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The Exact Neural Projection of the Visual Fields upon the First and Second Ganglia of the Insect Eye

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Summary. In apposition eyes with fused rhabdomeres, six retinula cells of one ommatidium have short axons which terminate in a single cartridge. Each ommatidium corresponds to one lamina cartridge, the two ganglion cell axons of which, together with the long axons from that ommatidium and a fifth axon, proceed as a bundle through the lamina-medulla chiasma to form a cartridge of the medulla. The projection of the lamina cartridges upon those of the medulla forms a series exactly in order but reversed about the vertical plane. In the fly *Calliphora*, in which the optical axes of the rhabdomeres of a single ommatidium diverge, the existing description of the projection to the lamina is confirmed and extended to the medulla. As in fused rhabdomere eyes, the lamina cartridges project by bundles, each of which contains five axons, in an exact series but in reverse order, to the cartridges of the medulla.

Therefore in each type of eye there is an exact projection of the external environment upon the optic medulla.

Introduction

A knowledge of the exact projection of the visual axons upon more central regions of the nervous system is of the greatest importance in understanding the mechanism of the compound eye. The regular geometrical array of thousands of receptors in the compound eye of insects, together with its regular pattern of afferent axons that are resolvable by light microscopy, makes this a particularly favourable system for analysis. Representative neuron types and axon pathways suggesting a projection of visual excitation upon progressively deeper layers are already known from early neuroanatomical work (Cajal and Sánchez, 1915). The problem is to refine techniques to reveal at the single neuron level a simple orderly anatomical arrangement of axons by which the visual fields of all insects are actually projected into their optic lobes.

The Major Anatomical Features

Behind each facet in the insect eye lies a group of (usually) eight receptor or retinula cells arranged together in an ommatidium. Most of

the retinula cells have a specialized photoreceptive region, or rhabdomere, and all have an axon which terminates in the optic lobe of the brain.

Two principal types of apposition eye differ in the pattern of optical axes of the rhabdomeres of the constituent ommatidia. In one type, as in the bee, locust and dragonfly, the rhabdomeres of retinula cells belonging to one ommatidium are fused into a long central rod. The experimental evidence that the fused rhabdomeres act together as a single optical path or light guide is of several kinds.

a) With two electrodes fixed 10 μm apart Shaw (1967) obtained dual records which could only be interpreted as coming from two cells of one ommatidium. These two cells shared the same field of view but were optimally sensitive to different planes of polarized light.

b) Some butterflies have a tracheal reflector at the proximal end of the rhabdom (Miller and Bernard, 1968). The presence of a bright spot of reflected light at the centre of the pseudopupil in these forms can only be explained if the rhabdom acts as a light guide. By the principle of reversibility of light the angular divergence of this light emerging from the facet is the same as the acceptance angle curve for light entering the facet.

c) An increase in the width of the acceptance angle curve, as opposed to a sensitivity increase, upon dark adaptation of the locust eye was explained as the effect of a reduction in refractive index in the region (Schaltzone) immediately surrounding the rhabdom. This explanation implies that light received by the retinula cells of one ommatidium is governed by internal reflection within the rhabdom and that the visual axes of all these cells are identical (Tunstall and Horridge, 1967).

d) Finally, in eye slices, the cut ends of the rhabdoms appear as bright sports when light shines into the eye e. g., in the locust (Wiedemann, 1965).

In the other type, found in the fly and called open rhabdomere eyes, the rhabdomeres of the retinula cells of one ommatidium are not fused but lie at different positions in the optical system behind one facet. Other retinula cells, however, in certain other neighbouring ommatidia, share a common optical axis as described below.

In all insect eyes the axons of the retinula cells pass through the basement membrane to the first optic neuropile, the lamina (Fig. 1). Here the axons are arranged in bundles called cartridges each of which contains several retinula axons and two second-order axons which have cell bodies above the lamina. The paths of axons from retina to lamina and from lamina through the chiasma to the next neuropile region, the medulla, have been traced in the following way.

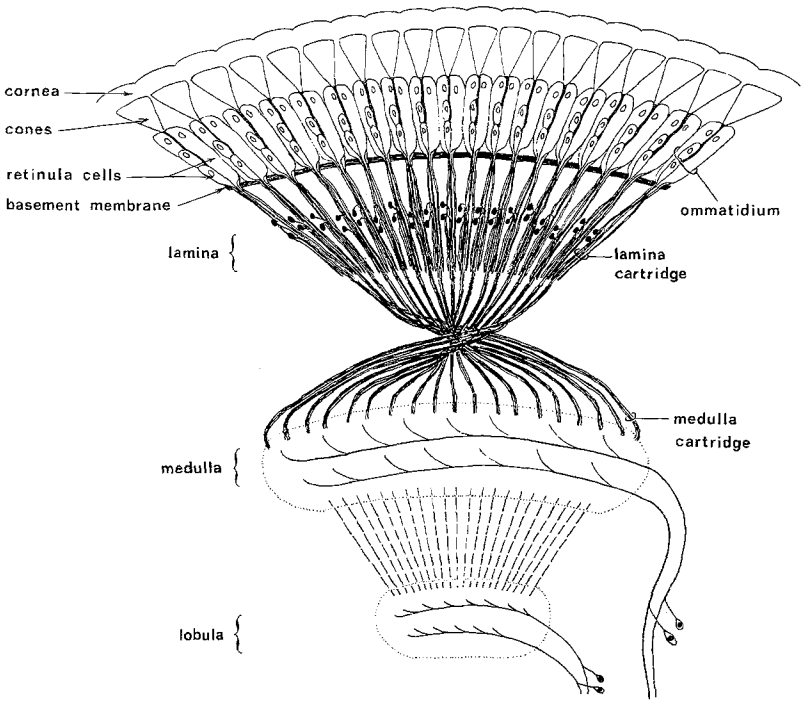

Fig. 1. A diagram of the retina and optic neuropile of a representative compound eye with fused rhabdomeres, cut in the horizontal plane, showing the regions discussed and in particular the projection across the chiasma between the lamina and the medulla. Structures lying central to the chiasma are indicated but not further analysed here

Materials and Methods

The following insects were employed: *Schistocerca gregaria*, *Apis mellifera* (drones), *Notonecta glauca*, *Aeschna cyanea* and *Calliphora vomitoria*. Eyes, together with adhering optic lobes were either fixed in phosphate buffered osmic acid (Millonig, 1961 in Pease 1964) for up to 12 hours at 4° C, or alternatively large eyes were fixed for up to 4 hours in phosphate buffered 5% acrolein at 4° C, dissected, and small pieces postfixed in phosphate buffered osmic acid as before. The material was then dehydrated in an acetone series at room temperature, embedded in Araldite and sections 1 μm thick cut on a Porter Blum microtome. Series of up to 1,500 consecutive sections were mounted on microscope slides and stained with toluidine blue.

It is essential that the fixation and staining produce good contrast of the axonal membranes. Often this was achieved by the relatively long period of fixation. Treatment of the sections for two minutes at room temperature in 2% sodium hydroxide in ethanol (Berkowitz *et al.*, 1968) also improved the contrast in some cases. It is also essential that the sections are transverse to the axons of

interest and that few sections are folded or lost. Consequently a successful series is selected from a number of inadequate ones.

The sections were then photographed with Zeiss Planapochromat ×40/1.0 or ×100/1.3 oil immersion objectives so that the maximum resolution available was obtained and axons down to $^1/_2$ μm in diameter could be traced with certainty.

At one end of the series the axons are numbered and as many as possible are traced through the stack of micrographs. The method is tedious and contains no new principles. Additional aids to tracing are provided by subtle differences in axoplasmic staining intensity in some axons compared with others from the same ommatidium or lamina cartridge, differences in axon diameter and by the constant spatial positions of axons. Errors are easily spotted during tracing because many axons are traced together and if one axon is carried over incorrectly between two photographs another axon cannot be accounted for. Any lack of agreement between the pathways of axons of the different ommatidia also leads to the discovery of errors in tracing.

Results and Discussion

Retina Projection upon Lamina

In fused rhabdomere eyes we have found that the eight axons from each ommatidium always keep together as a bundle as far as the lamina without interweaving with axons from other ommatidia. This is true for the locust, the drone bee, and for large dragonflies and their nymphs. In the first two of these examples at least, only six of the retinula axons terminate at the lamina, while a pair of axons, presumably but not yet proven to be of the two basal retinula cells, pass right through the lamina to the medulla. There is therefore a perfect projection so that the receptor cells of a single ommatidium with a common visual axis converge upon a single lamina cartridge (Fig. 2). In addition, the array of lamina cartridges exactly corresponds to the ommatidial array of the retina.

The more complex retina-lamina projection in the fly has already been described (Braitenberg, 1967). The eight retinula cells of each ommatidium in the eye of *Musca* receive light from seven different directions, i. e. the eight rhabdomeres of these cells have seven different optical axes, the rhabdomeres of the two centrally situated cells, Nos. 7 and 8, having the same axis (Kirschfeld, 1967). The two central cells have long axons that pass through the lamina and whose presumed terminals have been observed in the medulla (Melamed and Trujillo-Cenóz, 1968); the axons of the other six are short and terminate in a special pattern in the lamina (Braitenberg, 1967).

The angle between adjacent ommatidial axes is the same as the angle between the visual axes of two neighbouring receptor cells within an ommatidium, and Kirschfeld has shown that eight retinula cells of seven neighbouring ommatidia receive light from the same direction relative to the animal's head. Of these eight, six are receptor cells with short axons which undergo a complete interweaving before they make contact with

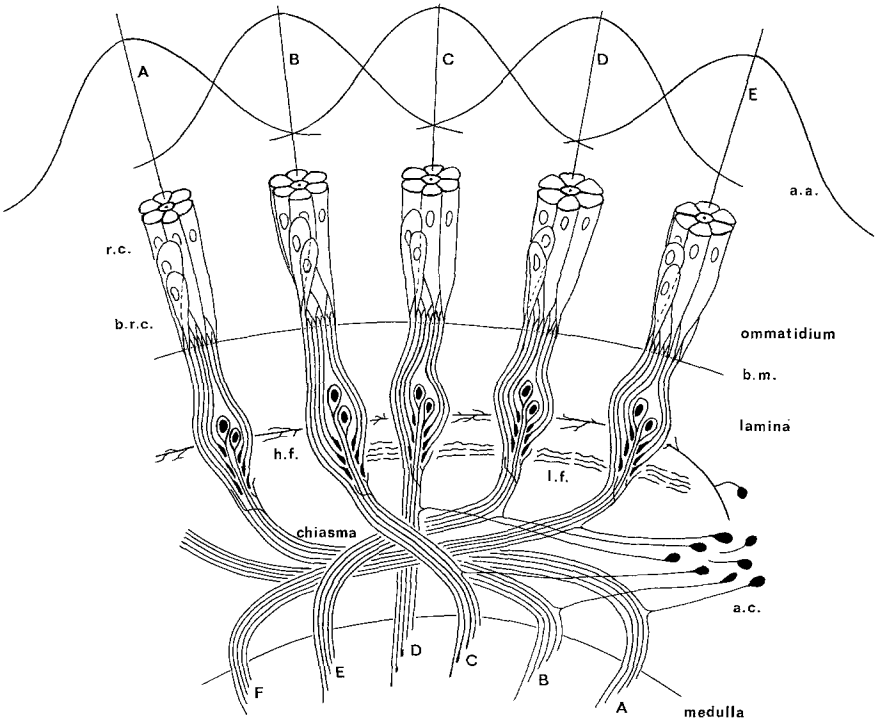

Fig. 2. The projection of visual axes in a typical fused rhabdomere apposition eye, as in the drone bee or locust, in horizontal section. In each ommatidium the eight cells all share a single axis. Six of them terminate upon one pair of lamina neurons. The other two continue with the bundle from their own lamina cartridge. The fifth axon in this bundle has an anteriorly placed cell body. The chiasma results in a reversed projection upon the medulla that is anatomically accurate at single neuron level

the second-order neurons of the lamina. The consequence of this interweaving is that six short-axon receptor cells with parallel visual axes have axons that converge upon a single pair of second-order neurons to form a lamina cartridge (Fig. 3). The lamina is composed of an array of these cartridges, each of which sums excitation from six cells with a single visual axis (Kirschfeld, 1967).

We have now followed both long and short retinula cell axons from about a hundred neighbouring ommatidia of *Calliphora* and confirm the above. Thus in open and fused rhabdomere eyes each cartridge of the lamina is a summing point for those short retinula axons that carry excitation from a single visual axis (Figs. 2 and 3). The pattern is perfect in that no errors have been found (Horridge and Meinertzhagen, in press).

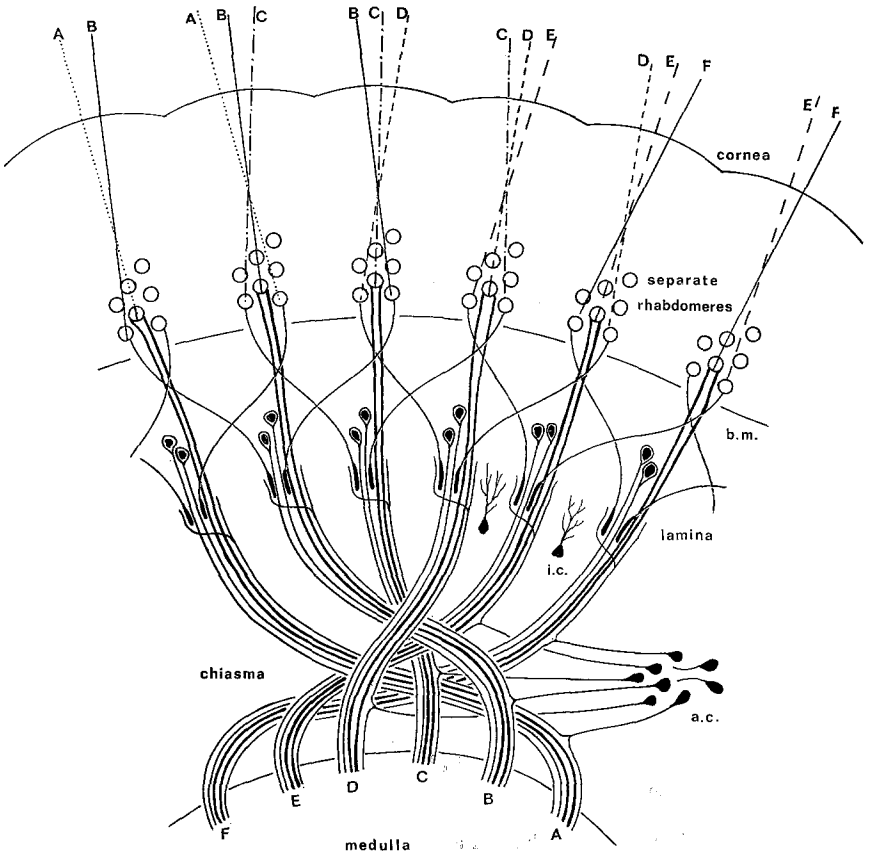

Fig. 3. The projection of visual axes upon the medulla in a dipteran fly as seen in horizontal section. In the retina three rhabdomere axes are shown out of the seven which each facet contains. The interweaving of the retinula axons ending in the lamina results in a summation of like axes BBB, CCC, DDD, upon the second-order neurons of the lamina. The projection through the chiasma upon the medulla results in an exactly reversed anatomical representation of that in the visual fields and lamina

Projection of Lamina Cartridges upon Medulla Cartridges

Situated between the lamina and the second optic neuropile, called the medulla, is a complex chiasma with a horizontal plane of decussation. Bundles of axons that have passed through this chiasma enter the medulla as a pattern of cartridges (Fig. 4) which are superficially similar to those of the lamina. The following facts emerge from our tracing through the chiasma region.

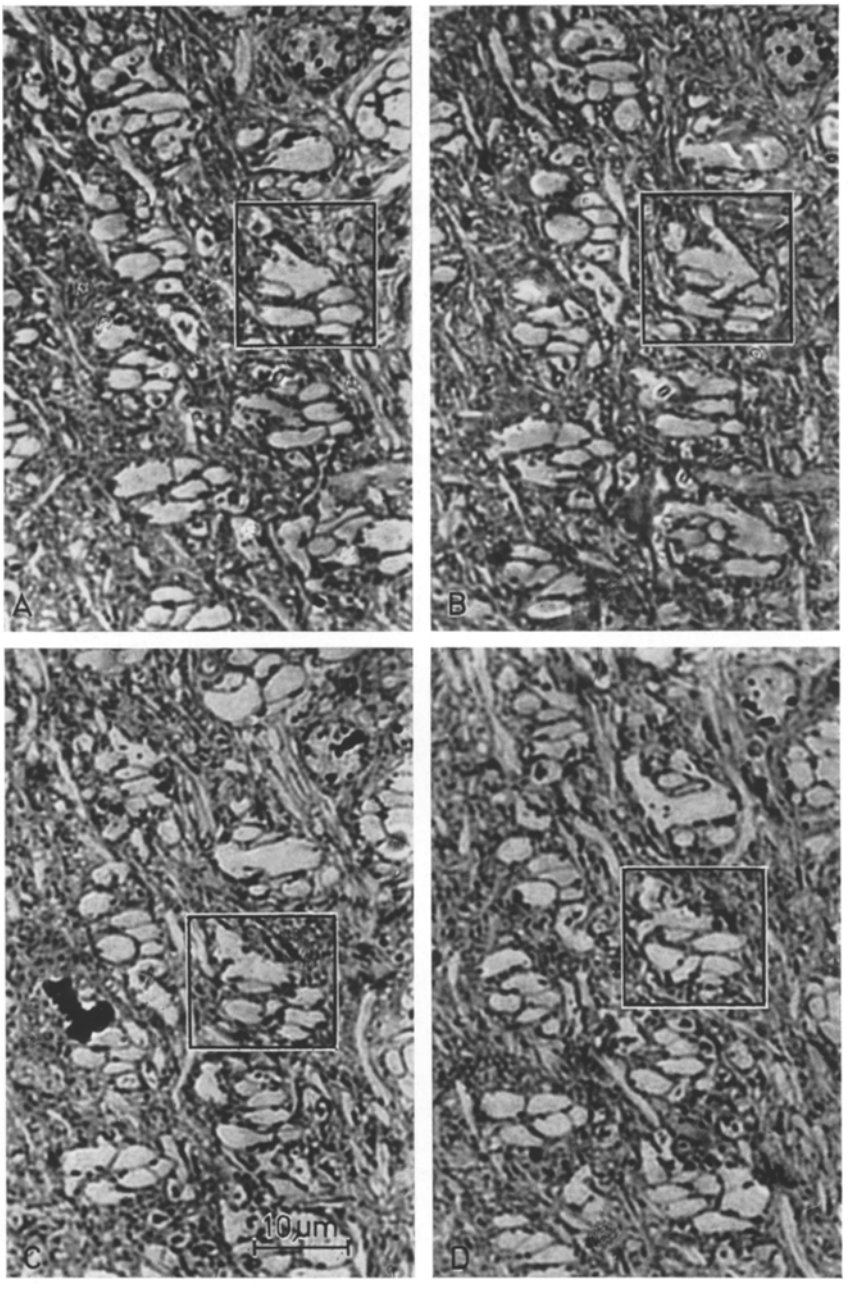

Fig. 4. Cartridges of the medulla of *Notonecta*. Four consecutive transverse sections through the bundles of axons that form the chiasma between lamina and medulla, cut at a level just within the medulla. The material has been embedded in Araldite, sectioned at 1 µm and stained with toluidine blue, and the micrographs are typical of many others used for tracing axons. Note from the scale that axons of $^1/_2$ µm diameter are distinguishable and that near maximum resolution available by light microscopy is obtained

In the several groups of insects mentioned above, the bundle of axons which travel together from a lamina cartridge to a medulla cartridge contains two long retinula fibres, two lamina ganglion cell fibres and at least one axon which probably has its cell body in an anterior cell mass (Cajal and Sánchez, 1915). This fifth axon has thin branches at both ends, which make tracing of it difficult within the cartridges.

In the fly the two long retinula axons pass between the rows of lamina cartridges, each pair proceeding together along the equatorial side of the cartridge that lies directly below its own facet. Together with the three other larger axons from this cartridge they pass through the chiasma and enter a single cartridge of the medulla. Therefore excitation from the eight receptors with a common field of view, namely the six receptors numbered 1 to 6 in six adjacent facets and from receptors 7 and 8 in a seventh facet (situated in the centre of the group of six) *converges upon a single cartridge of the medulla* (Fig. 3).

In the eye of the drone bee and of the water boatman, *Notonecta*, a bundle of five axons from each lamina cartridge runs through the chiasma to a single cartridge of the medulla (Fig. 2). As in the fly the bundle contains two long retinula cell axons, two lamina ganglion cell fibres and a fifth axon that branches at both ends. In the bee, as in the locust, the two long retinula axons enter at the centre of their own lamina cartridge.

In the fly, the drone bee and *Notonecta*, the projection of the lamina cartridges upon the medulla cartridges forms a perfect mirror image about a vertical plane, i. e. there is a chiasma in which a sequence ABCDEFGHIJ horizontally across the lamina (and retina) is exactly reversed to a sequence JIHGFEDCBA across the medulla (Figs. 2 and 3).

Limitations of the Present Findings

The anatomical basis for connectivity between the first- and second-order axons in the lamina cartridge is the observation of synaptic structures by electron microscopy. This is known in detail only for the fly (Trujillo-Genóz, 1965). Interpretation of records inferred to be of locust lamina ganglion neurons (Shaw, 1968) agrees closely with the anatomical projection presented here. The further synaptic connections of the five axons entering a medulla cartridge as one bundle are not known anatomically or physiologically but the fact that they are in close association in one place suggests that a higher order cell is excited by all with an afferent function, and the projection is preserved to third order neurons. The final resolution of the synaptic connections cannot be attained by the present method and awaits both electrophysiological recordings from second- and third-order units and electron microscopy of the cartridges of the lamina and medulla.

Functional Consequences of the above Findings

1. There is an exact topographical projection of the visual fields of the receptors first upon the lamina cartridges and then upon the medulla, with a medulla cartridge for each rhabdomere optical axis. The projection is perfect for the connection of every single neuron and (in the fly) very nearly perfect even for the positions of their synaptic terminals upon the second-order neurons.

2. Down to and including the axons of the medulla cartridges no class of axons visible by light microscopy diverges to an adjacent pathway: the axons, including those of basal retinula cells, come back to the connections corresponding to the optical axis of their retinal excitation. Because a motion perception apparatus requires elaborate interaction between excitation that derives from receptors with diverging fields of view, it must be located in a mechanism within the medulla, or in a more central neuropile.

3. In the open rhabdomere eye of the fly, information on the polarization plane of incident light presumably could be provided by the central retinula cells 7 and 8, but not by the other six, which blend together their different inputs at the lamina cartridges (Melamed and Trujillo-Cenóz, 1968). Information on the colour of incident light is available through the comparison of the respective inputs from the central retinula cell pair, with peak absorption in blue wavelengths, and the lamina ganglion cells, which have retinula axon inputs with peak absorption in the green (Langer, 1966). Thus, in the fly, information about polarization plane and colour of incident light for one particular angle is available among the five axons running to each medulla cartridge. For fused rhabdomere eyes information about the polarized light sensitivity of the long fibre retinula cells or about the spatial distribution of retinula cells of different spectral sensitivities within and between ommatidia is not available. Therefore the total information available in the medulla cartridges cannot yet be defined for fused rhabdomere eyes.

4. The lamina-medulla chiasma has the effect of exactly reversing the projection so that the anterior edge of the visual field is represented on the posterior edge of the medulla, and vice versa, with accuracy at single axon level. The significance of the chiasma is not known.

5. Aspects of the projection that remain uninvestigated could be of functional importance. In the Diptera the lamina is reported as containing a class of intrinsic neurons (Fig. 3). In other orders there are horizontal fibres of the lamina (Fig. 2). In several orders with fused rhabdomeres the lamina ganglion cells have lateral dendrites that reach adjacent cartridges (Fig. 2). The fifth axon in the bundles to the medulla could possibly be carrying efferent excitation from the medulla.

6. The anatomical tracing of axons shows that the maximum information about the angular origin of details in the visual fields is carried in an accurate spatial array right into the medulla in both types of insect eye.

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