total RNA [method of Scherrer *et al.* (*12*) which also employed a 30-minute pulse-labeling period].

The results as outlined in Table 1 showed that all three antimetabolites that we used depressed the mean recorded magnitudes of the olfactory responses to home water, particularly the higher dose of puromycin. However, it is even more significant that the ability of the olfactory bulbar response to differentially respond to home versus nonhome waters was strongly impaired by all of the three antimetabolities. Generally, olfactory discrimination was more strongly inhibited a short time (4 to 7 hours) after antimetabolite administration than it was for a longer period (9 to 28 hours). Thus, blockage of olfactory long-term memory by blockage of RNA and protein synthesis appears to be a temporary phenomenon, and recovery is already well advanced in the 9- to 28-hour interval. With the dosages used, there was relatively little dosage effect upon olfactory discrimination, since such discrimination was almost completely lost at the 4- to 7-hour interval.

The tentative conclusion that may be drawn from these data is that expression of long-term olfactory memory in homing salmon, as defined under our experimental conditions, requires continuous protein or RNA synthesis, or both. If this thesis should be sustained by further research, it would indicate that long-term memory is a continuous metabolic process, not merely a stamping out of long-lived residual template RNA or protein. Since the inhibition was only temporary and long-term olfactory memory was partially restored about 1 day later, it would appear that a residual basis for olfactory memory function outlasted the interruption in RNA and protein synthesis. The nature of this residual factor cannot, of course, be deduced from these experiments. Such a hypothesis adds a new aspect to the study of the chemical basis of memory and it deserves further active investigation.

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13. We thank Dr. L. R. Donaldson and S. B. Fallert for the spawning salmon. This work was aided by research grants NB04881 and AM10902 from NIH.

22 January 1969; revised 25 March 1969

Optics of Arthropod Compound Eye

Abstract. *The extent to which light can escape from one ommatidium into its neighbors in the compound eye has been examined by recording from single receptors during stimulation of single facets. In the "apposition" eye of the drone honeybee and locust, optical interaction is extremely small. In the "superposition" eye of the crayfish, more than half the light captured by the average cell gets in through neighboring facets, even when screening pigments are in the fully light-adapted position.*

Each optical subunit of the typical arthropod compound eye consists of a corneal lenslet of moderate thickness, followed by a crystalline tract: this latter is short in many diurnal animals, but can be extremely long in nocturnal Crustacea and Lepidoptera. Exner (*1*) classified the extreme types with long optics as "superposition" eyes, in the belief that light rays entering many facets could constructively combine to form one erect image on the receptor layer behind, at least in the dark-adapted eye. Upon light exposure, a pigment screen moves to partly surround each optical element in some eyes, supposedly eliminating the superposition process. Exner classified eyes with short optics as "apposition" eyes, these usually having an intervening pigment screen between ommatidia in all conditions of adaptation. The optics and rhabdoms of apposition ommatidia were supposed to act as individual light-guides, so that light entering one facet was confined to that ommatidium by internal reflection (*1*).

Two other variants on the superposition theme exist. First, it has been proposed (*2*) that some kind of usable image may result from the interference of diffraction patterns, supposedly produced by successive facets. Second, Parker (*3*) thought that Exner's idea of cooperative imaging in superposition eyes was implausible, but still believed it possible for light to scatter from one ommatidium to the next, without images being formed.

Each of these concepts shares the feature that light entering one facet can escape into neighboring ommatidia. This was widely believed to be so for the eye of the firefly (*1, 4–6*), but has recently been disputed (*7*). Similarly, Parker's claim (*3*) to have detected the spread of light between ommatidia of dark-adapted crayfish eyes has since been suggested unlikely (*5*) and could not be confirmed (*4*). Finally, apparent receptor sensitivity changes have been reported during migration of screening-pigment in nocturnal moth eyes (*8*), and these changes might well be explained partly by a corresponding change in optical interaction between ommatidia. But because of the gross recording and stimulating techniques used, these effects might equally well be interpreted as showing alterations in either size of the receptive field or the light-attenuating power of individual ommatidia; in similar moth eyes, the crystalline tracts are said to act as light guides (*9*).

It is difficult to evaluate these conflicting observations in the absence of any objective measurement of the extent of light spread between superposition ommatidia (*10*). Such measurements are discussed here, made with electrical recordings from single photoreceptors in response to light flashes delivered to single facets of the eye. The apposition eyes of the bee and locust are compared with the superposition eye of the crayfish.

Individual retinula cells were penetrated with micropipettes filled with KCl,

and initially examined with a perimeter system to find the receptive field centers (*11*). Drone honeybees were mounted in a moist chamber after removal of their legs and wings; isolated heads of the locust *Schistocerca gregaria* or isolated eyestalks of the crayfish *Astacus fluviatilis* were similarly mounted. Single facets were stimulated in each case through a small light guide, held by a micromanipulator against the facet surface. Light guides were made from glass microelectrodes dipped in black paint, dried, and broken to give tip diameters of 20 to 30 μm. The light source was a subminiature bulb pushed down to the constriction of the electrode barrel. Because neutral filters could not be used with this arrangement, curves of response versus light intensity were usually plotted with the perimeter system (*11*).

Using first a small light spot demagnified on to the cornea, it was possible in each case to show that only one small locus of excitability existed over a large area of corneal surface. Therefore, only facets adjacent or subadjacent to the most excitable one were normally examined with the light guide. A drone bee receptor is excited (Fig. 1) by light entering through one corneal facet (facet 0), but hardly at all by light entering the six adjacent ones. Of these six, only at facet 3 does illumination produce a measurable potential. An estimate of the relative amounts of light captured via facets 0 through 6 can be had by referring each record to the appropriate position in the response/log-intensity series (Fig. 1C). The response at facet 3 could be matched (*12*) by a 2 to 3 log-unit reduction in intensity at facet 0, and by a $\geqslant 3$ log-unit reduction for the other facets, because signals are not distinguishable above background at log $I = -3$. It thus appears that only 0.1 to 1 percent of the light caught by the receptor comes through other than one facet.

Similar results were obtained with the locust eye, but peak responses at the central facet were usually nearer the receptor potential saturation level. Discriminability of the potentials produced by stimulating adjacent facets was therefore greater, so that in one case it was possible to say that less than 0.1 percent of the light captured by the cell originated through adjacent facets (*12*).

All the crayfish cells examined showed evidence of light spread between facets. Large potentials appear in response to stimulation of facets adjacent and even subadjacent to the central excitable one

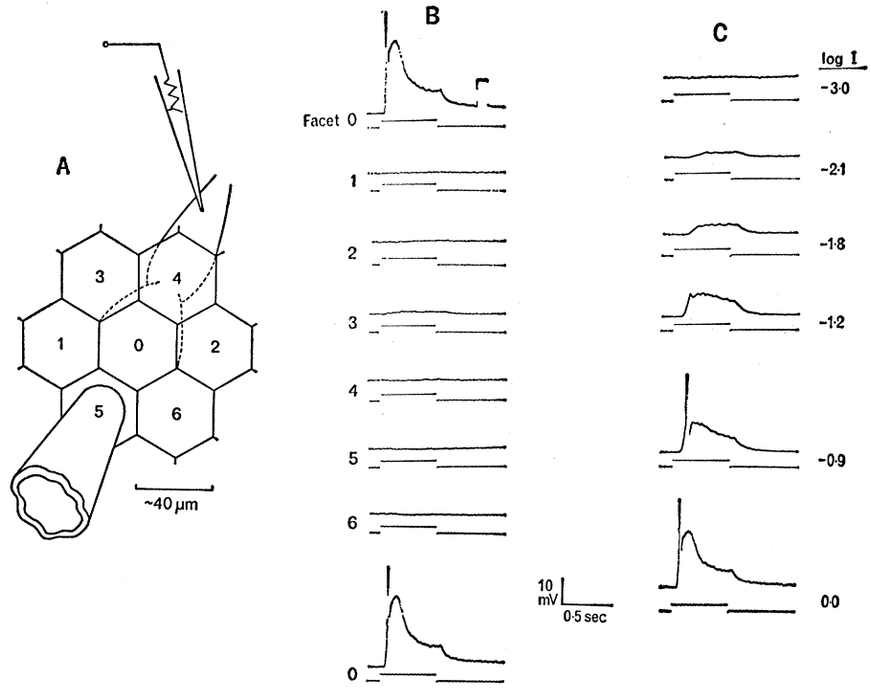

Fig. 1. Records from a drone bee retinula cell (B), as a light guide stimulates individual facets of the eye, numbered in sequence of examination (A). Only at facet 0 is any appreciable response seen, which consists of the characteristic slow potential with a single spike on the rising phase. (C) The effects of increasing light flash-intensity (*I*) at facet 0, in the steps shown.

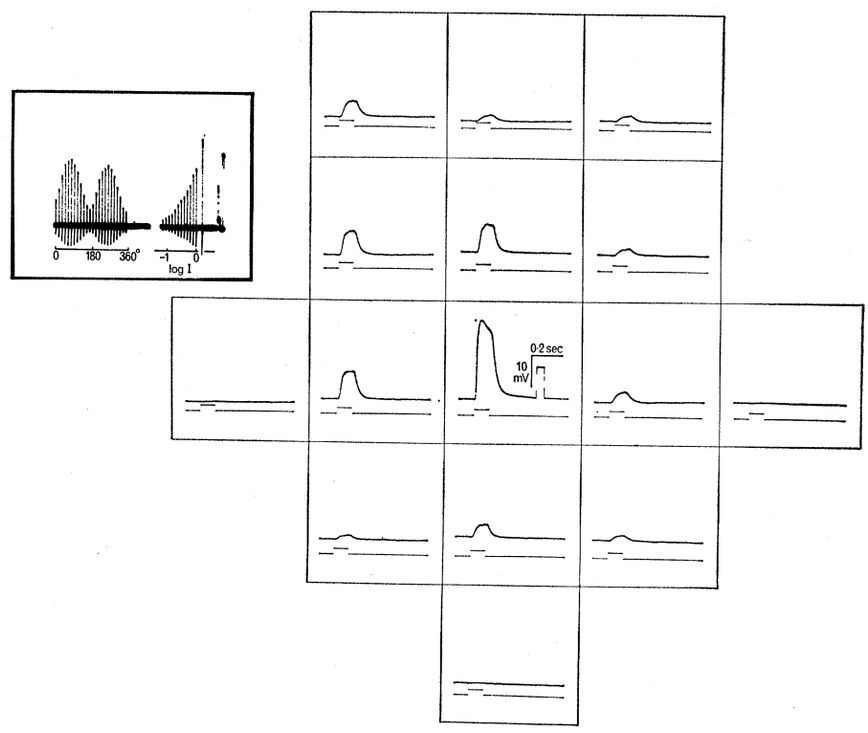

Fig. 2. Slow potential responses from a crayfish retinula cell as successive facets were stimulated with a light guide. The potentials are displayed inside the appropriately juxtaposed facets, which are squares of side about 60 μm in this eye. Large responses are seen when the guide is at facets adjacent and even subadjacent to the most excitable one (identified by the calibration pulse). (Inset) Polarized-light response and corresponding voltage/log *I* curve from this cell, plotted at about twice the amplification on a slow time-base, with a perimeter system [as in (*11*), but with a-c coupling to eliminate slow drift]. Flashes were delivered about 4 seconds apart, between which was rotated a Polaroid in 15° steps, or a neutral filter wheel in approximately 0.1 log-unit steps. There is a slight adaptational trend at this flash frequency.

(Fig. 2). Differing sizes of response at surrounding facets is attributable to location of the light guide, which in this case was facing more toward the upper ommatidia, and to the nonuniformity of flux emitted from the guide tip. An analysis of 37 directly adjacent facets, taken from six maps, was made by referring each potential to the appropriate voltage/log-intensity curve for that cell. This showed that the average effective light intensity coming through each adjacent facet was only 7.2-fold less than that coming through the central one. Since there are eight such adjacent facets, this means that less than half the light captured by the average cell comes through its own facet (*13*)—even this estimate is too high, because a certain amount of light is caught via subadjacent facets (Fig. 2). Light scattered directly into the central facet from the light-guide tip is a negligible contributor to this measurement, as the results on insect eyes show. Additional controls, using the guide tip to dimple crayfish facets inward, failed to reveal any such scatter.

Figure 2 shows also that the polarized-light response of the receptor is large. This was typical of most cells (*14*), but about 5 percent of those examined in both *Astacus* and the crab *Carcinus* showed much shallower modulations, with four instead of two peaks per rotation of the polarizer. One of these crayfish cells was mapped with the light guide, which revealed that two adjacent facets produced large, equal-sized potentials. Another unusual cell had a skewed, shallow polarized-light curve, and also produced such a map. Evidently recordings can be taken with one microelectrode from a pair of receptors in adjacent ommatidia, in these cases having orthogonal or skewed rhabdomere tubules. It is not known whether this occasional coupling of receptors reflects some accident of morphogenesis, or merely a fracture behind the tip in a few microelectrodes.

Mapping experiments were performed initially upon eyes from crayfish that had been kept in the dark an hour or more previously, since Parker (*3*) had been able to detect light spread only between ommatidia of fully dark-adapted eyes. Eyes were subsequently examined with the light microscope, using thin sections of material embedded in Araldite that had been fixed for 3 minutes in hot water to arrest pigment migration (*3*). In every case, both the distal and proximal pigments were found to occupy the extreme light-adapted position described by Parker. Measurements were then made between midnight and 2 a.m., in order to avoid competing with the daily cycle of pigment migration (*15*). But although these eyes were dark-adapted at the outset, as evidenced by their pronounced orange "glow" in dim illumination (*15*), later examination again showed the pigments in the light-adapted position; the facet maps were also not discernibly different. Such light-adaptation may be brought on by anoxia (*16*), but eyes excised and kept dark here for more than an hour retained their pigments in the dark position. The dim lights that were used during dissection of the eye and to find and test the facets must therefore have caused the pigment migration.

Because their interommatidial screening pigments are retracted, dark-adapted crayfish eyes would be expected to show even more optical interchange between facets than is demonstrated here (*3*), where the technique used may furthermore underestimate the amount of interchange as it is (*12*). It thus seems probable that when these "superposition" eyes have dark-adapted, considerably more than half the light captured by one receptor enters the eye through adjacent ommatidia. In "apposition" eyes, by contrast, the amount of optical mixing is very small. Even if this has been underestimated as much as tenfold for the locust eye, still, less than 1 percent of the captured light comes in through other facets. "Diffraction images" can therefore contribute very little, if anything, to visual processing in this insect [compare with (*2*)].

This demonstration of optical interaction between ommatidia of what is anatomically a superposition eye, should not be taken as an endorsement of Exner's original theory (*1*). This theory derives its support largely from observations on the firefly eye, but Horridge (*7*) has pointed out that the imperfect optics and refractive heterogeneity of the intact eye preclude any type of collective image-formation. Superposition images cannot be observed behind a relatively intact firefly eye (*7*), which has a crystalline tract light-guide system as in other arthropods (*17*). These findings most likely apply to other species, including the crayfish. The usefulness of the optical mixing demonstrated here thus becomes obscure, because it must broaden each receptor's visual field and so reduce acuity (*18*). Perhaps it is a penalty paid for an incomplete pigment screen (*3*), plus a long optical system which facilitates even more light spread in the dark, this in turn increasing the light-gathering power of each cell. Whether optical interaction does in fact increase following pigment retraction remains to be determined.

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10. In two species with apposition eyes, the fly *Musca* and the horseshoe crab *Limulus*, very little optical interaction between ommatidia could be measured (J. H. Scholes and H. K. Hartline, personal communications). Optical interaction may occur in pigmentless eye mutants of Diptera [however, see, for example, Y. Washizu, D. Burkhardt, P. Streck, *Z. Vergl. Physiol.* **48**, 413 (1964)].
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12. Matching the responses is a valid procedure only if the light-guide tip acts as a perfect radiator, which was not the case. It was found, by exposing calibrated film to the light-guides and scanning the resultant negatives with a microdensitometer, that a variable and often irregular diffraction pattern issued from the tip. Because more of the energy was concentrated to the center of the pattern, records from surrounding facets will *underestimate* the extent of light spread, if obliquely incident rays are the ones that can most readily escape from the ommatidial optics.
13. It is assumed that the penetrated receptor and the most excitable facet formed part of the same ommatidial unit. In agreement with this, the estimated position of the electrode tip served as a guide to this facet. One locust retinula was transiently depolarized when the light-guide was prodded against this facet, but not against adjacent ones; this procedure probably shifted the electrode slightly in the cell, thus suggesting that cell and facet were in mechanical, and therefore anatomical, continuity.
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19. I thank G. A. Horridge and the Science Research Council, United Kingdom, for support and G. Hacking for advice on histology.
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19 December 1968; revised 24 March 1969