CELLULAR INTERACTIONS AND PATTERN FORMATION IN THE DEVELOPMENT OF THE VISUAL SYSTEM OF *DAPHNIA MAGNA* (CRUSTACEA, BRANCHIOPODA)

II. Induced Retardation of Optic Axon Ingrowth Results in a Delay in Laminar Neuron Differentiation¹

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Abstract

Groups of embryonic photoreceptors in one side of the compound eye of *Daphnia* were irradiated with an ultraviolet microbeam at a stage when the cells were postmitotic but had yet to elaborate axons. Immediately after irradiation, the embryos were placed under fluorescent illumination. On the average, 16 of the irradiated photoreceptors were killed by the exposure. Previous observations suggest that an approximately equal number were rescued by the post-irradiation fluorescent illumination. The schedule of differentiation of the rescued photoreceptors was affected such that their axons arrived at the target region in the optic lamina from 2 to 10 hr after they would have normally. Serial section electron microscopic analysis showed that differentiation of laminar neurons contacted by the delayed axons also was delayed by a length of time corresponding to the delay in axon arrival. These and previous observations indicate that the differentiation of laminar neurons is triggered by contact with optic axons and can be initiated over a period of several hours after these cells become postmitotic.

It has been amply demonstrated that the eye exerts a strong influence on the development of visual centers in the central nervous system of both vertebrates and invertebrates. The experimental evidence (reviewed by Jacobson, 1978) shows that optic nerve fibers affect the survival, growth, and differentiation of central target neurons but probably not their proliferation. Although, in general, removal of afferent inputs eventually results in tissue atrophy and cell degeneration, significant species differences are observed in the extent to which target cell differentiation is affected. For example, if innervation is withheld by early eye ablation, target regions differentiate extensively in avian optic tecta (Kelly and Cowan, 1972; Whitehead, 1979) but much less in fish (Schmatolla, 1972) and amphibian (Eichler, 1971; Currie and Cowan, 1974) tecta. In arthropod optic ganglia, target neurons show some evidence of morphological differentiation when deprived of interactions with optic axons in the

butterfly *Danaus* (Nordlander and Edwards, 1969) but none in *Daphnia* (Macagno, 1978, 1979) or the locust (Anderson, 1978). In this report, I present further evidence that differentiation of target cells is triggered directly by growing optic axons in the crustacean *Daphnia magna*.

The single, bilaterally symmetric compound eye of *Daphnia* consists of 22 ommatidia, each containing 8 photoreceptors. The more lateral ommatidia on each side begin to mature first (at about 30 hr of embryonic development) and the more medial ommatidia mature later. As a consequence of this spatiotemporal organization of eye development, optic axons arrive at the target region in the optic ganglion (the lamina) in a temporal sequence which reflects the mediolateral positions of the ommatidia from which they originate (Lo Presti et al., 1973). During normal development, laminar neurons begin to differentiate morphologically a short time after they are contacted by growing optic axons, and a mediolateral gradient of differentiation also is observed in the lamina (Lo Presti et al., 1973, 1974). Deletion of ommatidia leads to a situation in which some laminar neurons are not contacted by growing optic axons. These laminar cells do not begin to differentiate morphologically when they would normally do so and soon degenerate (Macagno,

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1978, 1979). In the experiments reported here, the growth of some optic axons was delayed as a consequence of ultraviolet (UV) irradiation, causing a corresponding lag in the time of contact between these axons and target cells. The results show that the differentiation of target cells also is delayed by a corresponding amount of time.

Materials and Methods

The experimental procedures used were essentially the same as those described in detail in a previous paper (Macagno, 1979) and will only be summarized here.

The 20 embryos used in these experiments were a single parthenogenetic brood of an adult female selected from a continuous laboratory culture maintained at $22 \pm 1^\circ\text{C}$. The embryos were staged in terms of the number of hours after the extrusion of the eggs from the ovaries into the brood pouch (Lo Presti et al., 1973). At 24 hr, the embryos were removed from the brood pouch and placed in a Petri dish for further development *in vitro*.

The embryos were irradiated individually with a UV microbeam (Macagno, 1979) between 29.5 and 30.5 hr of development. At this stage, all of the photoreceptors in the compound eye are postmitotic. The irradiated photoreceptors were those occupying the two rows of ommatidia closest to and on the left of the midplane of the eye. At the time of irradiation, these cells had yet to begin to elaborate axons. Irradiation lasted 15 sec and was followed immediately by continuous exposure to fluorescent light for photoreactivation (Cook, 1970). Four control animals were treated identically except that wavelengths in the microbeam below about 365 nm were attenuated by a glass filter. In addition, since the irradiation was unilateral, the unexposed side of the eye served

as a built-in control for each experimental animal. Both control animals and control halves of irradiated animals were found to be normal.

To observe the process of recovery of the exposed cells, groups consisting of four experimental animals and one control each were fixed at approximately 12, 15, 18, and 25 hr after irradiation. Standard procedures were used for fixation, dehydration, and embedding (Lo Presti et al., 1973). Two or three animals from each group then were thin-sectioned serially (at about 90 nm) for electron microscopy, and one or two animals were thick-sectioned (at 1 μm) for light microscopy. Complete series through the compound eye were obtained for each of the thick-sectioned specimens. The photoreceptors were counted and the appearance of shielding pigment granules was assessed on serial light micrographs of each of these specimens. For thin-sectioned specimens, the series extended through the eye and optic ganglion. Thin sections were photographed at various magnifications in a Zeiss EM9-2S electron microscope. The number of bundles of optic axons reaching the lamina and the number of axons per bundle were determined from low power electron micrographs, and the state of differentiation of both photoreceptors and laminar cells was examined on higher power micrographs.

Results

Number of photoreceptors that survive UV irradiation. A previous paper (Macagno, 1979) reported that, in animals receiving a 20-sec exposure to the UV microbeam, an average of 141 photoreceptor axons (out of a total of 176) reach the lamina in the adult if post-irradiation illumination is used, but an average of only 104 reach

TABLE I

Delayed pigment maturation, number of photoreceptors in the compound eye, and number of photoreceptor axons and axon bundles found at the lamina as a function of embryonic stage

Specimen	Stage Fixed	Section Thickness ^a	Number of Photoreceptors ^b		Number of Axons (Axon Bundles) ^c	
			Control Side	Irradiated Side	Control Side	Irradiated Side
Control	hr	Thin	88	88	88 (11)	88 (11)
1	42	Thick	88	74		
2	42	Thick	88	76		
3	42	Thin	88	72	88 (11)	52 (7)
4	42	Thin			88 (11)	47 (6)
5	45	Thick	88	72		
6	45	Thick	88	74		
7	45	Thin			88 (11)	58 (8)
8	45	Thin			88 (11)	64 (8)
9	48	Thick	88	71		
10	48	Thin			88 (11)	60 (8)
11	48	Thin			88 (11)	67 (9)
12	48	Thin			88 (11)	68 (9)
13	55	Thick	88	69		
14	55	Thin			88 (11)	62 (8)
15	55	Thin			88 (11)	71 (9)

^aThick: 1- μm sections, light microscopy; thin: 90-nm sections, electron microscopy.

^bPhotoreceptor somata in the compound eye.

^cAxon bundles which have reached the lamina. Some bundles in the irradiated side have fewer than 8 axons.

the lamina if it is not used. This implies that some 37 irradiated photoreceptors are rescued by this procedure out of an average of 72 which are affected. The present experiments used the shorter 15-sec unilateral exposure, along with post-irradiation fluorescent illumination, in order to reduce the amount of cell death. Since the experimental animals were fixed at various embryonic stages and the number of axons reaching the lamina varies with time (see below), the photoreceptor somata in the eye, rather than the number of photoreceptor axons reaching the lamina, were counted to measure survival at each stage. The results, presented in Table I, show that an average of 72 photoreceptors (out of a normal complement of 88 for half an eye) survive on the irradiated side and 16 (the equivalent of 2 ommatidia) are deleted. Although the effects of not using post-irradiation illumination were not measured in the present experiments, we can presume from the earlier experiments that some irradiated photoreceptors are rescued by this procedure. As we shall see below, 2 or 3 ommatidia appear to be delayed under these experimental conditions, which is consistent with this presumption.

Maturation of irradiated photoreceptors. The earliest features of morphological differentiation of a *Daphnia* photoreceptor are the elaboration of shielding pigment granules (SPGs) in the cytoplasm and the extension of a single fiber (the optic axon) in a medial and posterior direction. These two events are followed by an increase in cytoplasmic volume, establishment of contacts between target laminar neurons and the growing axon, and formation of the rhabdom (E. R. Macagno, manuscript in preparation). Any particular group of photoreceptors in the compound eye undergoes this process at a specific time in development. I have examined some of these features in detail in order to ascertain any changes in the course of maturation of the photoreceptors which survive irradiation in these experiments.

The most readily studied feature is the elaboration of SPGs. Both the density and the size of the SPGs increase as a function of time. Since the size increases from an average of less than $0.1\ \mu\text{m}$ to over $1\ \mu\text{m}$ in the adult, it is possible to observe this process with the light microscope. Figure 1 shows examples from light micrographs of serial sections through the eyes of four irradiated

Figure 1. Examples of light micrographs from serial sections of four different experimental embryos irradiated on the left side of the midplane. *a*, Transverse section through the eye of an embryo fixed at 42 hr; the open arrow indicates the position of the midplane and the orientation is dorsal side up. For comparison, the left circle indicates an area within the region that was irradiated; the right circle is a mirror-symmetric area on the control side. Shielding pigment granules (SPGs) appear as black dots of various sizes. *b*, Coronal section through the eye and nervous tissue of an embryo fixed at 42 hr; the open arrow indicates the midplane and the orientation is anterior end up. The circles are the same as in *a*. Note the absence of SPGs in the left circle. *lam*, lamina. *c*, Same as *b* for an embryo fixed at 45 hr. SPGs in the left circle are fewer and smaller than those in the right circle. The bar at lower right is $20\ \mu\text{m}$ long. *d*, Same as *c* for an embryo fixed at 48 hr. The SPGs on the left are still smaller than those on the right, although, on the average, they are larger than those in *c*. The arrows at right indicate the approximate levels of the transverse sections shown in Figure 2, *a* (upper arrow), *c* (middle arrow), and *h* (lower arrow).

embryos which were fixed at 42 (two specimens), 45, and 48 hr of development (about 12, 15, and 18 hr after irradiation). Analysis of the serial micrographs shows that, at these times, SPG maturation in the irradiated region lags behind that in a mirror-symmetrical region on the control side. By 55 hr (25 hr after irradiation), this difference between the irradiated and control sides has essentially disappeared.

A second feature studied was the number of ommatidial axon bundles which are found at the lamina as a function of time. Normally, all axon bundles reach the lamina by about 40 hr of development (Lo Presti et al., 1973). From the data presented in Table I, we can see that the 11 bundles on the control side have arrived at the lamina by 42 hr as expected. On the irradiated side, however, only 6 and 7 bundles are found in two animals fixed at 42 hr; 8 in each of two at 45 hr; 8, 9, and 9 in three at 48 hr; and 8 and 9 in two at 55 hr. Moreover, in specimen 3 (fixed at 42 hr), where both the number of photoreceptor somata in the eye and the number of axons reaching the lamina were determined, 22 photoreceptors had yet to send axons to the lamina. These data indicate that 2 or 3 bundles of axons from the irradiated side which fail to reach the lamina by 42 hr do so by 48 hr

and therefore behave in a manner consistent with a temporal delay of at least 2 hr and probably no more than 8 hr. Previous observations on adults with similar but somewhat larger embryonic lesions showed that all surviving photoreceptors had grown axons which reached the lamina and made synaptic connections with target neurons (specimens d, e, f, and g in Table 2; Macagno, 1979).

The degree to which axon bundles have grown into the lamina is additional evidence of a delayed arrival of optic axons at the lamina in the irradiated side. Specimens 7 and 8 (fixed at 45 hr) each show ommatidia (2 and 1, respectively) in the irradiated region for which only the lead axon has entered the lamina and contacted target cells. In comparison, all 8 axons from each ommatidium in the control side have entered the lamina by this time. In the specimens fixed at 48 hr, both the lead axon and its followers have entered the lamina in every case, but very clear differences in the degree of maturation of some ommatidia can be observed. A particularly clear example from specimen 11 is presented in Figure 2.

It can be seen in Figure 2, *a* and *b* that the photoreceptors of the ommatidium on the left side of the midplane (the irradiated side) are less mature than those of

Figure 2. Electron micrographs of transverse serial sections through the eye and lamina of an embryo irradiated on the left of the midplane and fixed at 48 hr. The orientation is dorsal side up. *a*, Low power micrograph of a section through the eye (level indicated in Fig. 1*d*). The boundaries of an irradiated ommatidium on the *left*, of a symmetrically located control ommatidium on the *right*, and of the glial cells which form the midplane of the eye have been drawn in for clarity. *r*, photoreceptors; *GC* and *open arrows*, glial cells and midplane; *LE*, lenses. The bar at lower right equals 10 μ m.

the mirror-symmetric ommatidium on the right side. On the basis of four morphological criteria (smaller SPGs, fewer SPGs, smaller cell diameter, and lack of a rhabdom), it is possible to state that the ommatidium on the left side is between 6 and 8 hr less advanced develop-

mentally than the one on the right side. This is further supported by the morphology of the photoreceptor axons (see Fig. 2*c*). On the left, they have reached the lamina (Fig. 2, *d* and *e*), but only the lead axon extends into it and contacts more than one target cell (Fig. 2, *f* and *i*).

Figure 2. Continued. *b*, Micrograph of a section adjacent to the one shown in *a* at higher magnification. *r*, photoreceptors; *gc* and open arrow, glial cells and midplane; *pg*, shielding pigment granules; *rh*, rhabdom. The smaller number and smaller size of the pigment granules, the smaller size of the photoreceptors (due to less cytoplasm), and the lack of a rhabdom in the irradiated ommatidium on the left side of the midplane all indicate that it is less mature than the ommatidium on the right side. The bar at lower right equals 5 μ m. *c*, Micrograph of a section through the optic nerve (level indicated in Fig. 1*d*). The 8 fibers from each of the 2 ommatidia indicated in *a* are labeled *f*. Note the smaller size and flattened cross-section of the fibers on the left, which are also indicative of less mature photoreceptors on the left than on the right. The bar at lower right equals 2 μ m.

Furthermore, the lead axon appears to have a growth cone (Fig. 2*i*; Lo Presti et al., 1973). We normally would have expected to see this morphology some 8 hr earlier. In contrast, all 8 photoreceptor axons on the right have grown well into the lamina (Fig. 2, *f* and *k*), have contacted 5 laminar neurons, and have branched and formed a neuropil (Fig. 2*k*). There is no morphological evidence, in this or other cases which show a delayed maturation, to suggest that the affected cells have ceased to grow and will degenerate.

These observations, taken together, support the hypothesis that the schedule of differentiation of groups of photoreceptors in the irradiated side of the eye has been delayed by some hours and that, as a consequence, the

arrival of the axons of these photoreceptors at the target region has been delayed as well.

Effects on laminar cell differentiation. The state of differentiation of laminar neurons contacted by delayed axon bundles was studied in detail in the three irradiated animals fixed at 48 hr and sectioned serially for electron microscopy. In each specimen, there was evidence of a delay in the differentiation of laminar cells. An example of this delay can be seen readily in the micrographs of specimen 11 which appear in Figure 2, *d* through *k*. In the left side, only 4 laminar neurons have been contacted by the growing axons. Of these, only the first one contacted (*L1*) has begun to extend a process (Fig. 2*e*); the second (*L2*) is in the process of wrapping around the lead

Figure 2. Continued. *d*, Micrograph of a section about 3 μ m posterior to the section shown in *c*. The fiber bundle on the left of the midplane (circle) has entered the left lamina and is in contact with laminar cell *L1*. The fiber bundle on the right (fibers indicated by *f*) has not entered the right lamina at this level. The separation between the eye and right lamina is somewhat greater than that between the eye and left lamina. Since this separation increases with the stage of maturation of these structures, this is a further indication that the left side lags behind the right side. Other fiber bundles are labeled with diamonds. Small arrowheads in this and subsequent panels indicate the midplane. The bar at lower right equals 5 μ m and also applies to *f* below. *e*, Detail of *d* showing the 8 photoreceptor fibers and laminar cell *L1*. The lead fiber is labeled *lf*; the 7 followers are marked *f*. The open arrow at top points to a process of cell *L1* which follows the fiber bundle for a short distance. *gc*, laminar glial cell. The bar at lower right equals 2 μ m and also applies to *g* below. *f*, Micrograph of a section about 5 μ m posterior to the section shown in *d*. Of the fiber bundle on the left, only the lead fiber is still seen at this level in contact with a second laminar cell, *L2*. The bundle on the right (circle) has entered the lamina. Other fiber bundles are labeled with diamonds. *g*, Detail of *f* showing that *L2* is wrapped around the lead fiber and is attached to it by a desmosome. (*de*).

Figure 2. Continued. *h*, Micrograph of a section about 2.5 μm posterior to the section shown in *f* (level indicated also in Fig. 1*d*). On the *left*, the lead fiber is in contact with an additional laminar neuron, *L3*. At this level, *L1* has disappeared; no processes of this cell are found. On the *right side*, the fiber bundle is surrounded by two laminar neurons, *L1* and *L2*. The bar at lower *right*, which also applies to all remaining panels, equals 5 μm . *i*, Micrograph of a section about 0.5 μm posterior to the one shown in *h* showing that the lead fiber is in contact with a fourth laminar neuron, *L4*. The increased diameter and filopodia (open arrows) of the lead fiber indicate that this is the growth cone. Cell *L2* has nearly disappeared and also lacks processes. *j*, Micrograph of a section about 4.5 μm posterior to the one shown in *i*. At this level on the *left*, the lead fiber and laminar cells *L3* and *L4* have disappeared. Both *L3* and *L4* lack processes. Other undifferentiated laminar neurons are labeled *L*. On the *right side*, the fiber bundle has been joined by processes from cells *L1* and *L2* and is in contact with 2 additional laminar neurons, *L3* and *L4*. *k*, Micrograph of a section about 7 μm posterior to the section shown in *j*. Unidentified cells (possibly medullar) are seen on the *left* of the midplane at this level. On the *right*, cell *L5* has completed the cartridge. The photoreceptor fibers, as well as the processes of *L1* to *L4*, have branched and an immature neuropil is observed. The largest processes of *L1* to *L4* are indicated.

fiber (Fig. 2*g*), which indicates that it has been contacted recently by the lead fiber (Lo Presti et al., 1973); and the others (*L3* and *L4*) show no morphological evidence of differentiation. In contrast, all 5 laminar neurons in the right side have well developed processes that branch into the neuropil (Fig. 2, *j* and *k*). These features indicate that the differentiation of the laminar cells in the group in the left side lags behind that of the group in the right by 6 to 10 hr. In the two other cases that were studied in detail, the delay was estimated to be 2 to 4 hr and 4 to 8 hr.

In comparison, in an animal fixed at 55 hr (specimen 15, see Fig. 3), all remaining bundles in the irradiated side and their associated laminar neurons have matured

to the extent of forming a neuropil. No gross temporal differences between control and irradiated sides are apparent on inspection of the electron micrographs of this animal. However, the occurrence of axosomatic synaptic sites in an optic cartridge on the irradiated side (see Fig. 3*b*) indicates that this group is at a less differentiated stage than its neighbors. Axosomatic synapses are found only at early stages of cartridge maturation (V. Lo Presti, unpublished observations).

Discussion

Effects on photoreceptor differentiation. The results presented above show that UV irradiation plus post-irradiation fluorescent illumination can affect the sched-

Figure 3. Electron micrographs of sections through the lamina of a specimen fixed at 55 hr (specimen 15 in Table I). *a*, Section near the anterior surface. The midplane is indicated by two arrowheads. Four bundles of optic axons have been labeled on each side of the midplane (A through D). The bundle labeled A on the left (the irradiated side) shows an immature neuropil at this level. Two laminar neurons associated with this bundle have been labeled, and a synaptic site between an optic axon terminal and L2 is indicated (open arrow). All axonal bundles and target cells are at a more advanced stage of development than those shown in Figure 2; the existence of synaptic sites on the soma of L2 (see also *b* below), however, suggests that the development of the group labeled A on the left lags behind that of its neighbors. The bar equals 5 μ m and applies also to *c*. *b*, Higher magnification micrograph of a portion of the group labeled A on the left of the midplane in *a* above from a section 0.2 μ m anterior to the one shown in *a*. A number of synaptic sites between an optic axon terminal (*t*) and laminar neuron (L2) are indicated by open arrows. L1 and L2 are the same cells so labeled in *a*. The bar equals 2 μ m.

Figure 3. Continued. *c*, Micrograph of a section 2 μ m posterior to the one shown in *a* showing that some bundles other than *A* on the left (in particular *B* and *C* on the right) have formed a neuropil at this level.

ule of differentiation of *Daphnia* photoreceptors. The observations are not sufficient to decide whether the effect is to slow down the rate of differentiation, to delay the time when morphological differentiation begins, or both. In any case, the end result is to delay, by a few hours, the time of arrival of photoreceptor axons at the target region.

Although the cellular or molecular mechanisms which are responsible for these effects are unknown presently, some possibilities are indicated by our observations. The requirement for post-irradiation illumination suggests that the repair of radiation-induced pyrimidine dimers (photoreactivation) is necessary for rescuing some of the exposed cells (Cook, 1970). It is possible that this repair phase is the cause of the temporal effects observed if, for example, differentiation cannot proceed until all of the DNA damage is repaired. We are currently testing this hypothesis by looking at the effects of reducing the photoreactivating flux and delaying photoreactivation

exposure after UV irradiation. An alternative possibility, since the cells are exposed to all wavelengths produced by the mercury arc source, is that the temporal effects are produced by longer wavelength photons (>300 nm) than those responsible for dimer formation (≈ 260 nm). Support for this idea comes from the observation that UV radiation of these wavelengths can produce a delay in the mitosis of micro-organisms, with a peak effect at 340 nm (Ramabhadran and Jagger, 1976). Although we have no *a priori* reason to believe that mitosis and differentiation are affected similarly by UV radiation, we are currently studying the wavelength dependence of these effects on *Daphnia* photoreceptors. A third possibility is that the temporal effects are not due directly to exposure to UV radiation but are rather a secondary result of the deletion of some number of photoreceptors in these experiments. For example, the death of adjacent photoreceptors could interrupt temporarily the arrival of an inductive signal from the older, more lateral ommati-

dia that triggers differentiation of those more medial. Removal of degenerating debris, which takes a few hours, then would allow lateral cells to contact medial cells and trigger them. In this case, photoreactivation would be required only in order to prevent cell death but would not affect the timing of differentiation. A last possibility is that degeneration products directly affect axonal development in nearby photoreceptors. This is unlikely because the delaying effect is not observed in all photoreceptors adjacent to degenerating cells. It is also unlikely that degeneration products in the eye affect the differentiation of particular laminar cells, since the eye and lamina are separated physically by a narrow extracellular sinus and no correlation between the distribution of degeneration products and delayed laminar cells is observed. Working at dosages such that no cell death occurs will provide evidence for or against these last two hypotheses.

Effects on the differentiation of laminar cells. Observations reported previously (Macagno, 1978, 1979) showed that laminar cells fail to differentiate when deprived of contact with ingrowing optic axons and degenerate some 10 to 12 hr after such contact would have occurred normally. The data presented here show that, if the contact is delayed by some hours (2 to 10), differentiation of laminar cells is initiated at a correspondingly later time.

On the basis of these observations, two developmental rules can be proposed:

1. Differentiation of laminar cells can be initiated over an extended period of time.
2. Differentiation of laminar cells is triggered by contact with growing photoreceptor axons.

During normal development at 22°C, laminar cells become postmitotic 1 to 2 hr before they are contacted by growing optic axons (Flaster and Macagno, 1980; M. S. Flaster, unpublished observations). Since a delayed contact can still initiate differentiation 8 hr later, it would appear that precise timing is not necessary, allowing for some variability in the relative time of arrival of the optic axons. Such variability could occur, for example, as a consequence of daily changes in environmental temperature in the natural habitat of *Daphnia*, provided that rates of mitosis and extension of axons have different temperature dependences. Preliminary observations indicate that a 2 to 3°C decrease in temperature during embryogenesis results in a 2- to 4-hr delay in the arrival of axons at the laminar anlagen; observations on the temperature dependence of mitotic rates are currently underway.

Several developmental functions can be proposed for the triggering action of the photoreceptor axons. First, it provides a mechanism for insuring that the maturation of axons and target cells is coordinated temporally, which may play an important role in the attainment of a particular pattern of synaptic connectivity. Second, it is a means for recruiting a group of target cells for each bundle of growing axons and for removing these cells from the general pool available to other incoming optic axons. Third, it insures that only the required number of target cells differentiate; those lacking the contact interaction degenerate.

As pointed out in the introduction, the degree to which target cells of optic axons differentiate when afferents are deleted early in development varies considerably from species to species, ranging from no morphological differentiation in *Daphnia* to apparently extensive differentiation in birds. A wide range in the effects of afferent deprivation also has been reported for other sensory systems (see Jacobson, 1978, for a review). The observations of Hildebrand and collaborators on the development of deafferented antennal and optic lobes in the moth *Manduca sexta* provide an excellent example of different effects in a single species. The first order interneurons in the antennal pathway survive and form a reduced but otherwise apparently normal neuropil in the absence of antennal sensory fibers (Sanes et al., 1977; Hildebrand et al., 1979). In contrast, as in *Daphnia*, the first order interneurons of the optic lamina are affected profoundly when optic axons from the compound eye are prevented from reaching the target region (Maxwell and Hildebrand, 1981). A probable explanation for these differences is that different classes of cells have different degrees of autonomous differentiation and that certain features of the differentiated neuron require interactions with a variety of other cells in order to develop. Thus, the laminar neurons of *Daphnia* and other arthropods represent an extreme case, in which the initiation of morphological differentiation requires an interaction with other cells. To what degree neurons in general differentiate autonomously can be ascertained only when our knowledge of what and how many interactions they undergo during development is more complete.

References

- Anderson, H. (1978) Postembryonic development of the visual system of the locust, *Schistocerca gregaria*. J. Embryol. Exp. Morphol. 45: 55-83.
- Cook, J. S. (1970) Photoreactivation in animal cells. Photo-physiology 5: 191-232.
- Currie, J., and W. M. Cowan (1974) Some observations on the early development of the optic tectum of the frog (*Rana pipiens*), with special reference to the effects of early eye removal on mitotic activity in the larval tectum. J. Comp. Neurol. 156: 123-142.
- Eichler, V. (1971) Neurogenesis in the optic tectum of larval *Rana pipiens* following unilateral enucleation. J. Comp. Neurol. 141: 375-396.
- Flaster, M., and E. R. Macagno (1980) Correlation between cell birthdates and synaptic connectivity in a neuronal array. Soc. Neurosci. Abstr. 6: 410.
- Hildebrand, J. G., L. M. Hall, and B. C. Osmond (1979) Distribution of binding sites for [¹²⁵I]-labeled α -bungarotoxin in normal and deafferented antennal lobes of *Manduca sexta*. Proc. Natl. Acad. Sci. U. S. A. 76: 499-503.
- Jacobson, M. (1978) *Developmental Neurobiology*, ed. 2, Plenum Press, New York.
- Kelly, J. P., and W. M. Cowan (1972) Studies on the development of the chick optic tectum. III. Effects of early eye removal. Brain Res. 42: 263-288.
- Lo Presti, V., E. R. Macagno, and C. Levinthal (1973) Structure and development of neuronal connections in isogenic organisms: Cellular interactions in the development of the optic lamina of *Daphnia*. Proc. Natl. Acad. Sci. U. S. A. 70: 433-437.
- Lo Presti, V., E. R. Macagno, and C. Levinthal (1974) Structure and development of neuronal connections in isogenic orga-

- nisms: Transient gap junctions between growing optic axons and lamina neuroblasts. *Proc. Natl. Acad. Sci. U. S. A.* 71: 1098-1102.
- Macagno, E. R. (1978) A mechanism for the formation of synaptic connections in the arthropod visual system. *Nature* 275: 318-320.
- Macagno, E. R. (1979) Cellular interactions and pattern formation in the development of the visual system of *Daphnia magna* (Crustacea, Branchiopoda). I. Trophic interactions between retinal fibers and laminar neurons. *Dev. Biol.* 73: 206-238.
- Maxwell, G. D., and J. G. Hildebrand (1981) Anatomical and neurochemical consequences of deafferentation in the development of the visual system of the moth *Manduca sexta*. *J. Comp. Neurol.* 195: 667-680.
- Nordlander, R. H., and J. S. Edwards (1969) Postembryonic brain development in the monarch butterfly *Danaus plexippus plexippus* L. II. The optic lobes. *Arch. Entwicklungs-mech. Org.* 163: 197-220.
- Ramabhadran, T. V., and J. Jagger (1976) Mechanism of growth delay induced in *Escherichia coli* by near ultraviolet radiation. *Proc. Natl. Acad. Sci. U. S. A.* 73: 59-63.
- Sanes, J. R., D. J. Prescott, and J. G. Hildebrand (1977) Cholinergic neurochemical development of normal and deafferented antennal lobes during metamorphosis of the moth *Manduca sexta*. *Brain Res.* 119: 389-402.
- Schmatolla, E. (1972) Dependence of tectal neuron differentiation on optic innervation in teleost fish. *J. Embryol. Exp. Morphol.* 27: 555-576.
- Whitehead, M. C. (1979) Growth of dendrites in the optic tectum of the chick embryo following destruction of the eye primordium. *Neuroscience* 4: 379-390.