Disconnected Mutants Show Disruption to the Central Projections of Proprioceptive Neurons in *Drosophila melanogaster*

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**ABSTRACT:** We used a P[GAL4] enhancer-trap line, C161, in conjunction with the UAS-lacZ reporter construct to visualize the central projections of a defined set of thoracic and abdominal sensory neurons in a disconnected (disco) mutant background. The results show defects in the organization of sensory axons in the larval and adult central nervous system. The defects are indicative of problems with axon growth and development and include (a) poor axon fasciculation, (b) aberrant axon growth, (c) excessive terminal branching, and (d) ectopic innervation. Sensory neuron identity appears to be normal. The defects are comparable to those previously described for larval photoreceptor and adult retinular cells in disco mutants and extend the known effects of this mutation. Reduced larval and adult viability are likely to result from locomotory defects related to the disruption of the sensory system.

The central projections of insect sensory neurons are highly organized. This organization is such that sensory axons segregate in the central nervous system (CNS) according to their modality, creating a functional layering of the sensory neuropiles (Murphey et al., 1989). Further ordering within these layers is also apparent; for example, tactile axons show somatotopic ordering (Murphey, 1981; Levine et al., 1985; Newland, 1991; Merritt and Murphey, 1992). It has also been shown that this ordering of sensory axons plays a significant role in determining the patterns of synaptic connectivity (Bacon and Murphey, 1984; Shepherd et al., 1988; Bacon and Blagburn, 1992). Despite the obvious functional significance of this anatomical order, we are only just beginning to understand the mechanisms that control the developmental assembly of these sensory arrays.

One way in which the developmental mechanisms that shape sensory organization have been uncovered has been to use *Drosophila* to identify mutations that disrupt the assembly of sensory arrays. This strategy has proven successful and uncovered a number of genes involved in specifying sensory neuron identity, e.g., *cut* (Bodmer et al., 1987; Blochlinger et al., 1991) and *px-neuro* (Nottebohm et al., 1994; Hassan and Vaessin, 1996). Despite these successes, we still know little about how a sensory neuron’s identity is translated into ordered central projections. One reason for this is the difficulty faced in screening large numbers of *Drosophila* mutants for defects in sensory projections using conventional single-neuron staining techniques.

Despite its small size, conventional neuroanatomical techniques have been used to study sensory...
axons in Drosophila and shown that their organization is comparable to other insects (Murphey et al., 1989; Merritt and Murphey, 1992). More recently, however, the enhancer-trap technique (Brand and Perrimon, 1993) has been used to create a simple and reproducible method for visualizing identified sensory neurons. Using a UAS-lacZ reporter construct, the central projections of identified sensory neurons in both adult (Smith and Shepherd, 1996) and larval (Shepherd and Smith, 1996) CNS of Drosophila have been described in detail. With this development, it became possible to use enhancer-trap expression patterns to screen mutant stocks to identify those with disrupted sensory projections (Phillis et al., 1996).

To begin this process, we selected a well-characterized GAL4 line (C161) to examine the central organization of sensory axons in the thoracic and abdominal neuromeres of a mutation known to affect CNS development. The mutation chosen, disconnected (disco), was originally isolated on the basis of a degenerate eye phenotype that was shown to be the consequence of axon guidance defects in both larval photoreceptor and adult retinular cells (Steller et al., 1987). This work suggested that there were also defects in the peripheral routing of sensory axons in the trunk segments of disco mutant embryos, implying that there may be disruption of sensory axon projections other than in the cephalic neuromeres. We therefore examined the CNS of disco mutants for defects in sensory axons.

The results demonstrate that disco mutants show severe defects in their sensory projections in larvae and pupae. These defects are consistent with those observed in the visual system and suggest that they may be the consequence of large morphogenetic movements during CNS development. We also show that defects to the sensory system may underlie poor larval motility.

**MATERIALS AND METHODS**

**Genetics**

Flies were raised on standard medium at 23–25°C. To generate the C161 staining pattern in a disco background, disco/FM7c; +; + females were crossed to +; UAS-lacZ; C161/TM6B males. Progeny have the following genotype: All females are heterozygous (disco/Y or FM7c/+ ) and are phenotypically wild type. Half the males are hemizygous disco (disco/Y) and exhibit the mutant phenotype, and the remainder are hemizygous FM7c/Y and phenotypically wild type for disco. The two male genotypes were distinguished by the FM7c markers (y<sup>11d</sup>, B).

**X-gal Staining and Immunohistochemistry**

Tissue staining was performed exactly as described by Smith and Shepherd (1996).

**Photomicroscopy**

Tissues were examined on a Zeiss Axioskop microscope and photographed with Kodak Ektachrome 160T or Kodak Tech Pan film. Images were digitized onto Kodak photo CD and montages assembled using Adobe Photoshop 3.0 on a Macintosh IIci computer. Images were adjusted for brightness and contrast only.

**Quantifying Defects Observed in the CNS**

To quantify defects in axonal organization, preparations were scored blind according to two criteria: axon bundling and number of aberrant axons. The absence of defects was scored as 1 = normal; 2 = minor defects (<3); 3 = moderate defects (3–5); 4 = major defects (>5); and 5 = severe defects (unquantifiable).

For control data, homozygous Oregon-R flies were scored.

**Determining the Lethal Phase**

Embryos were counted onto plates, 30/plate, containing yeast paste, and the timing and number of deaths were recorded. Wild-type (OreR) embryos were used as a control. The temperature was maintained at 22 ± 2°C.

**Behavioral Analysis**

Adult flies (0–5 days old) from the disco/FM7c stock were transferred to fresh cornmeal-agar food medium, allowed to lay for 5 days, and removed. Temperature was maintained at 22 ± 2°C. This stock was used for two reasons. First, progeny with several genotypes (disco mutant males and females, FM7c/Y males, disco/FM7c and FM7c females) are produced. Control individuals (e.g., disco/FM7c) were raised in direct competition with mutants (e.g., disco<sup>1</sup>). Second, as disco<sup>1</sup> mutant males are only distinguishable from FM7c males by denticle band color, males could be tested blind for motility before being distinguished by their phenotype. Females with black denticle bands (disco and disco<sup>1</sup>/FM7c), once tested, were placed in containers to pupate. Only mutant females that reached late pupal stages could be distinguished by their normal eye phenotype. OreR adults, also 0–5 days old, were used to founder a separate
Motility Test

Wandering third-instar larvae were placed in the center of a petri dish on moist filter paper over a square grid (1mm²). Two light sources were placed above the dish to cast even light on the test area. As each larva moved, the number of times its posterior crossed a line was recorded over a 1-min period. Following the count, the larva was placed in a drop of saline and retested after 30 min. Once tested, larvae were sexed and placed in separate dishes with moist filter paper to pupate (where mutant females were determined by eye phenotype). The incubator was set for a 12-h light cycle, 09:00–21:00, and trials commenced at midday (±2.5h). The results were pooled and analyzed using a one way Student t test.

RESULTS

Organization of Proprioceptive Central Projections in Wild-Type Background

Larval CNS. In wild-type larvae, the axons from expressing neurons form a segmentally repeated pattern of projections within the CNS that is indistinguishable from that described for line C161 by Shepherd and Smith (1996). The projections of the expressing neurons form the rigidly arranged ladder-like pattern that is almost identical in all neuromeres of the trunk segments [Fig. 1 (A,C,E)]. In wild-type larvae, the axons are tightly bundled and the projections run parallel to each other and perpendicular to the midline [Fig. 1 (A,C)]. There is little or no evidence of aberrant axons, and all visible axons are within these tightly defined bundles. Blind scoring for axon aberrations of eight wild type larval ganglia showed no more than one error per individual, and all were scored as normal (Table 1).

Pupal CNS. Within the thoracic neuromeres of wild-type pupae, the axon projections from the legs, wings, and halteres are identical to those described by Smith and Shepherd (1996). Axons from the external sensilla on the leg form the typical crescent-shaped projection which completely outlines the neuropile of each thoracic hemineuromere [v-ant, v-post; Fig. 2 (A,C)]. Axons from the femoral chordotonal organ project medially to terminate close to the midline [med; Fig. 2 (A,C,E)]. Axons from dorsally located sensilla, on wing and haltere, form more complex projections restricted to dorsal neuropile (not shown). Blind examination for de-
Table 1  Defects in Sensory Axon Fasciculation and Growth in Larval CNS of disco1 Mutant Males and Control Genotypes

<table>
<thead>
<tr>
<th>Genotype for X Chromosome</th>
<th>n</th>
<th>Aberrant Projections</th>
<th>Fasciculation</th>
<th>Total</th>
<th>Average (Total/2n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type (+/+ and +/Y)</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>Females (discol/+ and FM7c/+ )</td>
<td>12</td>
<td>15</td>
<td>14</td>
<td>29</td>
<td>1.21</td>
</tr>
<tr>
<td>Males disco1/Y</td>
<td>16</td>
<td>55</td>
<td>46</td>
<td>101</td>
<td>3.16*</td>
</tr>
<tr>
<td>FM7c/Y</td>
<td>11</td>
<td>14</td>
<td>12</td>
<td>26</td>
<td>1.18</td>
</tr>
</tbody>
</table>

Scoring was on a scale from 1 to 5 where 1 = no defects; 2 = minor defects (<3); 3 = moderate defects (3–5); 4 = major defects (>5); and 5 = severe defects (unquantifiable). The mean for each genotype is the total/2n, where the total is calculated as the combined score for aberrant projections and fasciculation.

* Statistical significance (p < 0.01).

Defects in the ganglia of nine individuals showed that seven had no observable defects and two had slight defects, giving a mean of 1.11 (Table 2).

**disco Disrupts the Organization of Sensory Axon Projections in Larvae and Pupae**

To assess the effects of the disco mutation on sensory projections, the GAL4 enhancer trap line was used to drive the expression of a UAS-lacZ construct in a disco1 mutant background. The central projections revealed by this line were examined in both larval and pupal CNS. Mutant specimens and controls were examined blind and scored for the extent of observable defects.

**Larvae.** In female controls (disco1/+ and FM7c/+), the central projections of proprioceptive sensory neurons were indistinguishable from wild type, and of 12 specimens examined, 11 were scored as normal (Table 1). One exceptional female scored as moderately defective as a result of diffuse bundling in the v-ant and v-post pathways of one hemineuromere. This gave an average score for defects of 1.21 (Table 1).

In disco1/Y males (n = 16), the central sensory projections scored as either major or severely defective in seven specimens, moderately defective in a further seven, and with few or minor defects in just two. This gave a mean of 3.16 (Table 1). This score is significantly different (p < .01, chi-square test) from wild types, disco1/Y females, and FM7c/Y males. FM7c/Y males (n = 11) showed few defects, were indistinguishable from wild type, and scored as normal (Table 1).

**Pupae.** In females (both disco1/+ and FM7c/+), no major defects were seen and were indistinguishable from wild type. All specimens were scored as either normal or with minor defects, giving averages of 1.18 (disco1/Y) and 1.27 (FM7c/Y) (Table 2).

The sensory projections in disco1/Y males were clearly disrupted. Of 17 males examined, 11 scored moderate to severe disruption, two scored as minor abnormalities and four scored normal. This gave an average score for defects of 2.97, which is significantly different (p < .01, chi-square test) from heterozygous females (Table 2). Figure 2 shows examples of typical central defects of axons from the legs [Fig. 2(B,D,F)] in contrast to their wild-type counterparts [Fig. 2(A,C,E)].

FM7c males also exhibited defects in central projections. Of five specimens examined, all scored high for defects, giving a mean score of 3.90, which is significantly different (p < .01, chi-square test) from heterozygous females (Table 2). The nature of the defects were, however, distinguishable from those seen in disco mutants. For example, whereas the projections were diffuse, there did not appear to be any observable groups of aberrant axons weaving through the neuromere in the manner seen in disco mutants.

**Organization of Sensory Axons in the disco Mutant Background**

The results indicate that the central projections of the sensory neurons revealed by line C161 are severely disrupted in the disco1 background. To examine the nature of these defects, a detailed analysis of the sensory projections in both larval and pupal CNS was made.

**Larval CNS.** In disco1 mutant larvae, although gross disruption of the central projections is evident, the basic segmentally repeated pattern seen in wild-type specimens is still recognizable [Fig. 1(B,D,F), disco1; compare with Fig. 1(A,C,E), wild type].
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pathways, although evident in most neuromeres, are not rigidly aligned but meander toward the midline [black arrowheads, Fig. 1(D)], in contrast to the situation in the wild type where the medially directed projections are typically perpendicular to the midline [arrowheads, Fig. 1(C)].

**Axon Bundling.** A similar type of defect is also seen in the bundling of the axons in these main projections. In wild type, the axons are tightly grouped and form clearly defined axon bundles [Fig. 1(A)]. In disco mutants, however, this bundling is occasionally lost and results in a poorly defined array [Fig. 1(B)].

**Axon Tangles.** Adjacent to the midline, in the region where axons normally terminate, axons show excessive growth and often appear to form large axon tangles [white arrowheads, Fig. 1(D); white arrow, Fig. 1(F)], compared with the same region in wild-type neuromeres [Fig. 1(C,E)].

**Aberrant Projections.** Some axons also make incorrect projections. For example, within the abdominal segments, proprioceptive axons normally project close to the midline and bifurcate to send axonal processes anteriorly and posteriorly within the longitudinal connectives (Merritt and Whitington, 1995). In the specimen in Figure 1(D), however, axons entering the left hemineuromere of the third abdominal segment do not take this projection and cross the midline to enter the contralateral hemineuromere (upper white arrow). Furthermore, all axons entering the left side of one neuromere (A4) project to the midline and then posteriorly along it (lower white arrow). Thus, there is an absence of some axons that normally project in the longitudinal connective between these two hemineuromeres [black arrow, Fig. 1(D)]. A more extreme aberration is seen in the terminal neuromeres (A7–A8) of the individual shown in Figure 1(F). The nerve roots and longitudinal connectives on the right-hand side show excessive axons (white arrows), whereas contralaterally, there is a relative lack of stained axons [asterisk, Fig. 1(F)]. The most likely explanation for this phenotype is that axons that normally innervate the left-hand hemineuromere have ectopically entered the CNS through the contralateral nerve.

**Pupal CNS.** In disco pupal CNS, the observed disruption to the central projections is comparable to that seen in larvae. Hence, at a superficial level, the basic organization of the wild-type pattern is still recognizable and it is possible to identify all of the

**Figure 2** C161 pattern of expression revealed immunohistochemically in the pupal CNS of wild type (A,C,E) and disco (B,D,F) showing the central axonal projections of proprioceptive neurons. Anterior is at the top. (A–F) Thoracic neuromeres. (A,B) Prothorax. Arrows in (B) indicate distinct groups of axons that weave toward the midline. In wild type, all axons that project in the med pathway form a tight bundle [med in (A)] as do those in v-ant and v-post. (C,D) Mesothorax. Arrows in (D) show the diffuse projections of axons in a mutant, as compared with the typical wild-type pattern (C), in which the three projections (v-ant, v-post, and med) are clearly evident. (E,F) Metathorax. Arrow in (F) shows axons in the v-post pathway that form tangled projections in lateral regions of the leg neuropile. None of the axons that normally project to deeper regions of the neuropile in this pathway [compare with (E)] do so, but project back on themselves. Scale = 75 μm.

Within this organization, however, a number of distinct defects could be identified:

**Axon Weaving.** The most obvious defect is that the stereotypical rigid organization of the segmental arrays is disturbed. In defective specimens, these
major axon pathways (Fig. 2). Detailed examination of the projections does, however, show disruption of axons from sensory neurons on the leg, wing, and haltere.

The disruption of the leg projection is the most striking. As with the larval preparations, one characteristic defect is that the axon pathways are no longer cleanly aligned, but show signs of meandering growth. For instance, Figure 2 shows three examples in which it is clear that the projections are not as rigidly aligned as in their wild-type counterparts [compare left side (wild type; Fig. 2[A,C,E]), with right (disco1 mutant; Fig. 2[B,D,F])]. It is also apparent that the pathways are less tightly bundled and there is a loss of axon fasciculation. This can be seen in two ways: First, Figure 2(B) shows a prothoracic hemineuromere in which one projection has separated into at least three distinct axon bundles (arrows) in contrast to the same projection seen in wild type [Fig. 2(A)]. Another consequence of the poor axon fasciculation is that many of the projections appear to be diffuse. For example, Figure 2(D) shows details of the mesothoracic hemineuromere of a mutant in which the three central arrays (v-ant, v-post, and med) are hard to identify owing to the diffuse nature of the projections (arrows) in each [compare with wild type; Fig. 2(C)].

The pupal CNS in disco1 mutants also shows signs of aberrant growth and the formation of neuronal tangles. In Figure 2(F), the majority of axons projecting along the v-post tract, in a metathoracic hemineuromere, form a dense neural tangle with many, if not all, axons failing to make the correct projection to deeper regions of neuropile. Ectopic innervation of the CNS is also apparent in the thoracic neuromeres. In the specimen shown in Figure 2(B), the leg nerve does not enter the CNS via its normal route, but instead enters more posteriorly and medi- ally, compared with the wild type [Fig. 2(A)]. The ectopic location of this leg nerve may, in part, explain the subsequent defects observed in the central projections of afferents in the right prothoracic leg neuropile of this particular individual.

Defects are not restricted to the projections of sensory neurons on the legs. Similar defects are seen in the projections of axons from sensory neurons on the wing and haltere. Although less severe than defects from the leg, the wing projection in disco1 pupae shows meandering axons, poor fasciculation, and ectopic central projections (not shown).

Defects are also seen in axons from abdominal sensory neurons. The most striking of these are the projections of axons from a large multiscolophorous organ in the anterior abdomen (Smith and Shepherd, 1996). Axons from this structure normally project anteriorly in a single bundle [arrows, Fig. 3(A)] to terminate in the prothoracic neuromere [arrowheads, Fig. 3(A)]. In mutant individuals, the tight bundling of this projection is less evident [arrows, Fig. 3(B)] and the axons do not terminate normally, showing excessive branching [arrowheads, Fig. 3(B)].

**FM7c/Y Males.** FM7c/Y pupae show severe defects, in particular relating to axon bundling (Table 2). It is believed that the diffuse nature of this bundling may result from a secondary consequence of degeneration of the fly as a whole. Alternatively, it is possible that the multiple inversions present on this chromosome affect the activity of genes that underlie some aspect of the bundling of these axons.

### Sensory Neuron Fate

It is possible that the defects in the CNS are due to misspecification of sensory neuron fates in the disco1 background. To test this, the peripheral organization of the expressing sensory neurons was examined.

<table>
<thead>
<tr>
<th>Genotype for X Chromosome</th>
<th>n</th>
<th>Aberrant Projections</th>
<th>Fasciculation</th>
<th>Total</th>
<th>Average (Total/2n)</th>
</tr>
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<tbody>
<tr>
<td>Wild type (+/+ and +/Y)</td>
<td>9</td>
<td>11</td>
<td>9</td>
<td>20</td>
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<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>disco1/+</td>
<td>19</td>
<td>23</td>
<td>22</td>
<td>45</td>
<td>1.18</td>
</tr>
<tr>
<td>FM7c/+</td>
<td>11</td>
<td>14</td>
<td>14</td>
<td>28</td>
<td>1.27</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>disco1/Y</td>
<td>17</td>
<td>56</td>
<td>45</td>
<td>101</td>
<td>2.97*</td>
</tr>
<tr>
<td>FM7c/Y</td>
<td>5</td>
<td>15</td>
<td>24</td>
<td>39</td>
<td>3.90*</td>
</tr>
</tbody>
</table>

Scoring was as in Table 1.

* Statistical significance (*p* < 0.01).
Sensory Axons in disco Mutants

343 mutant appendages [legs \( n = 35 \), wings \( n = 25 \), halteres \( n = 13 \)], and abdomen \( n = 7 \), the morphology and positioning of sensory neurons were indistinguishable from wild type. Axons from these neurons form normal peripheral nerves to the CNS [Fig. 4(C)].

Developmental Time of Lethality

disco mutant and wild-type animals were examined to determine the lethal phase of the disco\(^1\) mutation [see Steller et al. (1987) for previous descriptions]. These data are summarized in Table 3. Of the wild-type controls, only 1 of 88 (1.1%) hatched individuals died before reaching adulthood (table 3), of the 82 hatched disco mutants, 32 (38.6%) died during larval stages, and of the 50 that pupated, 33 (39.8%) eclosed as adults and 17 (20.5%) died as pharate adults (Table 3). In a second trial, 30% (27 in 90) of mutants died as larvae. Earlier reports also show variable lethality (Steller et al., 1987) and suggested that the predominant lethal phase was as pharate adults. Here, we show that approximately one third of mutants die as larvae. These are likely to be those individuals with the most severe defects to the larval sensory system.

Larval Motility

It was suspected that mortality of disco mutants may result from coordination or motility defects. To investigate this, larval motility was tested (Table 4). Each larva was scored twice and the mean values for trial 1 and trial 2 for all individuals of each genotype were consistent (Table 4).

Both male and female disco mutants show significantly lower motility \( (p < .001, t \text{ test}) \) than their FM7c/Y and disco\(^1\)/FM7c siblings. Thus, disco/Y males had an average motility of 10.1 compared with an average motility of 24.6 for FM7c/Y males and 22.2 for wild-type males. disco females (disco/disco) scored 15.3 and 12.3 as compared to 37.2 and 36.9 for wild-type females. Wild-type females (+) performed significantly better that their male counterparts (Table 4), showing that a true indicator of reduced motility is valid only when comparing male mutants with male siblings and female with female. The design of the experiment catered to this. disco\(^1\) males show poor motility compared to their FM7c/Y male siblings, as do disco\(^1\) females with disco\(^1\)/FM7c female siblings.

Figure 3 Pro- and mesothoracic neuromeres, showing the anterior axon projections from the multiscoloporous organ in the anterior abdomen. In wild type (A), these axons project anteriorly in a single tight fascicle (arrow) to terminate near the midline (arrowheads). In the disco mutant (B), these anterior projections are diffuse and at least three separate groups of axons are evident (arrows show two). The terminal processes of these axons are also diffuse [arrowheads; compare (A,B)], and some branch prematurely [bottom pair of arrowheads (B)]. An asterisk marks the position of the anterior dorsal median nerve for reference. Scale = 75 \( \mu \text{m} \).
Table 3 Developmental Stages at Which Mutants Die

<table>
<thead>
<tr>
<th></th>
<th>+ Wild Type</th>
<th>disco1 Mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. embryos plated</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>No. hatched</td>
<td>88</td>
<td>82</td>
</tr>
<tr>
<td>No. unhatched</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>No. unfertilized</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>No. dead embryos</td>
<td>0</td>
<td>1 (1.2%)</td>
</tr>
<tr>
<td>No. pupae</td>
<td>87</td>
<td>50</td>
</tr>
<tr>
<td>No. die as larvae</td>
<td>1 (1.1%)</td>
<td>32 (38.6%)</td>
</tr>
<tr>
<td>No. adults</td>
<td>87 (98.9%)</td>
<td>33 (39.8%)</td>
</tr>
<tr>
<td>No. die as pupae</td>
<td>0 (0%)</td>
<td>17 (20.5%)</td>
</tr>
</tbody>
</table>

Percentages are calculated from the number of fertilized embryos.

DISCUSSION

This work has shown that the central projections of proprioceptive sensory axons in the trunk segments of larvae and adults are disrupted in a disco mutant background. The defects observed are indicative of a general failure in axon growth/guidance and demonstrate that the effects of the disco mutation are not restricted to the central projections of eye neurons. This work extends our knowledge of the disco phenotype and demonstrates interesting parallels with defects previously described in the visual system. Overall, the results suggest that there may be a widespread requirement for Disco during CNS development and that analysis of its role in the ventral nerve cord may provide insights into its developmental role.

Are Defects to the Proprioceptive Sensory System Comparable to Those in the Visual System?

Previous descriptions of the disco phenotype have concentrated on the organization of retinular axons from the eye to the optic lobe (Steller et al., 1987). This revealed two distinct phenotypes. The unconnected phenotype, in which the larval optic nerve (Bolwig’s nerve) fails to connect with the optic lobe, is the most common and results in the failure of adult retinular cells to innervate the optic lobes. In the less common connected phenotype, the larval

![Figure 4](image_url)  
**Figure 4** Peripheral organisation of sensory neurons. (A,B) Proprioceptive sensory neurons in two abdominal hemisegments of wild-type (A) and disco1 mutant male (B) third-instar larvae revealed by X-gal staining of line C161. Anterior is up, v = ventral; l = lateral; d = dorsal sensory neuron clusters; lbd = lateral bipolar dendrite neuron. All sensory neuron clusters are present and in normal spatial arrangement in both wild-type and disco mutant examples. (C) Sensory neurons in the prothoracic legs of a disco1 mutant male revealed by X-gal staining of line C161. All sensory neurons are present and in normal locations. FCO = femoral chordotonal organ; CoHPs = hair plates of the coxa; TrHPs = hair plates of the trochanter. The stained axons from all expressing neurons project as normal to the CNS via the leg nerve. Scale: (A,B) = 60 µm; (C) = 30 µm.
optic nerve does innervate the optic lobe, sometimes ectopically, and the adult retinular cells innervate the optic lobe but show morphological disruption. In this study, we have shown that in the thoracic and abdominal neuromeres, the majority of sensory neurons innervate the CNS normally, although there are signs of missing axons which could be interpreted as axons failing to innervate the ganglion in a manner comparable to the unconnected phenotype. Interestingly, the unconnected axons were most commonly found in the more posterior neuromeres. The vast majority of axons, however, innervate the CNS normally and are comparable to the connected phenotype. These connected axons, like axons in the connected eye phenotype, show variable central morphological defects. Furthermore, the variability of the neuromeres in which defects occur suggests that all neuromeres are susceptible to disruption in disco mutants.

This work therefore provides insight into the general role of Disco in sensory axon development. Whereas the unconnected phenotype is predominant in the eye of disco mutants, in the thorax and abdomen it is rare. We are using unconnected to describe the absence of all axons within a neuromere—for example, all axons in the v-post projection shown in Figure 1 (f). At this level of investigation, it was not possible to determine if one or two axons were absent from a particular projection, although earlier work on peripheral axon growth in embryos by Steller et al. (1987) would suggest that such defects do occur. When we see the unconnected phenotype, it is usually in the terminal neuromeres. This is of interest because the ventral nerve cord, like the cephalic neuromeres, undergoes a morphogenetic change during the latter stages (stage 16) of embryogenesis and shortens with the posterior neuromeres migrating anteriorly. The unconnected phenotype in the eye has been attributed to the breaking of the larval eye optic lobe connection during head involution (Steller et al., 1987; Campos et al., 1995). Thus, the larval photoreceptor axons initially innervate the optic lobe, but owing to forces created during head involution (stage 14), they are unable to maintain this connection. As a result, the axons frequently detach from the optic lobe and project ectopically.

A similar effect may explain the higher incidence of the unconnected phenotype in the terminal abdominal neuromeres. Because of condensation of the CNS, these axons undergo a more extensive migration than their more anterior homologues and are more likely to become detached. One caveat is that a defect of this nature could equally result from initial axon outgrowth abnormalities. For instance, one axon within the nerve may act as a pioneer for others, and if outgrowth of this axon is disrupted, then follower axons may also project incorrectly. To test this, it will be important to look in detail at staged disco\(^1\) mutant embryos to see (a) if initial axon outgrowth to the CNS is normal, and (b) if the peripheral defects previously observed in the trunk segments by Steller et al. (1987) result from detachment of axons from the CNS. This should show if the peripheral defects result solely from target recognition abnormalities in the CNS. Importantly, Steller et al. (1987) showed defects in peripheral axon growth in embryos before condensation of the CNS. Prior to this stage, however, the germ band contracts and it is possible that tensile forces present during this morphogenetic process may cause axon defects in a similar way.

**How Is disco Causing This Effect?**

It is apparent that the defects we see in disco mutants are comparable to those seen in the eye. Are these morphological similarities thus attributable to similar requirements for Disco during the development of these sensory axons? Expression of the Disco protein has been characterized in the optic lobes and ventral nerve cord (Lee et al., 1991). In the optic lobe, Disco is expressed in a small group of central neurons termed optic lobe precursor cells (OLPs). Campos et al. (1995) showed that in wild-type embryos, photoreceptor afferents of the larval optic nerve make early stable contacts with two OLPs (the corner OLPs) prior to their sending an

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**Table 4 Larval Motility Test**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Trial 1</th>
<th>Trial 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± 1 SD</td>
<td>Mean ± 1 SD</td>
</tr>
<tr>
<td>Males disco/Y</td>
<td>15 10.1 ± 11.6**</td>
<td>9.9 ± 8.5*</td>
</tr>
<tr>
<td>FM7c/Y</td>
<td>9 24.6 ± 9.9</td>
<td>24.7 ± 10.9</td>
</tr>
<tr>
<td>Females disco</td>
<td>4 15.3 ± 10.1</td>
<td>12.3 ± 8.6*</td>
</tr>
<tr>
<td>FM7c</td>
<td>6 10.2 ± 4.9*</td>
<td>10.5 ± 6.1*</td>
</tr>
<tr>
<td>disco/FM7c</td>
<td>44 24.3 ± 16</td>
<td>24.5 ± 10.4</td>
</tr>
<tr>
<td>Unknown</td>
<td>2 2.5 ± 0.5</td>
<td>3.0 ± 3.0</td>
</tr>
</tbody>
</table>

Wild type

<table>
<thead>
<tr>
<th></th>
<th>Trial 1</th>
<th>Trial 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males (+/Y)</td>
<td>26 22.2 ± 18.4</td>
<td>26.4 ± 19.3</td>
</tr>
<tr>
<td>Females (+/+ )</td>
<td>14 37.2 ± 26.5</td>
<td>36.9 ± 23.1</td>
</tr>
</tbody>
</table>

Scores are the mean (±1 SD) of the distance traveled (squares/min) of all larvae for a particular genotype in two separate trials.

* Statistical significance (p < 0.01).
axon into the optic lobe. They also showed that in disco mutant embryos, the OLPs do not differentiate normally and the photoreceptor axons cannot make this early stable connection. Consequently, the axons are susceptible to detachment during head involution (unconnected phenotype), arguing that the initial contacts are vital for correct innervation. In the thoracic and abdominal neuromeres, Disco is also expressed in a small group of cells (≈30/neuromere), but no function or identity has been attributed to them (Lee et al., 1991). It may be that disruption of these cells in disco mutants causes defects in the proprioceptive axon projections in a related way. This leads to two questions: Do proprioceptive axons make early stable connections with these Disco-expressing cells? and Are these cells abnormal in mutants? With this knowledge, it should be possible to determine whether these cells perform a function for proprioceptive axon guidance analogous to the OLPs in the optic lobe. Interestingly, axons from the OLPs and photoreceptor axons project together into the optic lobes to reach their central targets. Although the identity of Disco-expressing cells in the ventral nerve cord is unknown, it remains possible that the disordered axon growth phenotype of connected axons reflects a requirement for these cells in central axon guidance, and that there may be disruption of central neurons in addition to sensory neurons.

**disco Mutant Larvae Show Poor Motility**

Steller et al. (1987) showed that in disco mutant larvae, many sensory systems function normally at the behavioral level, e.g., geotaxis and chemotaxis. The only reported behavioral abnormality was blindness due to degeneration of the eyes and optic lobes. In view of the disruption of the thoracic and abdominal neuromeres we have described, we suspected that disco mutants would show poor coordination and locomotion. Our locomotor tests clearly show that disco mutants do have impaired mobility which could be attributable to defects in sensory axons. It is, however, important to ensure that it is not due to disruption of other developmental processes in disco mutants. In this respect, it is important to note that Disco expression is predominately restricted to the embryonic CNS including glial cells (Lee et al., 1991). This suggests that reduced mobility, and ultimately viability, does result from the defects in the CNS, as few other cells express Disco. Whether this is due to disruption of the sensory system alone is less clear, because there could also be as yet unobserved defects in central neurons which could equally cause reduced motility and viability. Obviously, it would be wise to examine the organization of central neurons in the disco mutant background.

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**REFERENCES**


MERRITT, D. J. and WHITINGTON, P. M. (1995). Central projections of sensory neurons in the *Drosophila* em-
bryo correlate with sensory modality, soma position
and proneural gene function. J. Neurosci. 15:1755–
1767.

of a somatotopic map in crickets: the cercal afferent

MURPHEY, R. K., POSSIDENTE, D. R., POLLACK, D. G.,
and MERRITT, D. J. (1989). Modality specific axon
projections in the CNS of the flies Phormia and Dro-

organisation of the central projections of afferents from
tactile hairs on the hind leg of the locust. J. Comp.
Neurol. 312:493–508.

NOTTEBOHM, E., USUI, A., THERIANOS, S., KIMURA, K.,
DAMBLY-CHAUDIERE, C., and GYSEN, A. (1994). The
gene poxn controls different steps of the formation of
34.

PHILLIS, R., STATTON, D., CARRUCCIO, P., and MURPHEY,
R. K. (1996). Mutations in the 8kDa Dynein light-
chain gene disrupt sensory axon projections in the Dro-
sophila imaginal CNS. Development 122:2955–2963.

SHEPHERD, D. and SMITH, S. A. (1996). Central projec-
tions of persistent larval sensory neurons prefigure adult
sensory pathways in the CNS of Drosophila. Develop-
ment 122:2375–2384.

The synaptic origins of receptive field properties in the
cricket sensory system. J. Comp. Physiol. A. 162:1–
11.

SMITH, S. A. and SHEPHERD, D. (1996). The central affer-
ent projections of proprioceptive sensory neurons in
Drosophila revealed with the enhancer-trap technique.

disconnected: a locus required for neuronal pathway
formation in the visual system of Drosophila. Cell
50:1139–1153.