Comparison of Stimulus Energies Required to Elicit the ERG in Response to X-Rays and to Light

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ABSTRACT The retina of *Rana pipiens*, the leopard frog or grass frog, is shown to be an extremely sensitive detector of x-rays. Its sensitivity to x-rays equals in some respects its sensitivity to visible light. The energy required for the response to visible light is so low that the reaction has long been known as one of the most sensitive in biological systems. An exact comparison is made of the amount of energy required in the stimulus to elicit an electroretinogram (ERG) in response to x-rays and in response to light. ERG's from threshold responses to maximal responses obtainable with x-rays and with light are reproduced. The rods of the retina are shown to be responsible for the production of the ERG. The actual amount of energy absorbed in the rhodopsin from x-ray and from light stimulation over a wide range of intensities and durations has been determined and has been related to the amplitude of the ERG. To the question whether light or x-rays are more efficient in eliciting an ERG, no simple or unequivocal answer can be given. The three dimensional relationship of amplitude of response, intensity of stimulus, and duration of stimulus shows rather unexpectedly that in certain regions light is more efficient while in other regions x-rays are more efficient.

INTRODUCTION

In a report from this laboratory (1) the techniques for producing an electroretinogram (ERG) in response to x-rays and in response to light, as well as a preliminary comparison between the x-ray response and the light response, were presented. In response to inquiries seeking information concerning the relative efficiency of the retinal receptors for x-rays and for light, the present paper compares the energy required to produce an ERG in response to x-rays with that required to produce an ERG in response to light. This comparison is based on the relative efficiency of x-rays and of light in producing the on response of the ERG, which response can be elicited by flashes of light

or of x-rays of sufficiently short duration that no damage to the photoreceptors attributable to the exposure to x-radiation could be detected.

MATERIALS AND METHODS

Responses were obtained from the retina of *Rana pipiens*, the leopard frog or grass frog. Records were made from intact living animals, freshly collected from the field to insure that the animals were in excellent physical condition. The animal was restrained in such a way that either the light beam or the x-ray beam could be focused on the eye without the necessity of moving or disturbing the animal. Flashes of light were produced by remote control of an Alphax heavy duty synchromatic shutter (Wollensak) and flashes of x-rays were produced by means of a remotely controlled focal plane lead shutter. Two photoelectric cells, one sensitive to light and one sensitive to x-rays, were placed in the path of the light beam and in the path of the x-ray beam to monitor the duration of the stimulus.

The response of the animal was displayed on one beam of a Tektronix 502 dual beam oscilloscope, and the signal from the photoelectric cell was displayed on the other beam. Photographic records were made of these traces. Records were also made with a Grass model III-D electroencephalograph, used with the EKG setting, which gave a time constant of 0.37 second, and with a direct-coupled Grass model 5 polygraph. A comparison of these records showed that the *b* wave of the ERG, on which all measurements were made in this study, was not distorted. The model III-D electroencephalograph was generally preferred because of its greater stability and because of the difficulty of contending with large DC potentials produced by attempts of the animal to move, potentials which often exceeded the magnitude of the ERG potential by factors of several hundred. The records shown in this paper are from this instrument.

A wide range of x-ray intensities was produced by a Picker Vanguard deep therapy x-ray generator operated at various voltages and currents up to 280 kv and 20 ma, with different degrees of filtration. The target-object distance remained constant at 12 cm. The x-ray beam was collimated so as to expose only the eye of the animal. Flashes of x-rays were produced by inserting a remotely controlled, focal plane lead shutter in the collimated beam. The dose was measured at the exact position of the retina with the components of an isolated eye serving as filtration. The intensity of the x-ray beam used for stimulation was determined by the use of two Victoreen condenser *r*-meters (model 70 with model 132 chamber and model 570 with model 652 chamber). These chambers were chosen because their sensitive volumes were of the same diameter as the eyes used in the experiments. This permitted an accurate dosage measurement of the collimated x-ray beam used in irradiating the eye. In order to determine the dose delivered to the retina itself, it was necessary to take into account the filtration of the beam by the ocular material overlying the retina. The Victoreen dosimeter was positioned under the x-ray tube at a distance corresponding to the distance from the tube to the retina of the eye in the experimental setup. An eye of the frog was then inserted into the collimator, with the sensitive portion of the dosimeter in precisely the same position as that occupied by the retina and with the

same filtration of the x-ray beam by cornea, lens, and ocular fluids as that of the actual experiments. The doses read on the meter were thus exactly the doses received by the retina after the beam had been filtered. The dose rate was determined for each of the intensities used in the experiments. The differences in the values read from the two meters fell well within the limits of accuracy claimed for the instruments by the manufacturer.

Conventional methods for light stimulation were followed. A General Electric No. 1493 bulb served as the source of light. The intensity of the light source was calibrated by referring the source to a standard lamp obtained from the National Bureau of Standards. An inside frosted, General Electric T-20 100 watt bulb was used as the standard. The National Bureau of Standards has shown that, with this lamp, the inverse square law holds at the intensities used in the present experiments without introducing errors of more than 1 per cent.

The standard lamp and the lamp sources used in the experiments were both referred to the same photocell in the following manner. An International Rectifier Corporation model B2M photocell was placed in the position normally occupied by the eye during an experiment. The light was focused on the cell in the same manner as was done on the eye. The output of the photocell was recorded on a sensitive voltmeter as the intensity of the light falling on the cell was changed from the lowest intensity to the highest intensity utilized in the experiments. This same photocell was then placed at definite distances from the standard lamp and the output of the cell again recorded on the voltmeter. The inverse square law was then used to determine the illumination at each position. The output of the photocell in millivolts was plotted as a function of the meter-candles of illumination by the standard lamp. The absolute values in meter-candles for the various stimuli used in the experiments were then determined from the output of the photocell when exposed to the stimulus. This procedure was followed to determine the intensity of light falling upon the surface of the eye. It was next necessary to measure the intensity of light falling on the retina itself.

In order to determine the intensity of light at the retinal surface, an isolated eye was placed in the path of various light beams corresponding to the various intensities used, and readings were made with a calibrated photocell. The choroid coat with pigment layer was removed from the retina. The eye was placed in such a position that light passed through the cornea, aqueous humor, lens, vitreous humor, and retina, and fell on the photoelectric cell. These values formed the basis for computations given in the next section. It was necessary to take readings immediately from the photoelectric cell as soon as the light was turned on, since the contraction of the iris reduced the transmission of light in a matter of seconds. Sufficient time was allowed between each reading to enable the iris to return to a dark-adapted condition.

RESULTS AND DISCUSSION

Energy Required to Generate the X-Ray ERG

It is possible to compute the amount of energy absorbed in a given biological entity during exposure to a definite dose of x-rays. From the dose rate of

x-rays used as a stimulus, the duration of the stimulus, the amount of energy absorbed per roentgen in a gram of tissue (a non-specific absorption identical for all soft tissue), and the amount of rhodopsin in a retina, it is possible to

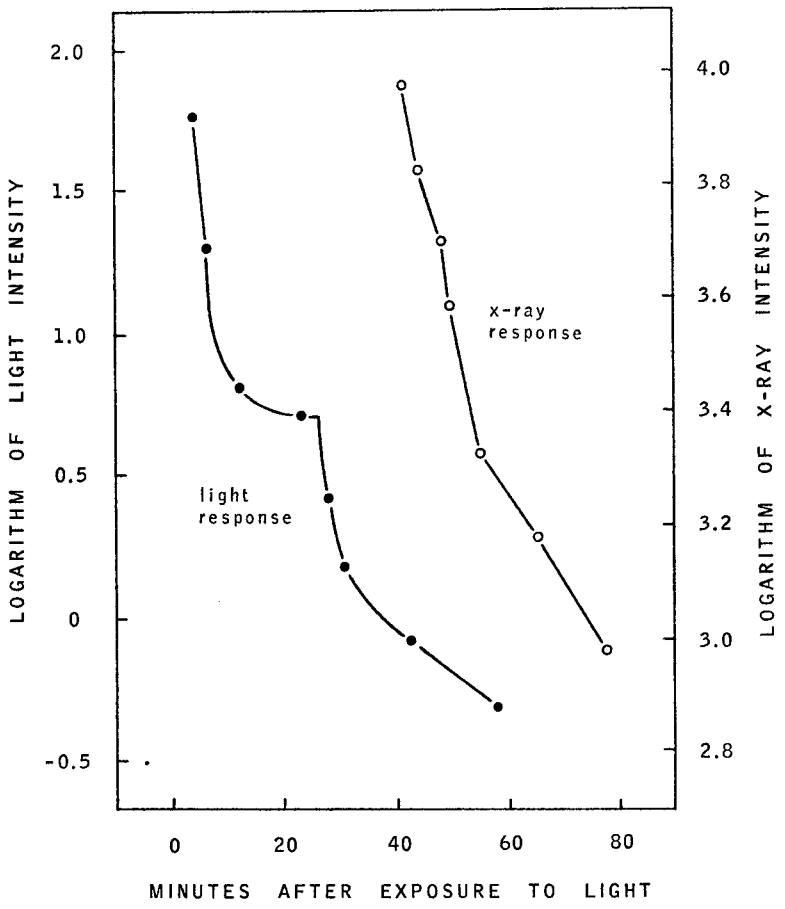

FIGURE 1. Dark adaptation curves for frog retina as determined by the production of the ERG in response to light and in response to x-rays. The curves show the adaptation time required to produce an ERG of 200 microvolts amplitude after 5 minutes of exposure to a bright light. The break in the curve for the light response corresponds to the shift from cone to rod function. This shift is apparently lacking in the x-ray response. Further details are given in the text.

compute the amount of energy absorbed in the rhodopsin of the retina during the flash of x-rays.

Three lines of evidence indicate that rhodopsin, the photosensitive pigment of the rods, is sensitive to x-rays and leads to the production of the x-ray ERG. First, no electroretinogram in response to x-rays could be elicited in this laboratory from the horned toad, an animal which lacks rod vision. Second,

in experiments designed to test the effect of dark adaptation on responses to x-rays and to light, there was no indication of a shift from cone function to rod function in the x-ray ERG as was the case for the light ERG. When the logarithm of the brightness of light necessary to produce a constant response was plotted as a function of time in the dark, the resulting curves for dark adaptation showed a break which characteristically occurred during the early stages of adaptation. This break was similar to breaks which have been reported in curves for dark adaptation in human beings and which have been shown to indicate a shift from cone to rod function. Such a break was observed in the response to light but not in the response to x-rays (Fig. 1). Third, when the retina was exposed to high intensity light for a period of 5 minutes, no response to x-rays could be elicited for a period of 8 to 10 minutes after the

TABLE I

ENERGY UTILIZED IN PRODUCTION OF ERG IN RESPONSE TO X-RAYS

r/sec.	Ergs absorbed per gm rhodopsin in 1 sec.	Ergs absorbed by rhodopsin of retina in 1 sec.	Ergs absorbed per flash by rhodopsin of retina: Duration of flash, *sec.* 0.015	0.04	0.08	0.4	1.0
6.5	637	0.041	0.0006	0.0016	0.0033	0.016	0.041
16	1568	0.10	0.0015	0.0040	0.0080	0.040	0.10
36	3530	0.23	0.0034	0.0090	0.018	0.090	0.23
66	6470	0.41	0.0062	0.017	0.033	0.17	0.41
127	12450	0.80	0.012	0.032	0.064	0.32	0.80
162	15900	1.02	0.015	0.041	0.081	0.41	1.02

end of the light exposure, whereas light stimulation of high intensity during this period produced a response, attributable to the functioning of cones.

In order to establish the amount of energy absorbed from the x-ray beam which contributes to the ERG during each flash of x-rays, it is necessary to determine the amount of rhodopsin in each retina. Broda *et al.* (2) extracted rhodopsin from ten retinas, which, in 1 cc of solution, gave an optical density of 0.814. The extinction coefficient for rhodopsin as established by Wald and Brown (3) is 40,600 cm^2 per mole equivalent of retinene. This value is defined by the equation, $\log_{10} I_o/I = e \cdot c \cdot l$, in which I_o is the intensity of light incident on the solution, I the intensity transmitted, e the molar extinction coefficient, c the concentration in moles per liter, and l the depth of the solution in centimeters. From this we calculate that the rhodopsin extract of Broda *et al.* had a concentration of 2.005×10^{-5} gm mol/liter. Since the rhodopsin was secured from ten retinas and the rhodopsin was contained in 1 ml, there was actually 2.005×10^{-9} gm mol/retina. In order to determine the actual amount of rhodopsin in one retina, one needs now to know the molecular weight of

rhodopsin. Using the molar extinction coefficient of Wald and Brown (3), one arrives at a molecular weight of about 47,000 for frog rhodopsin, from the values established by Broda *et al.* (2) for frog rhodopsin. Hubbard (4) arrived

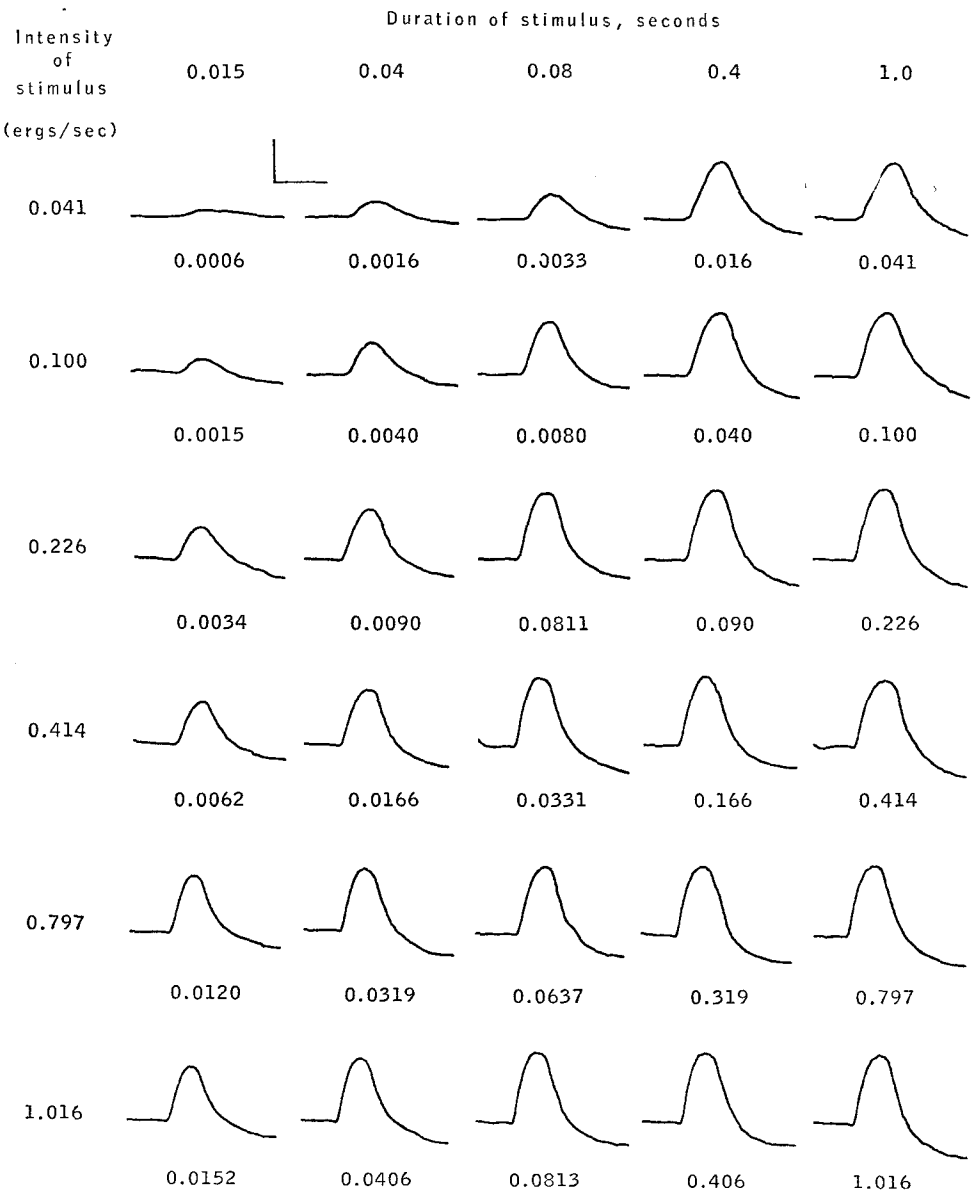

FIGURE 2. Photographic reproductions of electroretinograms in response to x-rays. These records correspond to the values given in Table I. The number under each record indicates the number of ergs of energy absorbed in the rhodopsin of the retina from the stimulus used to elicit the response. Calibration values, 500 microvolts and 400 milliseconds.

at a value of 40,000 for cattle rhodopsin. Krinsky (5) prepared samples of cattle rhodopsin in which the content of protein impurities was considerably reduced; for these solutions he obtained a molecular weight of 32,000. Since Hubbard recognized that her value might be high if protein impurities were present, we have adopted the value obtained by Krinsky for the molecular weight of rhodopsin. It appears, moreover, that the molecular weight of frog rhodopsin is similar to that of cattle rhodopsin (4). All computations in this paper can be readily changed if subsequently different values for the molecular weight of rhodopsin should be established. The amount of rhodopsin in one retina, therefore, is equal to 6.4×10^{-5} gm for the frogs used in the present experiments.

A value of 98 ergs per roentgen of x-rays was adopted as the amount of energy absorbed per gram of rhodopsin. This value was computed from information contained in the Atomic Energy of Canada Limited Radioisotope Handbook (6) which gives the amount of energy required to produce an ion pair in soft tissue and the number of ion pairs produced in soft tissue by 1 roentgen of x-rays.

Table I gives values and computations for a series of x-ray intensities and several stimulus durations. These values correspond to responses shown in Fig. 2, a typical series of responses to x-ray stimulation.

Energy Required to Generate the Light ERG

It is necessary to convert the intensities employed in the light flashes to ergs absorbed per retina per flash in order to compare them with the values for x-ray stimulation. From Walsh (7) we have taken the following values: One watt is equivalent to 682 lumens for radiation of wavelength 555 mμ; the mechanical equivalent of light at 555 mμ is, therefore, 0.001467 watt/lumen. Since 1 watt is equivalent to 10^7 ergs/sec. and 1 meter-candle is equivalent to 1 lumen per square meter, it follows that a flash of 1 sec. delivers 1.467 ergs/cm^2 when the illumination is 1 meter-candle.

Since the rhodopsin of the frog retina absorbs approximately 70 per cent of the incident light falling upon it (8), a flash of 1 sec. delivers 1.03 ergs/cm^2 when the intensity is 1 meter-candle. The actual area of the retina of the frogs used in the present experiments was determined by measurement to be 0.6 cm^2. According to Denton and Wyllie (8), the rods represent 67 per cent of the total area of the retina, or a net area of 0.4 cm^2 for the frogs used in the present experiments. Therefore a flash of 1 sec. duration at an intensity of 1 meter-candle corresponds to the absorption of 0.41 erg by the rhodopsin of one frog retina. From this value, one can easily compute the energy absorbed by the rhodopsin of the retina at any light intensity and any duration of stimulus. Table II gives typical values employed in these experiments, and Fig. 3 shows actual electroretinal responses recorded over the same range of intensity and duration of stimulation.

TABLE II

ENERGY UTILIZED IN PRODUCTION OF ERG IN RESPONSE TO LIGHT

Meter-candles at surface of retina	Ergs per cm² at surface of retina in 1 sec.	Ergs absorbed per cm² in 1 sec.	Ergs absorbed by rhodopsin in 1 sec.	Ergs absorbed per flash by rhodopsin of retina Duration of flash, *sec.* 0.015	0.04	0.08	0.5	1.0
0.012	0.0175	0.0122	0.005	0.00008	0.0002	0.0004	0.003	0.005
0.036	0.0528	0.0370	0.015	0.0002	0.0006	0.001	0.008	0.015
0.124	0.182	0.127	0.051	0.0008	0.002	0.004	0.026	0.051
0.45	0.660	0.462	0.19	0.0028	0.007	0.015	0.093	0.19
2.92	4.28	3.00	1.2	0.18	0.048	0.097	0.61	1.2
36	52.8	37	15	0.22	0.59	1.2	7.4	15
2460	3608	2526	1020	15	41	81	510	1020

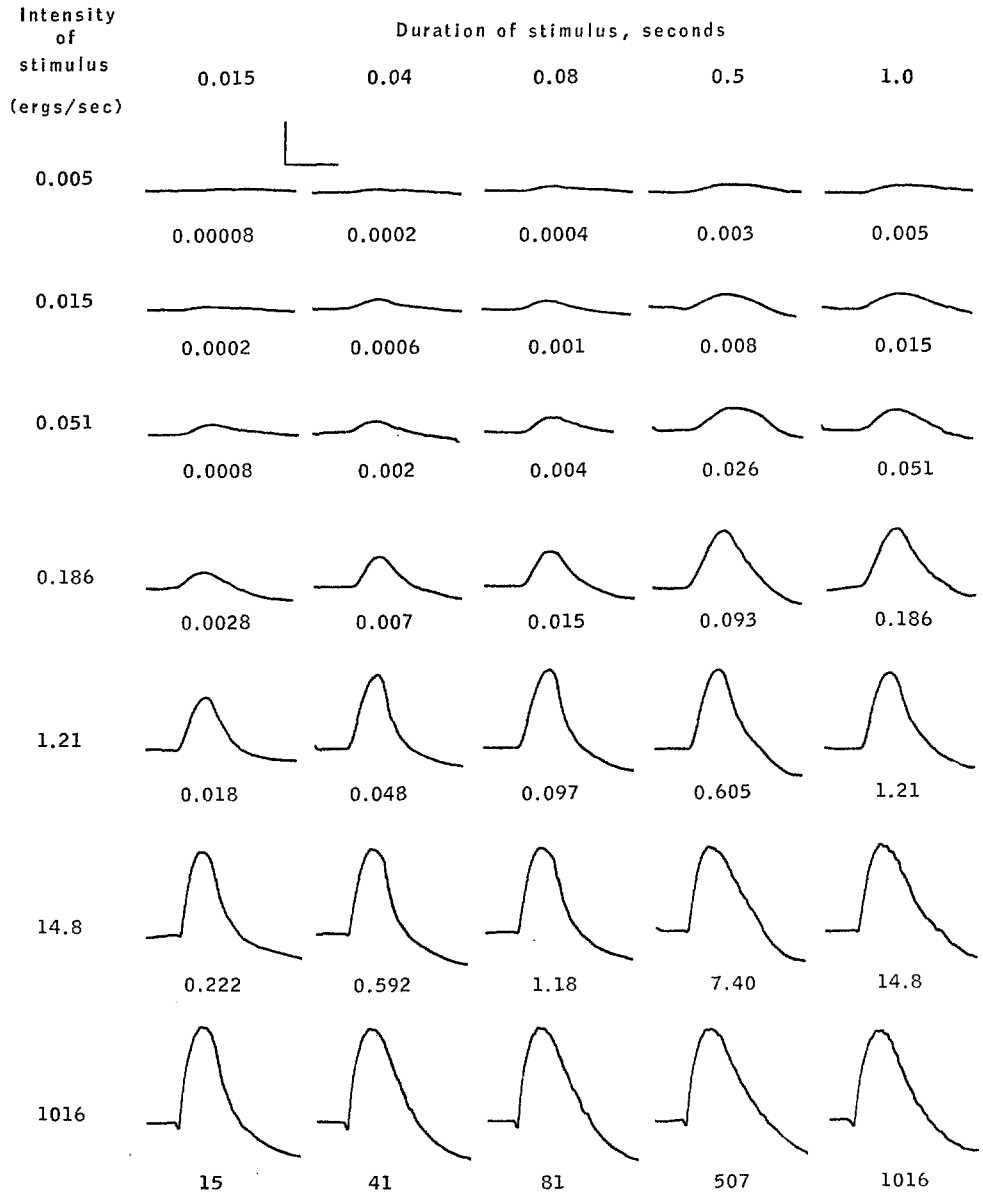

FIGURE 3. Photographic reproductions of electroretinograms in response to light. These records correspond to the values given in Table II. The number under each record indicates the number of ergs of energy absorbed in the rhodopsin of the retina from the stimulus used to elicit the response. Calibration values, 500 microvolts and 400 milliseconds.

Comparison of Energies of X-Rays and of Light Required for ERG

Since it is possible to elicit an ERG of the same amplitude by stimulating with lower intensities and longer durations as well as with higher intensities and shorter durations, one must consider both aspects of the stimulus (intensity and duration) in making an evaluation of the efficiency of x-rays and of light in eliciting the ERG. The relationship of amplitude of response as a function

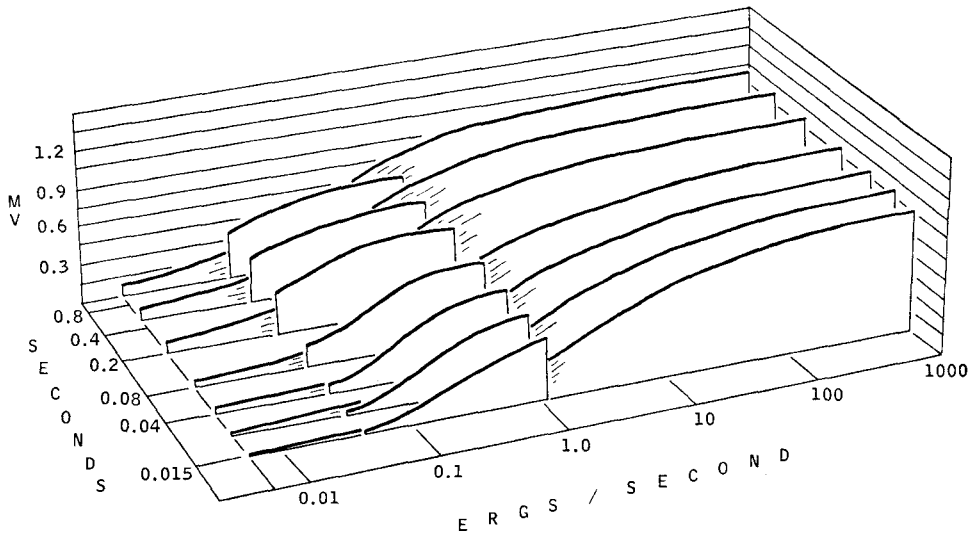

FIGURE 4. Amplitude of ERG plotted as a function of duration of stimulus and intensity of stimulus. Amplitude is expressed in millivolts, duration of stimulus in seconds, and intensity of stimulus in ergs of energy absorbed per second by the rhodopsin of one retina. There are two sets of curves, one for the light ERG and one for the x-ray ERG. The curves for the light ERG extend continuously from 0.0005 erg/sec. to 1000 ergs/sec. Superimposed on the curves for the light ERG are the curves for the x-ray ERG, which extend from 0.041 erg/sec. to 1.016 ergs/sec.

of duration and intensity of stimulus is shown in Fig. 4. The fact that the intensity of the x-ray stimulus covers a 25-fold range, while that of light covers a 200,000-fold range, is brought out by the very short intensity axis for x-rays in comparison with that for light. One should note, however, that even the log scale for intensity tends to obscure the true proportions of the intensities involved.

An examination of Fig. 4 shows that one cannot give a simple, unqualified answer to the question whether x-rays or light is more efficient in producing an ERG. At the lowest intensity of x-rays (0.04 erg absorbed by the rhodopsin of one retina in 1 sec.), and a duration of 0.015 sec., the amplitude of the response to x-rays and to light is essentially the same. At the highest intensity

of x-rays (slightly over 1 erg/sec.), and the same duration, 0.015 sec., the amplitude of the response to x-rays is actually higher than the amplitude of the response to light, indicating a greater efficiency of x-rays, judged by the amplitude of the response. At the lowest intensity of x-rays, as the duration of the stimulus increases, the amplitude of the x-ray response increases more than does the amplitude of the light response. This is particularly noticeable at an exposure of 1 sec. duration, at which value the amplitude of the x-ray response rises to almost twice that of the light response. On the other hand, at the highest intensity of x-rays, as the duration of the stimulus increases, the amplitude of the x-ray response fails to rise appreciably, whereas the amplitude of the light response continues to rise, surpassing the amplitude of the x-ray response at the longest durations.

In short, at certain combinations of duration and intensity of stimulation, the amplitude of the x-ray response is greater, whereas at other combinations the amplitude of the light response is greater. Noteworthy is the fact that the amplitude of the light response rises as the intensity of the stimulus increases, over a very great range, whereas the amplitude of the x-ray response levels off at much lower intensities.

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