brain profile shown in frontal view.

in this sensory system. Furthermore, the use of mosaics allows us to unravel the autonomous role of the growth cone during these aspects of neural development.

In contrast to most axonal projection phenotypes described so far, *gigas* yields full penetrance and fairly constant expressivity in the neurons studied. *gigas* is a remarkable tool for examining connectivity issues, because the phenotype manifests only after the normal mitotic program has been completed. Although formally possible, it is unlikely that the mutant condition would manifest earlier in the development of the cell. It should be noted that homozygous mutant larvae live without detectable severe abnormalities until metamorphosis. Only cells that normally would have ceased the synthesis of DNA show the *gig* phenotype (Fig. 1). At present, glia and sensory and motoneurons in the homozygous larvae have been found to be abnormally large in their somata, axons, and terminals; however, muscles, in which the normal way of growth is by polyploidy (Smith and Orr-Weaver, 1991), do not manifest the mutant phenotype. Although the *gig* protein is not yet known, it is plausible that it might be involved in the clock signal to stop DNA synthesis in postmitotic cells, as described in some yeast mutants (Hartwell et al., 1974; Broek et al., 1991).

Axon growth, stop signals, and target choice

Our data show that *gigas* neurons sprout more collaterals and extend their projection beyond their usual targets despite

Figure 8. Unusual projections from the antennal mechanosensory neurons. HRP tracings of wild-type antennae (*A*) ($n = 30$) show single neuron mechanosensory projections into the thoracic neuromeres in addition to the normal projection at the brain mechanosensory center. This unusual projection is found in $\sim 13\%$ of cases in various genotypes, including the wild type. In *gigas* mosaic antennae (*B*) ($n = 33$), however, these cases are more frequent (27%), sprout more branches, and extend more posterior into the thoracic ganglion.

growing over a normal substrate. Although the termination point is subject to variation in the wild type, these are never as great as the phenotypes observed in the mutant. The extended projection reproduces the normal features in terms of general pathway and branching pattern. Neurons with a single major branch (HU) or two clearly different branches (ASC) still maintain their characteristic profile. Neurons with two equivalent branches (ANP, PNP) show the extension through either branch, but never along both of them. In these cases, it seems that the growth dynamics of the mutant can be randomly drained by either growth cone. Once this choice takes place,

the growth continues along suitable pathways until another compatible target is reached. The phenotype of the *gigas* mosaics challenges the determinism of the substrate-derived growth inhibitory factors as stop signals for the growth cone. The experiments reported here show that the growing axon can override these putative signals. However, the *gigas* axons do not continue their growth indefinitely. They do stop, and their extended projections are kept within the territorial domains of mechanoreceptor endings.

Concerning target recognition, it is important to realize that the mutant axon seems to establish synapses at the normal targets, judging by the fine branching at the proper site and the normal behavioral responses that are elicited. It could be envisioned that a *gigas* axon has a quantitative change in its repertoire of receptors for target-derived stop signals. In this context, the extended projection could reflect a hypersensitivity of the mutant cell toward attractants located farther away (e.g., the brain). This is unlikely, because neurons such as HU never extend in that direction despite being located closer to the brain and also because neurons with two equivalent branches (ANP, PNP) do not always extend toward the brain. In the same way, the possibility that the *gigas* neuron could downregulate the expression of stop signals in the normal target cell can be ruled out. First, in that case the normal target cell would not stop other neighboring nonmutant projecting axons, and this has not been observed in the coincident HRP tracings of mutant and adjacent normal sensillae (data not shown). Second, this possibility would not explain why the *gigas* neuron eventually stops at other compatible targets. At this point we cannot provide a testable hypothesis about the mechanism that makes the *gigas* axon stop. In any event, the mechanism would have to account for the halt of DNA replication in the nucleus as well.

A plausible interpretation of the phenomenology unveiled by *gigas* suggests that axon growth can proceed according to the intrinsic capacity of the cell until this is exhausted. This effect might be triggered when a given threshold of stop signaling is received from the target. Under normal conditions, depletion of this growth capacity in the sensory neuron and appearance of target identification signals in the substrate would be coincident in time and space. Alternatively, the incoming axon and the target could be tuned to express matching levels of receptors and stop

Table 1. Grooming behavior in *gigas* mosaics

	Normal	Enhanced	Reduced	Absent	Different	Totals
High responders $\sim 100\%$						
HU	5	2	2	2	2	13
ANP/PNP	5	3	4	1	4	17
PPA	1			1	3	5
Medium responders $\sim 50\%$						
ASC	2		1	2		5
PSC	1	1				2
PS	3	1	1			5
APA	1	1	2			4
Low responders 0–10%						
ADC	2			1		3
PDC	2					2
PSA	2					2
Totals	24	8	10	7	9	58

Numbers of mutant bristles and their grooming response. The bristles analyzed and their normal probability of response (%) are indicated on the left (Vandervorst and Ghysen, 1980).

signals, respectively. In the mutant, neurons would follow the normal path and recognize the normal target, but in addition they would be able to reach and recognize farther targets until their presumed excess of receptors would be saturated at the “stop threshold.”

Glial cells are known to play a key role in the guidance of many axon projections (Hidalgo et al., 1995), although the embryo pioneers aCC and pCC exhibit normal projections on a *glial cells missing* mutant background (Y. Hotta, personal communication). Mechanosensory neurons are clonally related to their peripheral glia (Ferrús and Kankel, 1981; Giangrande, 1994), and consequently each mutant mosaic includes one glial cell along with the neuron. This cell, however, does not follow the axon in its full length along the corresponding nerve because other, CNS-born, glial cells cover this space. Thus, the mutant condition of this unique glial cell cannot be the cause of the abnormal neural profile. Also, the CNS glia does not seem to play a major role in restricting mechanosensory target domains, nor does it appear to react to the invading mutant branches (Fig. 4). Targets of this sensory modality might be defined by another type of mechanism not requiring a physical delimitation. Different cellular systems, however, may exhibit additional or alternative features. The observations in the tactile system described here contrast with the case of photoreceptors in which the mutant axons do not extend their branches either outside of lamina cartridges (R1–6) or beyond the normal medulla layers (R7–8) (Canal et al., 1994). This contrast would support the role of glia in the establishment of territorial domains in the visual centers and suggests an alternative mechanism for the mechanosensory centers. It is quite likely that the glia in the optic ganglia imposes severe constraints on the potential outgrowth of the *gig* photoreceptor axons (Saint Marie and Carlson, 1983; Winberg et al., 1992). By contrast, mechanoreceptor centers in the thorax as well as in the brain do not seem to be as compartmentalized by the glia, because the mutant axons can project to both ganglia.

Behavior modifications after single neuron change in connectivity

Mechanosensation is triggered by the movement of the bristle, although the subsequent transduction steps are still unknown (Kernan et al., 1994; García-Añoveros and Corey, 1997; Tavernarakis and Driscoll, 1997). It is remarkable that a single neuron change in connectivity is able to cause detectable changes in a behavioral response. This observation argues against the existence of a large degree of redundancy in this type of sensory perception. On the contrary, it points toward the existence of a detailed somatosensory map in which the projection of each neuron in the CNS represents a unique body site. The modified behavioral responses are still context-coherent, in agreement with the homologous nature of the new projection targets. It might be relevant to note that in mammals somatosensory representations of amputated limbs can be maintained only by the newly extended projections from compatible populations of axons (Florence et al., 1997). A similar case of constancy in the response of CNS interneurons after increments in the number and size of afferents during development has been described in crickets (Chiba et al., 1992). The modified response in *gigas* cannot be attributed to the abnormal morphology of the mutant bristle, because all of them show the same type of enlargement but 40% of them did not manifest a modified behavior (Table 1). However, the possibility of electrophysiological changes in the transduction process attrib-

utable to modifications of the biophysical properties of the enlarged whole sensilla (Hill et al., 1994) cannot be ruled out, and this is currently under study. In the eye, enlarged mutant cells show a threefold increase in the number of synapses. The increment in synapse number elicits a change in the phototactic response, indicating that the mutant retina conveys a higher or modified (or both) light input to the normal postsynaptic neurons (Canal et al., 1994). In the proprioceptive system we find that the change in behavior correlates reasonably well with the degree of abnormality in the site of projection. The fact that not all changes in connectivity could be revealed as changes in behavior is attributable, most likely, to the different levels of resolution between morphology and behavior.

Taken together, the structural and functional features observed in mechanosensory *gigas* neurons emphasize the autonomous component of the projecting axon during the formation of this sensory map and prove that growth cone arrest and target recognition are two different processes.

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