05 THE RETINA, SENSITIVITY AND RESOLUTION[1]

The combination of anatomy, optics and electrophysiology of the honeybee eye provides a splendid illustration of science in action and the way to figure out the mechanisms of processing in vision. It is a mature topic, with a wide variety of approaches—notably, optics, pigment movements, transduction, signal detection, successive arrays of nerve cells in parallel pathways, compromises between receptor sensitivity and resolution, distinctions between line-labelled channels and the interesting compromise between crowding-in more processing versus simplicity for speed of action.

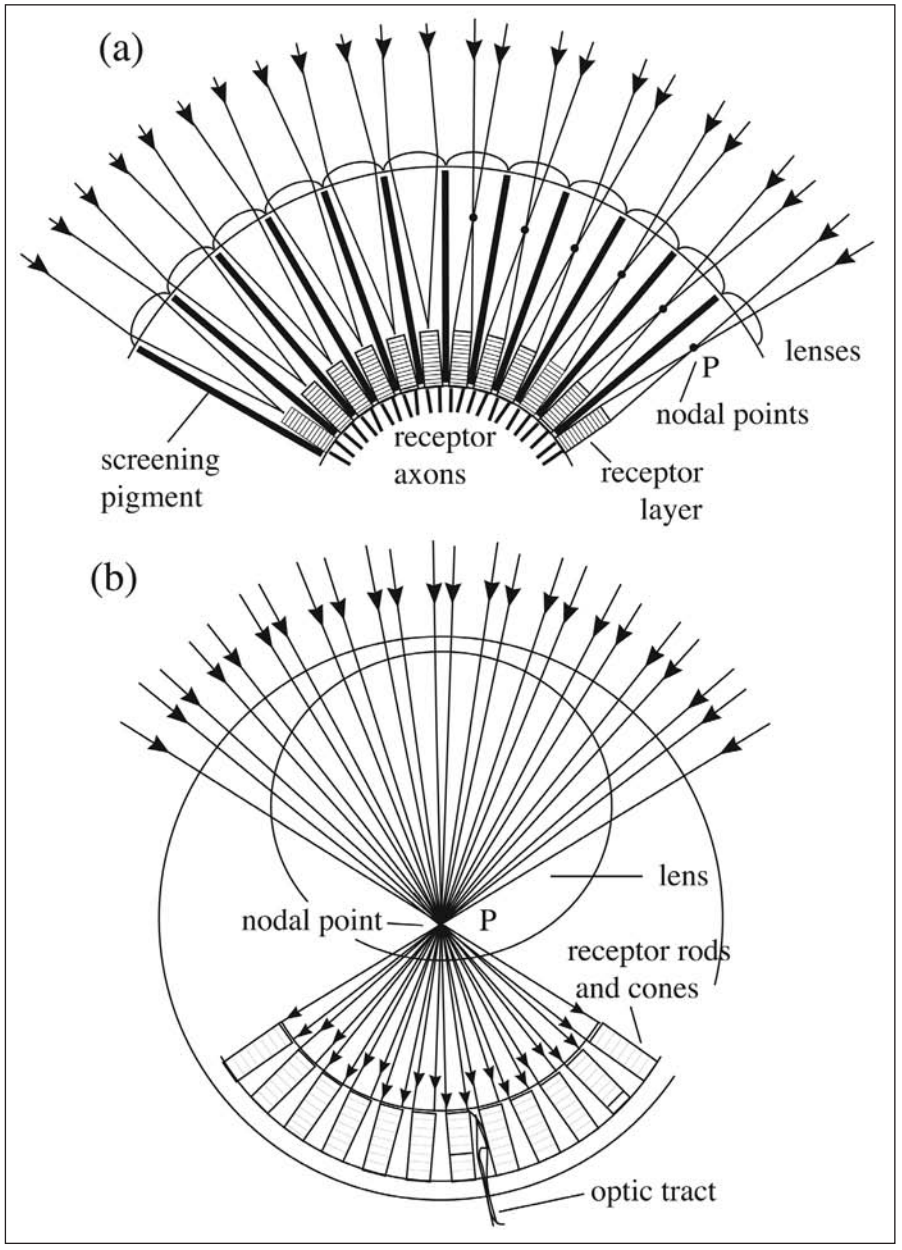
The compound eye is composed of numerous simple eyes, called ommatidia, which are arranged side by side at a small angle to their neighbours. Together, the whole forms a diverging hexagonal array of visual axes that samples the visual world in angular coordinates (Figure 5.1a), in a different way to the vertebrate eye (Figure 5.1b), but with similar results. In directions where vision is most vital, the hexagonal pattern of facets is most regular. The wide field of view assists the detection of prey and predators and is essential for the recognition of a place by a relatively simple brain. The compound eyes of crustaceans and insects are similar in detail and in their functional arrangements, besides being controlled by similar genes, although there is no continuous series of fossil compound eyes between them.

Early observations

More than three centuries ago, it was proposed that the insect retina divided the image into small, separate receptor fields. In his book of 1665, the versatile English scientist Robert Hooke (1635–1703) inferred that each facet was a convex lens that formed a minute reversed image on a sensitive layer below. Hooke did not see the images but understood that they could not form a smooth composite image because they were reversed. Only the rays close to the optical axis could be effective. He was followed in 1695 by the father of biological microscopy, Antonie van Leeuwenhoek (1632–1723), who vividly described the

reversed images in the flattened cornea of a fly. This led to a common view that the compound eye divided the panorama into an array of little pictures. The illustrations, however, were further confused because each facet showed the same image, making multiple views. The flattening of the cornea on the microscope slide caused this awkward artefact.

Figure 5.1 The compound eye (a) and the simple lens eye (b). In both, the panorama is projected to an array of receptors.



The little images were repeatedly mentioned in subsequent centuries, until Johannes Müller (1801–58), Professor of Physiology at the University of Berlin, simply bypassed them. Following Hooke, in his textbook of 1826, Müller assumed that the light passing through a single facet was concentrated to one receptor, so each facet must look in a single direction (Figure 5.1a, left side), but he also assumed that the panorama was divided without overlaps and without gaps (Figure 5.1a, right side). Both models were wrong in detail and Müller's theory was a simplification, but he carried sufficient authority to inhibit alternatives for 130 years or so.

As soon as new histological techniques were invented, very small, dense inclusions in each receptor cell, called rhabdomeres, were inferred to be the light-sensitive particles (Grenacher 1879). In many common large insects that fly by day—notably, bees, wasps, butterflies, locusts, crickets, mantids, dragonflies and the primitive wingless insect *Machilis*—the rhabdomeres were fused to a single rod, called a rhabdom, that extended inwards from the tip of the cone. As Exner noted later, Grenacher, like Müller, inferred that these eyes could have only one directional sensation for each ommatidium, but possibly more than one sensation of colour.

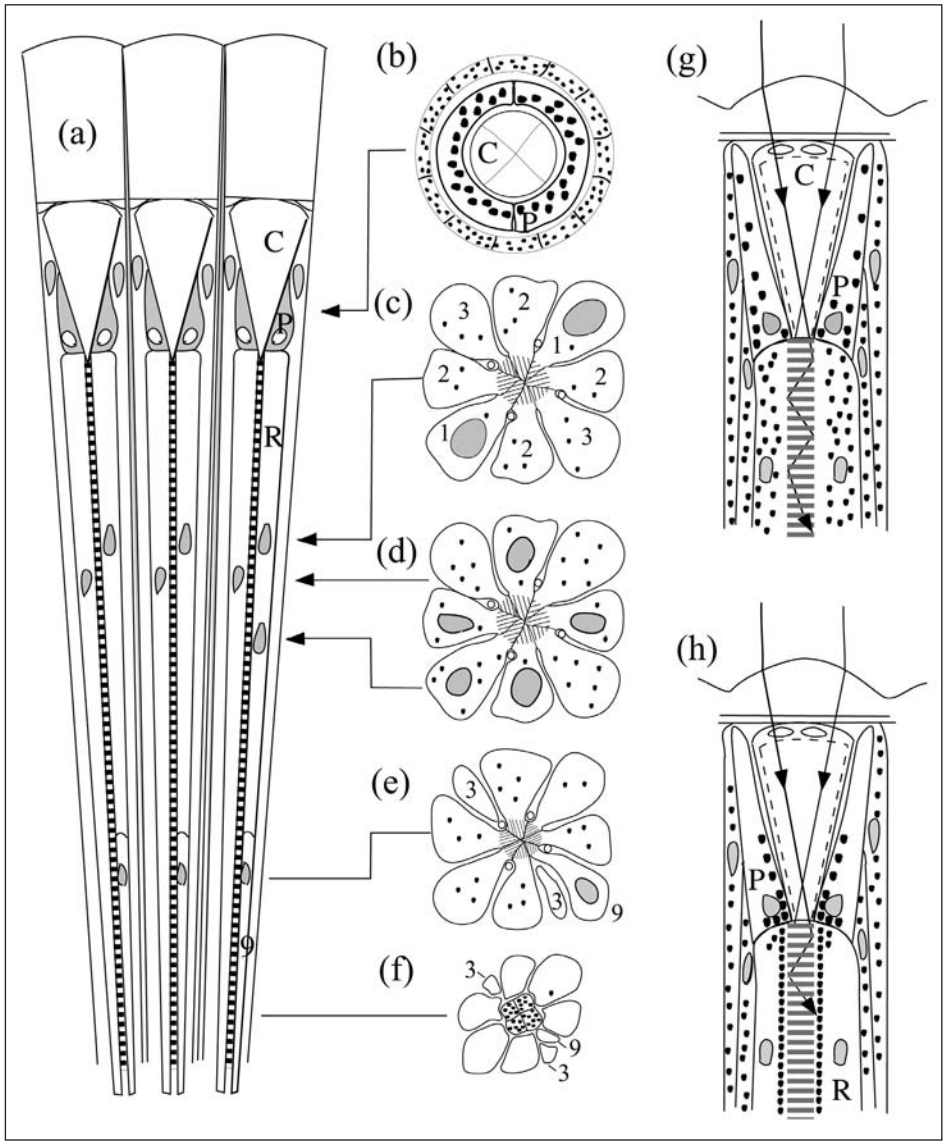
Many groups of insects—notably, bugs, flies, the primitive wingless insect *Lepisma* and many beetles and lower-order insects—however, had six, seven or eight separate rhabdomeres in the right place to receive the inverted image, so they could divide it into a few separate parts. Exner (1891), in his influential book, assumed Müller's theory and that the light was absorbed along the length of the rhabdom, like a single light guide. He examined only one species with separate (open) rhabdomeres—the drone fly *Eristalis*—but did not illustrate it, and subsequently the separate rhabdomeres were scarcely mentioned in twentieth-century texts, although they were the commonest type. The loss of a single gene, dubbed 'spacemaker', converts the open rhabdoms to the closed one.

Grenacher also inferred that broad rhabdomeres would function at lower light levels than narrow ones, that the field size would depend on the size of the rhabdomere and that ommatidia with large facets would be more sensitive than those with small facets. These relations between sensitivity and resolution were neglected until Kuno Kirschfeld rediscovered them in the late 1960s.

Advance was slow with sudden spurts. In a curious coincidence, Vigier (1907, 1909) in France, Cajal (1909) in Spain and Dietrich (1909) in Germany described the axons coming from the seven separate receptor cells of the fly ommatidium, and inferred that they looked in different directions. Each axon, however, meets with six others in the lamina layer. The effect of this intricate convergence of the axons is to sum together the parallel axial rays that enter the eye through each group of six facets, so making the optimum use of the little images by rotating and combining them. This amazing work was neglected because it appeared

in journals that were little read and few were interested. The convergence was observed again in silver-stained sections, worked out in detail, and published by Valentino Braitenberg (1967) and Kuno Kirschfeld (1967). In all the sciences, we find this neglect of a topic for decades and then another sudden simultaneous interest—like goldminers rushing from one strike to another.

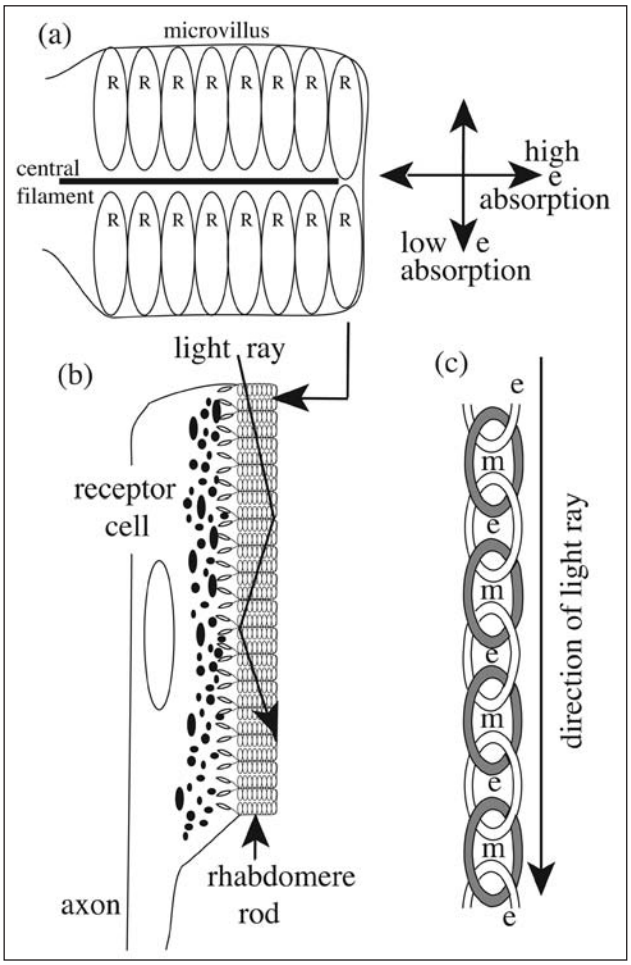
Figure 5.2 The structure of the retina of the worker bee. a) Vertical section; c = cone; p = principal pigment cell. b–f) Transverse sections at the different levels shown. g) and h) Details of the light path at the cone tip in the dark and light-adapted states, with exaggerated migration of the pigment grains.



Functional anatomy of the bee ommatidium

The bee has a common type of compound eye in which each ommatidium has its own convex lens, formed by the cuticle of the cornea (Figure 5.2). Accessory pigment cells and trachea screen the ommatidia optically from each other. Light rays near to the axis pass through the lens then through a transparent region, called the cone, formed by four cells, and are focused on the distal tip of the rhabdom (Figures 5.2g and 5.2h). The principal pigment cells, identified by large pigment grains (Figure 5.2b), surround the cone and secrete the corneal lens in the embryo. In the bee, there are sensory hairs between the facets.

Figure 5.3 Absorption of light in the rhabdomere. a) A microvillus with oriented rhodopsin molecules and the preferred direction of absorption of the electric vector. b) The light path with internal reflection. c) A representation of light with electric and magnetic vectors in planes at right angles to each other. The polarisation plane is defined as that of the electric vector, e.



The retinula cells (usually eight, but nine in the bee) each secrete a rhabdomere, which is one sector of the long thin rhabdom that acts as an absorbing light guide down the middle of each ommatidium (Figures 5.2a–f) and have an axon at the base that runs to the next neuron layer, the lamina (Figures 6.3 and 6.5). Each receptor cell is therefore a sensory neuron with a receptor organelle. The rhabdomeres are composed of parallel tubules, called microvilli, that are each packed with about 1000 molecules of the visual pigment rhodopsin (Figure 5.3b), which progressively absorbs light that passes down the rhabdom rod. The rate of absorption is about 0.7 per cent per micron, so that at least two-thirds of the light is absorbed in the first 100 microns.

Retinula cells usually contain black, brown or red pigment grains, which can migrate close to the rhabdom by day and away from it at night (Figures 5.2g and 5.2h), so altering its absolute sensitivity and spectral sensitivity. In the bee, these changes are small. The ommatidia are not all the same; often the dorsal ones contain more blue and UV-sensitive cells while ventral and lateral ones are more green sensitive. Types of retinula cells differ according to spectral sensitivity and direction of their optimum sensitivity to polarised light. Some mainly aquatic insects that live in moist habitats have spectacular regular patterns of orientated rhabdomeres across the eye, as though they discriminate particular patterns of polarisation. Like the hand of a whale or the arm of a bird, the functions of the huge differences between ommatidia of different insects should be obvious, but most remain a puzzle. Several groups including bees have specialised UV-sensitive ommatidia with oriented microvilli along the dorsal rim of the eye for navigation using the polarisation pattern of the sky (Chapter 8).

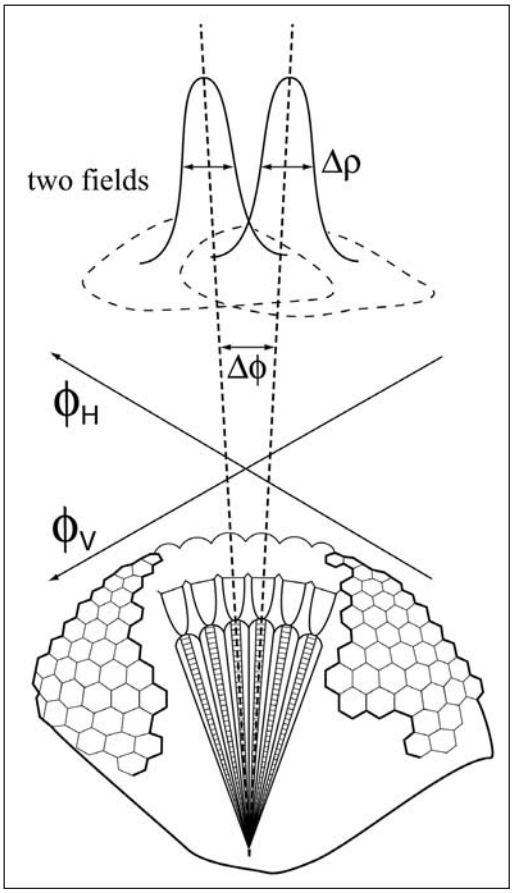
Early theory

The convex corneal lens with the photoreceptor in its focal plane turns each ommatidium into an optical instrument like a camera with a single pixel. At the end of the nineteenth century, it was well known that resolution was limited by the aperture (D) and the wavelength of the light (λ). Incident parallel rays are concentrated to a 'blur circle' in the focal plane, not to a point. From the theory of diffraction, the minimum full width of the blur circle was calculated as $2.4\lambda/D$ radians or λ/D radians at the 50 per cent intensity contour (Figures 5.4 and 5.5). Mallock (1894) argued that the interommatidial angle ($\Delta\phi$) should be matched to the full width of the blur circle ($2.4\lambda/D$) so that the receptor would slide smoothly from one blur circle to the adjacent one as the eye moved. So, $\Delta\phi$ in radians equals $2.4\lambda/D$, and $D\Delta\phi$ equals $1.2\mu m$ for green light, with $\lambda = 0.5\mu m$.

Mallock's survey of 18 insects of different sizes gave a relation between $\Delta\phi$ and λ/D that was compatible with $D\Delta\phi = 1.2\mu m$, but he was ignored for 60 years. The theory assumed that there was a single rhabdom of negligible width on the

axis, an inverse relation to the wavelength and contrast sensitivity similar to a human eye—none of which was actually realised, and there was no test of the resolution.

Figure 5.4 The two angles that define the compound eye, the field size of the ommatidium, $\Delta\rho$, and the interommatidial angle, $\Delta\phi$.



Mid-twentieth century

Unaware of Mallock's work, Barlow (1952) also assumed a match between the blur circle and the interommatidial angle. He considered three ommatidia in a row, with $\Delta\phi$, such that two distant point sources excited the two outer ommatidia sufficiently more than the central one. He predicted that $\Delta\phi$ should be less than λ/D but greater than about $0.5\lambda/D$. Again, there were only two factors—namely, the best focus and the optimum separation of neighbouring inputs—because he ignored the size of the rhabdom. Since, by geometry, $\Delta\phi = D/R$ radians, where R is the radius of the compound eye, $D\Delta\phi = D^2/R = 1.2\mu m$, so the radius should be proportional to the square of the facet diameter. Barlow was unaware of measurements of $\Delta\phi$ by Baumgärtner (1928) and assumed an

isotropic eye for the bee. Although the data were incorrect, in a number of eyes of species of bees of different sizes, the average eye radius was proportional to D^2, which was compatible with the theory.

The anatomy of light capture

The width of the field of view of each receptor cell at the 50 per cent level of sensitivity is called the acceptance angle ($\Delta\rho$) and the angle between the axes of adjacent ommatidia is the interommatidial angle ($\Delta\phi$). These angles can be specified in the vertical and horizontal directions (Figure 5.4).

When there is one fused rhabdom, as in the bee, there is one optical axis in each ommatidium. In a single ommatidium, with a small circular lens, the spherical and chromatic aberrations are negligible and the distribution of intensity in the blur circle (sometimes called an Airy disc when well focused) can be approximated by a Gaussian function, which simplifies the theory but omits a weak halo of light around the edge. A convenient approximation is that the angular diameter of the blur circle at its 50 per cent intensity contour is $\Delta\alpha = \lambda/D$ radians (Figure 5.5a).

To achieve the best compromise, the distribution of photon absorption at the distal tip of the rhabdom must match the distribution of photons in the blur circle (Figure 5.5b). Larger receptors (Figure 5.5c) subtend a larger angle in the outside world and therefore catch more light, but waste the lens resolution—exactly as happens with large pixels in a cheap camera that make the image grainy however good the camera lens. Receptors subtending less than λ/D radians in diameter (Figure 5.5a) simply throw away sensitivity with no extra gain in resolution.

Because light is absorbed by the visual pigment at a rate of only about 0.7 per cent per micron along its length, the rhabdom is a long rod with the incoming light focused on its distal tip, like the rods in vertebrate eyes. To catch rays optimally, the rod points directly at the nodal point of its lens. When separated from each other by a medium of lower refractive index, rhabdoms or rhabdomeres act as separate light guides. When light guides are about 1–2μm, their capture cross-section for light can be approximated by a Gaussian distribution of diameter d at the 50 per cent level of sensitivity, where d is the diameter of the rhabdom. Therefore, to match the resolution of the lens to the capture cross-section of the receptor, we have Equation 5.1.

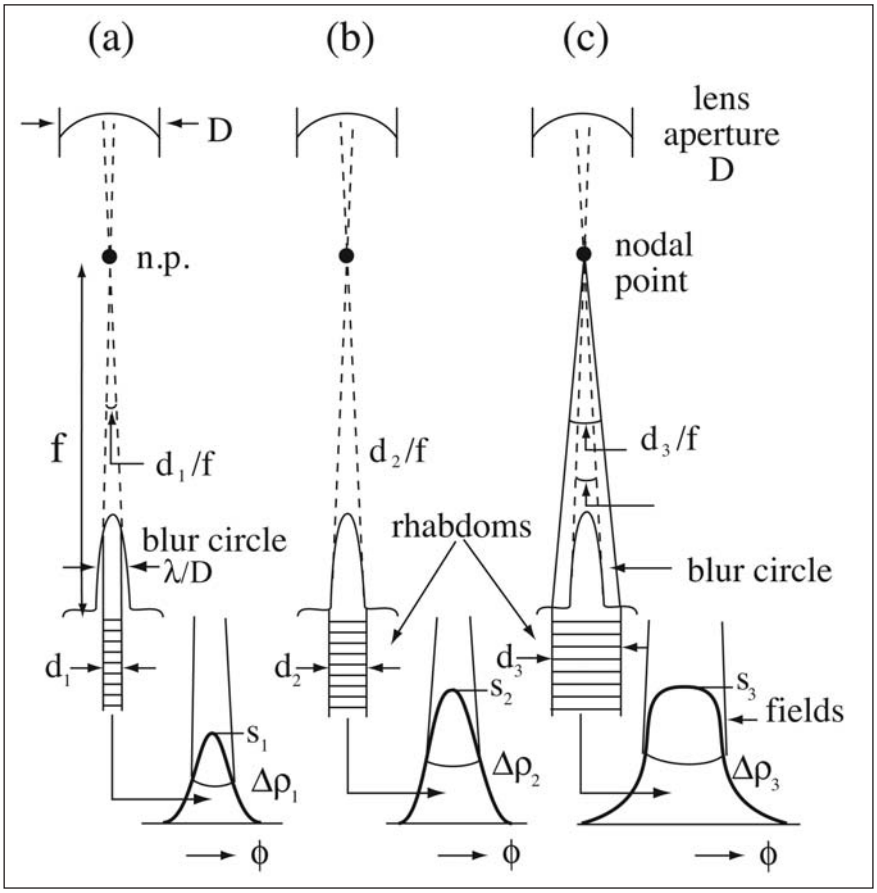
Equation 5.1

$$\lambda/D = d/f \text{ radians}$$

In Equation 5.1, f is the focal length, measured from the tip of the receptor (at the focal plane) to the nodal point of the lens (Figure 5.5b).

The nodal point is defined as the point through which rays pass as straight lines through the lens (Figures 5.1 and 5.5). The power of the lens of most non-aquatic insect eyes lies in the curvature of the outside surface—all the internal surfaces having much less power—so that the nodal point in an insect ommatidium usually lies near the centre of curvature of the surface of the cornea.

Figure 5.5 The effect of the rhabdom width d (μm) on the field of view of a single receptor. A point source in the outside world generates a blur circle, which subtends an angle of $\Delta\alpha$ at the posterior nodal point. The field of the receptor is generated as the blur circle moves over the receptor of angular width d/f radians, so the receptor field, $\Delta\rho$, is the convolution of the blur circle and the receptor absorption distribution. a) For narrow receptors, d_1, this reduces to $\Delta\rho_1 = \lambda/D$ when d_1/f is negligible and the sensitivity, s_1, is suboptimal. b) When diffraction and receptor width give an equal contribution, $\Delta\rho_2 \approx \sqrt{2}\, d_2/f = \sqrt{2}\,(\lambda/D)$. c) For wide receptors, $\Delta\rho_3 \approx d_3/f$ when $\Delta\alpha$ is negligible.



Rhabdom optics

A glass or transparent plastic fibre surrounded by a material with a lower refractive index transmits light along its length, and radar waves can be piped along a rod of polystyrene or wax. When the rod is thick, one can think of the process as total internal reflection of the waves, and classical optics based on ray tracing and absorption within the rod explains most of the properties of photoreceptors.

When the diameter of the light pipe approaches the wavelength of the light, the rays can fit into the pipe in only a limited number of ways, called modes, and the rod is properly called a wave guide. The number of modes carried in a wave guide is governed by the value of the mode parameter V, as in Equation 5.2.

Equation 5.2

$$V = \pi d/\lambda \, (n_1^{\,2} - n_2^{\,2})^{1/2}$$

In Equation 5.2, d is the rhabdom diameter, λ is the wavelength and n_1 and n_2 are the refractive indices inside and outside the light guide, respectively.

In fact, n_1 and n_2 are difficult to measure and the value of V is very sensitive to their difference. So, instead of calculating V from this equation, the first few modes have been observed in the light-adapted state in a few insects, from which the value of V has been inferred to lie between 1.5 and 4. Very approximately, the value of V and the number of modes in an insect rhabdom are equal to the diameter in microns, from which we can calculate that $n_1^{\,2} - n^2_{\,2} = 0.05$, so the refractive index of the rhabdom, n_1, is approximately 1.39.

In butterflies, rays are reflected by the tapetum at the base of the retina and return through the rhabdom and out of the eye. When the cornea is optically neutralised with a little oil, the first few modes can be seen under a microscope. The effect of the increase in light intensity can be directly observed as a loss of the third and then the second modes as they are absorbed by pigment migration.

With thinner wave guides, a greater fraction of the energy lies outside, where it is absorbed by pigment grains within a range of 2μm. When the receptor rod is <1μm, light travels longitudinally along it without internal reflection—called the first mode of vibration. As the mode parameter V is increased by shortening the wavelength, more modes are accepted (Equation 5.2). A light guide carrying only the first mode accepts only photons that are near axial; the more modes, the greater is the angle of the accepted cone of light, up to the critical angle.

Mode theory applied to photoreceptors in the early 1970s led to the conclusion that the thinnest of the photoreceptor rods in small insects, about 1μm diameter, carried only the first mode for green light. More modes can be carried if the receptor is broader, but that throws away some lens resolution. The situation

is quite different in a camera-type eye, where photoreceptors must also be as narrow as possible so that as many as possible can be packed in to optimise the spatial resolution.

A remarkable coincidence is revealed when we calculate the diameter of the receptor rod from two sets of assumptions. On the one hand, from the optics of the eye (Figure 5.5), d/f is approximately equal to λ/D, and f/D is the F number of the lens, so $d = F\lambda$. Since F ranges from 2 to 4 for insect eyes, and λ is 0.5μm for green light, the receptor diameter, d, is predicted to be between 1 and 2μm for all of the receptors that operate in bright light. So, a photoreceptor between about 1 and 2μm wide makes full use of the resolution of light. On the other hand, observations in butterflies, for example, reveal that a rhabdom between about 2 and 4μm wide is exactly the right size for the modes to be controlled by pigment migration outside, and the first and second modes carry most of the power. In fact, photoreceptor rods that operate in bright daylight are commonly about 2μm in diameter.

Absorption by the rhabdomere

The rhabdomere consists of numerous tubules (microvilli) of lipid-rich cell membrane, 80–90μm in diameter (Figure 5.3), on the inside walls of which are attached molecules of the visual pigment rhodopsin. The rhodopsin molecules, 12nm apart when packed in the microvilli, consist of a protein, called opsin, combined with a carotenoid related to vitamin A. There are about 1000 rhodopsin molecules per micron of microvillus. The alternating double bonds of the carotenoid capture passing photons, so the receptor acts as a photon counter and the light must be calibrated in photon flux rather than in energy units. The complexity of the vibration patterns of the rhodopsin molecule broadens the absorption spectrum—70 to 110nm wide at the 50 per cent level. This unusual property of the molecule is essential for its function in vision. The position of the peak of the spectral sensitivity depends on the particular opsin in the rhodopsin.

Perception of the plane of polarised light was popularised in the 1950s by publications from von Frisch on the bee, and simultaneously demonstrated by recordings from retinula cells (Autrum and von Zwehl 1962; Burkhardt and Streck 1965). The mechanism is that light in polarisation planes at right angles is absorbed differently by the rhabdomeres (Giulio 1963), called dichroism. It took another 25 years before it was shown that the polarisation pattern of the sky was detected by the line of specialised ommatidia along the dorsal rim of the bee eye, and not by ordinary ommatidia.

Polarisation sensitivity arises because the rhodopsin side chains are asymmetrical and elongated. The ratio of absorption along the preferred plane to that in the polarisation plane at right angles is up to 10:1 for each molecule. If the molecules lie at random in the plane of the walls of the microvilli, they will

appear to be oriented because the incoming light strikes some of the membrane edge-on. This effect of the microvillus geometry alone yields an absorption ratio of 2:1 to the plane of polarisation. The electrical response of the receptor is not linearly related to the absorption of photons, so the ratio of the maximum to the minimum absorption of the receptor, called the dichroic ratio, cannot be calculated from the electrical responses, but must be measured optically or by electrical recording of individual photon captures (Doujak 1985). Even then, we do not record the value for the molecule.

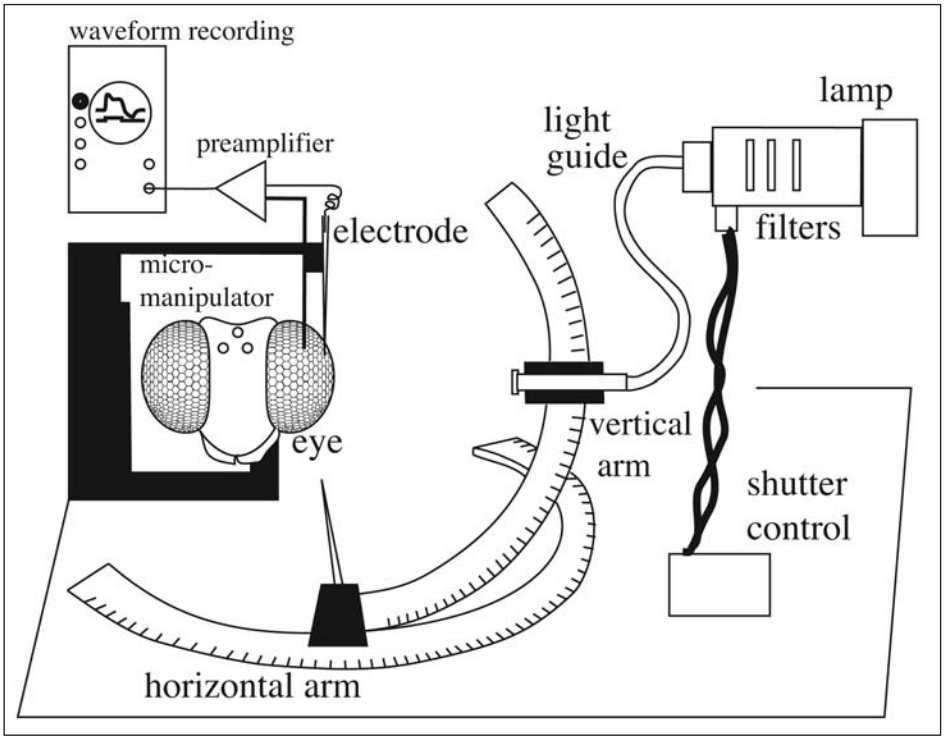
The capture of a single photon by a single rhodopsin molecule causes a molecular change, which initiates a chain of amplifying reactions within the cell and finally results in the opening of channels in the receptor cell membrane, giving an electrical response. The initial unit of action appears to be the microvillus. One photon capture causes one positive-going miniature potential (called a bump) caused by the entry of a pulse of mainly Ca^{++} ions. Photon arrivals are distributed randomly in time, so when the light intensity is increased, the bumps come closer together in an irregular noisy summation and eventually fuse into a (still noisy) receptor potential. Bumps are very small in the bee, and are usually not seen in recordings.

The powerful amplification from a single photon to the electrical response of a capacitive membrane requires a great deal of energy, as indicated by the large number of mitochondria in the photoreceptor cells. This is another reason why eyes are small. Unlike vertebrate rhodopsins, insect rhodopsins, to save some energy, are bleached to metarhodopsin by the normal absorption of a photon. They are reconverted back to rhodopsin by photons of a different (usually longer) wavelength. This reaction, called photoregeneration, leads to an equilibrium concentration of available rhodopsin, depending on the wavelength content of the ambient light. One consequence is that screening pigments are often red or yellow and admit long-wavelength solar power for the regeneration of the rhodopsin without loss of resolution in vision.

The response of the photoreceptor cell

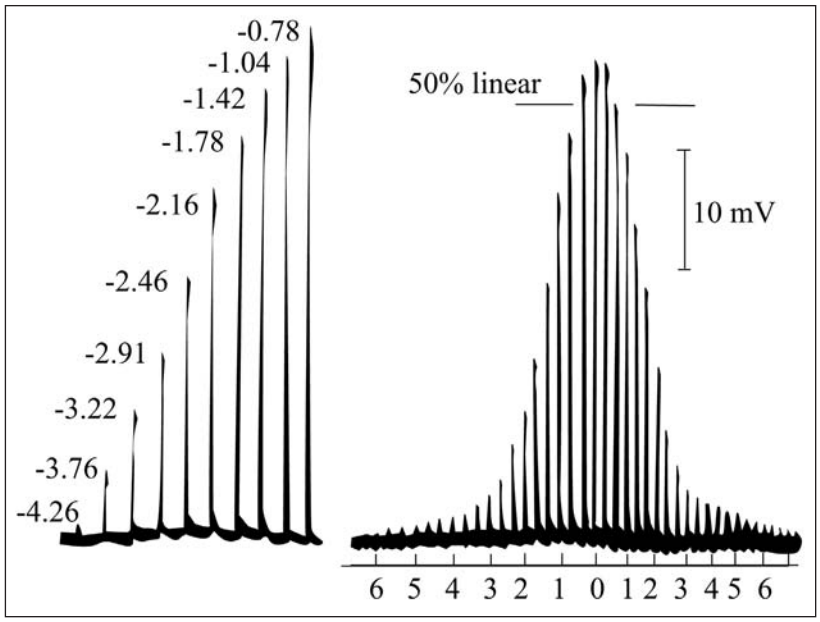
In some insects when dark adapted at night—notably, the locust, praying mantis and some beetles—single photon captures cause quite large depolarising responses, called ‘bumps’, so that it is possible to calibrate the transduction in the receptor cell by counting bumps and measuring the incident light intensity at the same time. Sensitivity can then be defined as the reciprocal of the number of photons required to generate a bump. It is possible to show that about 50 per cent of the incident photons are usefully captured, and also that an optical gain greater than 1 can be measured in some types of nocturnal eyes with optical overlap between ommatidia.

Figure 5.6 The method of measuring field sizes of single retinula (receptor) cells. A small source (the end of a quartz light guide) is moved around the eye on a calibrated cardan arm that measures to an accuracy of 0.1° in two coordinates. Flashes of the source at each angle cause responses that are picked up by a microelectrode from a single cell, amplified and recorded. Angular sensitivity is the reciprocal of the number of photons required to give a constant response at each angle on the eye. The light guide can be replaced by a lens to check from the shape and position of the pseudo-pupil that the optics of the eye are not damaged and to identify which eye region is stimulated.



Rhodopsin molecules absorb photons as they pass down the rhabdom, causing a chain of amplification that eventually results in a depolarisation of the resting potential across the receptor cell membrane. The receptor potential is readily recorded with a microelectrode in many large insects (Figures 5.6 and 5.7a). Any measure of it on the oscilloscope screen is arbitrary; the real response is that measured by the next neurons downstream, and there are several of them responding in different ways. The receptor response is usually measured as the height of the peak to a brief flash or the initial peak at light 'on'. The shortest integration times over which light is summed linearly is about 10msec for common large diurnal fast-flying insects at 20ºC. The peak depolarisation response increases with increasing light intensity in a smooth sigmoid curve

Figure 5.7 Responses of a receptor cell obtained with the apparatus in Figure 5.6. a) Responses to the point source on the axis as successive neutral density filters were removed from the light path. The logs of the filter densities are shown. b) Responses as the constant light source was moved in steps of $^1/_3°$ in the horizontal plane of the eye.



(called the V/log I curve), with a dynamic response over an intensity range of about 1000-fold. As in most other sense organs, in the eye, all response properties are relative and depend on the previous stimulation.

Adaptation to light moves the response curve to the right (Figure 6.5, Circles 1, 2 and 3), raises it upwards by the amount of the maintained response to background illumination and often makes it steeper. Repeated flashes cause a shortening of the response and a decline in the height. A sudden onset of a maintained light causes a rapid rise to a plateau that slowly declines. These changes are caused by a combination of several effects of pigment migrations, changes in membrane properties and probably extracellular potentials from the lamina that back off the steady-state response. Light-adapted eyes have higher flicker fusion frequency than the same eyes when dark adapted.

The responses of the receptors are graded—that is, without spikes. This makes transmission less noisy and faster over distances of less than a few millimetres. In evolution, the ganglia of the insect nervous system tend to fuse together and spike transmission is reduced.

One definition of sensitivity is the reciprocal of the number of photons that give a constant response—usually 50 per cent of peak. On this measure, the honeybee has a relatively insensitive eye by day, but behavioural experiments

reveal a 1000-fold increase in sensitivity at night. Because there is no reliable relation between the intensity and the depolarisation of the receptors at any one time, one might suppose that this is a hindrance to accurate vision. Certainly, that would be the case in a camera. Visual systems, however, are interested in detecting features, not in measuring light intensity, or even relative intensity, except in colour vision.

Recent work has shown that the electrical properties of the cell membranes are tuned to the ecological requirements. Transduction is more sensitive and decays more slowly in slow eyes than in fast eyes. The retinula cell membrane acts as a low pass filter that smoothes the photon and transduction noise and increases sensitivity at the expense of speed. Fast photoreceptors have lower input resistances and voltage-sensitive potassium channels with delayed rectification that cut off the response with a large inward current and speed up the frequency response. This great expenditure of energy is necessary to charge the large membrane capacity of the rhabdom quickly. In slow eyes, these large currents do not occur (Laughlin and Weckström 1993).

The principle of univariance

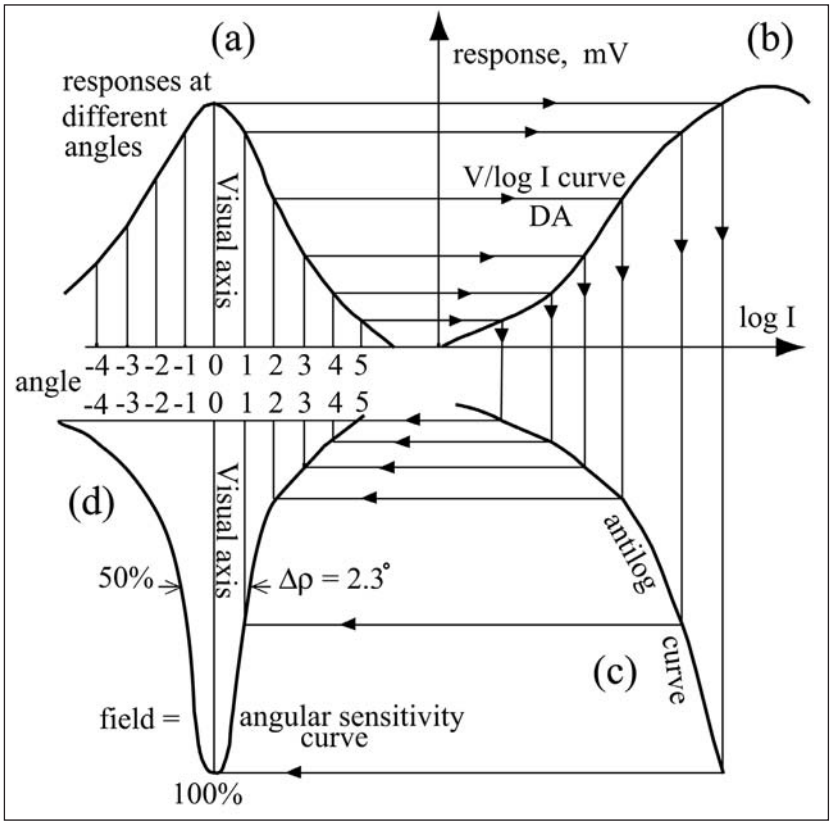
The rhodopsin family of visual pigments has broad spectral sensitivity and, when the pigment molecule is excited by a photon, there is nothing in the response to indicate its wavelength, plane of polarisation or direction of origin. Effectively, the receptors are coarsely tuned photon counters with a smoothing filter. The response to flashes of increasing intensity is a monotonic graded increase in the peak and plateau (Figure 5.7). Responses to flashes of different wavelength, polarisation plane or direction on the eye can therefore be calibrated and related to each other in terms of the equivalent intensity on the optical axis that would give a criterion response (at a constant mix of wavelengths). In this way, the fields of the receptors can be plotted in terms of relative sensitivity for each independent variable and the resulting fields can be used to predict the relative receptor responses to other stimulus patterns.

Univariance means that the effect of intensity, angle, wavelength and polarisation can be calculated once the responses have been transformed to a sensitivity scale, because the variables are independent. Then, having measured sensitivity to various physical variables, we can discuss how the different compromises affect the receptors in terms of the laws of physics. Absolute sensitivity is best measured by counting bumps at calibrated low-light levels or by measurement of the photon flux required to yield a 50 per cent response in the V/log I curve to axial rays at the spectral peak of the receptor. Other measures of sensitivity are the slope of the V/log I curve and the noise amplitude as a fraction of the signal amplitude. All these measures are useful in their own way.

The field of the receptor

The concept of 'field' is fundamental to the analysis of nervous systems. The spatial field of a photoreceptor is defined as the angular distribution of sensitivity when a point source is moved outside the eye (Figure 5.6). The field of a neuron is the plot of the sensitivity to all its inputs, in all the dimensions in which they exist. In this case, sensitivity is defined as the reciprocal of the light intensity required to give a constant response. The field might thus depend on the choice of this arbitrary constant response and on other factors such as the polarisation plane or wavelength, so that even for a primary photoreceptor the field is dependent on the kind of stimulus. The optical axis of the receptor is defined as the axis of symmetry of the field. The responses also have important temporal properties. The field must be obtained by tedious exploration, which is why microelectrode recording is one bottleneck in the advance of knowledge of nervous systems.

Figure 5.8 The method for calculating the field size. a) Dark-adapted responses as in Figure 5.7b. b) The dark-adapted V/log I curve as in Figure 5.7a. c) Taking antilogs, one obtains the linear value in (d) at the corresponding angle in (a).



The spatial field is measured by moving a flashing point source at measured intervals in front of the eye (Figure 5.7b) while recording the heights of the graded electrical responses of a retinula cell with a microelectrode (Figure 5.7a). The point source is then kept stationary near the axis and its intensity at successive flashes is controlled with a series of neutral density filters. The graph of response versus intensity on the axis is called the V/log I curve and this curve acts as a calibration whereby the response measured at each angle is converted to a sensitivity relative to the maximum response on the axis (Figure 5.8). A more accurate but slower way to measure the field is to adjust the intensity of the point source at each angle relative to the axis until the same response is obtained at each angle, and then plot the reciprocals of these intensities. A problem can arise from the adaptation to the test light and the control of screening pigments can be via a separate pathway.

In a compound eye, there can be overlaps of the receptor fields of adjacent ommatidia, which increases the overall sensitivity, whereas in a lens-eye the receptors cannot be closer than side by side.

Convolution

We now come to a difficult but important concept that is not usually taught in school. When the field of an optical detector of any kind sweeps across a contrast in the panorama, the spatial distribution of the sensitivity of the field is multiplied point by point and moment by moment with the spatial distribution of the intensity in the image. So, if we plot the response to a contrast as a function of time, we see at first the background state in the receptor, then the initiation of a response that rises to a peak as the field moves on the contrast, and then decays as the field passes over. The process of continuous multiplication as the field passes is called convolution. If the field of the detector is very narrow, a reasonably faithful contour of the contrast (in one dimension) can be recorded. If the shape of the field and the shape of the contrast and their relative velocity and response times are known, the exact result of the convolution can be calculated, but the reverse is not true because many situations give rise to the same response.

In general, this is the situation in vision. The fields of the receptors and feature detectors are adapted to their tasks but the real shape of the image is not known. Therefore, the representation is never completely accurate. The fidelity of the response is also reduced by the adaptation to a repeated stimulus, the lack of control over the light intensity and the unknown detail of the motion of the eye—and we have a photographer's nightmare. Nevertheless, visual systems are remarkably effective because they are dedicated to the expected tasks and have many channels in parallel.

The theoretical size of the field of the receptors can be calculated approximately. The blur circle is nearly a Gaussian function of angular width $\Delta\alpha = \lambda/D$

radians (Figure 5.5a), and the optical absorption at the tip of the rhabdom is also approximately a Gaussian function of angular width d/f radians. The convolution of these two Gaussians is another Gaussian of angular width, $\sqrt{\{(\lambda/D)^2 + (d/f)^2\}}$. Therefore the field width at the 50 per cent sensitivity level—usually called the acceptance angle, $\Delta\rho$—can be calculated from the anatomy of the ommatidium. In some insects that fly in bright sunlight, $(\lambda/D) \approx (d/f)$, in which case $\Delta\rho = \sqrt{2}(d/f)$. In most insects, $(\lambda/D) < (d/f)$ because the rhabdomeres are larger than the blur circle, to help catch more light from diffuse sources, so $\Delta\rho = (d/f)$ radians.

Modulation at the photoreceptor

Contrasts always have a spatial as well as a temporal component that is generated by a moving eye. Spatial contrast is not detected directly, but simultaneous receptor responses can be correlated with each other at a deeper level in the visual system. A line, spot or edge modulates all the receptors as they pass, but each single receptor has no way to distinguish between them. The modulation frequency is a useful cue that depends on the density of edges in the pattern. It is the simplest and most frequent cue by which honeybees recognise a place.

Assuming that the receptive field is Gaussian in shape, from the work of Götz (1965), the relative modulation of light intensity caused by the movement of a spatial sine wave stimulus of period $\Delta\theta$ outside the eye (as in Figure 5.9) is given by Equation 5.3.

Equation 5.3

$$M = (I_{max} - I_{min})/(I_{.max} + I_{min}) = m.\ I.\ \exp\ [-3.56\ (\Delta\rho_{LA}/\Delta\theta)^2]$$

In Equation 5.3, m is the relative intensity modulation in the stimulus, I is a measure of the luminance of the stimulus and $\Delta\rho_{LA}$ is the width of the field of the receptor when it is light adapted to the mean intensity of the stimulus, not of the dark-adapted receptor, which is the measurement usually available. This equation is the result of a convolution of the sinusoidal input with the Gaussian receptor field, showing again that every operation in spatial vision involves convolution.

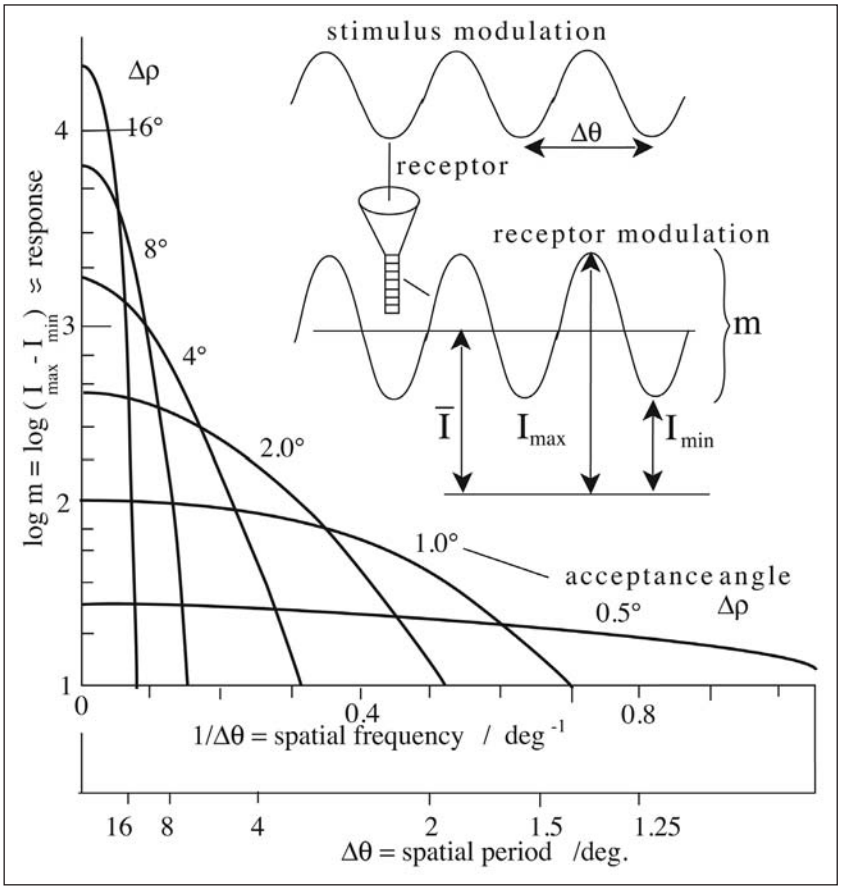
Fused rhabdomeres, as in the bee

In most insects, the commonest type of retinula cell has a peak that matches the most abundant environmental background colour. Frequently, as in the bee, four of the seven to nine retinula cells are green sensitive except in the dorsal part of the eye, where more are blue sensitive to match the sky.

If a photoreceptor absorbs all the light that falls on it, it will be black, so that discrimination of colour or polarisation will be impossible. To avoid this, vertebrate cones are short and absorb only a small fraction of the incident

light, but sacrifice sensitivity. Some insects have rhabdomeres that are fused to form a long central rod along the axis of the ommatidium, as in the bee (Figure 5.2). Light passing down the composite rhabdom is absorbed approximately in proportion to the volume of each sector. The most abundant rhabdomeres, with peak absorption for green light, absorb less blue or ultraviolet. Each rhabdomere absorbs its own preferred kind of light, with the result that each cell retains its own sensitivity to colour and plane of polarisation, but all the light can be used. Theoretically, the spectral sensitivity curve of each receptor type is narrowed by the absorption of light in the others.

Figure 5.9 A regular striped pattern laid out in angular space in front of each facet can be represented by its sine-wave fundamental of period $\Delta\theta$. As the eye moves relative to the pattern, each receptor generates a modulated response, which is an oscillation *in time*. The graph shows the log modulation as a function of the pattern period for typical values of the acceptance angle, $\Delta\rho$. High sensitivity, as in dim light, requires large fields, and high resolution requires small fields, as shown in a different way in Figure 5.5.



A different result follows when the retinula cells are tiered—that is, one before another along the light path, as in many insects. In the bee, one cell—number nine—at the base of the rhabdom, receives light that has passed through all the other rhabdomeres. The effect could be a marked sharpening of its spectral or polarisation sensitivity.

These effects of optical coupling are difficult to separate because there is also an unknown amount of electrical coupling between neighbouring retinula cells. The electrical coupling can be purely resistive leakage, which makes the sensitivity curves more similar, or it can be an antagonistic current flow, so that, for example, the activity of large numbers of neighbouring green-sensitive cells can hyperpolarise the blue-sensitive cells and modify their spectral and polarisation sensitivity curves.

The dimensions of the rhabdoms in the eyes of many large diurnal insects are exactly in the range where the light is controlled by very small radial movements of pigment grains. They act like a shutter around the outside of the rhabdom, with migration of pigment grains to within 1μm of the rhabdom surface in the light and away from the rhabdom in the dark (Figures 5.2g–h). They are able to absorb some of the light within because they reduce the internal reflection. Each cell acts independently, so their differences in spectral sensitivity can be detected histologically.

The principal pigment cells around the cone tip contain large black pigment grains that form a variable diaphragm controlling the entry of light to the rhabdom. When the corneal lens is neutralised with a little oil, the resulting closure of this diaphragm can in fact be seen from outside the eye by examination with an epi-illumination microscope.

One of the important lessons of the retinal array is that so few of the factors acting on the retinula cells are measurable that one has to be content with a list, such as the outline of the optics, the anatomy and the absorption curves of rhodopsins with different peaks. The consequences of all interactions on the cell are bundled together and conveyed as a single variable in the electrophysiological response, which after all is the output that acts on the next stage. In the nervous system in general, on account of unknown interactions, the redefining of the signal at each stage can be observed only by microelectrode recording, not by calculation. This is an important fundamental lesson for the study of any nervous system.

Sensitivity

The human eye, with an F number near 10, is not especially sensitive in dim light. Eyes or cameras that function in daylight usually have an F number between 2 and 16. Insect ommatidia are in this range. For example, if $\lambda = 0.5\mu m$ and $d = 2\mu m$, $d/\lambda = 4$, and, because $\lambda/d = D/f$, it follows that $F = f/D = 4$ (see

Figure 5.5). A rhabdom of diameter d = 2μm is exactly in the size range where the light passing down it is controlled by pigment grains that act as a sleeve diaphragm around the outside (Figures 5.2g and 5.2h).

When the honeybee eye is dark adapted at night, pigment grains within the retinula cells move away from the rhabdom (Figures 5.2g and 5.2h) and, at the same time, sensitivity to axial light is increased 1000-fold. The receptor field sizes are little changed but those measured behaviourally are greatly increased, so there is summation at a deeper level. Bees are not diffraction limited, they are not specially adapted for vision in bright sunlight and they commonly work in the shade. Rhabdoms of nocturnal bees are further enlarged. As the rhabdom width is increased, there is increasing sensitivity to a diffuse source, with sacrifice of resolution. Some insects that are active both by day and night—for example, locusts and mantids—increase the rhabdom diameter at night by a factor of at least 10, greatly increasing their sensitivity to diffuse sources but retaining lens resolution by day.

Spectral sensitivity

Colours are discriminated by collaboration between several receptor cells. In the bee, as in many insect orders, there are three types with spectral sensitivity peaks in the ultraviolet, blue and green (Figure 6.7b); the relative stimulation of these types gives the insect the opportunity to distinguish a range of colours. Bees have nine retinula cells in each ommatidium, one of which is basal and UV sensitive. Four of the other retinula cells have spectral sensitivity peaking near 540nm, two are ultraviolet (near 340nm) and two are blue sensitive (near 440nm). Most of the vision needed for mobility, obstacle avoidance, edge detection and so on, is colourblind and uses only the green receptor channel. For worker bees, black and white patterns on paper are just another set of colours, depending on their UV reflectance.

Polarisation

In the bee, electron microscopy shows that the microvilli of four of the retinula cells lie at right angles to the others in cross-sections, from which von Frisch et al. (1960) inferred that they detect the polarisation plane of the blue sky as part of the sun compass. This caused a lot of confusion because two directions of microvilli were not sufficient to detect all directions of polarisation without ambiguity.

Opinions differ about whether the rhabdom is twisted as a specialisation to prevent the colour vision being disturbed by polarisation of light that has been reflected at natural surfaces. The ommatidium is not twisted when carefully frozen before being fixed for sectioning. Electrophysiology shows that retinula

cells of normal ommatidia are indeed sensitive to the plane of polarisation and that the polarisation sensitivity of single receptors is confounded with spectral sensitivity. The ninth (basal) retinula cell is also a puzzle.

Detection of the compass direction from the main axes of the polarisation pattern of the blue of the sky is done by specialised ommatidia along the dorsal edge of the eye, where the spatial resolution is poor but the microvilli are oriented in the pattern of a preset filter that matches the polarisation pattern around the position of the sun. By rotating itself, the bee receives a maximum or minimum stimulus from the sky (see Chapter 8).

The interommatidial angle ($\Delta\phi$)

When the eyes of many insects are examined with a lens, a small black spot appears to follow the movement of the observer. This is the place, called the pseudo-pupil, where light is not reflected back to the observer's eye because it is absorbed by the ommatidia. The angles between the ommatidial axes can therefore be measured by observing the eye on a goniometer stage (Figure 5.10). Many native bees have an obvious pseudo-pupil, but the honeybee eye has none, so its visual axes have been mapped with illumination from the back of the eye outwards through the optics. The result is a map (Figure 5.11) that provides fundamental data for any eye. Insects reveal their habits in their eye maps, especially in those that include facet size. This is a topic where the physics of the retina is related to ecology and behaviour (Figure 5.12), as documented in several earlier reviews related to spatial sampling and gradients of $\Delta\phi$ and sensitivity (Horridge 1978, 2005a).

Figure 5.10 Equipment for measuring D and $\Delta\phi$ to make a map of the eye. The centre of the pseudo-pupil is the visual axis looking at the centre of the camera. Dust grains are used as markers on the surface of the eye, which is photographed every 5° or 10° around the eye in two dimensions. The angular coordinates of the pseudo-pupil are then marked on a linear map of the facets, from which a map of the visual axes is made in angular coordinates (as in Figure 5.11).

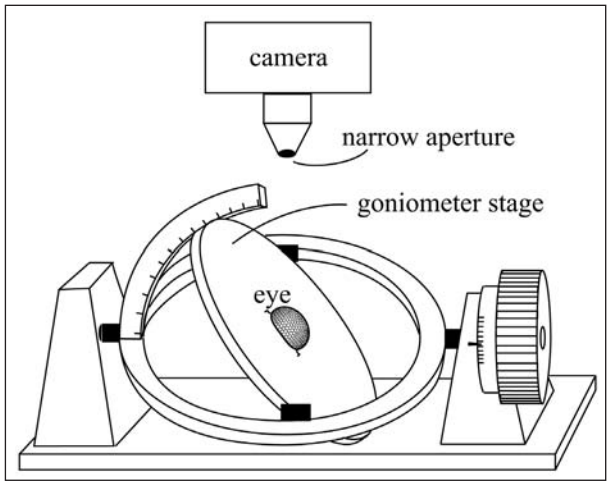
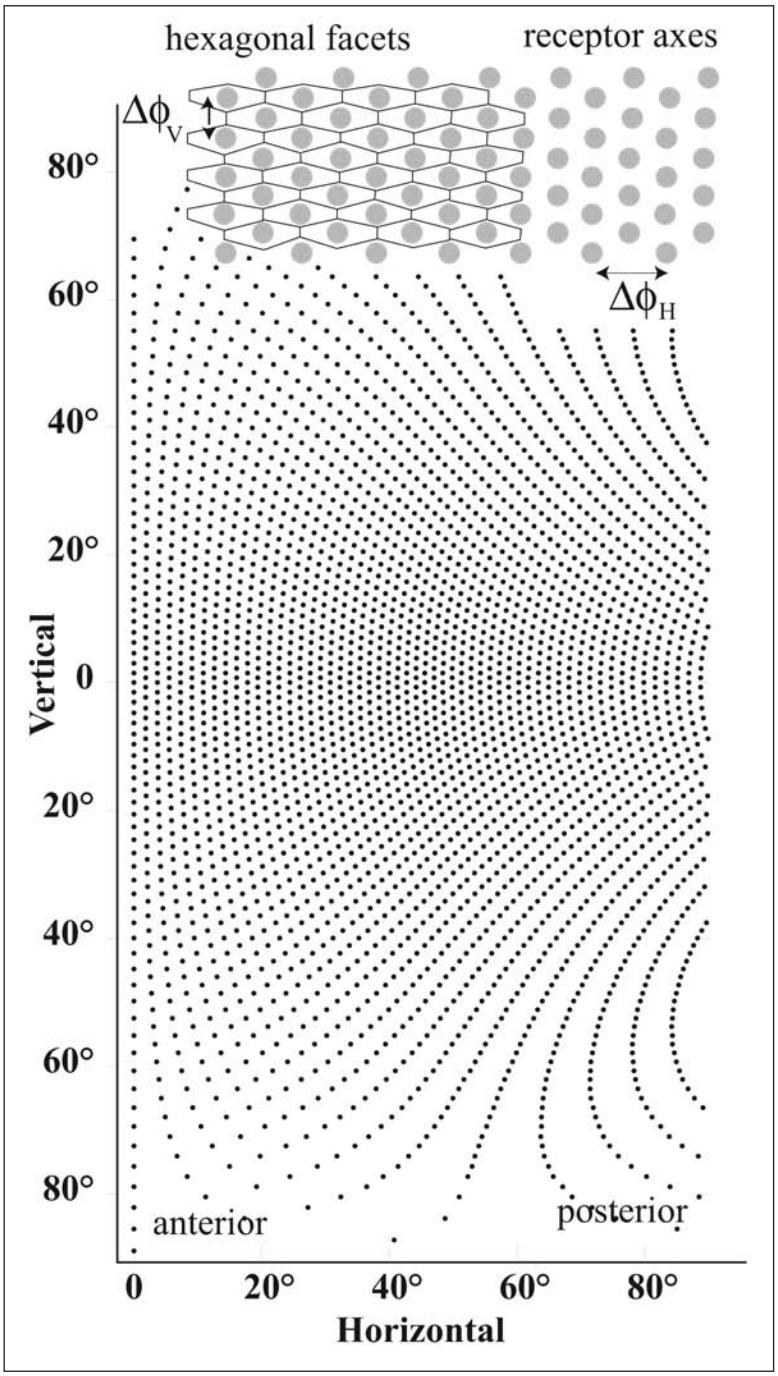


Figure 5.11 Map of the axes of the worker bee eye in isotropic angular coordinates as far as the mid-line (on the left). Inset: An enlarged map of a portion, also in isotropic angular coordinates, to show how the vertical compression of the facets produces a pattern of vertical rows.



Most day-flying insects, including bees, have horizontal rows of facets at the front of the eye, but flies have vertical rows. Many authors use $\Delta\phi$ as the angle between nearest neighbours irrespective of the direction on the eye, which is convenient for eye maps (Figure 5.11). The convention usually followed is to use $\Delta\phi$ in this way in isotropic regions, but $\Delta\phi$ measured in the vertical and horizontal planes is commonly found in the literature. The use of the term interommatidial angle ($\Delta\phi$) must always be defined. An eye like that of the honeybee has very different values of $\Delta\phi_H$ and $\Delta\phi_V$, because the facets are hexagons.

Early measurements of $\Delta\phi$ in the honeybee eye were few and suspect, partly because the eye showed no pupil, except in the pupal stage. From sections, Baumgärtner (1928) had measured the interommatidial angles of the honeybee, ranging from $\Delta\phi = 2.4^o$ in the horizontal direction at the front of the eye to 2^o at the side, 4^o at the back and 1^o in the vertical direction. These measurements were not corrected in the literature for 70 years and led astray several researchers, including Srinivasan and Lehrer (1988) and Giurfa et al. (1997). Seidl (1982) surveyed the whole eye and located the real optical axis of each ommatidium (Figure 5.11), but Seidl's data remained unpublished until they were reworked by Andy Giger (1996) and published by Mike Land (1997a, 1997b).

The bee's isotropic pattern with vertical rows of axes is achieved by vertical compression, which is common in insects that fly by day. Bee eyes are not spherical and the radius of a horizontal row is different from that of the vertical row at the same place. Further, the optical axes are not perpendicular to the cornea. These complications reduce the validity of measurements of $\Delta\phi$ except by optical methods. In the worker honeybee, $\Delta\phi = 1.65–1.7^o$ in all directions in the region around the centre of the eye (Figure 5.11). There is overlap of about 15° between the fields of the two bee eyes along the mid-line looking forward (Figure 1.3).

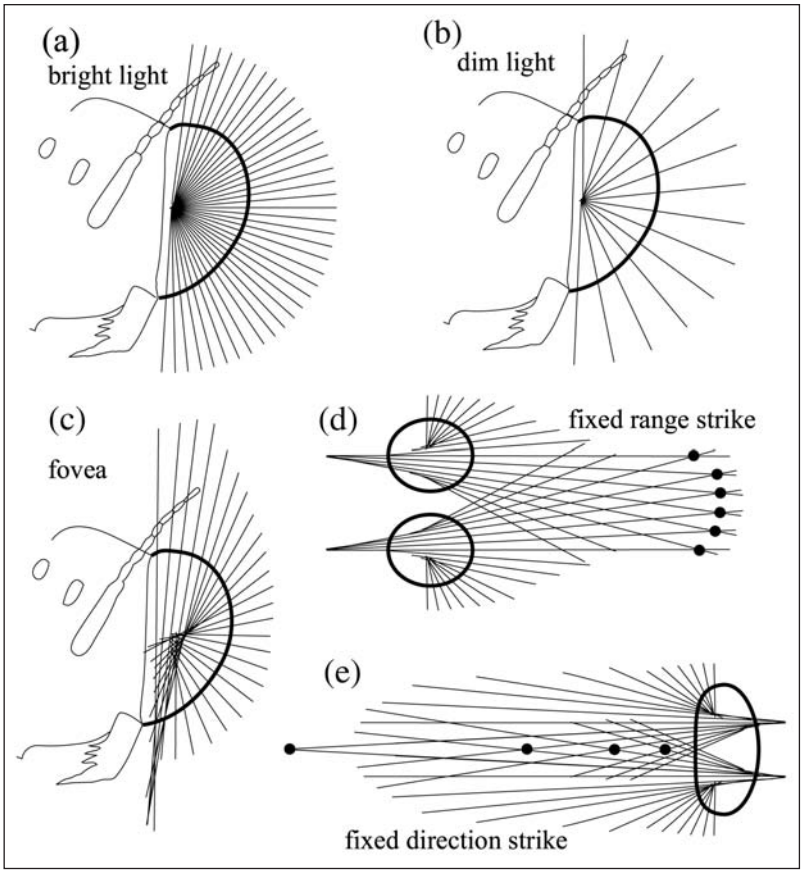
The effect of transduction noise

The theory so far might have conveyed the message that the lens resolution of individual ommatidia of insects, particularly those that fly in strong sunlight, can be limited by the diffraction of light, but this is not exactly so. A few large insects that chase prey in bright sunlight, with facet diameters less than 30μm, have acceptance angles near 1^o, implying that the rhabdom diameter contributes little to the field. On the other hand, most insects have sacrificed some spatial sampling for greater receptor size and have therefore sacrificed both receptor resolution and sampling resolution for increased sensitivity.

There is a variety of evidence suggesting that even the eyes of diurnal insects that function in bright sunlight are not at the diffraction limit. First, direct measurement on most insects reveals that the rhabdoms and the field sizes are

larger than calculated from the aperture. In flies, the acceptance angle depends on rhabdom width, as direct measurements of fields demonstrate. Second, measurements show that interommatidial angles are often larger than expected from the apertures and behavioural resolutions are better than expected because of lateral inhibition in the lamina—that is, the eyes under-sample, meaning that there are gaps between the receptor fields. Third, many insects with apposition eyes are active in sunlight but have mechanisms of dark adaptation that remove screening pigment and increase sensitivity in dim light at the expense of lens resolution, but without change in the interommatidial angle. The bee is peculiar in that the eye is 1000 times more sensitive to modulation when dark adapted with little obvious change in the retina. The fields of the lamina cells are narrowed by strong lateral inhibition when in sunlight but perhaps rapid synaptic changes enable the bees to see in dim light.

Figure 5.12 Diversity of eye geometry for different ambient intensities and visual tasks. a) A diurnal eye that functions in bright light with small facets and high spatial sampling frequency. b) An eye for dim light has fewer, larger facets. c) An acute zone (a 'fovea') is made by local increases in eye radius, as in dragonflies. d) Coincidences of certain visual axes, as in the praying mantis, for prey capture at a fixed range. e) Coincidences of visual axes for discrimination of range along the head axis—almost always associated with the grabbing action of the mouthparts, as in dragonfly larvae.



These observations are all supported by direct measurements of the noise. The arrivals of photons are Poisson distributed in time and therefore photon noise is proportional to the square root of the mean number of photons arriving in the sampling period. So, the signal/noise ratio is proportional to $N/\sqrt{N} = \sqrt{N}$. For the bee to see better in dim light, the signal must be increased relative to the noise, so this implies that the photon capture must be increased.

Each photon gives rise to a bump, and bumps fuse, so another way to reduce noise is to increase the integration time for the electrical response, as in 'slow' eyes, but this means a reduction in the speed of the response. The ways to gather more photons are to increase the aperture, D, the receptor volume, reduce the F number f/D or combine the signal from several ommatidia. The last possibility implies optical pooling with a superposition eye or neural pooling within the eye. Examples of all these ways occur in various insects, but the situation in ants and bees requires more work.

Direct measurements of the signal/noise ratio in retinula cells show that the diffraction-limited, noise-free eye is unattainable even in the brightest light. At low luminance, the noise comes mainly from the random arrival of photons, called shot noise, but at luminance of more than 10^4 cd m^{-2} (daylight), photon shot noise is negligible compared with the photoreceptor transduction noise that originates in the variety of sizes of potentials produced by single photon captures. So-called dark noise—the spontaneous activation of rhodopsin molecules in the dark—is negligible in insects.

The receptor capacity of the retinula cell to give a further increment of electrical response depends ultimately on the number of simultaneously active channels per receptor. In white-eyed *Drosophila*, the photon capture rate saturates at about 2.5×10^4 events, which overlap, which is about three orders of magnitude less than the number of rhodopsin molecules. Recordings from single locust receptors show that the signal/noise ratio follows the square root rule that is the consequence of shot noise up to moderate intensities, but then saturates at about $S/N = 40$. Saturation of the signal/noise ratio implies that Weber's Law holds ($\Delta I/I$ is a constant).

Eyes suited to low luminance are all dominated by the lower signal-to-noise ratio caused by more photon noise. Increase in facet size to increase sensitivity and resolution implies reducing the number of facets and therefore under-sampling. Compound eyes suited to bright environments have no way to increase sensitivity in the short term except by moving screening pigment, increasing receptor size or by having superposition optics of some kind. The bee is relatively insensitive when light adapted, but often flies in shade (Wolf and Zerrahn-Wolf 1935).

In the bee, the noise levels of the three receptor types together with their colour opponency predict very well the shape of the photopic spectral sensitivity

curve measured behaviourally (Vorobyev and Osorio 1998). The noise in the receptor cells sets an absolute limit on the behavioural discrimination of different wavelengths (Vorobyev et al. 2001). Vorobyev et al. (2001) combined the skills of different specialists on bee training, physiology and computation as a foretaste of what must be done throughout the whole subject, including detection of motion and each of the feature detectors for edges. Interestingly, for a bee to discriminate a colour in a field of 60 ommatidia requires a photon flux per receptor cell about 1000 times that required by a fly to detect the direction of motion in a large field (Dubs et al. 1981), so the bee is insensitive by day.

Anomalous resolution

In the early 1960s, the resolution story took an extraordinary turn. From the work of Edmund Burtt and Bill Catton at Newcastle, it had been known for a decade that the locust was sensitive to the movement of a small spot or black/white edge by as little as 0.1°. At that time, there was still some confusion between the lens resolution, the spatial resolution of a grating or a spot and the least-detected motion.

A large neuron (the DCMD unit) of the locust ventral cord responded to a small movement of a grating of period 0.3°, which was 10 times smaller than the minimum calculated from the width of the blur circle (Burtt and Catton 1962, 1969; Catton 1998, 1999). Burtt and Catton inferred a larger aperture than a single facet and suggested that rays entering by several neighbouring facets summed behind the cornea to generate intensity patterns that improved the resolution.

Sections of the eye, however, show that the region behind the lens is packed with pigment grains so that each ommatidium is optically separated from its neighbours. John Palka (1965) and Horace Barlow (1965) published rebuttals that pointed out that the locusts had probably responded to a low harmonic of the period of the grating that moved behind a window, as one bar disappeared from one side of the grating and reappeared at the other side.

At the time, I had electrophysiological equipment, a few spare students and Vincent Wigglesworth had sent me some locusts. We placed the locust head at the centre hub of an arm on which was mounted a lamp behind a pinhole and recorded from the retinula cells while flashing the lamp. The angular sensitivity curve approximated to a Gaussian curve that had a width, $\Delta\rho = 4.4°$, in a region where $\Delta\phi$ was 2.4° (Tunstall and Horridge 1967). $\Delta\rho$ was the acceptance angle (or half-width) at the 50 per cent level of sensitivity. As shown later, this value was too large because the optics were damaged by the insertion of the electrode. Later, in 405 measurements of $\Delta\rho$ at the front of the locust eye, the average

minimum $\Delta\rho$ was 1.16° in bright light in mid-afternoon and the maximum was 2.64° when dark adapted at night (work in Canberra published by Dr Wu in Chinese).

The whole matter was aired at an international symposium held in Stockholm in October 1965 (Bernhard 1966). Unfortunately, the revised edition of Wigglesworth's (1965) influential textbook on insect physiology carried the erroneous story, so a few generations of students worldwide were misled. That, however, was not the end of it. Claims of extremely high resolution of a small target persisted (Catton 1999). Further work popularised the adjustable strength of the lateral inhibition between second-order nerve cells in the insect lamina (Srinivasan et al. 1982). One of my students, John Scholes (1964, 1965), had shown that the night eye of the locust easily responded to single photon arrivals in single receptor cells, so that cells in a wide surround would deliver lateral inhibition. The peak at the centre of each field would be squeezed narrower, so improving the apparent resolution, but at the expense of an increase in noise and loss of absolute sensitivity. The earlier results with gratings were never explained.

As well as supposing that rays passing through several facets could sum together in the thick mass of dark pigment cells between the ommatidia, and the error of testing the eye with a grating of limited size with hidden harmonics, there was an error of understanding. Locusts are obviously not adapted to looking at gratings, so why the high resolution to them? The measured resolution of motion of a grating was the modulation summed over a large area, but in popular accounts, it was assumed to apply to any small object.

To estimate range before they jump, locusts move their head and measure the small relative movement of objects in front of them. Similarly, humans have evolved (sub-pixel) vernier acuity to infer the range of surrounding objects by measuring the parallax when the head is moved. These life-saving functions demand extreme sub-pixel resolution of a small movement of an edge, which is done by lateral inhibition and sophisticated summation along the edge, resulting in narrow edge-detector fields and even narrower modulation detectors. The feature detector for modulation is at the working edge of natural selection for sharp vision, as shown by the continual adjustment to light intensity to optimise the signal. In fact, for several vital reasons, such as having few facets or catching small prey, there are many insects in which $\Delta\rho$ is much smaller than $\Delta\phi$, and the field of the modulation detector could be narrower still.

Measurements of resolution in the bee

The resolution as measured in behavioural tests is related only indirectly to the interommatidial angle because the response depends on which feature detectors are in action. Baumgärtner measured the minimum angular sizes of

blue and yellow rectangles that were detected or discriminated from a distance by flying bees and found that a coloured rectangle was detected more easily if the long side was vertical rather than horizontal. From his own measures of $\Delta\phi$, he inferred that the critical factor was the number of ommatidia involved. The minimum areas subtended about 8° at the eye, but for decades, Baumgärtner's measurements were misrepresented as evidence that the resolution was limited directly by $\Delta\phi$. Later, Gould (1985) found that bees discriminated flower patterns with two or three colours when the patches each subtended at least 10°, and he also commented that the resolution in memory was poorer than that of the retina. The resolution of a coloured patch and discrimination between two colours are both limited by the noise in the signal and, the lower the light intensity, the greater is the number of ommatidia involved.

The resolution of the bee eye was also measured by allowing each bee to walk freely on a glass plate beneath which a regular grating moved (Hecht and Wolf 1929). The bees turned against the direction of the motion, so this was directed locomotion, not an optomotor response. The minimum stripe period was near 2° in bright light, irrespective of the direction on the eye. Hecht and Wolf calculated from the optics that the minimum blur circle width was $\approx 1.14°$. Referring to Baumgärtner, they saw that $\Delta\phi$ was not the limiting factor, which must be the modulation generated by the field size of the receptor, $\Delta\rho$. In dim light, the minimum period increased to 30°, so they postulated other receptors with wide fields and directional motion detectors with a wide span.

When trained honeybees were tested for discrimination of gratings against a plain grey target of the same average intensity, the minimum period in daylight was near 2.5° for horizontal and vertical gratings tested separately against grey (Srinivasan and Lehrer 1988). Referring to Baumgärtner again, the resolution was inferred to depend on the modulation—that is, $\Delta\rho$ and not $\Delta\phi$. When coloured gratings were used, with no contrast with the green receptors to eliminate motion detectors, the bees could still discriminate, although the resolution was not as good. Modulation was therefore detected by blue and green receptor channels. There was nothing to show whether the bees really detected the layout of the gratings.

Later, Giger and Srinivasan (1996) found that edge orientation was detected by modulation of the green receptors only. If, however, orthogonal gratings are oblique and without green contrast, they cannot be discriminated even when stationary (Horridge 2003c). Therefore, with vertical versus horizontal gratings with no green contrast, the cue must have been the difference in induced temporal modulation of blue receptors, irrespective of measurements of $\Delta\phi$.

Do the bees see the grating?

When no other cue is available, bees trained on a checkerboard versus grey, or with alternating vertical and horizontal gratings versus grey, learn only the modulation cue and the lower limit of resolution is 2.5° irrespective of the type of pattern (Horridge 2003c). As it flies, a bee scans in the horizontal (yaw) plane so a grating with vertical bars generates a lot of flicker (modulation) at the eye, but a horizontal grating generates much less, so the bees could rely on the difference in modulation, which is the preferred cue anyway. When trained on these gratings, however, they also learn the edge orientations.

When bees were trained on a single black-and-white grating versus white paper, they responded almost as well to a pattern of black spots versus white paper as they did to the grating, so they cared little for the pattern (Horridge 2006b). Tests showed that they had learned the modulation and the orientation, as well as to go to anything black.

Bees also learned to discriminate between an oblique grating at 45° from a similar grating at 135°, with no difference in the modulation caused by scanning in flight, so the edge orientation alone was the cue. When the resolution tests were repeated on these trained bees with oblique gratings of various periods, the limit was at a period of about 3.5° for orientation, not 2.5° for modulation. As the training patterns were rotated, the bees switched from the modulation to edge orientation as the cue.

More convincingly, when trained on a grating at 45° versus the same grating at 135°, with no contrast to the green receptors, the bees could not detect the orientation cue, and no other difference was available, so they failed to learn. Clearly, they did not remember the stripes.

The gratings provide another example showing that bees detect and learn cues, not patterns, and the results expand the concept of resolution of the bee eye. From now on, we must think of resolution of edges in terms of the feature detectors, including modulation. When the positions of areas of colour or black are learned, larger regions of the eye are involved (Chapter 10).

Measurement of sensitivity and optical gain

Educated guesses from models are instructive but a measurement by microelectrode recording is definitive. Sensitivity can be measured as the photon flux per facet required to give a threshold or a 50 per cent of maximum response, or as the slope of the curve of the response in mV plotted against intensity.

In the dark-adapted locust, the retinula cells can also be calibrated by counting the effective photon captures (bumps) by intracellular recording at fluxes less

than 10 per receptor per second. It is then observed that a reasonable signal/noise ratio is reached at a flux of approximately 100 photons per receptor per second, which is approximately 1000 photons per facet per second or 10 photons per facet per 10ms period. At these light levels, individual photon captures are seen as bumps of about 1mV in the recording. Lillywhite (1977) showed that 50 per cent of the axial photons arriving on a facet were captured by the rhabdom. In the gyrinid water beetle *Macrogyrus*, which has a superposition eye, there are two photon captures for every photon (of green light at 552nm) that falls on the facet belonging to the receptor that is recorded from (Horridge et al. 1983).

For a facet of 500μm^2, full sunlight provides 5 x 10^5 photons per facet per 10ms period, which is more than the transduction can use. In shadow, when intensities are down by a factor of 100, an eye will cope quite well, but in deeper shadow or at sunset, we reach a flux of 10^8 useful photons/cm/s at the cornea, which is approximately 5 photons per receptor per 10ms period—that is, the lowest limit for useful vision. Plenty of insects, however, find it necessary to fly in luminance lower than moonlight, which is a factor of 10^7 less than sunlight. The increased sensitivity is found in two ways.

First, parallel rays passing through several facets are deflected across a clear zone by the optics and converge on the layer of rhabdoms below, forming a superposition eye, as in skipper butterflies and some moths, *Neuroptera*, many night-flying beetles and a few others. Alternatively, they are absorbed in adjacent ommatidia and the convergence is done by convergent axons ending on lamina ganglion cells, as in flies and maybe all others with separated rhabdomeres. Second, there might be a large rhabdom that during the day is screened by pigment cells or pulled down deep into the retinula cell, as in many eyes with open rhabdomeres. Alternatively, the rhabdom diameter could increase tenfold, as in the mantis and locust.

The temporal resolution of compound eyes usually decreases in dimmer light. Long ago, Autrum distinguished 'fast' and 'slow' eyes—the latter characteristic of species that were active in dim light. The temporal properties of the membranes and synapses of the receptors and lamina cells (like the whole nervous system) depend on the mix of ion channels of different types in the membranes (Laughlin and Weckström 1993). This control of integration times at the front end, like all the other physical mechanisms in the retina, is felt through the whole visual system.

Comment

The analysis of the retina illustrates what happens when serious scientists get their hands on a versatile subject that yields hard results and promotes worthy discussions. In fact, the supporting philosophy of this science was not fundamentally different from the 400-year-old use of data to calculate the tracks of the planets or the use of costs and prices to run a business. There is similar convergence of concepts and experiments, essential training, the expert use of

complex equipment and familiarity with a large reservoir of previous studies—however full of errors they might be—but observation, imagination and logic are still the main foundations.

Endnote

1. The study of the retina does not tell us what the bees see, but it is an excellent example of how a variety of techniques, combined with a lot of hard work, expose the optimisation of the mechanisms by long evolution.