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RESPONSES OF CAT RETINAL GANGLION CELLS TO BRIEF FLASHES OF LIGHT

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SUMMARY

1. The responses of cat retinal ganglion cells to brief flashes of light have been illustrated and described with a view to providing material for comparison with psychophysical experiments in the scotopic (rod-dominated) range of performance.

2. There is a minimum response duration of 50–70 msec no matter how brief the flash is made. This duration is reached with stimuli lasting 32 msec or shorter.

3. Reducing the background illumination obviously increases the latency of responses to stimuli at 4 times threshold intensity (about 10 msec increment per log. unit decrement) but has no obvious effect on the minimum response duration.

4. The relation between intensity and duration of a flash for threshold responses closely resembles that in human psychophysical experiments. The Bunsen–Roscoe law is applicable for flash durations up to 64 msec.

5. If equal amounts of energy are delivered in the form of a pair of flashes of varying separation rather than by rectangular pulses, the shape of the response changes more abruptly with the temporal factor.

6. Non-linear performance is apparent for stimuli as weak as 4 times threshold.

7. A method is developed for quantitative analysis of individual responses. It is based upon cross-correlation of the train of impulses with a Gaussian smoothing function and represents local impulse frequency as a smooth function of time. The method also improves the signal-to-noise ratio of post-stimulus time-histograms of the sum of many responses.

8. The measurement of variability of individual responses is the main

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result of the method; its magnitude indicates that it is a significant new factor limiting temporal resolution with suprathreshold stimuli.

INTRODUCTION

Effective vision places conflicting demands upon the visual system. On the one hand there is the need for the response to reproduce faithfully the rapid fluctuations of retinal illumination which occur as the visual scene changes. On the other hand, the system must be very sensitive in order to detect small local differences of retinal illumination. The temporal pattern of quantal absorptions in visual pigment is quite irregular even though the illumination is nominally constant; therefore, reliable estimation of local differences of mean quantal flux demands that the quantal shower be averaged over some finite period of time to provide useful signals. This immediately places a limit upon the speed with which the output can follow an abrupt change in the light intensity. Accuracy and reliability are obtained at the expense of speed of response, an effect familiar to those who use photomultiplier tubes.

If the response at any instant is to depend upon the light flux incident during some finite period, then it will ordinarily be the case that the response to an extremely brief (and very bright) flash of light is spread over some finite period of time. By analogy with electrical and mechanical systems one may refer to the time course of the response to a very brief flash containing a finite amount of energy as the 'impulse response' of the visual system.

In the case of linear systems, the impulse response is an economic description of performance from which the behaviour to an arbitrary input may readily be predicted. Although non-linear behaviour of the visual system may supervene at quite modest stimulus levels (Enroth-Cugell & Robson, 1966; Easter, 1968), the performance is approximately linear over a small range of stimulus increments near threshold and here the impulse response has predictive power. The purpose of this paper is to report on the impulse response of retinal ganglion cells under various conditions and to examine its relation to threshold, temporal summation and discrimination of stimuli which differ in intensity and duration.

METHODS

Preparation. Extensive recordings were made from the cell bodies or axons of twenty-one on-centre and four off-centre retinal ganglion cells in eleven cats. Additional observations were made on other units in other cats, but not in as great detail. The weights of the cats ranged from 2·6 to 4·2 kg. After initial screening to exclude gross corneal defects or gross visual-motor behavioural defects, the animals were anaesthetized with ethyl chloride and ether followed by thiamylal sodium (20 mg/kg

given over a period of 3–5 hr) for surgical procedures. A level of light anaesthesia was maintained in the Berkeley series of cats by infusion of a mixture of urethane (18·75 mg/kg.hr) and α-chloralose (0·625 mg/kg.hr) and muscular relxation was obtained by addition of gallamine triethiodide (5 mg/kg.hr) and *d*-tubocurarine (0·5–1 mg/kg.hr) to the infusion fluid. The animals were artificially ventilated with 97 % oxygen–3 % carbon dioxide mixture through a tracheal cannula at 37 ml./stroke, 31 strokes/min. In the Canberra series a gas mixture consisting of nitrous oxide 70 %, oxygen 28·5 % and carbon dioxide 1·5 % was used with the urethane–chloralose mixture omitted. There did not appear to be any significant differences in the results. Anaesthetic level was checked periodically by temporary withdrawal of the supply of neuromuscular blocking agents. There were brisk reflexes but no organized responses to pain-producing stimuli. Body temperature was maintained at 37·5° C by an electric heating blanket controlled by the temperature.

Recording. Tungsten-in-glass electrodes were introduced into the eye via a small cannula which penetrated the coats of the globe just in front of its equator. The cat's own optical system was undisturbed by the procedure. Electrodes approached the retina from its inner (anterior) surface and suitable cell-body recordings were obtained usually after several placements. Amplification, display and recording arrangements by cathode-ray oscilloscope, loudspeaker and camera were conventional. Details of special equipment are described elsewhere (Barlow & Levick, 1969*a*). Briefly, post-stimulus time-histograms of the sums of twenty responses were accumulated in a modified multichannel scaler (Nuclear Data Inc., Schaumburg, Illinois); such histograms give the time density of impulses (ordinate) as a function of the time after a stimulus (abscissa). The data from selected runs were subsequently processed on an IBM 360/50 computer. To avoid cumulative effects of stimulation, a delay of at least 3·2 sec was used between successive stimuli. At stimulus energies well above threshold, a 12 sec delay was employed.

Stimulation. A fluorescent-light source (modified from Gerbrands & Stevens, 1964) consisting of a bank of four tubes (Sylvania type F4T5/CWX U.S.A.), each with a series ballast resistor and common filament supply, were connected in parallel as the anode load of a power-tetrode vacuum-tube. Rectangular voltage wave forms applied to the grid of the power tube produced abruptly rising and falling wave forms of light from the fluorescent tubes which were monitored by a vacuum photocell (RCA 929) with an interposed visual correction filter (Kodak Wrattan 106). The time constants of rise and fall were approximately 0·2 msec, so that a pulse of 2 msec or longer looked rectangular for all practical purposes. The tubes were mounted side by side in a chassis which was closed except for a variable aperture above the tubes. Kodak neutral density gelatin filters could be placed between the aperture and the tubes to control flash intensity. Closely applied to the aperture and extending over the rest of the top surface of the chassis were several layers of frosted acetate sheeting. Overhead incandescent sources illuminating the front of this surface provided an approximately homogeneous background field in the centre of which the flash was added from underneath.

Because it is convenient to plot receptive fields and align stimuli on a horizontal surface, an adjustable mirror was placed about 20–30 cm in front of the eye. It was centred on the line of sight of each receptive field and tilted so as to project the latter on to the centre of the stimulus field. The distance along the line of sight from eye to stimulus plane was 57 cm, so that 1 cm subtended approximately 1° at the eye. Receptive fields were mapped by the usual methods. Light intensities were measured against a standard using a Gamma Scientific Photometer Model 721B with scotopic correction filter and checked regularly with a calibrated SEI photometer. All light units are scotopic quantities. A corneal contact lens, 3 mm artificial pupil (area =

7 mm²) and appropriate spectacle lens were routinely employed. Accommodation was paralysed by atropine eye drops (1 %) and the cervical sympathetic trunk was cut on the experimental side.

Several terms will occur frequently in this paper. The *luminance*, *luminous intensity* or *intensity* of a flash is the time rate of emission of luminous energy. The flash *energy* is the total luminous energy, the integral of luminance with respect to time. In the case of a rectangular pulse, it is the product of intensity by duration.

RESULTS

Diffuse flashes applied to large areas of retina stimulate both the central zone and the antagonistic surround of the receptive field of a ganglion cell, so that complicated responses (Grüsser & Kapp, 1958; Grüsser & Rabelo,

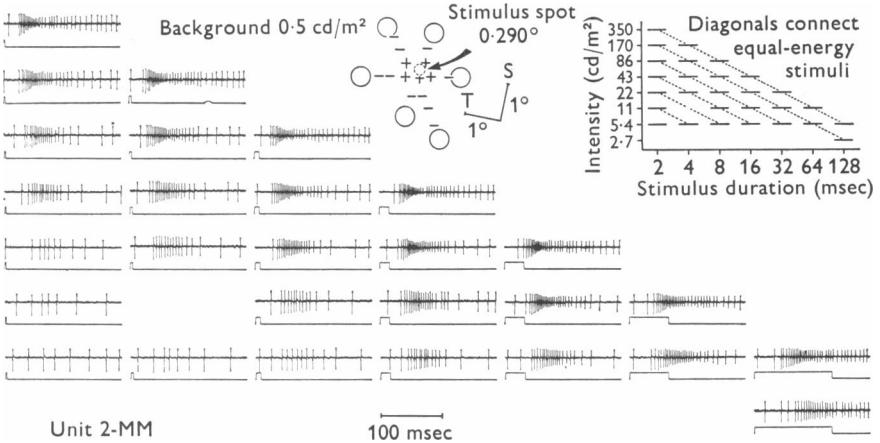

Fig. 1. Individual responses to flashes of different intensities and durations. Inset at top right explains the arrangement of the records. Each column corresponds to a particular stimulus duration, each row to a particular intensity; intensity and duration increase by factors of two proceeding upward and to the right respectively so that records along a diagonal correspond to stimuli having constant intensity-duration products. The upper trace of each record is the spike train (cell body recording, negativity upward), the lower trace is the output of a photocell monitoring the stimulus. At top, centre is a map of the receptive field; symbols: '+' means response at light on, '−' at light off, 'O' no response (also indicates size of mapping spot). Superior (S) and temporal (T) meridians in the visual field are shown together with 1° calibration marks. The position of the 0·29° stimulus spot is shown dotted within the receptive field centre.

1958) would be expected from the interaction of the two components. Since we wished to unravel the temporal behaviour of simple responses before sorting out the interactions, stimuli were kept well within receptive field centres and were carefully centred (Fig. 1) and focused. The experi-

ments to be described have been carried out on a number of cells under a variety of conditions. The over-all picture of the results is best conveyed by describing the behaviour of a typical ganglion cell. Afterwards, some of the variations under different conditions of adaptation will be summarized. In all the experiments described in this paper, the background level of illumination was always in the scotopic (rod-dominated) range (Barlow & Levick, 1968).

Effect of shortening a light flash

It is a readily verifiable fact that as the duration of a flash of light is progressively shortened our sensory experience of the flash also shortens. At ordinary background luminances (e.g. 0·5 cd/m^2) and ordinary stimulus luminances (e.g. 10 times threshold for a 1 sec flash) this is true at least for the range of duration from 4 to 0·4 sec. However, for flashes shorter than about 100 msec it is difficult to decide on the duration of the sensory impression; the striking effect is that briefer flashes look decidedly dimmer. These subjective observations are paralleled by the responses of cat retinal ganglion cells. A typical example is shown in Fig. 1. The lowest complete row contains records of single responses to flashes of light, the durations of which decreased by factors of two from 128 msec at the right to 2 msec at the left. The duration of the packet of extra spikes also decreased stepwise until the 32 msec flash was reached. Further shortening no longer significantly shortened the packet, which simply became less densely populated and ultimately was lost in the maintained discharge. For the conditions illustrated the energy for a subjectively estimated threshold response would lie between the energies of the 4 and 8 msec flashes. Thus the record of the response to the 2 msec flash is essentially a sample of the maintained discharge. A clearer picture of the time course of a response is obtained by compiling a post-stimulus time-histogram (Gerstein, 1960) of the sum of responses to repeated presentations of the same stimulus. In such a display the horizontal co-ordinates correspond to successive blocks of time after the onset of a stimulus-locked marker and the ordinates give the number of impulses that have occurred in each time block, totalized over the number of stimulus presentations. The column on the left of Fig. 2 contains a set of such histograms of the sum of twenty responses for each stimulus. The time increments of the abscissae are 2 msec in order to resolve the detail of the wave forms. The continuous line drawn through each histogram is the result of applying smoothing by a method to be discussed in a later section. It captures the shape of the responses to about the same extent as drawing a line of best fit by eye. The features of the set of smoothed histograms confirm in a more objective way the impression gained from inspection of the individual responses.

Decreasing the stimulus duration decreases the duration of the response only down to about 32 msec; further decreases serve merely to reduce the number of extra spikes evoked by the stimulus within the minimum response duration.

A convenient measure of the averaged responses is the duration at one quarter of the maximum magnitude. Response magnitude is calculated as the height of the smooth curve describing average spike frequency above a level corresponding to the average ongoing discharge before the stimulus. We chose one quarter rather than the usual one half because the response to long flashes generally sagged below the latter level before the end of the stimulus. Also, such a measure avoids problems caused by the gradual beginning and differing magnitude of responses.

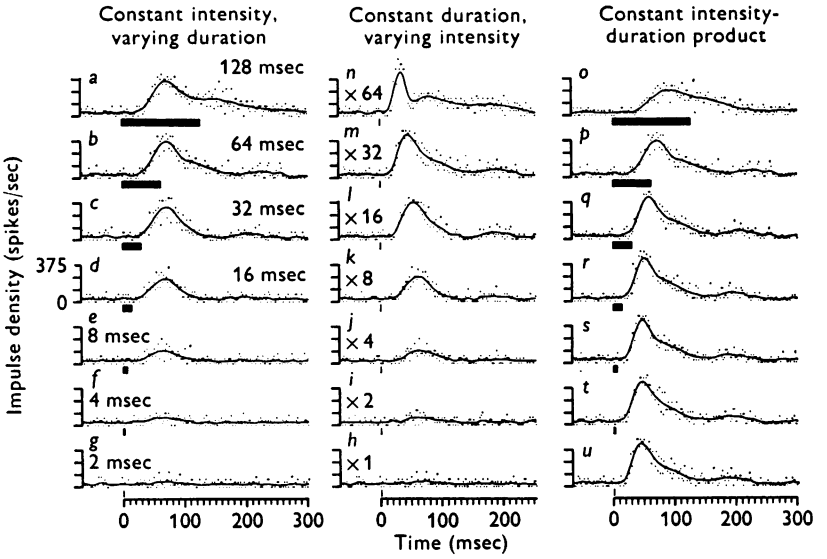

Fig. 2. Post-stimulus time-histograms of responses to flashes of different intensities and durations. The raw data of each record is the pattern of dots. Each dot plots the count of impulses occurring in 2 msec time blocks as a function of time after the stimulus, summed over twenty repetitions. The continuous curve is the result of smoothing the data by a Gaussian time weighting function (see text) of standard deviation 6 msec. Left-hand column, records *a* to *g* (corresponding to lowest complete row of Fig. 1) are responses to flashes of constant intensity (5·4 scotopic cd/m²), the indicated durations of which decrease by factors of two as one reads down; timing is given by black bars. Middle column, records *h* to *n* (corresponding to left-hand column of Fig. 1) show the effect of increasing the intensity (by the indicated factors) of a 2 msec flash; *g* and *h* are identical. In the right-hand column the energy of the flashes (0·34 scotopic cd/m² × sec) is kept constant by reciprocal variation of intensity and duration; record *u* is identical with *m*, and *p* with *b*. Same unit as in Fig. 1.

The measure of response duration is plotted against stimulus duration on a log. scale in Fig. 3*A* (crosses). The graph shows that the responses could not be shortened to less than about 70 msec by shortening the stimulus. An exceptional value of 50 msec for a 2 msec stimulus is the result of the response merging with the ongoing discharge thus obscuring the analysis. This was checked by examining responses to 2 msec flashes of progressively increasing intensity. The additional measurements of duration are plotted as filled circles in the Figure. The points cluster in the vicinity of 70–80 msec response duration over the thirty-twofold range

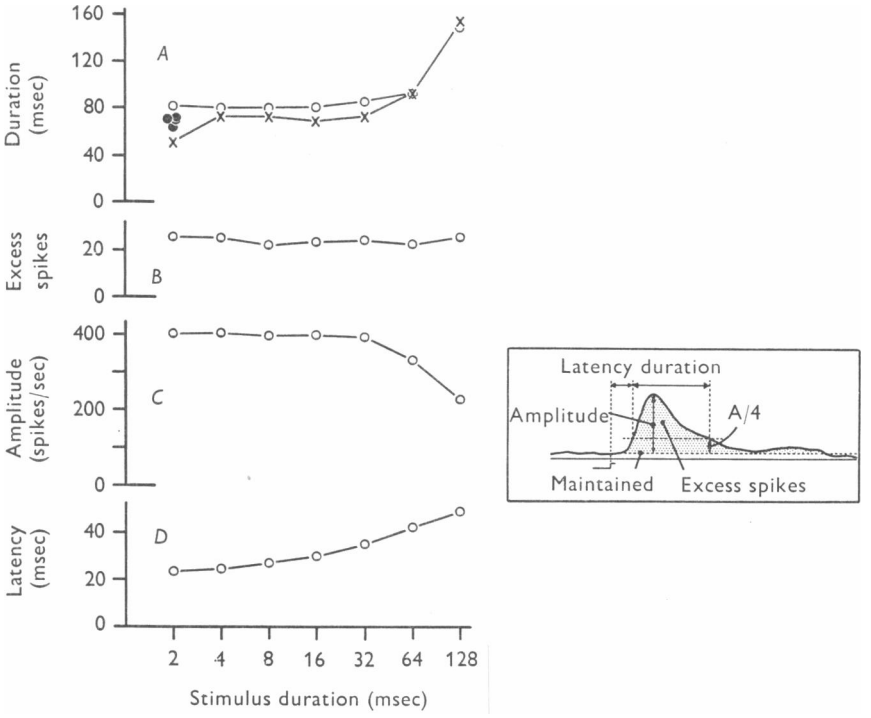

Fig. 3. Changes in features of response as functions of stimulus duration. Inset at right illustrates the method of measuring four response parameters. Latency (from leading edge of stimulus) and duration; measured to the points where the smoothed post-stimulus time-histogram rises to one quarter of response amplitude above the maintained discharge. Excess spikes (area shown dotted): the maximum value of the sum of the spikes less the extrapolated contribution of the maintained discharge. Amplitude: the height of the response above the maintained discharge. Crosses: measurements on left-hand column of records of Fig. 2 (constant intensity, decreasing durations of stimulus). Open circles: measurements on right-hand column (equal-energy flashes). Filled circles: measurements on centre column of records of Fig. 2 (constant duration, varying intensity of stimulus).

of intensities employed. The corresponding histograms are shown in the middle column of Fig. 2 and individual responses in the left-hand column of Fig. 1. The top record of the series is quite different in shape from the rest possibly on account of intervention by cones rather than rods; other experiments (Barlow & Levick, 1968) showed that the Purkinje shift occurred at comparable intensities. Stimulus light scattered on to the inhibitory surround might also be responsible. The measurement of this record was therefore omitted from Fig. 3*A*. The result is that increasing stimulus intensity increased the magnitude of the response but had little effect on its duration. The point to be made is that there is a definite minimum response duration regardless of the brevity of the stimulus.

Comparison of the histograms of the left-hand and middle columns of Fig. 2 also shows that increasing the duration of a stimulus had much the same effect as increasing its intensity by the same factor, at least up to 32 msec duration. The question naturally arises; to what extent can intensity and duration be traded to produce a constant response? Not unexpectedly, it turned out that the product of intensity and duration had to be constant provided the duration did not exceed some value. Post-stimulus time-histograms of responses to flashes having different durations but constant intensity-duration products are shown in the right-hand column of Fig. 2. Records of the corresponding individual responses lie along the diagonal of the matrix of Fig. 1 starting with the extra record in the bottom right-hand corner. For stimulus durations from 2 to 32 msec, responses are very much alike in size and shape. In psychophysical studies the reciprocity of intensity and duration to achieve a constant criterion response (equal detectability at threshold, equal subjective brightness above threshold), sometimes referred to as the Bunsen–Roscoe law (Brindley, 1960), has been taken to imply that the system is no longer capable of resolving the time course of stimuli over the range of durations. If retinal ganglion cells in humans behave as in the cat, our experiment shows that factors limiting over-all visual performance operate at or before the ganglion cell level.

At what minimum stimulus duration does the reciprocity relation supervene? It is not an easy question for a simple answer because of the difficulties in deciding that a particular response differs significantly from the set of responses to a comparison stimulus. A general approach to the problem will be developed in a later section of the paper. For present purposes, various measures of responses to the equal-energy stimuli of Fig. 2 have been plotted against stimulus duration in Fig. 3*A* to *D*. As with the constant-intensity series, response duration (open circles, Fig. 3*A*) stays relatively constant for equal-energy stimuli up to 32 msec

long. Similarly, the graph turns sharply upward beyond 64 msec of stimulus duration. Response amplitude, defined as the maximum value of the smoothed histogram above the pre-stimulus level of ongoing activity, also remains constant over the same range (Fig. 3*C*). It diminishes for stimuli longer than 32 msec in such a way that the number of extra spikes elicited remains substantially unchanged over the whole range of stimuli considered (Fig. 3*B*). The latency of responses increases progressively with

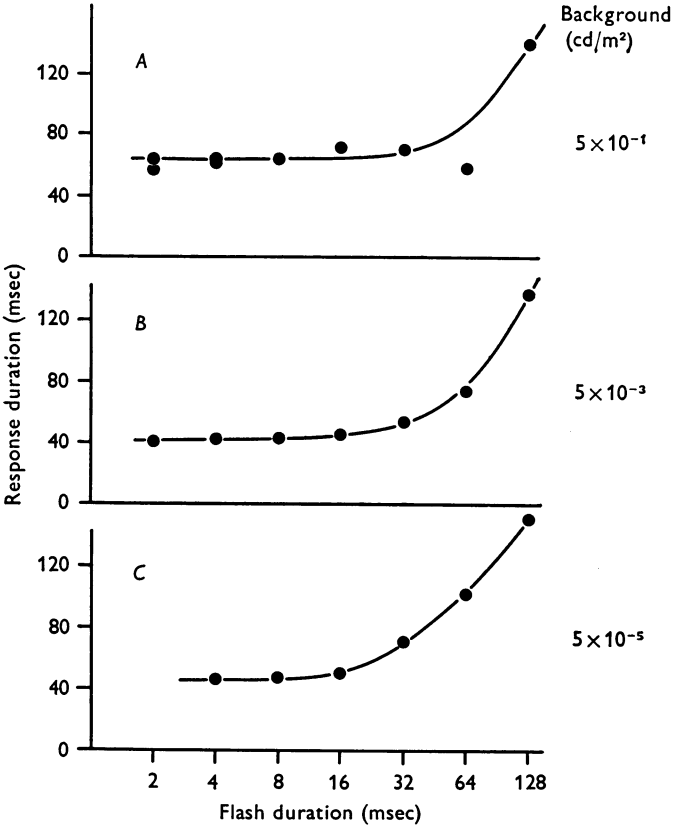

Fig. 4. Effect of adaptation level on durations of responses to equal-energy stimuli adjusted at each level to be about 4 times threshold for the 2 or 4 msec flash. Flash energies (in scotopic cd/m² × sec): $3{\cdot}4 \times 10^{-2}$ (*A*), $4{\cdot}3 \times 10^{-3}$ (*B*), $6{\cdot}8 \times 10^{-4}$ (*C*). Pupil area: 7 mm².

stimulus duration. This has important consequences for psychophysical measurements of the discriminability of equal-energy flashes. If the test and comparison flashes both begin at the same instant, then discrimination could merely be based on apparent time-order rather than on dissimilarity of time course.

The results so far may be summarized as follows. There is a definite minimum duration of response however brief the stimulus is made. For constant responses, intensity and duration are interchangeable stimulus parameters up to about 32 msec duration for this unit under scotopic conditions of adaptation.

Effect of adaptation level within the scotopic range

The durations of responses to sets of equal-energy flashes at different levels of background illumination are plotted in Fig. 4. The backgrounds were chosen to cover the range of pure scotopic behaviour (Barlow & Levick, 1968). In order to make useful comparisons the energy levels were adjusted to be approximately 4 times threshold for the 2 or 4 msec flash at each background. Contrary to expectations, the range of stimulus durations yielding minimum response durations did not increase at lower backgrounds; if anything, the trend was in the other direction. In other words, by analysing response duration one could infer the duration of a stimulus to much the same lower limit regardless of background level. The stimuli at the lowest background level delivered only about one fiftieth of the quanta at the highest level. Not only was the stimulus duration yielding minimum response durations not altered by adaptation level, but also the minimum response duration itself was not particularly altered by adaptation level (Fig. 5*C*).

Measurements on the responses of a number of units (Fig. 5*B*) show that there is considerable variability but no obvious trend with adaptation level within the scotopic range. This is in sharp contrast with measurements of latency (Fig. 5*A*) which are less scattered and show obvious lengthening with reduction of background. The slope of the line joining the means of measurements at 5×10^{-1} and 5×10^{-3} cd/m^2 is 9·5 msec decrement per log. unit of background luminance. The effect of background luminance is to move the response bodily along the time axis with little effect on shape (Fig. 5*C*).

Duration-threshold measurements

The setting of the intensity of a light flash to produce a statistically significant change in the ongoing discharge is a task which can be carried out simply and fairly reliably by listening to the ganglion cell discharge on the audio monitor (Barlow & Levick, 1969*a*). By carrying out the experiment for flashes of different durations, one obtains the relation between the duration and intensity required to produce a particular effect, namely a significant alteration in the ongoing discharge on 50% of the trials. Analogous threshold measurements in human psychophysical experiments (e.g. Graham & Margaria, 1935; Barlow, 1958; Baumgardt &

Hillmann, 1961) have been used to demonstrate the reciprocity of intensity and duration for the detection of threshold stimuli and to determine the duration up to which the reciprocity relation is valid.

Our results on a retinal ganglion cell are plotted in Fig. 6 and show remarkable similarity to the human data. Threshold responses required a

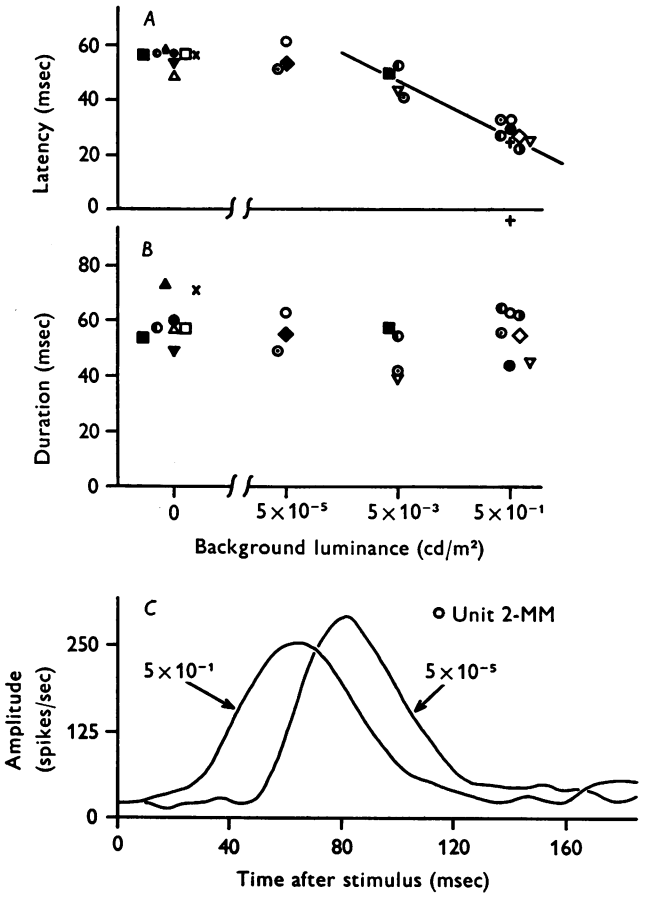

Fig. 5*A*, *B*. Latency and duration of responses of sixteen units at different backgrounds for 2 msec flashes adjusted to about 4 times threshold in every case. Each symbol refers to a particular unit. Measurements were made on post-stimulus time-histograms smoothed by a Gaussian function (S.D. = 6 msec). In *A*, the line drawn through the means of the latencies at the two higher backgrounds has a slope of 9·5 msec decrement per tenfold increase of background. Where necessary to avoid clutter, the points at each background have been shifted horizontally. *C*: superimposed tracings of smoothed post-stimulus histograms of one unit at backgrounds 4 log. units apart. Obvious changes in latency are not paralleled by changes in response duration.

constant product of intensity and duration up to a duration of about 64 msec as shown by the agreement of the points with lines having a slope of -1 on this log.–log. plot. The relationship held for the three background levels used which corresponded to most of the range of pure scotopic performance. Beyond 64 msec, threshold intensity continued to fall with

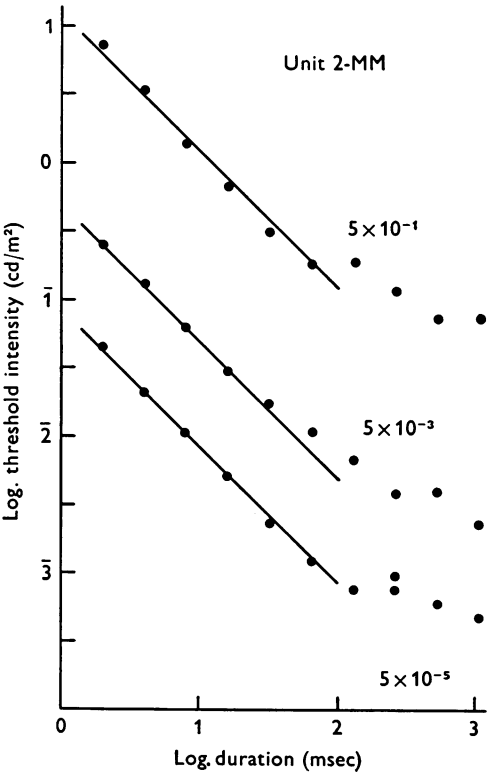

Fig. 6. Threshold intensity as a function of duration of the flash at different backgrounds (indicated near each set of points in scotopic cd/m²). The comparison lines at the left have slopes of -1 and therefore correspond with constant products of intensity and duration (Bunsen–Roscoe law) on this log.–log. plot.

increasing stimulus duration such that it was now inversely proportional to a fractional power of duration. It is difficult to determine the power precisely, but an inverse square root relation is not excluded.

Although each plotted point corresponds to a just-detectable response it would not be correct to assume that such responses were indistinguishable. In fact, threshold responses to flashes lasting 64 msec or more sounded distinctly longer on the audiomonitor than those to briefer flashes.

On the other hand, no reliable distinctions could be made between threshold responses to flashes of 32 msec or less in duration.

Two-flash experiments

Temporal resolution may also be studied by examining the responses to pairs of brief stimuli of identical intensity and duration as the time interval

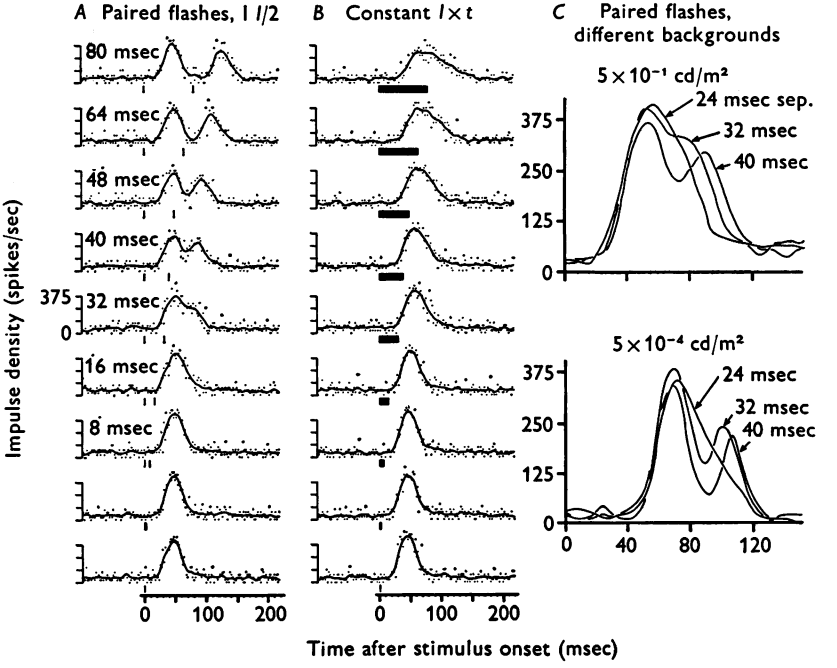

Fig. 7*A*. Responses to pairs of 2 msec flashes, the leading edges of which are spaced apart by the time intervals indicated on each post-stimulus time-histogram. In the cases of the lowest two runs, 2 msec spacing corresponded with a single continuous flash lasting 4 msec, and zero spacing corresponded with a single 2 msec flash of twice the intensity. *B*: comparison set of responses to flashes of constant intensity–duration product having the same energy as in *A*. Stimulus timing is indicated by black bars or vertical strokes. Background for *A* and *B* is 0·5 scotopic cd/m². Energy delivered by each form of stimulation = $8{\cdot}5 \times 10^{-2}$ scotopic cd/m² × sec, which was 4 × threshold. The bottom records are responses to identical stimuli, thus providing a control on the order of variability in replication of the experiment. *C*: superimposed tracings of smoothed post-stimulus time-histograms of responses of same unit as in *A*, *B* to paired flashes at two backgrounds. Traces are marked with the separations of their evoking flash-pairs. Temporal resolution appears to be improved at the lower background. All histograms of this Figure have been smoothed with a Gaussian function of 4 msec S.D. Energy delivered in upper traces is the same as in *A*, *B*; in lower traces, it is $5{\cdot}4 \times 10^{-3}$ scotopic cd/m² × sec.

between the flashes is progressively reduced. This is the analogue in the time domain of one of the methods of specifying the spatial resolving power of telescopes and other optical instruments.

The results of a typical experiment are shown in Fig. 7*A*. In the left-hand column is a series of post-stimulus time-histograms of responses to a pair of identical 2 msec flashes spaced apart by different intervals. Separation is defined as the interval between the leading edges of the pulses. At the top, flashes 80 msec apart produced responses separate from each other. They began to overlap for inter-flash intervals of 48 msec or shorter. The response became single-peaked for flash spacing of 32 msec or shorter. Limiting temporal resolution can be defined in various ways: for instance, the minimum flash spacing for which the response is significantly lengthened (32 msec in Fig. 7*A*); the minimum spacing for which the response is two-peaked (40 msec); or the minimum spacing for which the gap between two response peaks reaches as deep as one quarter of the maximum amplitude (64 msec). Resolution is more sharply defined in this experiment than by examination of responses to single flashes having constant intensity–duration products (Fig. 7*B*). In the latter series, response shape changes more gradually with stimulus shape.

All of the above limits for temporal resolution could be predicted from the form of the impulse response if the system were operating linearly. Unfortunately the records of Fig. 7*A* show that this is not the case. In the top record of the left-hand column the response to the second flash is obviously not the same as the response to the first, even though they are clearly separated. Also, comparison of the top and bottom records of the left column shows that the response to the first flash at the top is far from being half the size of the response at the bottom, even though the top flash was half as intense. The bottom stimuli were about 4 times threshold. Even at this relatively low level of incremental stimulus, non-linear effects are obvious.

Similar two-flash experiments were done at several adaptation levels including complete darkness. In confirmation of the earlier results, temporal resolution was not degraded at low backgrounds. Indeed, as shown in Fig. 7*C* the minimum spacing to produce a two-peaked response decreased from 40 msec at 5×10^{-1} cd/m^2 to 32 msec at 5×10^{-4} cd/m^2.

The basis for discrimination of responses

Up to this stage ganglion cell responses have been described in terms of twenty-sweep post-stimulus time-histograms. There is a degree of arbitrariness about implying differences of responses from differences in the histograms. One must take account of the random variations of individual responses to identical stimuli.

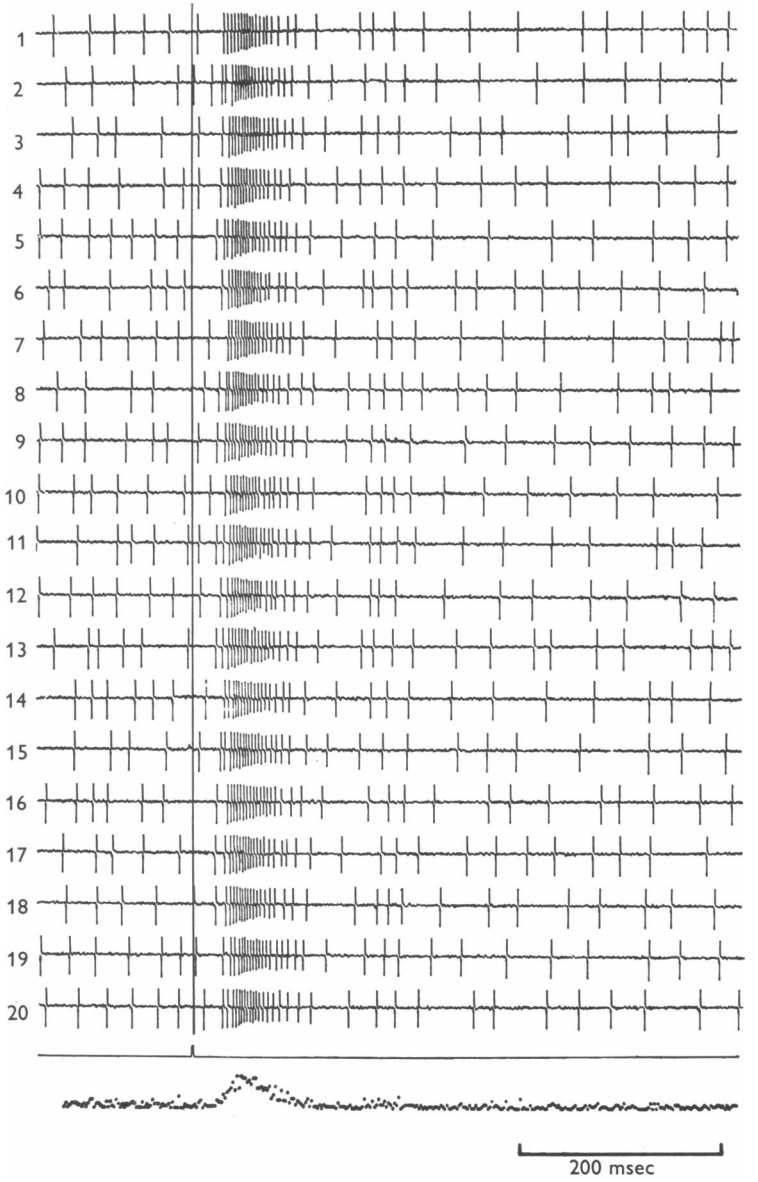

Fig. 8. Variations of twenty individual spike responses (base lines retouched) (numbered 1 to 20) to identical 2 msec stimuli at about 8 times threshold intensity. The photocell record of the light flash is shown below number 20. The vertical line is the time reference of the stimulus. Accumulated post-stimulus time-histogram of the twenty responses shown at bottom. Background 0·5 cd/m².

For example, a minimum response duration has been inferred from the shape of the histogram. Now, it might be the case that individual responses to brief flashes are in fact brief, but the latency of their appearances varies considerably. The width of the summed response would thus reflect the jitter of the individual responses and not their true width. However, observation of individual responses on the oscilloscope and audio monitor showed that their form was well reproduced by the histogram. Photographs of twenty individual responses to a 2 msec flash are lined up with the resulting histogram in Fig. 8. The responses are all different but fluctuation of their apparent latencies is much less than the duration of any of them.

Again, averaging (or summing) increases the signal-to-noise ratio; measurements of discrimination thresholds which are noise-limited will therefore depend upon the number of presentations. It may well be that the whole animal has access to many samples of the effects produced by a single presentation since the stimulus would be located within as many ganglion-cell receptive fields. Difficulties arise in attempting to ascertain the effective number especially when one questions how mutually independent their responses might be. In any case, the fundamental anchor point is the information available from a single response of a single neurone.

Analysis of single responses. When one subjectively appraises the spike-train response to a flash of light by listening to the audio monitor or by looking at a photographic record such as Fig. 8 (1), it is not easy to specify what operations underlie a judgement such as: this test response is longer in duration than a previously elicited standard response. The difficulty lies principally in the presence of an ongoing discharge upon which the response is superimposed. It is impossible to determine unambiguously which is the first or the last spike of the response. One might proceed by measuring the intervals between successive spikes and say that the response begins when the interval is less than a particular value, but such a treatment would strongly depend upon the character of the ongoing discharge and would generally give a nonsensical result if the ongoing discharge were of Poisson type, since the shortest intervals occur most frequently in this case. The output of retinal ganglion cells can be of this type, especially at low luminance levels (Barlow & Levick, 1969*b*).

Intuitively, it seems that measures which reflect the time-density of spikes reproduce more closely one's subjective appraisal of responses. One records the number of impulses occurring in a fixed interval of time, as this interval is moved along the train of impulses. In its simplest form, this sliding average gives a rather jagged output as a function of time since it abruptly steps up or down one unit as each spike enters or leaves the sampling window. A much smoother output is obtained by making a

weighted sum: the contribution of each spike to the output is made to diminish smoothly with its distance from the centre of the window. Application of this method transforms the spike record into a smooth curve whose height above the time axis reflects the local time-density of spikes of the original data. To perform this analysis we recorded each response on a single sweep of the multichannel scaler, thus quantizing spike timing to a precision of $\pm$ 1 msec because of the usual 2 msec channel width. As a starting point we have employed a Gaussian distribution of weights for its convenience as a familiar smooth function whose spread is defined by a single parameter. The weighting operation is not biologically implausible. It may be not unlike that applied by the next neurone on the path if one considers distributed conduction times in fine terminal ramifications, multistage chemical transmission and electrotonic spread along fine dendritic trees. An additional advantage is that the operation can later be approximated on-line with simple electrical networks, albeit with some delay in real time.

In the faithful reproduction of the time-density variations there is an optimum width of the weighting function: if it is too wide, the processed wave form looks much broader and flatter than the original packet of spikes; if it is too narrow the waveform generally reproduces the density of the packet but is excessively jagged due to irregularity of the spike packing. Figure 9*A* shows the effect of smoothing an individual response by Gaussian weighting functions having various S.D.s. The same operations applied to the post-stimulus histogram of the sum of twenty such single responses from the same unit under the same conditions is also shown for comparison (Fig. 9*B*). There seems to be no simple objective test for deciding on the best smoothing functions. Fitting based on the principle of least squares is inappropriate and optimizing the signal-to-noise ratio sacrifices much of the shape of the signal. On the basis of inspection, a S.D. of 4 or 6 msec (2 or 3 channels of the histogram) would be suitable for most neurones under most conditions.

Variability of response duration. Using the above method, one may analyse individual responses in exactly the same way as the summed histograms (cf. Fig. 3) and thus obtain an important piece of information which is lost in accumulating a 20-sweep histogram, namely the variability of individual responses. The duration of each response is measured and the values used to compile a histogram of the relative frequency of response durations (Fig. 10*A*). If it is accepted that the sample is drawn from a normally distributed population we could say that durations removed by more than 2·88 S.D.s from the mean would occur on average once in 500 presentations of a 2 msec flash. This duration can be used as a criterion for judging whether or not a given single response belongs to the set

evoked by 2 msec flashes. For any preassigned fallibility rate, there corresponds a definite criterion duration defined by the mean and standard deviation of the distribution of response durations.

We are now in a position to give a sharper answer to the question posed earlier in the paper, 'At what minimum stimulus duration does the reciprocity relation supervene?' Turning again to Fig. 10 we transfer the criterion duration derived from the variability of individual responses on to

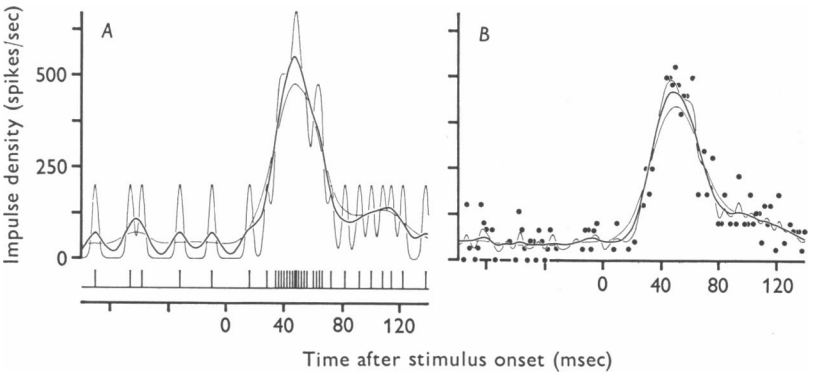

Fig. 9*A*. Transformation of the spike train of a single response into a smooth graph of time-density of impulses versus time. Diagram of the original spike train after quantization into 2 msec time bins is shown below. Smoothed representations are shown above. Heavy line is result of smoothing by a Gaussian function of 6 msec S.D., fine lines for 2 msec and 10 msec S.D. respectively. *B*: smoothing by same three functions of the post-stimulus time-histogram (filled circles) of the sum of twenty individual responses including the one in *A*. Increasing the smoothing S.D. evens out sampling irregularities but tends to reduce and broaden the representation of the response in both *A* and *B*.

the graph relating average response duration to stimulus duration for flashes of equal energy. If we define the threshold for successful discrimination to correspond with 50% correct responses, then this will occur at a stimulus duration of about 63 msec. The duration of responses to such flashes will be symmetrically distributed about a mean of 85 msec (again invoking a normal population) which is the criterion duration set by the scatter of response durations to 2 msec flashes and a false positive rate of 1 to 500.

The principles used in this section are precisely those developed by Barlow & Levick (1969*a*) in the objective determination of the neurophysiological threshold for detecting a flash. Only the domain of application (response duration) differs. Our Fig. 10 is the companion of their Fig. 5. The same types of controls were applied. For instance, the duration

of average response was used, although the average of the durations of individual responses is required. There is considerably more labour in the latter measurement, so we have been satisfied to use the former after empirically establishing the close approximation of the two by direct measurement in different units under various conditions. The use of many samples would improve our accuracy in estimating the criterion duration

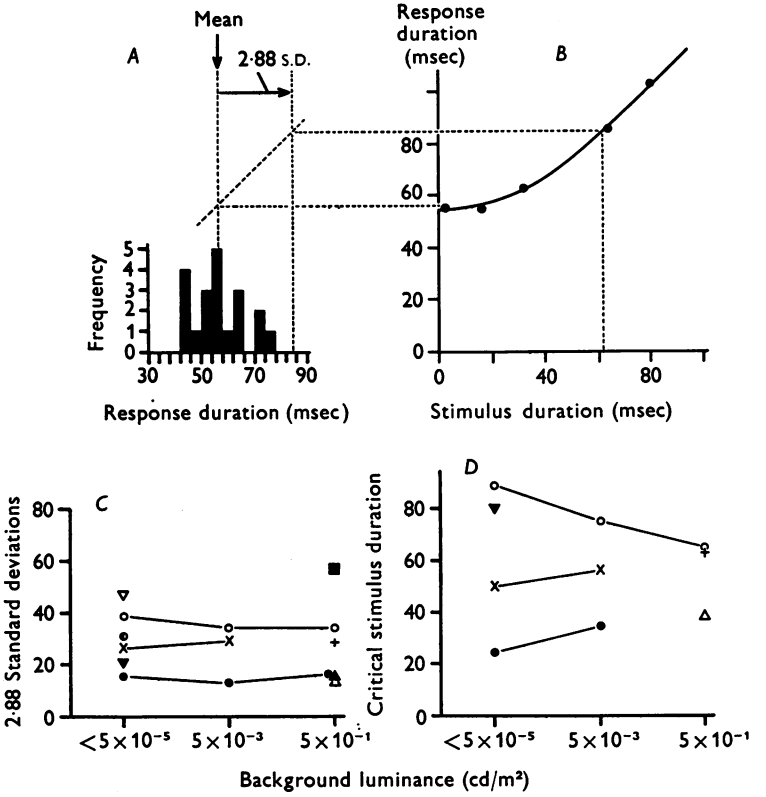

Fig. 10. Objective determination of 'critical duration'. *A*: frequency histogram of the durations of twenty smoothed individual responses which yields a measure of response variability (2·88 S.D.s of the distribution) and a criterion duration (mean + 2·88 S.D.) corresponding to an assigned proportion of false positive judgements. *B*: relation between average response duration and stimulus duration which enables the criterion response duration for discrimination to be stated in terms of stimulus duration. A stimulus of this duration would be at the threshold for discrimination from a 2 msec flash of the same energy. *C*: measurements of variability of response duration (2·88 S.D.) on 10 units at different backgrounds. *D*: measurements of 'critical duration' at different backgrounds. In *C* and *D*, symbols refer to particular units and the lines join measurements on the same unit at different backgrounds.

and the response-duration/stimulus-duration relation but would not spuriously lower the estimate of discrimination threshold.

Because of its magnitude, variability of minimum response duration turns out to be an important determinant of the threshold for discrimination. The measure of variability (2·88 × S.D. of the distribution) for 11 units at three adaptation levels is plotted in Fig. 10*C*. Lines connect measurements on the same unit. There is considerable scatter between units but no significant trend with adaptation level. The stimulus level for all measurements was 4 times threshold. Applying the method of Fig. 10*A*, *B* in those units for which complete data were available, we obtain the stimulus durations corresponding to threshold discrimination. These are plotted in Fig. 10*D*. The range of scatter is uncomfortably large. It is partly attributable to the small size of the sample for estimating the variability of response duration. Another factor is that some of the responses have long, gently sloping tails which challenge simple definitions of duration. We have tried other measures of single responses (peak firing rate, excess spikes), but found them less efficient and the results rather more scattered.

Despite the range of the results, the analysis is valuable for supplying an objective basis for asserting that duration begins to be a significant independent factor in the discrimination of equal-energy flashes at about 50 msec at scotopic adaptation levels.

DISCUSSION

In a remarkable paper, Hartline (1934) provided the neurophysiological underpinning for discussions of the intensity-duration relation as applied to the visual system. Studying responses in nerve fibres connected to single receptors in the eye of *Limulus polyphemus*, he showed that responses to flashes containing equal energy (intensity × duration) were very much alike provided flash durations did not exceed 100 msec.

It has always been recognized that minor modifications would be required in carrying over the results of this work on the marine arthropod to aid the interpretation of human visual function. In the light of our results various comparisons can be made. There are obvious quantitative differences in time scale: the latency and duration of responses in *Limulus* are about 4–10 times longer, as would be expected. However, there are important qualitative differences as well.

First, there was no ongoing discharge in the absence of a stimulus in the *Limulus* nerve fibre. There is no doubt that such activity is a normal feature of retinal ganglion cell performance in darkness and at other background levels (Kuffler, FitzHugh & Barlow, 1957; Bornschein, 1958);

its existence makes a substantial difference to the methods of analysing responses: the threshold criterion for detection is no longer a single impulse and it is no longer possible to determine the onset and cessation of responses unambiguously. Measurement now must depend upon averaging techniques which emphasize the signal component relative to the noise of the ongoing discharge or upon operations with single responses which take advantage of clustering of the impulses making up the signal. Secondly, it is not obvious from Hartline's results that one could establish the concept of a minimum response duration. Compare the Fig. 1 of his paper (Hartline, 1934) with our Fig. 1: reduction of flash intensity led to substantial shortening of response duration at all flash durations in the case of *Limulus*, down to the stage where the response is represented by only a single impulse. It is then important to know what is the fluctuation in latency, because this factor now substitutes for response duration. Even at a background of complete darkness and with stimuli weak enough to eject on the average only one extra spike over the ongoing discharge per flash, the histogram of the sum of many responses shows that the signal from a cat retinal ganglion cell is spread over 50–80 msec. A parallel result was observed in the massed discharge of the eel's optic nerve by Adrian & Matthews (1928).

The point of making these comparisons is that there may be subtle aspects of single receptor performance which do not attract attention until the outputs of a great many of them are pooled on to a ganglion cell. For instance, if the dark discharge of the latter were attributable to impulsive events in a pool of say 30,000 receptors, then the mean rate of such events in a single receptor would be less than 1 per 1000 sec and easily overlooked.

The existence of a minimum response duration has important consequences for threshold measurements. Barlow & Levick (1969*a*) have shown that the detection threshold is set by the scatter of the number of impulses contributed by the ongoing discharge in an interval corresponding to the duration of the response. While stimuli remain in the range where response duration is minimum, threshold is reached by a constant number of extra spikes; as Fig. 3*B* suggests, this requires a constant intensity-duration product for the stimulus, so that threshold intensity is inversely proportional to duration (Fig. 6). However, stimuli which produce responses longer than the minimum duration face a requirement for more extra spikes because of the increased scatter in the number contributed from the ongoing discharge over the longer comparison period. The scatter is measured by the standard deviation of the number distribution and was found to be proportional to the square root of the duration of the period. Thus the intensity–duration product of the flash must be correspondingly

increased for threshold. If the number of extra spikes were proportional to the flash energy, then threshold intensity would be inversely proportional to the square root of duration.

Critical duration

The concept of critical duration has figured prominently in discussions of visual performance. It is very convenient to have a single parameter which summarizes the temporal integrating capacity of the visual system or part of it under various conditions. However, it is an elusive measure to define satisfactorily. Graham & Kemp (1938) referred to it as 'the longest duration of stimulus which has an influence in determining a given aspect of the response' and treated it as the upper limit of the range of applicability of the Bunsen–Roscoe law. Others speak of the range of 'complete temporal summation' (Brindley, 1960; Barlow, 1958; Baumgardt & Hillmann, 1961, with reservations). The 'kritische Zeit' (critical time) of Grüsser & Kapp (1958) is not the same as the conventional critical duration discussed by most others. These authors found that the primary burst of impulses of the response to the second of a pair of brief flashes would *vanish* when flash spacing was equal to the critical time. Very bright flashes were employed, illuminating both centre and surround of the receptive field and adaptation level was not controlled, so the experiments are not really comparable with the bulk of the human psychophysical data. It is also questionable whether superposition (Überlagerung) may be validly used in the interpretation of such results, because of the manifest non-linear performance illustrated.

The common basis for measuring critical duration is to utilize threshold measurements; a threshold sensation is thus the 'constant effect' to which the Bunsen–Roscoe law refers. But are all these threshold sensations indistinguishable? The answer from our analysis of the neural responses seems to be yes, at least over the range for which the Bunsen–Roscoe law holds (Fig. 6). With single, weak, threshold responses one cannot do more than count the total excess spikes: the response is effectively confined to a single dimension.

However, for suprathreshold responses there are enough spikes to permit significant measurements of additional, independent response features such as duration and amplitude. It is therefore not surprising that the critical duration would be found to be shorter at higher energy levels (J. L. Zacks, personal observation) since indistinguishability is now required in more than one dimension of the response. Our analysis of the distinguishability of individual responses to equal-energy flashes is a first step in this direction. The general problem that is introduced is the relationship of critical duration to temporal resolution.

We propose that the definition of critical duration be refined as follows: it is the time interval within which arbitrary manipulation of the wave form of stimuli leads to responses indistinguishable from the response to a very brief flash of the same energy. The interesting point of this treatment is that it recognizes a neglected aspect of temporal resolution: the variability of individual responses to constant stimuli. In this connexion it is worth recalling that the degradation of high frequency signals by low-pass networks can be reversed by compensating operations such as differentiation; a limit to such restoration is set by the signal-to-noise ratio of the input.

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REFERENCES

ADRIAN, E. D. & MATTHEWS, RACHEL (1928). The action of light on the eye. Part II. The processes involved in retinal excitation. *J. Physiol.* **64**, 279–301.

BARLOW, H. B. (1958). Temporal and spatial summation in human vision at different background intensities. *J. Physiol.* **141**, 337–350.

BARLOW, H. B. & LEVICK, W. R. (1968). The Purkinje shift in the cat retina. *J. Physiol.* **196**, 2–3*P*.

BARLOW, H. B. & LEVICK, W. R. (1969*a*). Three factors limiting the reliable detection of light by retinal ganglion cells of the cat. *J. Physiol.* **200**, 1–24.

BARLOW, H. B. & LEVICK, W. R. (1969*b*). Changes in the maintained discharge with adaptational level in the cat retina. *J. Physiol.* **202**, 699–718.

BAUMGARDT, E. & HILLMANN, B. (1961). Duration and size as determinants of peripheral retinal response. *J. opt. Soc. Am.* **51**, 340–344.

BORNSCHEIN, H. (1958). Spontan- und Belichtungsaktivität in Einzelfasern des N. opticus der Katze. 1. Der Einfluss kurzdauernder retinaler Ischämie. *Z. Biol.* **110**, 210–222.

BRINDLEY, G. S. (1960). In *Physiology of the Retina and Visual Pathway*, p. 177. London: Ed. Arnold.

EASTER, S. S. (1968). Excitation in the goldfish retina: evidence for a non-linear intensity code. *J. Physiol.* **195**, 253–271.

ENROTH-CUGELL, CHRISTINA & ROBSON, J. C. (1966). The contrast sensitivity of retinal ganglion cells of the cat. *J. Physiol.* **187**, 517–552.

GERBRANDS, R. & STEVENS, J. C. (1964). A high-intensity flash-source. *Am. J. Psychol.* **77**, 643–646.

GERSTEIN, G. L. (1960). Analysis of firing patterns in single neurons. *Science, N.Y.* **131**, 1811–1812.

GRAHAM, C. H. & KEMP, E. H. (1938). Brightness discrimination as a function of the duration of the increment in intensity. *J. gen. Physiol.* **21**, 635–650.

GRAHAM, C. H. & MARGARIA, R. (1935). Area and the intensity-time relation in the peripheral retina. *Am. J. Physiol.* **113**, 299–305.

GRÜSSER, O.-J. & KAPP, H. (1958). Reaktionen retinaler Neurone nach Lichtblitzen. II. Doppelblitze mit wechselndem Blitzintervall. *Pflügers Arch. ges. Physiol.* **266**, 111–129.

GRÜSSER, O.-J. & RABELO, CARMEN (1958). Reaktionen retinaler Neurone nach Lichtblitzen. I. Einzelblitze und Blitzreize wechselnder Frequenz. *Pflügers Arch. ges. Physiol.* **265**, 501–525.

HARTLINE, H. K. (1934). Intensity and duration in the excitation of single photoreceptor units. *J. cell. comp. Physiol.* **5**, 229–247.

KUFFLER, S. W., FITZHUGH, R. & BARLOW, H. B. (1957). Maintained activity in the cat's retina in light and darkness. *J. gen. Physiol.* **40**, 683–702.