Formation of the retina–lamina projection of the cockroach: no evidence for neuronal specificity

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SUMMARY

There is a topographical mapping of neural elements onto the lamina neuropile of the optic lobe of the cockroach, such that adjacent ommatidia project to adjacent points (optic cartridges) in the lamina neuropile. Postembryonic growth of the compound eye occurs by addition of new ommatidia to its growing margin. Retinula axons grow from the newly formed ommatidia to the lamina. By transplantation experiments in which the position or the orientation of retinal material is altered, it is shown that retinula axons do not make connections in the lamina with respect to their old position and orientation, but rather, in keeping with their new situations, apparently maintaining a retinotopic mapping upon the optic lobe.

INTRODUCTION

Proper functioning of a nervous system depends on the establishment of precise neuronal connections during development. Identifiable cells in the peripheral nervous system can be shown to establish particular connections with specific central cells. For example, cercal afferent neurons establish connections with particular giant axons in the cricket central nervous system (Edwards & Palka, 1974).

Where sense organs consist of repeated identical units (as in the compound eye), the target may consist of a corresponding set of anatomically identical neuronal units. In the cockroach compound eye, each ommatidium contains eight retinula cells. A bundle of eight axons (one axon from each light receptor cell of the ommatidium) projects from the retina to the lamina, the first synaptic region in the optic lobe. Here, retinula axons from a single ommatidium together with a set of five or six second-order cells (plus centrifugal fibres and other neuronal elements) form the repeated structural units of the lamina neuropile, the optic cartridges. There is one optic cartridge per ommatidium.

The topographical mapping of the retina upon the lamina requires that

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particular first-order retinula cells make connections in particular cartridges. This paper is concerned with the way in which the retina–lamina projection arises in the development of the cockroach nervous system, to ask whether or not differential properties of the neurons are responsible for ordering in the projection.

According to a neuronal specificity model, retinula axons from ommatidia in various regions of the compound eye would have characteristic particularities of some nature which would permit synapses only with neurons having a complimentary label. As there is a topographical mapping of the retina upon the lamina in insects (Meinertzhagen, 1976), the neuronal specificity model requires there to be complimentary labels between neurons in corresponding regions of the retina and lamina. This model can be tested by transplant operations which relocate ommatidia in abnormal retinal loci.

Anderson (1978b) has suggested that neuronal specificity plays no part in the formation of connexions in the retina–lamina projection of another insect (Schistocerca gregaria). In that study, parts of the developing eye were transplanted heterotopically, and the tracts of nerve fibres which subsequently developed were examined after silver staining. Those results showed that the nerve fibre tracts do not appear to grow back to areas of the lamina to which they would have projected if no operation had been performed. Contact guidance of growing axons along previously projected fibres more readily explains her results. Since the claim is an important one, it is essential that it is substantiated. Describing regenerating tracts of fibres is suggestive, but only the demonstration of the appropriate pattern of regenerated terminals in the lamina can provide conclusive proof that connections are not established by a neuronal specificity mechanism. The purpose of this study is to provide such proof.

In the present study, a number of experiments to relocate ommatidia have been performed. By marking sets of graft-derived ommatidia and observing their retinula axon terminals in the lamina, the establishment of neuronal connexions between shifted ommatidia and topographically correct regions of the lamina is now clearly demonstrated. The results agree with Anderson’s (1978b) prediction that neuronal specificity does not operate in the establishment of the retina–lamina projection.

**MATERIALS AND METHODS**

Stocks of *P. americana* were maintained under standard laboratory conditions (12 h light and 12 h dark at 24 °C) and fed on rat pellets and water. Surgical techniques were the same as described previously (Nowel & Shelton, 1980). Grafts were held in place with a small droplet of melted insect wax (Krogh & Weis-Fogh, 1951).

Because the retinula axons from transplanted ommatidia degenerate and
do not regenerate (Anderson, 1978a), neuronal specificity had to be tested in another way. Instead of transplanting differentiated retina, portions of the growing eye margin (budding zone) were transplanted (Fig. 1). Host animals were newly-moulted nymphs (larval instars 3–5, or final/penultimate, depending on the experiment: see below). Grafted integument consisted of the eye margin (composed of the compound eye proliferation zone), the adjacent vertex epidermis, and the overlying cuticle. By the time the nymph host had developed to the adult, a portion of the operated eye was graft-derived and recognizable by a pigment marker. The stocks used for transplant operations to form these chimeras were wild type and lavender (Ross, Cochran & Smyth, 1964).

To trace connexion patterns in the optic lobe, small localized lesions were made in the compound eyes of anaesthetized adult insects. After immobilizing the cockroach as for a grafting operation (Nowel & Shelton, 1980), a silver earthing wire was inserted into the head through a hole cut in the cuticle at a posterior medial point. A tungsten microelectrode was connected to a function generator set to deliver 2 $\mu$A at a frequency of 1 MHz. After removal of narrow strips of the overlying cornea, the microelectrode was inserted into the exposed ommatidia to be electrolytically destroyed, and left in each point of insertion for 10 seconds. An ‘I’ or ‘L’ pattern (the lines approximately five to eight ommatidia wide) was cauterized in the eye by gradually moving the microelectrode along the incision. When a particular pattern of microcautery was completed, the wounds were covered with insect wax. Sixteen hours later the animal was killed and the eye and optic lobe were fixed in an aldehyde fixative (Karnovsky, 1965) in a 0-1 M phosphate buffer (Hayat, 1970) at pH 7-4 for 2–4 h. The tissue was post-fixed in phosphate-buffered 1 % osmium tetroxide for 2–12 h, then dehydrated in an acetone series and cleared in propylene oxide. Embedding was in Spurr’s resin following a long period of infiltration.

The optic lobe was serially sectioned in 1 $\mu$m sections with a Huxley Ultramicrotome and glass knives, mounted in order on subbed slides, stained with toluidine blue, and examined with a Zeiss compound microscope. Sections of lamina neuropile showing degenerating axon terminals (which appear dark blue following such treatment: Geisert & Altner, 1974) were drawn at a magnification of 450 x or 1100 x using a Zeiss camera lucida. Sections were also photographed using a Zeiss Photomicroscope II.

RESULTS

Grafting operations on P. americana

Following the exchange of graft material between lavender and wild-type individuals, tissue which develops from the grafts is recognizable by its distinctive pigmentation (Fig. 2). When the resulting chimeras had reached the imaginal stage, a pattern was traced in the graft-derived retina with a coagulating electrode to locally destroy a set of select ommatidia. The position and pattern
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(a) Controls

Lesions were made in the eyes of 14 unoperated wild-type *P. americana* adults. The lamina neuropiles were subsequently examined to determine the regions of retinula axon terminations for sets of (cauterized) ommatidia in different regions of the compound eye. Results from operated retinae were compared with those from the unoperated retinae and with control-operated retinae (those in which a graft was transplanted homotopically).

After approximately 50% of the experimental and control operations, cautery in the graft retina results in a complete pattern of retinula axon degeneration in the lamina neuropile. The rest of the lamina appears normal: the cell body layer appears undamaged, monopolar axons in the lamina neuropile are healthy, and the size and shape of the neuropile are unaltered. This evidence argues against the possibility that retinula axon degeneration is caused by direct injury to the optic lobe. In addition, the anatomical relationship between the lamina neuropile and that part of the retina receiving the lesion makes the possibility of direct damage to the lamina extremely unlikely, but does not rule it out completely. In at least 10% of the analysed specimens, the retinal degeneration pattern is only partially represented in the lamina. In approximately 40% of cases, there is no degeneration detected in the lamina. As retinula axon degeneration is observed in all of the unoperated animals following retinal lesions, it is concluded that, where cautery of graft-derived tissue does not result in degeneration in the lamina, the graft retinula fibres have failed to reach the lamina. In partial degeneration patterns, it is likely that only part of the graft projects to the lamina.

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Fig. 1. Operations to alter the position or orientation of compound-eye tissue. Squares of integument including both eye margin and head epidermis were exchanged between the eyes of newly-moulted wild-type and *lavender* nymphs of *P. americana*. (Results of these operations are shown in Fig. 2.) (a) A–P shift. Grafts from the dorso-posterior region were exchanged with those from the dorsoanterior region to result in a shift of the graft along the anteroposterior axis. (See Figs 2a, b.) (b) Reversal of A–P axis. Grafts from right eyes were exchanged with those from left eyes, resulting in the reversal of the anteroposterior axis. (See Fig. 2c.) (c) 90° rotation. Grafts including the anterior eye margin were exchanged with those including the dorsal eye margin, resulting in a 90° rotation of the tissue with respect to both the anteroposterior and the dorsoventral axes. (See Figs 2d, e.) (d) D–V shift. Grafts including the dorsal eye margin were exchanged between young nymphs and adults (or final/penultimate nymphal instars). (See Figs. 2f–h.)
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(b) Anterior-to-posterior shift

An area of integument including the posterodorsal eye margin was replaced by a graft including the anterodorsal eye margin (Fig. 1a). Fourteen resulting chimeric eyes were analysed (Fig. 2a). Of these, nine animals showed degeneration in the posterior lamina after a lesion was made in the graft-derived retina (Figs 3a, 4). No axon degeneration was seen elsewhere in the lamina. Similar lesions in the posterior portion of two unoperated retinae resulted in degenerating axon terminals in similar regions within the lamina neuropile (Fig. 3c). Thus, retinula axons project from anterior ommatidia (shifted to the posterior part of the compound eye) in keeping with their new positions. They do not exhibit an ‘anterior’ specificity.

(c) Posterior-to-anterior shift

In a similar way, posterodorsal eye margin was shifted to an anterodorsal position (Fig. 1a). Of 12 chimeras analysed, 9 showed axon degeneration only in the anterior part of the lamina after a lesion was made in the graft-derived retina (Fig. 3b). The positions of degenerating axons in the lamina of these operated animals were similar to those found after similar lesions were made in the two unoperated retinae (Fig. 3c). Again, shifted ommatidia project to targets appropriate to their new (rather than their original) positions.

(d) Anterior–posterior reversal

The dorsal eye margin of a left eye was exchanged with that taken from a right eye, thus inverting the A–P axis (Fig. 1b). Of 28 chimeric eyes analysed (Fig. 2c), 13 showed a pattern of projection in the lamina similar to that in unoperated animals based on analysis of four control animals (Fig. 5). In

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Fig. 2. (a) Chimera of *P. americana* following an anterior-to-posterior shift of eye margin between *lavender* and wild-type nymphs, as in Fig. 1(a). (b) Chimera produced by a posterior-to-anterior operation, as in Fig. 1(a). (c) Chimera produced following an inversion of the A–P axis, as in Fig. 1b. Axes of graft and host tissues are indicated: A, anterior; D, dorsal. (d) Chimera produced following a clockwise rotation of 90° of the dorsal eye margin, as in Fig. 1c. Axes of graft and host tissues are indicated: A, anterior; D, dorsal. (e) Chimera produced following an anticlockwise rotation of 90° of the anterior eye margin, as in Fig. 1c. Axes of graft and host tissues are indicated: A, anterior; D, dorsal. (f) Chimera produced following a ventral-to-dorsal shift, as in Fig. 1d. (g) A scanning electron micrograph of a compound eye similar to that shown in Fig. 2f, following a ventral-to-dorsal shift. Note the raised contour of the grafted material. This may reflect differences in curvature of the graft and host integument, or differences in adhesive characteristics of host and graft cells resulting in a shortening of the graft/host interface. g, graft-derived ommatidia; h, host-derived ommatidia. (h) Chimera produced following a dorsal-to-ventral shift of adult integument, as in Fig. 1d. Bars represent 0.25 mm.
Experimental Neuronal specificity prediction

Experimental Neuronal specificity prediction

Control

Control
addition, seven animals exhibited a partial projection from the grafted tissue, though the complete pattern of projection was impossible to determine.

\((e)\) \(90^\circ\) rotations

A length of dorsal eye margin was exchanged with a length of anterior eye margin (Fig. 1c). Of 15 chimeric eyes analysed (Figs 2d, e), five showed a pattern of degeneration similar to that in unoperated animals, based on analysis of three control animals (Fig. 6).

\((f)\) Ventral-to-dorsal shift

A length of dorsal eye margin of a final or penultimate larval instar was replaced by the dorsal eye margin of a young (third–fifth instar) larva (Fig. 1d). Of 14 successful operations, graft ommatidia in four projected to the most dorsal portion of the lamina only, in keeping with the graft’s new dorsal position in the host eye. It thus compares with the nerve projection from an unoperated compound eye as determined from the region of axon degeneration in the lamina following cautery of a group of dorsal ommatidia in the control eye (Fig. 7a).

A possibly significant observation is that whereas most grafts are incorporated within the plane of the host eye, the graft-derived ommatidia in the chimeric eyes formed by a ventral-to-dorsal shift commonly formed an elevated structure (Fig. 2g). A tenable explanation for this phenomenon is that a difference in adhesive properties between donor and host tissues causes a reduction in the extent of the graft/host interface.

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Fig. 3. These composite diagrams show the effects of a lesion made in graft-derived ommatidia following an anterior-to-posterior shift (a), or a posterior-to-anterior shift (b) (see Fig. 1a), and can be compared with the effects of similar lesions made in the anterior (c) or posterior (c\(u\)) portions of control (unoperated) eyes. Diagrams (a\(i\)) and (b\(i\)) show the pattern of degenerating axons (seen in the sectioned lamina as black dots) following the lesion (heavy line) in the graft portion of the compound eye (e). A similar set of diagrams (a\(u\), b\(u\)) shows how the pattern of degeneration would have appeared if the terminals derived from the transplanted retina had innervated loci of the lamina neuropile appropriate to their pre-operative origin according to a neuronal specificity model of axon projection, based on results from control animals (c\(i\), c\(u\)). Sheets of lamina neuropile (1) are shown, with lines representing the sections which are illustrated alongside. Shading indicates regions in the lamina where degenerating terminals are actually found (a\(i\), b\(i\) and control laminae) or where they are expected according to the neuronal specificity model (a\(u\), b\(u\)). The diagrams show that axons from transplanted pieces of the eye project to the lamina with no regard for their previous position. Illustrated sections are camera lucida drawings of sections of the lamina neuropile 20 \(\mu m\) apart and are from a complete 1 \(\mu m\) series. A and P indicate anterior and posterior regions of the compound eye and lamina.
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(g) Dorsal-to-ventral shift

Dorsal eye margin of a young (third–fifth instar) larva (which would form central ommatidia in the compound eye of the imago) was replaced by a length of dorsal eye margin of a newly-emerged adult (which would form the most dorsal ommatidia of the eye of the imago). Of 20 operations, five were successful (Fig. 2h). In the two analysed, one had projected to the central region of the lamina, in keeping with the new position of the graft within the host eye (Fig. 7b), based on results from two control eyes. No axon degeneration was observed in the lamina of the other animal.

DISCUSSION

In these experiments (Fig. 1a–d), the position and/or orientation of pieces of retina was altered. It is known that these alterations result in permanent changes in the final pattern of the insect retina. In Schistocerca gregaria, rotation of the eye margin results in rotation of the polarities of the graft-derived ommatidia (Nowel, 1979; P. M. J. Shelton, personal communication). Furthermore, the ‘dorsal spot’ (a set of characteristic and obviously different ommatidia which forms the most dorsal portion of the locust compound eye) appears at the ventral tip of the eye if the eye margin of the anterodorsal retina is shifted ventrally (Anderson, 19786; Nowel, 1979). For these reasons it is certain that graft-derived eye tissue retains its regional determination and polarity during grafting experiments.

In the developing insect compound eye, as new retinula cells are sequentially generated and contribute to the formation of new ommatidia, they send out axons into the developing optic lobe where the outer optic anlage is sequentially generating ganglion cells. Meinertzhagen (1975) and Shelton (1976) suggest that the growth phase of these postembryonically developing axons depends upon contact guidance of a growing axon tracking along a neighbouring bundle which has grown previously, based on observations of minimal filopodial activity and rapid elongation to the lamina.

On the basis of the present studies, the continued retinotopic projection (as deduced from the formation of connections between the lamina and eye tissue in keeping with its transposed rather than original position and/or orientation) shows that in the postembryonically developing retina–lamina projection, the matching of specific projecting and target cells (Sperry, 1936)

Fig. 4. Micrographs of semithin sections on which Fig. 3a is based, showing the appearance of degenerated axon terminals (arrows) in the lamina neuropile (ln). (a)–(d) correspond to sections nos. 1–4, respectively, in Fig. 3a. mn, medulla neuropile; ooaoa, outer optic anlage; ooc, outer optic chiasma. Bars represent 25 μm.
Fig. 5. These composite diagrams show the effects of a lesion made in graft-derived ommatidia following an anterior-to-posterior reversal (a) (see Fig. 1b), and can be compared with the effects of lesions made in control (unoperated) eyes (b), following the conventions set out in Fig. 3. The similarities between the pattern of degenerating terminals in the lamina of the operated animal (a) and that of the control animal (b) show that axons from graft-derived ommatidia project to the lamina with no regard for their pre-operative position or orientation. Sections of lamina are taken from a complete series and are 20 μm apart. A, anterior; P, posterior; e, compound eye; l, lamina neuropile.
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does not occur. Rather, the development of the projection which connects the set of light-receptor cells with the sheet of lamina neuropile can be explained by contact guidance of nerve fibres along previously projected axons to connect these two synchronously-growing organs, the retina and the lamina (Anderson, 1978b). This would explain both the observations of successful projections from grafted retinas reimplanted in various orientations or positions, and also many of the observations of abnormalities in these projections (such as whorls, and axon tracts parallel to the basement membrane) following operations which perhaps had damaged the already established nerve pathways (see Anderson, 1976, 1978b). This may also explain the occasional observation of patterns of degenerated axon terminals representing only a portion of the patterns which had been expected. No firm conclusions could be drawn from the specimens in question, but even here the incomplete degeneration patterns never supported a neuronal specificity model. The possibility exists that only a portion of the graft-derived retinula cells project to the lamina. The other axons may never reach the lamina neuropile. Rather than following previously-projected retinula axons to the lamina, axons from the graft may follow misleading pathways as a result of the disruptive effects of the surgical procedures. Thus, some axons may be prevented from making appropriate connexions with their target cells (see Wigglesworth, 1953).

Nardi (1977) notes that following shifts of grafts along the anteroposterior axis in the retina of Ephesia kühniella, there is a distortion of the surface contour of the chimeric eye, with a constriction of the graft-derived ommatidia into an elevated region. He suggests that there are differences in cell surface parameters arranged in the form of a gradient along this axis. It has further been suggested that these differences may specify cell position along at least one axis of the eye (the A–P axis), and may therefore be involved in the specification of connexions between retina and optic ganglia. Surface molecules on growing axons may be used to recognize specific target neurons within the brain (Nardi, 1977). Results similar to those of Nardi (1977) have been found in the present study (Fig. 2g). Regardless of whether graded cell adhesion variations or other factors cause the distortion of the graft tissue surface, the present experiments show that in P. americana, axon specification is not correlated with graft appearance. Axon projection proceeds according to the new position within the eye whether or not the surface contour of the graft is affected by transposition along an axis.

These results are in agreement with a number of other studies on insect nerve development and regeneration which conclude that growing nerve fibres appear to rely on mechanical guides (such as other nerves) or spatio-temporal cues rather than neuronal specification to reach their target loci. The establishment of the retina–lamina projection in the imaginal Drosophila is along the optic stalk which contains the larval ocellar nerve (Hanson, 1972; Meinertzhagen, 1973, 1975; Trujillo-Cenoz & Melamed, 1973; Melamed &
Experimental

Neuronal specificity prediction

Control

(b)
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Trujillo-Cenoz, 1975). All imaginal discs are connected to the central nervous system by larval nerves. This may indicate that the mechanical connexion between target and growing fibres is an important or even indispensible feature of sensory nerve development, and if these guides are disrupted, other nerves may be followed centripetally (Ghysen & Deak, 1978). Studies on homeotic mutants of Drosophila (Stocker, Edwards, Palka & Schubiger, 1976; Stocker, 1977; but see also Vandervorst & Ghysen, 1980), the abdominal sensilla of Rhodnius prolixus (Wigglesworth, 1953), the antennae of Manduca sexta (Sanes & Hildebrand, 1975), the ‘pioneer neurons’ of Locusta legs and antennae (Bate, 1976), and on cockroach eyes transplanted to the leg (Wolbarsht, Wagner & Bodenstein, 1966) show developing axons growing along previously projected nerves. Several studies on regenerating nerves indicate that much reliance is placed on the presence of a physical bridge at the site of the injury along which the severed processes can grow (Bodenstein, 1957; Boulton, 1969).

In a number of insect systems, however, specificity best explains the establishment (or re-establishment) of the very accurate projection of relocated or regenerating nerves to their target sites. Studies on both sensory (Edwards & Sahota, 1967; Schafer, 1970; Palka & Edwards, 1974; Palka & Schubiger, 1975; McLean & Edwards, 1976; Anderson & Bacon, 1979) and motoneurons (Pearson & Bradley, 1972) demonstrate an unequivocal matching of neuron with target. The consistent patterns of nerve terminals of the locust wing hinge stretch receptors despite ‘mistakes’ or ‘alternative’ courses argue in favour of the presence of labelled sites in target neuropiles to which the neurons project (Altman & Tyrer, 1977a, b).

This study has examined the establishment of the topographical projection of the set of retinula axons on the set of lamina ganglion cells in the cockroach. From the point of view of the ingrowing retinula fibres, optic cartridges in different regions of the lamina appear to be equivalent, with no labelled differences between them. Many questions remain unanswered. Whether or not the more complex retina-lamina projection patterns found in various dipterans, for example (Braitenberg, 1967; Zeil, 1979), can be explained by this sort of mechanism is unknown. In addition, the connections of the neurons within and between cartridges have not been examined. It is likely that particular retinula axons within one bundle make selective connexions with

Fig. 6. These composite diagrams show the effects of a lesion made in graft-derived ommatidia following a 90° rotation (a) (see Fig. 1c) and can be compared with the effects of lesions made in control (unoperated) eyes (b), following the conventions set out in Fig. 3. The similarities between the pattern of degenerating terminals in the operated animal (a) and that of the control animal (b, rather than b,) show that axons from graft-derived ommatidia project to the lamina with no regard for their pre-operative position or orientation. Sections of lamina are taken from a complete series and those shown are 40 μm apart. A, anterior; P, posterior; e, compound eye; l, lamina neuropile.
Fig. 7. These composite diagrams show the effects of lesions made in graft-derived ommatidia following a ventral-to-dorsal shift (a1), and a dorsal-to-ventral shift (b1), and can be compared with the effects of lesions made on control (unoperated) animals (a0), following the conventions set out in Fig. 3. The similarities between the patterns of degenerating terminals in the operated animal (a1) and the control (a0), and the differences between the operated animal (b1) and that expected according to the neuronal specificity model (b0) based on the control (a0) show that axons from graft-derived ommatidia project to the lamina with no regard for their pre-operative position. Sections of lamina are taken from a complete series and are 20 μm apart. A, anterior; P, posterior; D, dorsal; V, ventral; e, compound eye; l, lamina neuropile.
the various second order elements which themselves make specific interconnections (Boschek, 1971). The basis of this neuronal specification remains unknown.

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REFERENCES


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