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INTERACTIONS AMONG RODS IN THE ISOLATED RETINA OF *BUFO MARINUS*

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SUMMARY

1. The existence and extent of interactions among rods were analysed in the isolated retina of *Bufo marinus*. Intracellular recordings were made from the outer segments with micropipettes. Stimuli of precisely measured geometry were delivered to the outer segments using a compound microscope.

2. To demonstrate the existence of interactions two rods were simultaneously impaled; current injected into one rod resulted in a current-induced potential of like sign in the other rod.

3. When the diameter of a circular stimulus was increased from 30 to 500 μm the mean sensitivity of the rods increased by $1{\cdot}2 \pm 0{\cdot}3$ log units (S.D.), though the illuminance measured through the impaled rod was adjusted to be constant.

4. The extent of interactions was examined by presenting a dim, slit-shaped stimulus at each of several displacements from an impaled rod. This stimulus either passed through the retina before coming into focus on the outer segments (transillumination) or was focused directly on the outer segments (incident illumination). For each displacement both the amount of light scattered onto the impaled rod and the response of the rod were measured.

5. The amount of light scattered onto the impaled rod was assessed for each displacement of the stimulus. For incident illumination this assessment was made by measuring the distribution of bleached pigment about the slit stimulus. The bleaching could be described by an exponential that decreased with distance; this exponential had a space constant of 4 μm. For transillumination the assessment was made in two ways. The light that passed through the impaled rod was measured and was found to decrease exponentially with slit displacement; the mean space constant of this exponential was $9{\cdot}3 \pm 3{\cdot}2$ μm (S.D.). In a few experiments the distribution of bleached pigment about the slit stimulus was measured and was found to decrease exponentially with distance; this latter exponential had a slightly longer space constant than the exponential measured using transmitted light.

6. For each impaled rod a convolution of an exponential interaction function with the measured distribution of stimulus illuminance about the impaled rod could be fitted to the plot of response amplitude *versus* displacement of the slit stimulus. For stimuli

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presented by transillumination the mean space constant describing the interaction functions was 20 ± 6 μm (S.D.). For stimuli presented by incident illumination the mean space constant of the interaction functions was 22 ± 4 μm (S.D.).

7. This report presents new evidence that interactions among rods exist in the isolated retina and that the extent of interactions can be described by a space constant of about 20 μm.

INTRODUCTION

The receptor potential of a rod is initiated when light is absorbed by rhodopsin in the rod outer segment. However, because of interactions among rods (Schwartz, 1973, 1975, 1976; Fain, 1975; Fain, Gold & Dowling, 1976; Copenhagen & Owen, 1976*a*,*b*; Lamb & Simon, 1976; Leeper, Normann & Copenhagen, 1978; Werblin, 1978; Gold & Dowling, 1979; Gold, 1979) the response recorded in a single rod outer segment depends not only on the amount of light absorbed by that outer segment but also on the amount of light absorbed by the outer segments of neighbouring rods. Several studies have shown that intracellular injection of various substances into the outer segment alters the rod's response to light (Brown, Coles & Pinto, 1977; Miller & Nicol, 1979; Waloga & Brown, 1979). In order to interpret the results of such studies it is necessary to know how much of the response recorded intracellularly from a given rod is generated as a result of light absorbed by that rod. We therefore undertook a detailed analysis of the spatial extent of interactions among rods in the isolated retina of *Bufo marinus*.

The extent of interactions was studied by measuring the spatial distribution of the response evoked by a stimulus which was directed towards a single rod or row of rods. However, the stimulus illuminance was also distributed across the retina, presumably due to light scatter. Thus, the response recorded from a rod reflected both the effects of scattered light and the effects of interactions. To isolate the contribution of interactions to the recorded response one must therefore know the distribution of stimulus illuminance. In previous studies of the extent of interactions among rods the effect of scattered light was either estimated (Schwartz, 1973; Copenhagen & Owen, 1976*a*; Gold, 1979) or ignored (Fain *et al.* 1976; Leeper *et al.* 1978). Without knowledge of the amount of scattered light these previous results are not interpretable. In the present study, for each rod for which the spatial distribution of the response was measured the distribution of stimulus illuminance was *measured* by one of two independent methods. These measurements allowed a valid determination of the extent of interactions among rods.

METHODS

Preparation and recording

Healthy *Bufo marinus* were dark-adapted for at least 8 hr; a small section of the isolated retina was mounted, receptor-side up, in a chamber with a transparent bottom (Brown & Pinto, 1974). The preparation was continually superfused with oxygenated Ringer solution containing 108 mM-NaCl, 2·4 mM-KCl, 0·86 mM-$CaCl_2$, 1·3 mM-$MgCl_2$, 5 mM-glucose and 2·8 mM-HEPES (*N*-2-hydroxyethyl piperazine *N'*-2-ethanesulphonic acid), buffered to pH 7·8 with NaOH. The chamber was placed on the stage of a compound microscope, illuminated with infra-red light (> 850 nm. Wratten 87B) and viewed through an infra-red image converter (Varo 6914) or infra-red image

intensifier (Varo 8686/3). A portion of the retina was selected in which the long axes of the rods were parallel to the optical axis of the microscope.

Micropipettes were made from glass capillary tubing (o.d., 1·2 mm; i.d., 0·8 mm) on a flat-bed puller (J. B. Keefe, Islington, Mass.) and filled with 1 M-potassium acetate. The pipette tip was never advanced more than 70 μm proximal to the tips of the rod outer segments, and therefore all data were recorded from the outer segments. Electronic oscillations were produced at the tip of the pipette in order to impale a rod. In experiments in which two rods were simultaneously impaled, each micropipette was mounted on a separate manipulator and advanced independently. The signal from each micropipette was fed into an electrometer (WPI, Model M4) and subsequently stored on magnetic tape (Vetter Inc., Rebersberg, PA).

Optical stimulator

Two separate optical stimulators were used; the stimulus either passed through the neural layers of the retina before impinging on the rods (transillumination) or was incident on the tips of the rod outer segments. In each configuration the numerical aperture (N.A.) of the stimulus optics was set lower than the numerical aperture of the rods ($\sim$ 0·1) so that the rods, acting as light guides, captured and contained most of the light (Tobey, Enoch & Scandrett, 1975). For transillumination (see Fig. 1*A*) the stimulus was focused on the retina by the microscope condenser (N.A. adjusted to less than 0·1) and the illuminance in the plane of the tips of the rod outer segments was observed. Because the outer segments act as light guides, the stimulus image was probably formed first at the base of the outer segments and was also observed to be in focus at the tips of the rod outer segments. For incident illumination (see Fig. 1*B*) the stimulus was directed through a microscope objective (N.A. adjusted to 0·05) by a pellicle. The stimulus was focused on the retina by the objective using light reflected from the surface of the outer segment tips. The stimulus geometry was determined by an aperture stop, either a circular hole or slit. This stop was mounted on a translator so that the stimulus could be displaced across the retina.

Measurement of distribution of stimulus illuminance

Two different methods were used to measure the distribution of stimulus illuminance about the rod that had been impaled and studied. When the retina was transilluminated we measured the distribution of light which exited from the tips of the single outer segments (photometric method). In some preparations we also measured the distribution of bleached rhodopsin caused by a bright version of the stimulus. When incident illumination was used we measured the distribution of bleached pigment.

Transillumination. We used the photometric method to measure the distribution of light which passed through the retina and exited from single outer segments. The light was gathered by a microscope objective (N.A. 0·4 or 0·75) and focused onto a suitably masked photodetector. The mask permitted light from the image of only a single rod to be detected; the photometer assembly could be moved to detect light exiting from any rod in the field of view of the microscope objective.

To position the photometer and measure the stimulus distribution about the impaled rod, a small-diameter stimulus (which, because of light scatter, illuminated two or three rods) was first positioned so that the recorded receptor potential was maximized. An infra-red filter was then inserted and the mask centred on one of the illuminated rods under visual control (using the infra-red image intensifier). A photodiode (PIN 020A) or photomultiplier tube (1P28) was positioned behind the mask.

With the photometer centred over the chosen rod, a high-illuminance version of the transilluminating stimulus was presented. For circular stimuli we measured the illuminance at the centre of the stimulus; the illuminance at the centre of a small-diameter stimulus was normalized to the illuminance at the centre of a large-diameter stimulus to give the relative illuminance. Slit-shaped stimuli were presented at each of several displacements from the chosen rod, and the illuminance through the rod was measured at each stimulus displacement. A plot of illuminance *versus* displacement gave the line spread function of the slit-shaped stimulus. The line spread function was fitted by an exponential and characterized by a space constant, λ. The mean space constant describing the line spread functions was $9{\cdot}3 \pm 3{\cdot}2$ μm (S.D.) (see Table 1).

Our aim was to measure all of the flux that passed through the chosen outer segment. For most experiments a dry objective (N.A. = 0·4) was used to collect the light exiting from the tips of the rod outer segments. However, to ensure that the photometric measurements were not limited

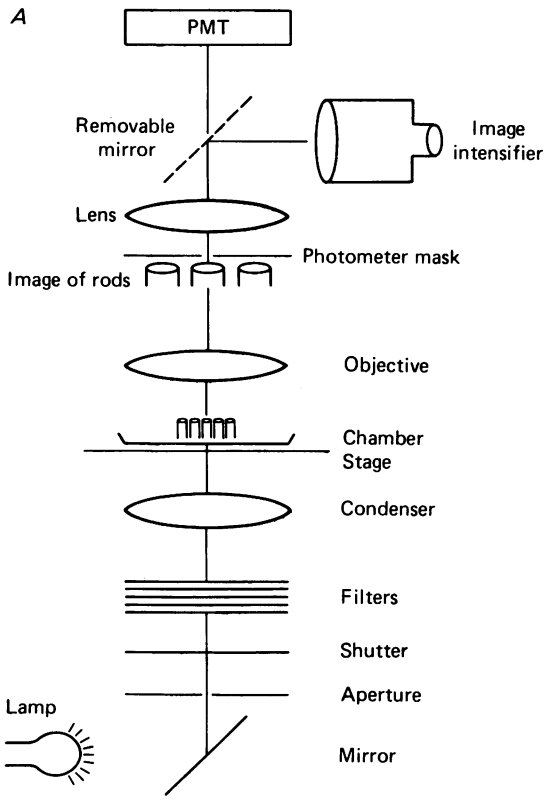

Fig. 1. Schematic diagram of apparatus. The retina was mounted receptor-side up in a transparent-bottomed chamber and placed on the stage of a compound microscope. The preparation could be viewed using an image intensifier.

A, transillumination. The stimulus geometry was determined by an aperture (a slit or circular hole) which was focused onto the tips of the outer segments by the condenser. The aperture was mounted on a translator to allow displacement of the stimulus. To measure the distribution of illuminance, the stimulus at the tips of the outer segments was focused by the objective onto a mask. A lens focused the image of the rods and the mask onto either the photometer or onto the cathode of the image intensifer. The mask allowed the light passing through one outer segment to be measured by the photometer. PMT, photomultiplier.

by the numerical aperture of the objective, a water-immersion objective of higher aperture (N.A. = 0·75) was substituted. The measured illuminance distribution was not different with this higher aperture.

However, objectives with high N.A.s necessarily have small depths of focus. Scattering at retinal depths proximal to the rods may be extensive, causing light to pass through the rods at angles greater than would permit total internal reflexion within the rods. This scattered light would nevertheless be effective in bleaching rhodopsin and in contributing to the receptor potential, but might not be focused onto the photodetector and measured. The distribution of stimulus iluminance obtained with the above photometric method was therefore re-checked by measuring the distribution of bleached pigment caused by the stimulus. Bleaching of pigment was detected by measuring the decrease in axial absorbance at 500 nm, the wave-length for maximal absorbance of red rods of *Bufo marinus* (Harosi, 1975), upon presentation of a bright and prolonged version of the stimulus.

The decrease in axial absorbance at 500 nm was measured for each of several positions across

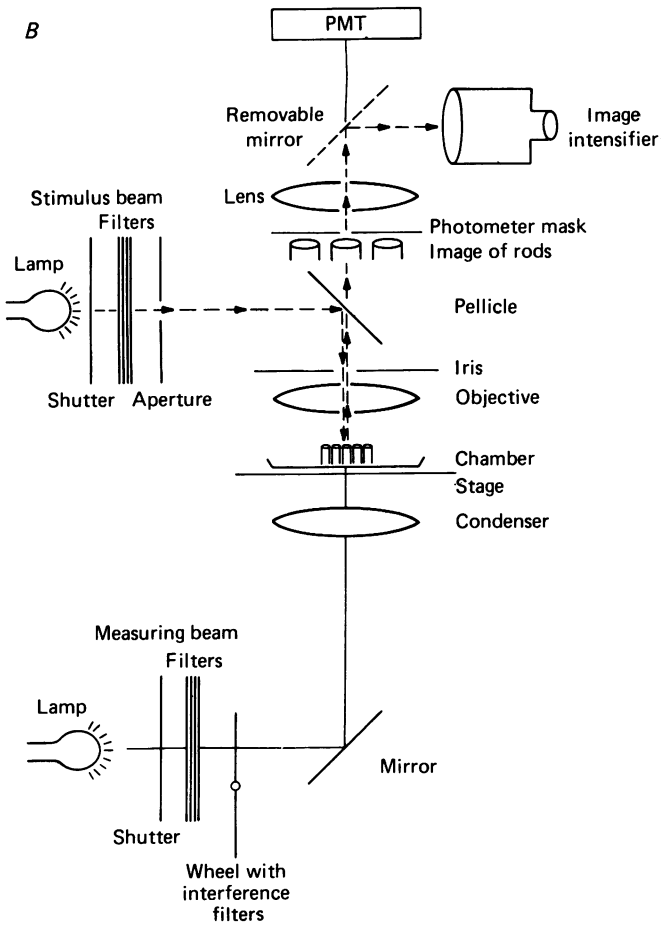

Fig. 1 (*cont.*).

B, incident illumination. The stimulus geometry was determined by an aperture which was focused onto the tips of the outer segments by the objective. A pellicle allowed the reflexion of the stimulus from the tips of the rod outer segments to be viewed with an image intensifier. The distribution of stimulus illuminance was measured spectrophotometrically (see text and Fig. 3). A measuring beam was directed through the retina by a mirror. A rotating wheel containing six interference filters sampled the spectrum at 430, 470, 500, 530, 580 and 660 nm. After traversing the retina the measuring beam was collected by the microscope objective and directed to the cathode of a photometer (S-20 cathode). A mask in the image plane of the objective was positioned to allow only the light from a selected rod to be measured. PMT, photomultiplier.

the retina and was plotted as a function of distance from the chosen rod; an exponential could be fitted to the data. The space constant of the 'bleached pigment function' was 9 μm, whereas the space constant of the line spread function measured using the photometric method for the same rod was 7 μm (see Fig. 2).

Incident illumination. Although the stimulus was incident on the outer segments, the light may have been scattered by intracellular debris, broken outer segments, or back-scatter from the retina. It was therefore necessary to measure the distribution of stimulus illuminance. This measurement was always made upon the rod that had been impaled. To identify the impaled rod a small circular stimulus (1·7 μm diameter) was presented under visual control to each of several rods. The amplitude of the receptor potential of one of the rods was often two to four times that of any of

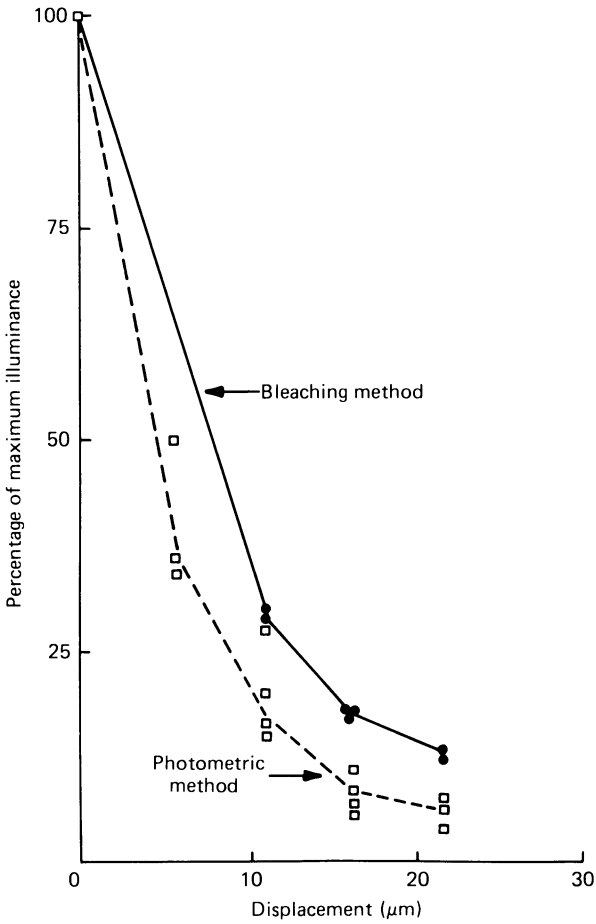

Fig. 2. Comparison of two measurements of the distribution of stimulus illuminance. A slit-shaped stimulus was delivered by transillumination. The line spread function of the stimulus was measured by two methods. First, the transmission of light (500 nm) through a single rod was measured as a function of displacement of the stimulus from that rod (open squares). Secondly, the distribution of bleached pigment was assessed by measuring the change in absorbance at 500 nm caused by presentation of a bright version of the stimulus (filled circles).

its neighbours; we assumed that this rod was the impaled rod. A suitably masked photometer was then centred over the impaled rod.

To measure the distribution of stimulus illuminance about the impaled rod we measured the distribution of bleached pigment caused by presentation of a bright, prolonged version of the stimulus. We chose not to present the stimulus in one location and then to measure the distribution of bleached pigment in each of several nearby rods because this scheme would have required re-positioning the photometer mask many times. Instead, we measured the bleaching of pigment that occurred in the impaled rod as a result of presentation of a bright, prolonged version of the stimulus at each of three distances from the rod: first, with the stimulus passing through an outer segment that was two outer segments away from the impaled one; secondly, with the stimulus passing through a nearest neighbour; and finally with the stimulus passing through the impaled rod itself.

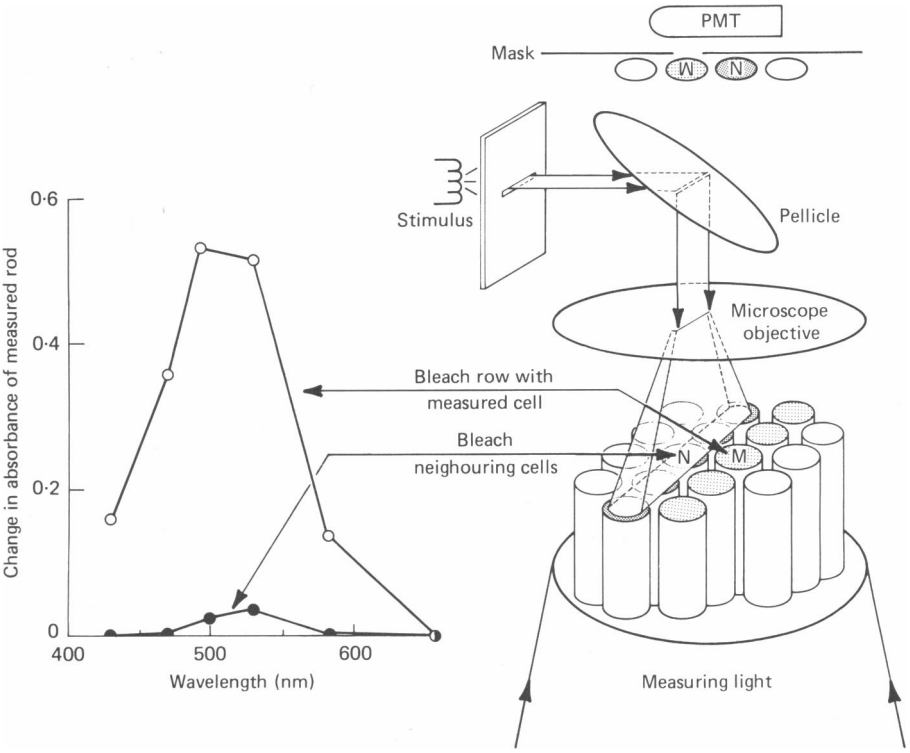

Fig. 3. Measuring the distribution of bleached pigment. A slit-shaped stimulus was directed at the retina by a pellicle and the stimulus focused onto the tips of the rod outer segments with a microscope objective. The distribution of stimulus illuminance was evaluated by measuring the distribution of bleached pigment. A photomultiplier (PMT) was centred over the image of the impaled rod (see text for details), which is indicated as M (measured cell). A measuring light was presented at each of six wave-lengths and the absorbance at each wave-length measured. The change in absorbance through the measured rod, M, caused by presentation of a bright version of the stimulus through the row that included a neighbouring rod, N, is shown by the filled circles. The change in absorbance through the measured rod caused by presentation of a bright version of the stimulus through the measured rod is shown by the open circles.

We measured the bleaching of rhodopsin in the impaled rod as follows. The measuring light passed through a rotating wheel (1 c.p.s.) that carried six interference filters (10 nm pass-band) which sampled the spectrum at 430, 470, 500, 530, 580 and 660 nm. After traversing the retina the measuring beam was collected by a microscope objective, passed through a mask that transmitted only light from the impaled rod, and was directed to the cathode of a photomultiplier (S-20 cathode). The negative logarithm of the photomultiplier current measured before presentation of the high-illuminance version of the stimulus was stored for each wave-length in an averaging computer (twenty presentations). From this stored information was subtracted the negative logarithm of the current for each wave-length obtained after presentation of the high-illuminance version of the stimulus; this difference was the difference spectrum. Since light of 660 nm wave-length will be absorbed insignificantly by rhodopsin and its photoproducts (Wald, Brown & Gibbons, 1963) the absorbance difference at 660 nm was taken to be zero. Bleaching of the rod was calculated from the decrease in axial absorbance at 500 nm. The energy of the high-illuminance and prolonged version of the stimulus was held to a value low enough to bleach no more han 20 % of the pigment in the impaled rod when directed at that rod. For each stimulus displacement the decrease in axial absorbance of the impaled rod at 500 nm was expressed as a fraction of the absorbance decrease

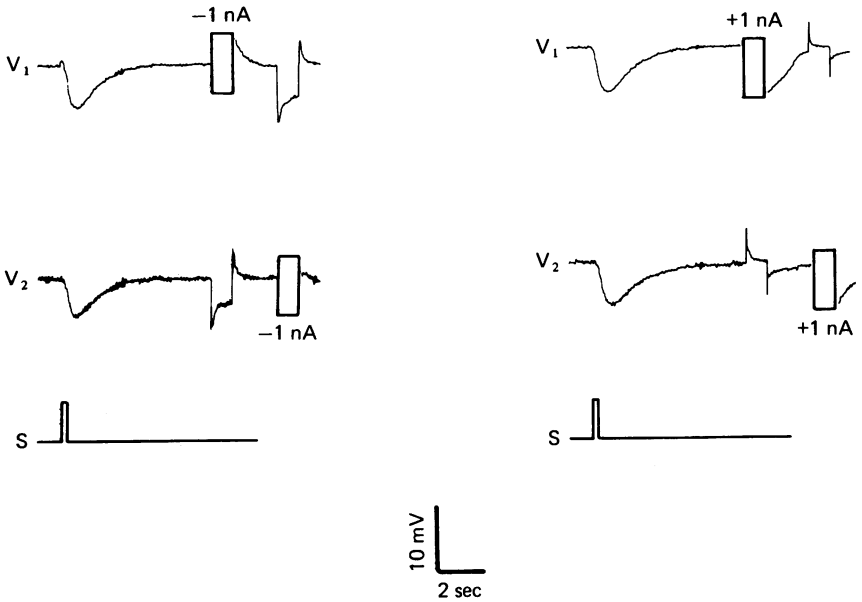

Fig. 4. Current-induced potentials between two interacting rods. Two rods were simultaneously impaled and their membrane potentials monitored as shown by traces V_1 and V_2. A 200 msec stimulus was delivered as indicated by trace S. When hyperpolarizing current was injected into either rod, as indicated by the rectangles, a hyperpolarizing potential was recorded in the other rod (left-hand column). When depolarizing current was injected into either rod (right-hand column), a depolarizing potential was recorded in the other rod.

that occurred when the stimulus was directed at the rod. The mean amount of pigment bleached by presenting the stimulus at a displacement of one rod was 14 % of the amount of pigment bleached by stimulating the impaled rod directly (see Fig. 3). When the stimulus was displaced two rods, the amount of pigment bleached in the impaled rod was below the limit of detectability (5–10 %).

RESULTS

Evidence for interactions among rods in the isolated retina

Simultaneous impalements of two rods. Direct evidence for interactions among rods was obtained by simultaneously impaling two rods (Griff, 1979). In about 20 % of the 980 simultaneously impaled pairs of rods, injecting current (±1 nA) into one rod of the pair caused a detectable polarization of like sign in the other rod of the pair (see Fig. 4). Similar results were obtained regardless of which rod was selected to be the rod into which current was injected. The injection of hyperpolarizing current into a given rod was more effective than injection of depolarizing current in causing a current-induced potential in the other rod of the pair.

To show that the above results were not caused by changes in voltage in the extracellular medium, one electrode was withdrawn from the rod it had impaled. Current passed into the extracellular medium did not cause a detectable potential change in the impaled rod, nor did current injected into the impaled rod cause a

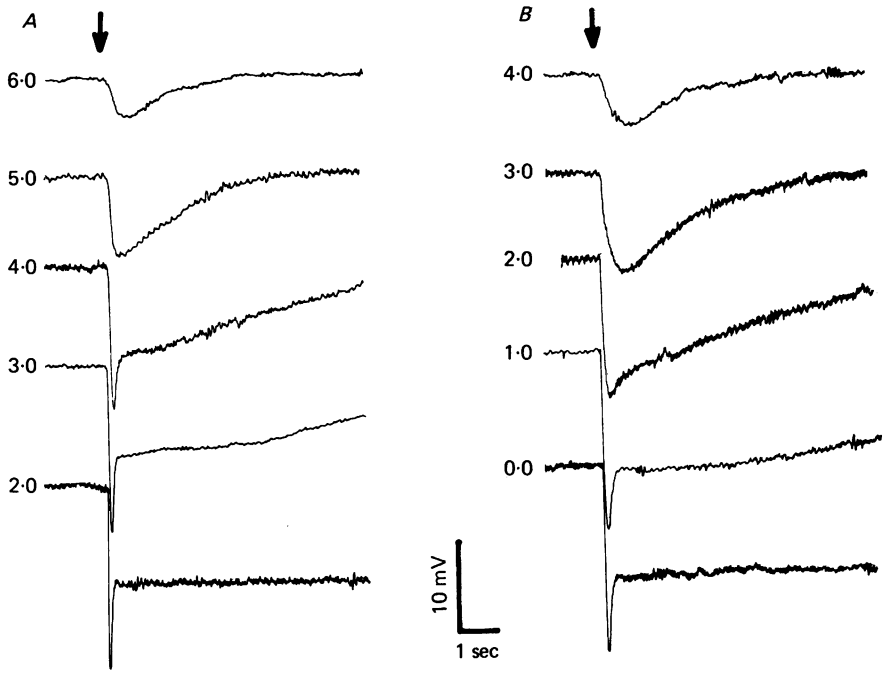

Fig. 5. Responses to large and small stimuli. Responses recorded from a single rod, evoked by stimuli of 500 μm diameter (*A*) and 30 μm diameter (*B*). The arrow above each set of responses indicates the time of a 20 msec stimulus. Filter densities are shown next to each trace. For this cell the measured illuminance at the centre of the small stimulus (30 μm) was 0·3 log units less than the illuminance at the centre of the large stimulus (500 μm).

detectable change in the voltage recorded in the extracellular medium (limit of detectability = 0·5 mV). Similar results were obtained in turtle rods by Copenhagen & Owen (1976*b*) and in tiger salamanders by Werblin (1978).

Responses evoked by large and small stimuli. An assessment of interactions among rods in the isolated retina was also made by examining the responses of single rods to circular stimuli of diameters 500 μm (large) and 30 μm (small). Rods responded with a graded hyperpolarization that increased in amplitude with stimulus illuminance. Responses of a single rod to large- and small-diameter stimuli are shown in Fig. 5. At low illuminance the wave-form of the receptor potential was a monotonic hyperpolarization. For high illuminances the responses to both large and small stimuli consisted of a rapid hyperpolarization to a transient peak which decayed to a plateau hyperpolarization.

Comparison of the responses evoked by large and small stimuli was most meaningful if both stimuli delivered the same amount of light to the impaled rod. Therefore, the relative illuminances of the large and small stimuli were measured for each experiment (see Methods). The illuminance in the centre of the small stimulus was less than the illuminance in the centre of the large stimulus, presumably due to scattering of the light as it passed through the retina. The amount that the illuminance in the centre of the small stimulus was decreased varied from preparation to preparation: the range was 0·3 to 0·7 log units (n = 15; mean = 0·4).

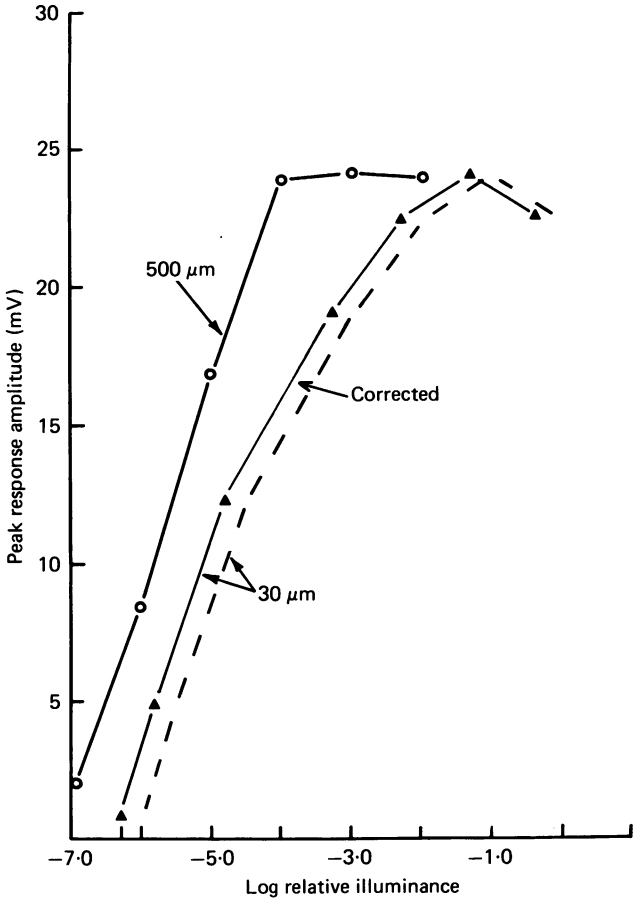

Fig. 6. Responses to large and small stimuli. Plot of peak response amplitude of a single rod *versus* log relative illuminance for large- (open circles) and small- (filled triangles) diameter stimuli. To correct for the measured attenuation of the small stimulus (see text) the curve for the small stimulus was shifted 0·3 log units to the left, to give the curve labelled 'Corrected'. Straight lines connect data points. (Data are from the same rod as in Fig. 5.)

We compared the sensitivity of a rod to large and small stimuli by plotting the peak response amplitude against the log relative illuminance (Fig. 6). The curve for the small stimulus was shifted on the illuminance axis by an amount equal to the attenuation of the small stimulus to give a corrected curve. For the cell shown in Fig. 6, the measured attenuation was 0·3 log units.

The plots of peak response amplitude *versus* stimulus illuminance were similar for large and small stimuli. However, a higher illuminance was needed to evoke a response of given amplitude (criterion response) with the small stimulus than with the large stimulus. The difference in illuminance needed to elicit a criterion response from a given cell is defined as the change in relative sensitivity. This difference in sensitivity is given by the horizontal separation between the plot of the response amplitude against illuminance from the large stimulus and the plot of response amplitude against corrected illuminance for the small stimulus. For twenty-five cells

the relative sensitivity, measured using half-maximal responses, increased by $1{\cdot}2 \pm 0{\cdot}3$ log units (S.D.) when the stimulus diameter was increased from 30 to 500 μm.

In order to compare our results more easily with similar results obtained in other preparations, we calculated the absolute sensitivity to large and small stimuli. The response of a rod to stimuli which resulted in fewer than 100 photo-isomerizations per rod per second was found to be linearly related to the stimulus illuminance. The number of photo-isomerizations per rod per flash was calculated by multiplying the stimulus illuminance by the effective collecting area of a rod (Fain, 1975). The absolute sensitivity was then calculated by dividing the amplitude of the response to these dim stimuli by the number of photo-isomerizations per rod per flash. Unfortunately, for these experiments the attenuation of the small stimulus was not measured. We therefore used the average attenuation of the small stimulus from previous experiments (0·4 log units) to account for the difference in illuminance at the centre of the large and small stimuli. For responses evoked by the large stimulus (500 μm diameter) the mean sensitivity was $523 \pm 233\,\mu$V (S.D.) per photo-isomerization per rod (twenty-two cells). For the small stimulus (30 μm) the mean sensitivity was $29{\cdot}1 \pm 12\,\mu$V (S.D.) per photo-isomerization per rod. The difference between these sensitivities agrees with that found from the half-maximal responses.

Changes in time course of receptor potential. Increasing the diameter of the stimulus also affected the time course of the receptor potential. When responses to large and small stimuli of matched illuminance (falling upon the impaled rod) were compared, the response to the large-diameter stimulus reached its maximum before the response to the small-diameter stimulus. Similar results have been reported in turtle rods by Detwiler, Hodgkin & McNaughton (1978). This difference in time course was observed over the entire range of stimulus illuminances used. When the stimuli delivered more than $9{\cdot}5 \times 10^5$ quanta sec^{-1} μm^{-2} more complex differences between the time courses of the responses to large and small stimuli became apparent (see Fig. 7). For the first 100 msec the membrane was more hyperpolarized in response to the large stimulus than in response to the small stimulus. The responses then 'crossed' and for the next 200 msec the membrane was *less* hyperpolarized in response to the large than the small stimulus. These differences were consistently observed in twelve rods. Similar results have been reported in turtle rods by Schwartz (1973). Thus, illuminating neighbouring rods (with a large-diameter stimulus) not only increases the sensitivity of a given rod but also alters the time course of the response of the rod. These experiments in which we compared responses to stimuli of measured equal illuminance but different geometry provide new evidence that interactions among rods exist in the isolated retina.

Spatial extent of interactions among rods

Transillumination. The contribution to the response made by rods at increasing distances from an impaled rod, i.e. the spatial distribution of the response of a single rod, was analysed using a slit-shaped stimulus (Lamb & Simon, 1976; Lamb, 1976). The response amplitude was plotted against the displacement of the stimulus from the impaled rod (see Fig. 8). Data points appeared to fall about a straight line when the data were plotted on semi-logarithmic graph paper. An exponential function was fitted to the data by the method of least squares. The displacement at which the

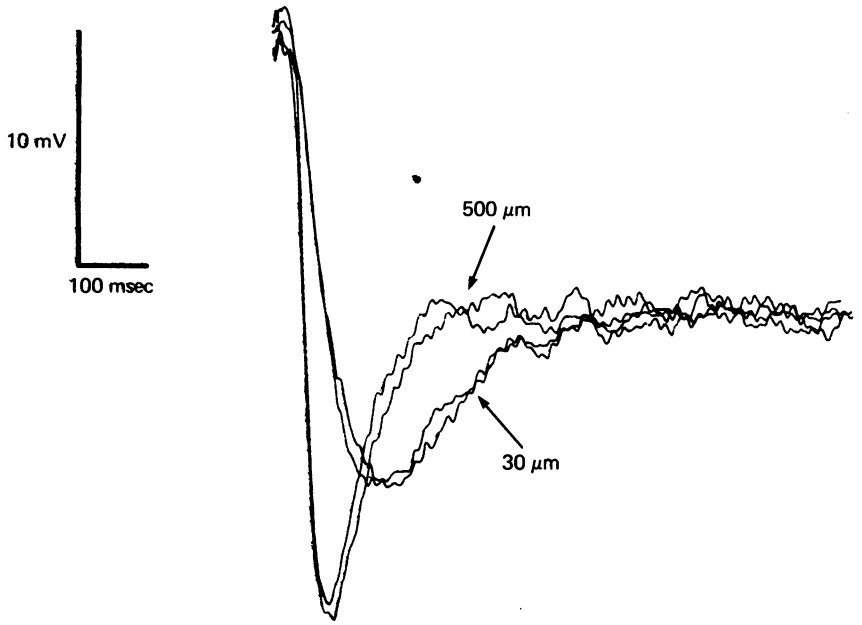

Fig. 7. Comparison of the response wave-forms of a single rod to large and small stimuli of matched illuminance ($6{\cdot}03 \times 10^6$ quanta sec^{-1} μm^{-2}). The small bar indicates the time of a 20 msec stimulus. Four responses to interleaved large (500 μm) and small (30 μm) stimuli are superimposed.

normalized response amplitude was 37% of maximum was defined as the space constant. The space constant was measured from the plots of response amplitude *versus* the displacement; for ten cells the mean space constant was $34{\cdot}5 \pm 6{\cdot}9$ μm (S.D.). Note that this value does not take the effects of scattered light into account.

A very dim stimulus, which delivered less than 100 quanta per rod per second, was used in the above experiments; the response to such a stimulus, centred on the impaled rod (displacement = 0) was linearly related to the stimulus illuminance. If the light scatter from the stimulus was known, the portion of the response that could be attributed to the scatter could be calculated from the proportionality. Therefore, the measured amount of light passing through the impaled rod at each displacement of the stimulus was used to determine the contribution of scattered light to the response.

The observed voltage response of an impaled rod is predicted mathematically by the *convolution* of a function describing the scattered light with a function describing the interactions (Lamb & Simon, 1976):

$$V(x) = \int_{-\infty}^{+\infty} L(\mu)\, I(x-\mu)\, d\mu,$$

or in short form

$$V(x) = L(x) * I(x),$$

where x is the distance from the rod to the slit stimulus, $V(x)$ is the voltage in a rod

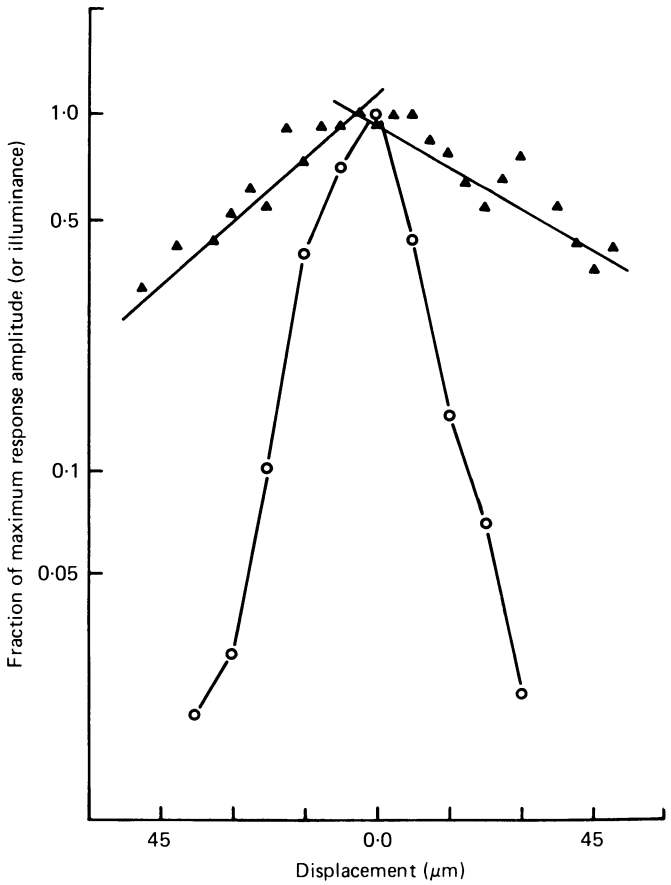

Fig. 8. Response to a slit-shaped stimulus passing through the retina. Plot of normalized peak response amplitude (filled triangles) or illuminance (open circles) *versus* displacement of a slit-shaped stimulus which passed through the retina. The stimulus was displaced from an impaled rod (at the origin) in 7·5 μm steps; at each displacement a response was recorded and the amount of light scattered onto the impaled cell was measured. These data were normalized against the response amplitude or the stimulus illuminance when the stimulus was centred on the impaled rod. An exponential, the transformation of which is shown by the straight lines, was fitted to the response data by the method of least squares.

as a function of distance, $L(x)$ is a function describing the distribution of illuminance (line spread function) of a slit-shaped stimulus, and $I(x)$ is a function describing the spread of voltage among rods via interactions. We will refer to $I(x)$ as the 'interaction function'.

The interaction function was assumed to be an exponential (Lamb & Simon, 1976). To determine the interaction function we convolved the line spread function, measured photometrically, with each of several exponential functions characterized by different space constants, λ. We then selected the calculated response function ($L(x)*I(x)$) which best fitted the measured response function (Gold, 1979). The calculated response function was fitted to the measured response function by eye. Neither the line spread functions nor the response functions were necessarily symmetrical about

TABLE 1. Extent of interactions with transillumination

Cell	Side	Stimulus (μm)*	Mean response λ (μm)*	Mean interaction λ (μm)
3-10-1	L†	7·0	> 30	29
3-10-1	R†	7·5	> 30	22·5
3-16-1	L	9	52	32·75
3-16-1	R	9	37	29
3-18-1	L	‡	33	18·75
3-18-1	R	‡	38	26·75
3-18-2	L	8	28	15
3-18-2	R	7·5	38	18·75
3-21-3	L	‡	25	15
3-21-3	R	‡	35	22·5
3-21-5	L	7·5	25	15
3-21-5	R	7·5	37	22·5
3-22-4	L	17	30	15
3-22-4	R	9	32	22·5
3-22-5	L	‡	15	11·25
3-22-5	R	‡	37	22·5
4-13-1	L	15	37	22·5
4-13-1	R	8	< 45	15
4-13-2	L	‡	45	22·5
4-13-2	R	‡	< 45	18·75
4-13-3	L	‡	30	18·75

* Displacement at which response falls to 1/e.
† L and R refer to displacements to the left and right of the origin.
‡ Measurement of stimulus illuminance on same date (same retina) was used.

the origin. Therefore functions to the right and to the left of the origin were calculated separately. The mean space constant of the interaction functions to the right and left of ten rods was $20{\cdot}3 \pm 6{\cdot}0\,\mu$m (S.D.).

Incident illumination. Using incident illumination the distribution of the response and the distribution of the stimulus illuminance could be measured about the same rod (see Methods). The contribution to the response made by rods at increasing distances from an impaled rod was again analysed using a displaced slit-shaped stimulus. A very dim stimulus was used in order to ensure that the response to the stimulus, when centred on the impaled rod, was linearly related to the stimulus illuminance. Therefore, a measurement of the amount of light passing through the impaled rod at each displacement of the stimulus allowed determination of the contribution of scattered light. We found that when the stimulus was displaced *one* rod away from the impaled rod the illuminance of the impaled rod decreased to 14 % of the illuminance measured when the stimulus was centred on the impaled rod. Displacing the stimulus any further caused an undetectable amount of scatter to the impaled rod (limit of detectability = 5–10 %). These data were approximated by an exponential function which decayed to 14 % at a distance of 8 μm (one rod) from the impaled cell.

The normalized response amplitude evoked by a very dim stimulus was plotted as a function of displacement from the impaled rod (see Fig. 9). The space constant

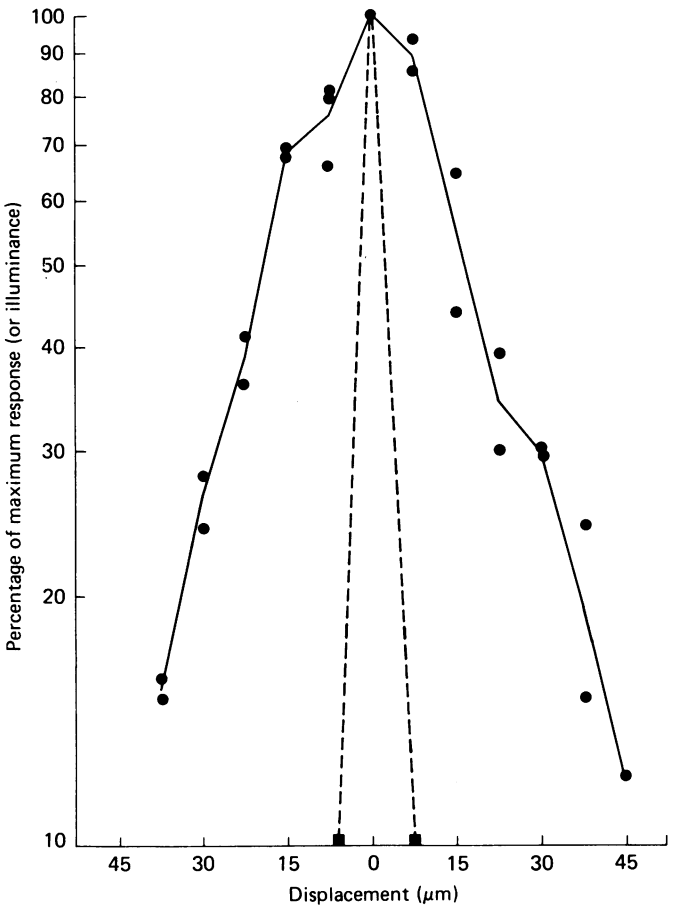

Fig. 9. The normalized response evoked from an impaled rod by a slit-shaped stimulus which was incident on the tips of the outer segments of the rod (filled circles). The distribution of stimulus illuminance was measured by the distribution of bleached pigment and is shown by the filled square symbols.

was measured from plots of response amplitude *versus* displacement for seven cells; the mean space constant was $26{\cdot}9 \pm 4{\cdot}5\,\mu$m (S.D.). When the interaction functions were calculated from the measured response functions and the measured scatter functions, as described above, the mean space constant of the interaction functions was $22{\cdot}3 \pm 4{\cdot}5\,\mu$m (S.D.) (see Table 2).

DISCUSSION

Much of the interpretation of the above results depends upon knowing the distribution of illuminance for stimuli of different geometries. We wanted to measure all the light that was capable of exciting *the impaled rod*. In experiments in which the stimulus transilluminated the retina our illuminance measurements were made

TABLE 2. Extent of interactions with stimulus incident on rod outer segments

Cell	Side	Mean response λ (μm)	Mean interaction λ* (μm)
11-6-2	L†	24·6	19·3
	R†	25	21
11-14-2	L	25·5	20·5
	R	36	27
11-15-2	L	27	26
	R	34	28
11-29-1	L	24	19
	R	24·5	19
11-30-1	R	24	19
11-30-2	L	30	30
	R	22	17

* Calculation of the interaction functions was based on a line spread which fell to 14% at a displacement of 8 μm (see text).

† L and R refer to displacements to the left and right of the origin.

either upon the impaled rod or in close proximity to the impaled rod. Because the portion of the retina containing the impaled rod was selected to be homogeneous, we conclude that these measurements represent the distribution of stimulus illuminance about the impaled rod. The light exiting from the rod outer segments was collected by an objective with high N.A. Since increasing the N.A. from 0·4 to 0·75 did not significantly increase the amount of light collected, we conclude that the aperture of the objective was sufficiently large to collect nearly all of the light which exited from the tips of the rod outer segments. However, because of the small depths of focus of these objectives we would not have measured light which passed through a rod at an angle such that the ray of light was not internally reflected within the rod. In fact, the slightly larger space constant for light scatter that was measured for transillumination with the bleaching method (p. 241) may indicate that the photometric method did indeed over-estimate image quality slightly.

In experiments in which the stimulus was incident on the tips of the outer segments the distribution of stimulus illuminance was evaluated by measuring the distribution of bleached pigment. This measurement depended only on the absorption of light by rhodopsin, and did not rely on capturing all the scattered light.

Finally, because light scatter was reduced and we could see the reflexion of the stimulus from the tips of the outer segments, we could determine accurately which rod was being stimulated. Thus, we could determine which rod had been impaled and we could measure the distribution of stimulus illuminance about that rod.

Our measurements of the distribution of stimulus illuminance across the retina would not have detected changes in the distribution of illuminance along the length of a rod outer segment. Studies of disc shedding and renewal (Young, 1971) suggest that the base of the rod outer segment is specialized for production of new discs and that the tips of the rod outer segments are periodically shed. Response characteristics may also vary as a function of position along the rod outer segments (but see Baylor, Lamb & Yau, 1979, and McNaughton, Yau & Lamb, 1980). Therefore, we cannot

TABLE 3. Reports of the extent of interactions among rods in *Bufo marinus*

Reference	Space constant of interactions (μm)
Fain *et al.* (1976)	121*
Normann & Pochobrasky (1976)	21 ± 6
Leeper *et al.* (1978)	24 ± 5
Gold (1979)	19 ± 7

* Calculated from expression relating response and stimulus diameter for a two-dimensional resistive network (see Fig. 2 in Simon, 1976).

rule out the possibility that the responses recorded from a single rod are also influenced by the distribution of illuminance along the length of the rod outer segments.

Extent of interactions

Because we could accurately measure the distribution of stimulus illuminance about the actual rod from which we had measured the distribution of the light response, we could calculate how much of the response at a given distance from the impaled rod was due to interactions and how much could be attributed to scattered light. For example, in experiments using transillumination the calculated space constant describing the interactions was $20{\cdot}3 \pm 6{\cdot}0\,\mu$m (S.D) whereas the apparent space constant uncorrected for light scatter was $34{\cdot}5 \pm 6{\cdot}9\,\mu$m (S.D.). The effects of light scatter clearly result in an over-estimation of the extent of interactions.

Table 3 summarizes various reports of the extent of interactions among rods in *Bufo marinus*, most of them uncorrected for the effects of light scatter. The very large value of Fain *et al.* (1976) is probably the result of light scatter and incorrect focusing of the stimulus (Gold, 1979). In more recent experiments Gold (1979) used incident illumination and assumed therefore that light scattering in the retina was insignificant. In this report we show that the amount of light scatter is minimal when the stimulus is incident and focused onto the tips of the outer segments of rods whose long axes are parallel to the stimulus beam. Thus, the space constant of $22{\cdot}3 \pm 4{\cdot}5\,\mu$m (S.D.) reported in this paper corroborates the $19 \pm 7\,\mu$m (S.D.) space constant reported by Gold (1979).

Normann & Pochobradsky (1976) and Leeper *et al.* (1978) both studied interactions in the eye-cup preparation of *Bufo marinus*. Their values for the space constant for interactions, uncorrected for scattered light, were $21 \pm 6\,\mu$m (S.D.) and $24 \pm 5\,\mu$m (S.D.) respectively. Recall that the space constant determined above with transillumination, uncorrected for scattered light was $34{\cdot}5 \pm 6{\cdot}9\,\mu$m. One explanation for this difference is that the presence of the apical processes of the pigment epithelium optically isolates the rods in the eye-cup preparation, thus diminishing the effects of light scatter. However, the paradox remains that nature would ensure the optical isolation of the rods at the outer segment only to have the signals from neighbouring rods interact.

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