Survival of photoreceptor neurons in the compound eye of *Drosophila* depends on connections with the optic ganglia

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Summary

The importance of retinal innervation for the normal development of the optic ganglia in *Drosophila* is well documented. However, little is known about retrograde effects of the optic lobe on the adult photoreceptor cells (R-cells). We addressed this question by examining the survival of R-cells in mutant flies where R-cells do not connect to the brain. Although imaginal R-cells develop normally in the absence of connections to the optic lobes, we find that their continued survival requires these connections. Genetic mosaic studies with the disconnected (disco) mutation demonstrate that survival of R-cells does not depend on the genotype of the eye, but is correlated with the presence of connections to the optic ganglia. These results suggest the existence of retrograde interactions in the *Drosophila* visual system reminiscent of trophic interactions found in vertebrates.

Key words: trophic interactions, visual system, cell death, retinal degeneration, *Drosophila*.

Introduction

Certain neurons are known to degenerate as a consequence of ablation of their target cells, or after deafferentation. These phenomena, known respectively as retrograde and anterograde transneuronal degeneration (Cowan, 1970; Purves and Lichtman, 1985), reveal the importance of cell-cell interactions for neuronal survival. A number of factors have been shown to mediate target-dependent survival in the nervous system (reviewed by Levi-Montalcini, 1987; Oppenheim, 1989; Barde, 1989).

In invertebrates, various instances of anterograde dependence during neural development have been described (e.g. Anderson, 1978b; Macagno, 1979; Schneiderman et al., 1982). The fruitfly *Drosophila melanogaster* provides a classical example of such an interaction in the development of the imaginal visual system (reviewed by Meinertzhagen, 1973). When eyes are reduced, due to surgical procedures or in certain mutant flies, hypoplasia of the underlying optic ganglia is observed (Power, 1943; Schoeller, 1964). Subsequent mosaic analysis, using eye mutants, demonstrated that innervation has an inductive effect on the development of the optic ganglia (Meyerowitz and Kankel, 1978). In fact, retinal innervation is required not only for the generation of the second order neurons of the first optic ganglia but also indirectly for the maintenance of higher order neurons in the optic lobes (Fischbach, 1983; Fischbach and Technau, 1984). More recently, it has been demonstrated that, in *Drosophila*, the birth of lamina neurons requires retinal innervation (Selleck and Steller, 1991).

In contrast to vertebrates, no example of retrograde dependence during development has been described in invertebrates. It has been shown that sensory organs as well as motoneurons can differentiate in the absence of interaction with their targets (e.g. Sanes et al., 1976; Whittington et al., 1982; Anderson, 1985; Costello and Wyman, 1986). The insect retina is particularly well suited to studies of retrograde dependence given its accessibility and the fact that it is not essential for viability under laboratory conditions. Experiments involving retina transplantation, optic lobe ablation and sectioning of the optic stalk have been carried out in various insects (e.g. Kopec, 1922; Chevais, 1937; Wolski and Wolski, 1971; Mouze, 1978; Anderson, 1978a) and have demonstrated that the retina is able to develop autonomously from the underlying optic ganglia.

In *Drosophila* the best evidence for autonomy of retinal development stems from analysis of supernumerary eyes present in flies carrying mutations in the extra-eye (ee) gene. These structures send projections that never reach the optic ganglia (Marcey and Stark, 1985). Morphological analyses of these extra eyes at the electron microscopy level showed that they develop appropriately, displaying the characteristic arrangement of photoreceptor cells seen in normal compound eyes (Marcey and Stark, 1985). However, these studies
did not address the importance of retrograde trophic input for the maintenance of the fully differentiated retina. Here, we investigate this problem by using recessive mutations which prevent the connections of the photoreceptor cells with their target cells in the optic lobes of the fruitfly *Drosophila melanogaster*. We examined the maintenance of the photoreceptor cells in flies mutant for either the *disconnected* (disco) gene or the *ee* gene.

Previous experiments have revealed that in disco mutants the imaginal R-cells form a relatively normal retina but generally fail to reach their target cells; the optic lobes are extremely reduced in these cases (Steller et al., 1987). Mosaic analysis demonstrated that this “unconnected” phenotype occurs independently of the genotype of the compound eye. Here we show that R-cells degenerate after eclosion in these “unconnected” disco flies. Using genetic mosaics, we demonstrate that the genotype of the retina does not affect R-cell degeneration, which is strictly correlated with the presence of connections to the optic ganglia. In ee flies, while the normal eye retains its morphology with age, R-cells in the supernumerary eyes progressively degenerate. We conclude that, while retinal differentiation proceeds in the absence of connections with the brain, R-cell survival after eclosion requires interactions with the underlying optic ganglia. We propose the existence of trophic interactions between the optic ganglia and the photoreceptor cells in the retina.

**Materials and methods**

*Fly stocks*

All flies were raised on standard cornmeal-sugar-agar-yeast medium supplemented with fresh yeast. *Drosophila* cultures were grown at 19°C or 25°C and 60-65% humidity. A mutation in the gene disconnected (disco) was used (Steller et al., 1987). This chromosome carried the visible mutations for the genes white (w) and forked (f) unless otherwise stated. These mutations confer white eyes and abnormal bristle morphology respectively which can be easily scored under the dissecting microscope. The extra-eye (ee) mutation was kindly provided by William Stark. The wild-type control used carried mutations for the genes yellow (y) and white (w).

*Generation of Mosaics*

Gynandromorphs were constructed using the unstable ring X chromosome (R(1)wvC, Hall et al., 1979). R(1)wvC/Binsn females were crossed to *w disco* /+*F* males. The mutant patches in the mosaic progeny were recognized with the aid of visible markers (w and f).

*Histology*

Fly heads were fixed for 1 hour in 1% glutaraldehyde, 2% paraformaldehyde in 0.1 M sodium phosphate buffer pH 7.4. The heads were postfixed in 1% osmium tetroxide in the same buffer, dehydrated and embedded in standard SPURRS medium (Spurrs, 1969). Semithin sections (0.5 to 1 μm) were mounted in Permout and inspected under phase-contrast microscopy. Cryostat sections of adult heads were prepared for mAb24B10 staining essentially as described in Steller et al., 1987. β-galactosidase activity staining on cryostat sections was performed as described by Mismer and Rubin (1987).

**Electroretinograms**

ERGs were recorded extracellularly essentially as described by Rendahl et al., 1991. For these experiments, flies less than a day old were used.

**Results**

*Photoreceptors in unconnected disco flies progressively degenerate after eclosion*

Previous studies have established that the development of the photoreceptor cells of the *Drosophila* retina proceeds independently from that of the underlying target cells (Chevais, 1937; Marcey and Stark, 1985). In the present study, we examined the role of the target cells for the proper maintenance of the photoreceptor cells after development is completed. In order to address this question, we assessed the integrity of retinula cells of adult flies which do not innervate the underlying optic ganglia. In flies mutant for the X-linked disco gene, the imaginal photoreceptor axons fail to reach their target cells during the third larval instar, which consequently disrupts optic lobe development (Steller et al., 1987). The phenotype of disco flies at various developmental stages has been described and discussed elsewhere (Steller et al., 1987). Briefly adult flies carrying mutations for the disco gene typically show only a rudiment of the optic lobes (“unconnected” phenotype). No structure resembling the first optic ganglia (lamina) is ever detected in unconnected flies (Steller et al., 1987). Instead the photoreceptor cell axons frequently terminate in a mass of muscle tissue which often but not always replaces the optic ganglia (Fig. 1B). In other cases the optic lobe in disco is replaced by hemolymph or non-neuronal cells of unknown identity. Externally these flies can be recognized by the deformity of their compound eyes. In some flies, during the third larval instar, the retinular axons of one or both eye imaginal discs are able to establish connections with the developing optic lobes (“connected” phenotype). In these cases, adult flies have optic ganglia of almost normal size, however, their structure is abnormal and clearly distinct from wild type (Fig. 1C and D). The variability in the morphology of the lamina in connected disco flies is illustrated by the two examples shown in Fig. 1C and D. In a homozygous disco* stock, approximately 90-95% of the flies have visual systems of the unconnected type and 5-10% have the connected type.

Adult flies hemizygous for the disco* mutant allele were aged at 25°C. Their heads were embedded in plastic and semithin sections were inspected under phase-contrast microscopy. The specimens were sectioned in a horizontal plane from dorsal to ventral such that the most superficial sections represent transverse sections of the most dorsal ommatidia. In all specimens, deeper sections were also obtained such that the morphology of the ipsilateral optic ganglia could be
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Fig. 1. Optic lobe defects in disco flies. All of the photomicrographs in this figure show horizontal semithin plastic sections of adult fly heads under phase-contrast microscopy. They are representative of the various types of optic lobes underlying the compound eyes analyzed in this study. (A) Wild-type optic lobe showing the organized neuropil centers of the Drosophila visual system. (B) Optic lobe area from a disco mutant displaying the unconnected phenotype. In these flies, the space normally occupied by the optic ganglia is replaced by non-neuronal tissue. The R-cell axons do not establish any connections with their normal target cells. (C and D) Optic lobes from disco mutant flies displaying the less severe connected phenotype. Although the optic lobe is almost normal size, its structure is clearly disorganized. In connected disco flies, the pattern of projection from the R-cells is almost normal (Steller et al., 1987 and Fig. 4).

Abbreviations: la, lamina; me, medulla; lo, lobula; lp, lobula plate. Scale bar is 20 µm.

unambiguously assessed for each of the sectioned compound eyes (as shown in Fig. 1B, C and D).

Fig. 2 shows cross sections of ommatidia from a newly eclosed (panel B) and from a 15-day-old disco fly (panel C); both flies exhibited the unconnected phenotype (Fig. 1B). Examination of ommatidia in newly eclosed flies indicates that development of the retina is not significantly impaired by absence of interaction with the underlying optic ganglia (Fig. 2B). The normal number of R-cells per ommatidium is present, and these cells appear to have terminally differentiated, as judged by the expression of photoreceptor-specific genes (like chaoptin and rhodopsins, Figs 4, 8 and data not shown), and the fact that newly eclosed unconnected disco flies are able to be depolarized in response to a light stimulus (see Fig. 6D).

In 15-day-old flies only a few R-cells are found in each ommatidium (Fig. 2C). The presence of pigment granules in a pattern resembling the one found in young disco flies suggests that, at this stage in the degenerative process, the pigment cells surrounding the R-cells have largely maintained their integrity.

These observations confirm and extend previous results that retina development proceeds largely inde-
Fig. 2. Photoreceptor cell degeneration in disco mutants. The photomicrographs in this figure show cross sections of the most dorsal area of adult compound eyes viewed under phase-contrast microscopy. (A) Ommatidia from a wild-type fly carrying the eye color mutation white (w); ommatidia in all of the subsequent panels are from flies carrying the wild-type w+ gene. Each ommatidium contains 20 cells, 8 of which are photoreceptor neurons (R-cells). The rhabdomeres of these R-cells are seen as dark spots and are arranged in a trapezoidal pattern. Because the inner R-cells, R7 and R8, are stacked on top of each other, only 7 rhabdomeres can be seen in any given plane of section. The boundary of each ommatidial unit is outlined by the membranes of the pigment cells. Deeper sections were always obtained in addition to these shown here in order to assess the presence of connections to the optic ganglia. (B) Ommatidia from a disco mutant that had eclosed approximately two hours prior to fixation. At this stage, R-cells are present and the typical number of rhabdomeres can be identified in spite of the slight irregularity of the ommatidial units. In this and the following specimens no connections to the optic ganglia could be found. (C) Ommatidia from an unconnected disco fly which was aged for 15 days at 25°C before sectioning. Only a few rhabdomeres can be found in most of the ommatidia. In this field, only one ommatidium still contains five rhabdomeres (arrow). The remaining rhabdomeres seem to correspond to both outer and inner R-cells. (D) Ommatidia from a connected disco mutant which was aged for 15 days at 25°C. Note that the rhabdomeres in each ommatidium are found in the correct number and are properly positioned. Scale bar is 5 μm.

Genetic mosaic analysis of photoreceptor cell survival in disco flies

Previous mosaic analyses demonstrated that the unconnected phenotype in disco flies is independent from the genotype of the compound eye regarding disco mutant alleles (Steller et al., 1987). However, these studies did not specifically address the requirement of disco gene function for R-cell survival. It remained possible that the R-cell degeneration in disco flies is due to lack of connections with the optic ganglia but is rather due to the requirement of disco gene function in the retina. In order to distinguish between these two alternatives, we examined the retinas of gynandromorphs generated by the unstable ring X chromosome system (Hall et al., 1976).

Mosaic flies were aged at 25°C for at least 10 days. The mutant disco chromosome carried a mutated copy of the white (w) eye color marker in order to assess the genotype of the cells in the eye. Adult heads of genetic mosaic animals were embedded in plastic as described in Materials and methods and horizontal semithin sections were inspected under phase-contrast microscopy. The presence of connections between R-cells and the optic lobe was assessed as described in the previous section.

Fig. 3A shows a section through a compound eye which was entirely mutant for disco but whose R-cells projected to a phenotypically wild-type optic lobe. In such cases, no degeneration of R-cells could be detected. This was in sharp contrast to the massive retinal degeneration seen in compound eyes of mosaics independently from interactions with the optic ganglia. However, they suggest that survival of R-cells is dependent on interactions with the optic ganglia.
where the underlying optic lobe displayed the unconnected phenotype (Fig. 3B, C). In these cases, R-cells degenerated irrespective of whether cells in the compound eye were mutant (Fig. 3B) or wild type (Fig. 3C) for disco function. Finally, no retinal degeneration was detected in compound eyes of mixed genotype as long as R-cells had established connections with the underlying optic lobe (Fig. 3D). Clones of disco cells in otherwise wild-type and connected eyes showed completely normal morphology in aged flies. In contrast, the contralateral eye of the fly shown in Fig. 3D did not connect to the optic ganglia and had suffered retinal degeneration (Fig. 3B). This demonstrates that R-cell degeneration in disco mutants does not depend on the genotype of cells in the eye, and is strictly correlated with the failure of R-cells to project to the optic ganglia.

Fig. 3. Degeneration of R-cells in disco mosaics. All of the panels in this figure show cross sections of the most dorsal area of adult compound eyes viewed under phase-contrast microscopy. All specimens shown in this figure have been aged for at least ten days at 25°C prior to fixation. The mutant patches were recognized by the absence of pigmentation; the disco chromosome used in these experiments carries a mutation in the white gene. (A) Ommatidia from a compound eye entirely mutant for the disco gene; this eye is associated with an ipsilateral optic lobe indistinguishable from wild type. No degeneration can be seen. (B) Ommatidia from a compound eye entirely mutant for the disco gene; the ipsilateral optic ganglion in this fly is of the unconnected phenotype. Massive degeneration can be observed (compare to Fig. 2C). (C) Ommatidia from a compound eye entirely wild type for the disco gene. The ipsilateral optic ganglion in this fly is of the unconnected phenotype. Despite the presence of disco* function in the entire retina, the degeneration observed is indistinguishable from that found in compound eyes that are entirely mutant for the disco gene (compare to Fig. 3B). (D) Ommatidia from a compound eye of mixed genotype. The ipsilateral optic ganglion in this fly is indistinguishable from wild type. No degeneration can be detected in this specimen. The arrowhead points to an ommatidium of mixed genotype. The arrow indicates an ommatidium of wild type. Scale bar is 5 μm.
We conclude that R-cells depend on interaction with the optic ganglia for survival after eclosion but do not autonomously require disco gene function for survival.

**Retinal degeneration in disco flies is rescued by the establishment of connections with disorganized optic lobes**

The results presented above strongly indicate that the R-cell degeneration observed in disco flies results from the lack of connections with the optic lobe. In about 5-10% of disco flies R-axons project to an optic lobe which is almost normal size but is significantly disorganized (the connected phenotype; Steller et al., 1987). The connections between the eye and the optic ganglia can be visualized in cryostat sections which have been stained with an antibody specific for the photoreceptor cells. Fig. 4 shows horizontal sections through heads of wild-type, disco connected and disco unconnected flies stained with the monoclonal antibody against the photoreceptor-specific protein chaoptin (Zipurski et al., 1985; Fig. 4 panel A, C and D respectively). A neuropil pattern resembling that of lamina and medulla of wild-type flies is found in connected disco flies (Fig. 4B,C). However in connected flies the overall morphology is significantly different from wild type as opposed to the virtual lack of optic ganglia in the unconnected phenotype (Fig. 4D). We were interested in determining whether the connections found in connected disco flies are able to prevent retinal degeneration.

Newly eclosed disco' flies of the connected type were selected based on the presence of a deep pseudopupil (Franceschini, 1972). These flies were kept at 25°C for at least two weeks after which the deep pseudopupil phenotype was reassessed. In 139 out of 145 eyes examined (96%) the deep pseudopupil was retained, suggesting that no degeneration had occurred. This conclusion was confirmed by semithin sections of these specimens. Connected compound eyes from disco flies did not display any signs of degeneration at that level of resolution. Fig. 2D shows a cross section through the compound eye of a disco fly which has been aged for 15 days. The morphology of R-cells and their rhabdomeres, and ommatidial structure in general is indistinguishable from wild type. In such cases, we often found that the contralateral eye displayed the uncon-
nected phenotype and had suffered severe retinal degeneration (data not shown).

**Retina-lamina connections in disco connected eyes**

The results described in the previous section demonstrated that R-cells can survive in disco mutants if they project to the optic ganglia (connected phenotype). Although the overall morphology of the optic ganglia is distorted in connected disco mutants, these flies possess a lamina which is organized into cartridges that appear relatively normal (Fig. 5A and 5B). However, we could not determine from this analysis if all elements of the lamina are present in connected disco flies, and whether these elements are still functional.

In order to investigate if R-cells can establish functional synaptic connections with the lamina in connected disco, we performed electroretinograms (ERG). ERGs measure the mass electrical response of the eye consisting of the total extracellular potential produced by retinula cells and by postsynaptic neurons in the lamina. The ERG waveform of a wild-type fly consists of a positive on-transient, a negative sustained potential and a negative off-transient (arrow heads). These transient potentials are observed at the beginning and end of stimulation. Newly eclosed disco flies of the connected phenotype as judged by the presence of a deep pseudopupil were used to record ERGs. A total of 12 ERGs were recorded. A fraction of these (n=4) had both transients (panel B) while the rest had only the OFF or a very small ON transient (n=3), (panel C) or neither one of the transient potentials (n=4) (not shown). In all cases the negative sustained potential was present. The scale bar in panel A is valid for panels B and C as well. (D) A typical ERG recording from a young unconnected disco fly. The arrows indicate when the light was turned on and off respectively. A total of three specimens were recorded. In all samples no transient potentials were observed. The presence of a negative sustained potential, albeit considerably smaller than that of wild-type flies, demonstrates that the R-cells in unconnected disco flies are able to respond to a light stimulus.

![Fig. 5. Lamina structure in disco adults of the connected phenotype. Semithin horizontal section through the lamina ganglion of a wild type (A) and connected disco (B) fly. In connected disco flies, the lamina cartridges can be readily identified. The overall structure of lamina cartridges in connected disco flies is very similar to wild type at that level of resolution. Scale bar is 10 \( \mu \)m.](image)

**Fig. 6. Electroretinogram recordings from disco flies.** (A) A typical electroretinogram (ERG) waveform of a wild-type fly consists of a positive on-transient, a negative sustained potential and a negative off-transient (arrow heads). These transient potentials are observed at the beginning and end of stimulation. Newly eclosed disco flies of the connected phenotype as judged by the presence of a deep pseudopupil were used to record ERGs. A total of 12 ERGs were recorded. A fraction of these (n=4) had both transients (panel B) while the rest had only the OFF or a very small ON transient (n=3), (panel C) or neither one of the transient potentials (n=4) (not shown). In all cases the negative sustained potential was present. The scale bar in panel A is valid for panels B and C as well. (D) A typical ERG recording from a young unconnected disco fly. The arrows indicate when the light was turned on and off respectively. A total of three specimens were recorded. In all samples no transient potentials were observed. The presence of a negative sustained potential, albeit considerably smaller than that of wild-type flies, demonstrates that the R-cells in unconnected disco flies are able to respond to a light stimulus.
transients were either very small or not detectable. This variability is in contrast to the survival of R-cells in the vast majority (96%) of the connected disco flies examined. It is possible that the abnormal morphology of the optic ganglia impairs the recording of the lamina neuron response due to shunting. Therefore, functional connections may be present even in these cases where small or no transients were recorded. These results demonstrate that functional connections between eye and lamina can be established in at least some of the connected disco animals.

Photoreceptors in ectopically located eyes degenerate after eclosion

The findings described in the previous sections indicate that the dependence of the R-cells on connections with the optic ganglia is a general phenomenon and not specific to disco mutant alleles. Therefore, we expected that other mutations that prevent the interaction between the retinal cells and the optic ganglia would also cause degeneration.

We tested this hypothesis by analyzing extra-eye (ee) mutants. The ee mutation causes the appearance of supernumerary eyes, on the dorsal side of the adult head. These structures develop properly and send projections that never reach the optic ganglia or the central brain and which terminate as plexuses within a disordered tissue mass composing predominantly of muscle cells, trachea and axon bundles (Marcey and Stark, 1985). Fig. 7 shows cross sections of ommatidia from supernumerary eyes of flies just after eclosion (panel A) or 15 days after eclosion (panel B). As an internal control, we inspected the ommatidia from the regular compound eye (Fig. 7C) of the same fly as shown in panel B.

As determined previously (Marcey and Stark, 1985), the ommatidia of the supernumerary eyes from young flies (Fig. 7A) show the usual characteristics of a fully developed retina. There are in general seven rhabdomeres in any cross-section through each ommatidium. They are arranged somewhat irregularly in a pattern very similar to the one seen in eyes of young disco flies (Fig. 2B). In contrast to young flies only a few rhabdomeres can be found per ommatidium in ectopically located eyes of aged flies (Fig. 7B). Again, as in disco flies, the pigment granules can be seen in a pattern resembling that of young ee flies, suggesting that the pigment cells surrounding the photoreceptor cells have remained after degeneration of the R-cells. The ommatidia of the normal compound eye in older ee flies are indistinguishable from wild type (Fig. 7C), indicating that the R-cell degeneration observed in the supernumerary eye is not due to lack of ee gene function in R-cells per se.

Degeneration affects both inner and outer R-cell types

The results presented above indicated that most of the R-cells degenerate in the absence of connections to the optic ganglia. However, we could not determine from these experiments whether all R-cell types, i.e. both inner and outer R-cells, are affected. Since a few R-cells are typically present in aged unconnected eyes, it was possible that these represent the R7 and/or R8 cell type. A different behavior of inner versus outer R-cells would not be entirely unexpected, because their axons terminate in different ganglion layers of the brain. We addressed this question by examining the expression of rhodopsin-lucZ fusions which are specifically expressed in different R-cell types (Mismer and Rubin, 1989;
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Fig. 8. Expression of Rhl and Rh4 in aged disco flies. In order to determine if the R-cell degeneration observed in unconnected disco flies affected both inner and outer R-cells, we analyzed the expression of an Rhl-lacZ fusion gene and an Rh4-lacZ fusion gene in aged (at least 20 days) disco flies of the connected and unconnected phenotype. In wild-type Rhl (A, B and C) is expressed in all outer R-cells while Rh4 (D, E and F) is expressed in ~30% of R7. (A) Wild type, (B) disco connected, (C) disco unconnected, (D) Wild type, (E) disco connected, (F) disco unconnected. Expression of either Rhl or Rh4 is not affected in connected disco flies (panels B and E respectively). Expression of Rhl and Rh4 is equally reduced in unconnected disco flies (panels C and F, respectively). Panel F shows the mirror image of the contralateral eye from the same fly shown in panel E. Abbreviations: re, retina; la, lamina; me, medulla. Scale bar is 50 μm.

Fortini and Rubin, 1990). A fusion between the Rhl-promoter and lacZ specifically stains outer R-cells (R1-6, Mismer and Rubin, 1989), and a Rh4-promoter-lacZ fusion labels a subset of R7 cells in the retina (Fortini and Rubin, 1990). These reporter gene constructs were introduced into a disco mutant background by crossing males which were homozygous for the rhodopsin-lacZ fusions to disco females. The male offspring of this cross are hemizygous for disco and heterozygous for the element containing the rhodopsin-lacZ fusion. These males were aged on average for 20 days at 25°C, sectioned and stained for lacZ activity. Both unconnected and connected compound eyes were obtained in these studies. Because we typically did not detect any degeneration in the retina of connected disco flies (see for example Fig. 2D), the lacZ expression in these specimens was used as an internal control for the effect of aging. In aged unconnected eyes, the intensity of the lacZ staining was significantly decreased for both the Rhl- and Rh4-lacZ fusion constructs (Fig. 8). The reduction in the lacZ activity appears more prominent towards the base of the retina. We conclude that degeneration is not restricted to the R1-6 cell type, but also affects R7 cells. We also used northern analysis to examine the steady state levels of mRNA from Rhl (Zuker et al., 1985, O'Tousa et al., 1985), and another rhodopsin gene expressed in R7 cells, Rh3 (Montell et al., 1987). The levels of both Rhl and Rh3 message were decreased in aged unconnected disco flies compared to young flies (data not shown). Taken together
these data suggest that both outer R-cells and at least one of the inner R-cell types, R7, degenerate in the absence of connections to the optic ganglia.

Discussion

Many instances of retrograde transneuronal degeneration, both during and after neural differentiation is completed, have been described in vertebrates (reviewed by Cowan, 1970; e.g. Hamburger, 1934; Hamburger and Levi-Montalcini, 1949; Crews and Wiggston, 1990). In contrast, only examples of anterograde dependence have been reported in invertebrates. This study provides evidence for the existence of retrograde transneuronal dependence in the adult visual system of the fruitfly Drosophila melanogaster.

We determined the integrity of retinular cells in situations where the photoreceptor cell axons do not reach the optic ganglia. For this, we examined cross sections of ommatidia from flies carrying mutant alleles for either the disconnected (disco) or the extra-eye (ee) gene. In aged flies, extensive R-cell degeneration was found in eyes that did not make connections with the optic ganglia. Flies that were aged in complete darkness showed the same degree of degeneration (data not shown). This degeneration is apparently restricted to the photoreceptor cells since non-neuronal support cells, e.g. cone cells (data not shown), and pigment granules of the pigment cells, are present well after the time when R-cells have degenerated. In most ommatidia only a few rhabdomeres are left 15 days posteclosion. This degeneration was shown to occur in all outer R-cells (R1-6), and in R7.

Our mosaic studies demonstrate that disco+ gene function is not required in the retina for the survival of R-cells. The ability of R-cells to survive is strictly correlated with the presence of connections to the optic ganglia. This conclusion is also supported by the observation that in non-mosaic disco animals degeneration is only observed in flies of the unconnected phenotype. Finally recent experiments using antibodies against disco protein product demonstrated that the disco gene is not expressed in the retina, and is only detected in a relatively small number of brain cells during the late larval, pupal and adult stages (Lee et al., 1991; Lee and Steller, unpublished observations). Taken together, these observations indicate that R-cells in the compound eye of Drosophila depend on interactions with cells outside the retina for continued survival after eclosion.

The results discussed above also confirm previous reports that retinal differentiation does not depend on interactions with the optic ganglia (Kopeck, 1922; Chevais, 1937; Wolski and Wolski, 1971; Mouze, 1978; Anderson, 1978a). Although the compound eyes of disco individuals are not completely normal at eclosion, displaying somewhat deranged positions of rhabdomeres and pigment cells, we have every indication to believe that R-cells successfully complete their terminal differentiation program in disco mutants. First, the normal structural features of the different cell types constituting an ommatidium are found in disco mutants, and terminal differentiation markers, like opsin, are properly expressed. In addition, our results indicate that functional photoreceptor cells can develop in the absence of connections with the optic ganglia: R-cells from newly eclosed unconnected disco flies are able to respond to a light stimulus, demonstrating that a functional phototransduction machinery has been correctly assembled. We believe that the imperfect eye geometry in disco is associated with the variable amount of deformations previously caused by the lack of an optic stalk (Steller et al., 1987). Due to defects in the eye's curvature, the precise alignment of retinal cells, most noticeably the rhabdomeres and pigment cells, is slightly out of order.

The retinal axons of unconnected disco eyes are sometimes seen contacting muscle tissue present in the space otherwise occupied by the optic ganglia. It could be argued that the degeneration observed in these cases was caused by a cytotoxic effect from the muscle cells. However, this explanation of our results appears very unlikely, since only some R-cells in disco mutants project to muscle tissue. In addition, we have seen at least 20 cases where the optic ganglia were replaced by either hemolymph or non-neuronal cells of unknown identity, yet R-cells degenerated in these cases as well. Therefore, degeneration cannot be a consequence of ectopic contacts between retinal axons and muscle tissue. Furthermore, the poisonous action of a putative diffusible toxin would somehow have to be restricted to the retina, since other neurons which are in direct contact with the muscle tissue or hemolymph, for example neurons of the central brain, continue to survive and function for long times. Finally, in extra eye mutants, which have normal optic lobes, retinal degeneration affects only the supernumerary, unconnected eyes, not the regular compound eyes. Therefore, it appears that R-cell degeneration is not due to cytotoxic effects from degenerating cells or contact with ectopic muscle tissue, but is caused by the lack of interactions with cells in the optic ganglia.

We have several reasons to believe that the proposed trophic support is contributed to the retina by cells in the optic ganglia, and not in some other tissue outside the visual system. Since the space underlying the eye is entirely occupied by the optic ganglia, any tissue outside the visual system would have to produce a factor(s) that can act over a considerable distance. However, such long-range effects are not consistent with the striking left/right asymmetries that we frequently observe in disco mutants or mosaics; we have observed at least 50 cases where retinal degeneration was restricted to only one (unconnected) eye, but did not affect the ipsilateral (connected or wild-type) side. This argues strongly against any long-range diffusible factor, since it is very difficult to imagine how the action of such a factor could always be restricted to the eye on that side that lacks the optic ganglia. Finally, retinal degeneration in the extra eye mutant affects only the supernumerary eyes but not the adjacent regular
compound eyes. We conclude that R-cell survival depends on interactions with the optic ganglia, and not the presence of some other structure(s) outside the visual system.

The results described in this paper do not reveal which cell type(s) in the optic ganglia is responsible for survival of photoreceptor cells. In particular, we do not know if neuronal or glial elements of the optic ganglia provide the proposed trophic support for photoreceptor cells. Although functional connections between R-cells and their target neurons in the optic lobe can be found in disco mutants, it is possible that these connections are not essential for R-cell survival. A significant portion of connected disco flies fail to show an optic lobe response to a light stimulus (i.e. they lack on and off-transients). Nevertheless, R-cells survived in the majority of connected disco flies examined. The caveat for such observations is that abnormal morphology of the optic ganglia may impair the recording of the transient components of the ERG tracing due to shunting. Since ERGs measure the overall electrical response of the eye and lamina with respect to a reference point in the body, the ability to obtain a normal ERG waveform depends on the existence of proper electrical barriers between the retina and the brain, and also on barriers within the different elements of the optic ganglia (Heisenberg, 1971; Shaw, 1977). We believe that these barriers are severely reduced in unconnected and also many connected disco animals, which display significantly disorganized optic ganglia (see Steller et al., 1987). Such a lack of proper electrical insulation, or "shunting", may account for both the reduced amplitude of the depolarization potential, and also the observed lack of on and off-transients.

We have not determined the degree of degeneration of the optic ganglia in the few connected disco flies that lost the deep pseudopupil after two weeks (6 out 145 animals, corresponding to ~4%). A more careful analysis of such cases may aid in establishing a correlation between optic lobe structure and survival of the R-cells. Mutations that cause well-defined defects in the optic lobe structure will be particularly useful in elucidating the underlying physiological and cellular mechanisms that play a role in the maintenance of the retina.

Glia cells are thought to play a diverse role in the invertebrate nervous system. A supportive role for glial cells has been reported in the crayfish where transglial channels have been implicated in the long-term survival of the distal stump of severed medial giant axons (Shivers and Brightman, 1976; Shivers, 1976; Meyer and Bittner, 1978a,b). Glial cells have also been suggested to be mediators in the induction of glomeruli by afferent axons in the olfactory system of the hawkmoth Manduca sexta (reviewed by Tolbert and Oland, 1989).

In the fly visual system, several types of glial cells which are intimately associated with the photoreceptor axon have been described (Trujillo-Cenoz, 1965; Saint Marie and Carlson, 1983). A subset of these cells establish specialized axo-glial associations called capi-

tate projections (Trujillo-Cenoz, 1965; Stark and Carlson, 1986; reviewed by Lane, 1981). These structures arise from the association of the epithelial glial cell with axons from the outer photoreceptor cells (R1 to R6), and it has been previously proposed that they might be involved in trophic interactions between the lamina and photoreceptor axons from R1-6 (Stark and Carlson, 1985, 1986). However, no direct experimental evidence for such a function is presently available.

In conclusion, the experiments described in this paper demonstrate the dependence of R-cells on interactions with the optic ganglia for survival after development is completed. These interactions are reminiscent of trophic interactions widely studied in vertebrates. Genes that encode molecules involved in the proposed trophic interaction should be uncovered by mutations that cause light-independent retinal degeneration. These genes may be involved in the expression of the signal or the receptor for the proposed interaction. Alternatively, this interaction may be mediated by a variety of molecules and cell interactions. In this case only mutations that completely abolish the optic ganglia will cause retinal degeneration.

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