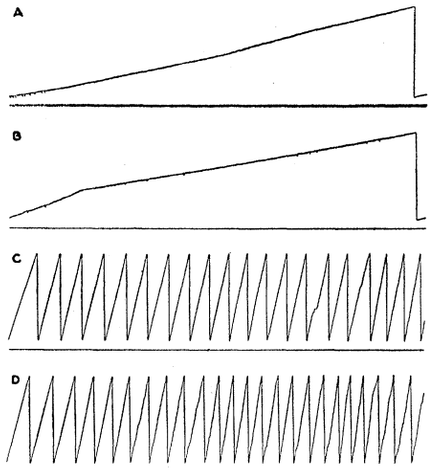

Fig. 2. Records of selected 2-hour test sessions from two subjects. *A*, 3rd session; *B*, 9th session of subject with lowest mean rate; *C*, 3rd session; *D*, 9th session of subject with highest mean response rate. The session previous to that shown in *B* required 12 responses per minute.

the room halfway through the period to give new instructions. On inquiry, 7 of the 11 subjects who experienced both conditions stated that losing money was the more distasteful, only three said the shock was more distasteful, and one was indifferent. Avoidance responses were developed equally often by those who were instructed to use the plunger and by those who were not. Those who were not so instructed sometimes displayed such bizarre behavior as standing in the corner or standing on the heads. The proportions of males and females who learned the avoidance response did not differ.

Three representative records from the first hour of testing are shown in Fig. 1. The record of an individual who did not develop an avoidance response is shown in Fig. 1*A*. Characteristically, some responses were made on each plunger during the first half-hour of the session, but all efforts were abandoned after the pennies were exhausted about midway in the session. Figure 1*B* shows a common pattern of abrupt change in response rate. Also common was the pause such as occurred at the point marked *T* in the record. Note that the irrelevant right-hand plunger was pulled throughout the session, and also that satiation phenomena appeared near the end of the hour. The individual of Fig. 1*C* showed initially overcomplex behavior that was later eliminated, but he continued to manipulate the irrelevant plunger throughout. Also of interest is his lack of generalization of the response between the two conditions; such a failure was the rule, occurring in five of six opportunities. Some individuals eliminated responses on the irrelevant plunger, but maintained an inappropriate rate, while others achieved an appropriate rate but failed to eliminate the irrelevant responses. No subject developed the most economical response pattern within the first hour's training.

After the first experimental session, 10 subjects were selected at random to participate in 20 additional hours of testing, and seven of them completed the entire series of tests. The loss of coins was used throughout this series as the aversive event, to investigate the effects of variation in the length of test session, in the interval between loss of coins, and in the magnitude of each loss.

All these subjects acquired the avoidance response by the end of the second hour, although some persisted with inappropriate rates and responses on the irrelevant lever throughout the entire 20 hours of testing. Typically from the third session on, each subject entered the experimental room and commenced immediately to pull the plunger at his characteristic rate. Only one subject showed the so-called warm up phenomenon that is often encountered in rats working under this schedule. Records of the third and ninth testing sessions (during which the response-loss interval was 20 seconds, and each aversive event cost 2 cents) for the subjects with highest and lowest response rates are shown in Fig. 2. Differences in response rates between individuals far exceeded those produced by alterations in the experimental conditions. Mean response rates for subjects under all conditions ranged from 6.21 to 119.8 per minute. In contrast, the median responses per minute for the three conditions of coin value were: 2 cents, 7.91; 10 cents, 8.22; and 50 cents, 8.88. The interval between a response and the loss of the next coin had a somewhat greater influence: at a 5-second interval the median rate was 22.84 responses per minute (12 was the minimum rate to protect all coins). Rates for the 20- and 80-second intervals were 4.46 and 3.06 respectively.

The basis for the large individual differences observed in the performance of nondiscriminated avoidance behavior by human subjects is now being investigated in this laboratory (*6*).

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6. This research was supported by grant No. R58-1.2-8 from the California State Department of Mental Hygiene. Much of the testing was done by Claire Elder.

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Electroretinogram in Response to X-ray Stimulation

Abstract. The retina of the grass frog, *Rana pipiens,* responds to flashes of high-intensity x-rays and produces an electroretinogram indistinguishable in form from the electroretinogram produced in response to light stimulation at low and intermediate intensities. At higher intensities the form changes and, for maximal responses, the electroretinogram in response to x-rays shows a lower amplitude and a longer latent period than that in response to light. The prolonged latent period indicates additional intermediate reactions for the x-ray response.

Invisibility is generally emphasized as one of the properties of x-radiation (*1*). The ability of x-rays to evoke some sort of retinal response, however, has been known from the fact that, in early years, men looked into x-ray beams and reported various visual sensations. Once the harmful nature of x-rays was recognized, this activity was suddenly curtailed.

Two successful attempts to produce electroretinograms by x-rays have been reported in the literature. Himstedt and Nagel (*2*) showed records from frogs and birds which indicate little more than some sort of electrical disturbance caused by x-rays; these records bear little resemblance to the electroretinogram as recorded today, in response to light, with modern equipment. Elenius and Sysimetsä (*3*) gave a brief report on low, threshold responses in human subjects suffering from cataracts.

Attempts to produce an electroretinogram in response to x-rays usually fail, due, apparently, in large part to inability to stimulate the retina with intense, quick flashes of high-energy x-rays. In the research reported here the difficulty was overcome with the experimental setup shown in Fig. 1. The high-intensity beam was built up, while the ½-in. lead shutter protected the eye of the frog from the beam. Slits of various widths in the lead shutter were passed over the eye, by remote control, much as a focal-plane shutter operates in a camera. The movement of the shutter gave exposure times proportional to the width of the slit and the speed of movement of the shutter. The duration of the exposure was monitored

by a photoelectric cell, sensitive to x-rays, placed in such a position that the beam of x-rays struck the cell at precisely the same time that it struck the eye. The whole eye was exposed to the x-ray beam.

A General Electric No. 1493 bulb, the source of light, was mounted inside a light-tight box, together with reflector, condensing lenses, and projecting lenses. An Alphax heavy-duty synchromatic shutter (Wollensak) was mounted on the front of the box. A small front-surface mirror, placed above the animal just outside the path of the x-ray beam, directed the horizontal light beam into the eye and provided total, uniform illumination of the eye. The instruments controlling the intensity of the light source were operated from an adjacent control room. The opening and closing of the shutter were remotely controlled by means of a shutter release with synchronizing circuits for triggering the oscilloscope and accessory photographic equipment. The output of the lamp was calibrated by reference to a standard lamp obtained from the National Bureau of Standards. The beginning and end of the light stimulation were indicated by the signal from a photocell mounted above the animal in a position such that the light passing the mirror fell on the cell.

The animal was supported on a block of Styrofoam, which had been carved to fit the shape of its body. This block was fitted in the plastic container, to which water was added to keep the animal moist during the course of the experiment. Not evident in the figure is the fact that the animal was restrained in order to avoid the possibility of movement, which might interfere with the alignment of the eye relative to the x-ray and light beams. Shielded leads from the two electrodes in the animal were taken to a Tektronix type 122 preamplifier. The response was displayed on one beam of a Tektronix type 502 dual-beam oscilloscope, the signal from the photocell was displayed on the other beam, and the traces were recorded photographically with a Grass Kymograph camera. The information obtained from the oscilloscope was supplemented by concomitant recording from a Grass Model III-D electroencephalograph; two additional leads were taken from the electrodes in the animal to this instrument, as well as leads from the photocells.

Records were made from animals in which the cornea and lens had been removed and in which an electrode (either Ag-AgCl or 0.005-in. platinum-iridium wire fused into the tip of a small glass capillary tube) had been placed in the vitreous humor. More commonly, in view of the greater stability of the preparation, records were made from the intact eye. Contact with the eye was made with a small stainless steel needle or with a sealed-wick-electrode assembly, shown in Fig. 2. The wick was placed in contact with the surface of the eye, at the periphery, so as not to interfere with the light or x-ray beam. Sealed-wick assemblies of this type posed no problem of drying for periods in excess of 8 hours. The

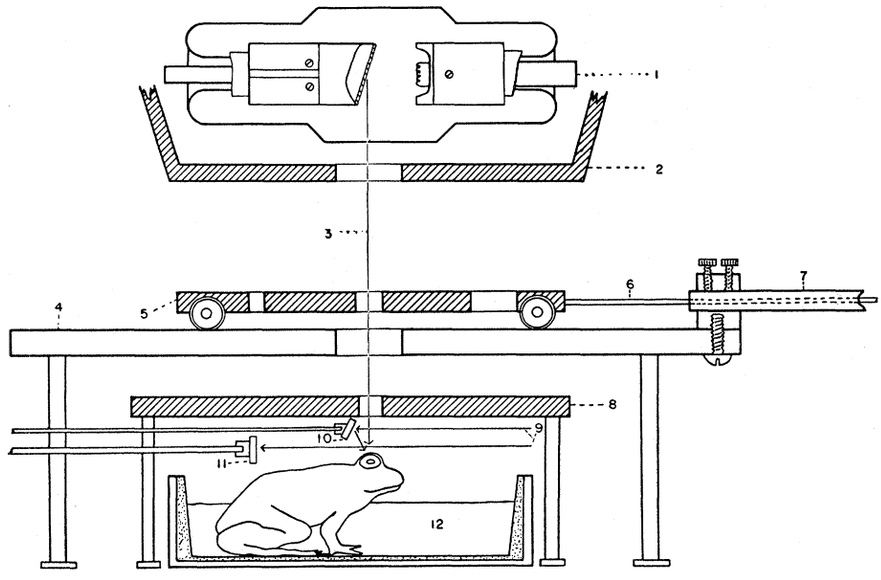

Fig. 1. Apparatus used for stimulation with light or x-rays, or both: 1, x-ray tube; 2, lead shield of x-ray tube; 3, x-ray beam; 4, track and support for lead shutter; 5, lead shutter; 6, shutter control shaft; 7, housing for control shaft; 8, lead shield over animal, with hole of same diameter as eye of animal; 9, light beam; 10, front-surface mirror; 11, photoelectric cell to record light signal; 12, plastic container with animal in water. Connections between animal and recording equipment are not shown. The photocell for recording x-ray signal is described in the text.

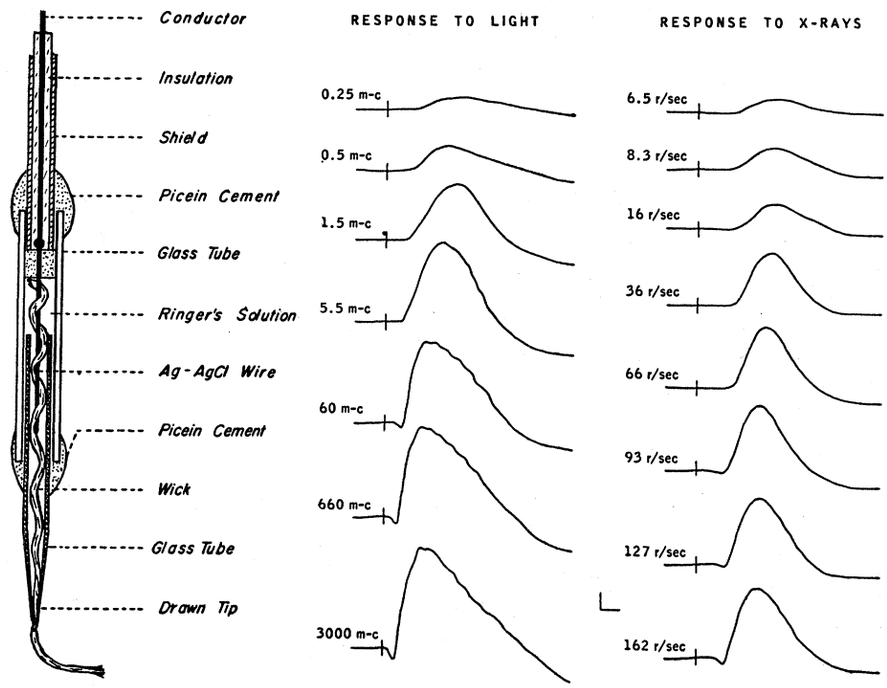

Fig. 2 (left). Wick electrode with sealed reservoir, designed to prevent leakage and permit use for extended periods of time. Fig. 3 (right). Electroretinal responses to light flashes of 0.08-second duration and intensities from 0.25 to 3000 m-ca, and to x-rays of 0.04-second duration and intensities from 6.5 to 162 r/sec. All responses are from the same animal during the same experiment. Calibration values: 150 μv and 100 msec. The duration of x-ray stimulus used here exposed the eye to a minimum of x-ray damage yet produced maximal responses at the higher intensities.

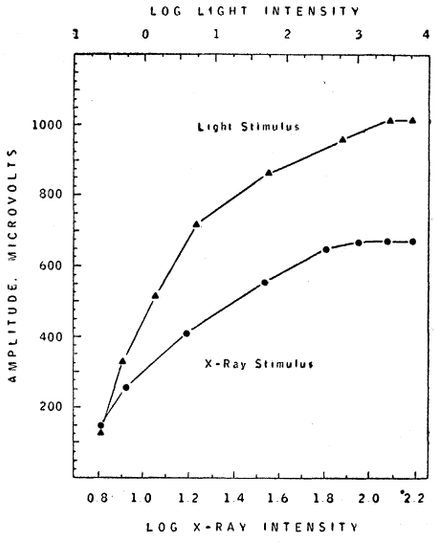

Fig. 4. Mean amplitude of *b*-wave in the on response to x-rays and to light, plotted as a function of the logarithm of light intensity in meter-candles and of x-ray intensity in roentgens per second. Duration of stimulus as in Fig. 3. The values indicate that a maximal response was obtained in both situations. Values are based on experiments involving 35 animals.

indifferent electrode in all cases was placed in contact with the skin over the nose. It was possible with this arrangement to record responses to light and responses to x-rays from the same animal under identical conditions without disturbing the assembly, since stimulating and recording equipment was controlled from a shielded room that was adjacent to the room containing the x-ray tube, the light source, and the animal.

A typical series of "on" responses to light and to x-rays is shown in Fig. 3, ranging from near-threshold responses to maximal responses obtainable for that particular retina. There is a striking similarity between the two sets of responses reproduced in Fig. 3, especially between the responses to light from threshold to 1.5 m-ca and the responses to x-rays from threshold to 66 r/sec. Lest it be concluded, therefore, that the mechanism of action of the two stimuli are the same, certain differences should be pointed out.

The mean maximal amplitude of the *b*-wave from 35 animals in response to x-rays was 665 μv, whereas the mean maximal amplitude obtained from the same animals in response to light was 1010 μv (Fig. 4). Increasing the magnitude of the stimulus in either case did not increase the amplitude beyond these maximal values. It is remarkable that the magnitude of the stimulus necessary to evoke the responses shown in Fig. 2 covered a 12,000-fold range for light, whereas the range for x-rays was only 25-fold. This point is emphasized in Fig. 4 by the different logarithmic scales for light intensity and for x-ray intensity.

The latent period for the response to x-rays in Fig. 2 varied from 135 msec for the maximal response to 190 msec for the minimal response; the latent period for the response to light, by contrast, varied from 60 msec for the maximal response to 150 msec for the minimal response. That Elenius and Sysimetsä did not detect a difference in the latent period of the two responses is probably due to the fact that they considered only a threshold response. At such low levels, the latent period of the two responses does not differ as greatly as at higher amplitudes, although, in the work under discussion, considerable difference was found even at the lower amplitudes. In studies on 35 animals, for example, the mean latent period for 120-μv responses to x-rays was 181 msec, whereas for comparable responses to light it was 140 msec. The mean latent period for maximal responses to x-rays for the same animals was 129 msec, whereas for maximal responses to light the latent period was 71 msec.

The period of latency of the two responses is of considerable significance. Differences in latency suggest different mechanisms in the interaction between the photoreceptors and light, on the one hand, and the photoreceptors and x-rays on the other. Absorption of x-ray photons by the rods is apparently responsible for the electroretinogram. This assumption is based on two lines of evidence. 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, when the logarithm of the brightness of light necessary to produce a constant response is plotted as a function of time in the dark, the resulting curves for dark adaptation show a break which characteristically occurs during the early stages of adaptation. This break is 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. In view of the results reported here, we propose that, in some way analogous to the manner in which the rods react with visible light, the pigment of the rods, rhodopsin, absorbs x-ray quanta, undergoes chemical change, and leads to excitation which produces the electroretinogram. In this sequence of events the delay, and hence the difference in the response, is to be found in the chemical change involved in the bleaching of rhodopsin. It is suggested that the reaction of radicals produced by x-rays (*4*) is involved in the chemical change (*5*).

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Micromanipulation in Control and Handling of Zygiella x-notata as an Experimental Animal

Abstract. The spider *Zygiella x-notata* may be brought under direct control analagous to that of the common laboratory animals for an important group of experimental investigations on the nerve, muscle, or secretion of digestive glands. Without anesthetics, chilling, or damage the animal can be fixed for prolonged periods and microinstruments (which include feeding pipettes), positioned by a standard micromanipulator.

The spider *Zygiella x-notata* has been used as a sensitive biological test animal for a number of psychopharmacologic and hallucinogenic drugs (*1*). Characteristic disturbances in web-building activities and patterns reflected the effects of the drugs on the central nervous system of the animal. Important applications of the test to body fluids of man have been made in the search for hallucinogenic substances in schizophrenia by Witt and Weber (*2*).

Administration of psychotrophic substances has been either by direct injection (with a high mortality due to irremediable chitinous damage), or by the ingenious indirect technique of Wolff and Hempel (*3*); the latter consisted in the injection of the drug dissolved in sugar solution into the abdomen of a desiccated fly which was weighed and thrown on the web, which was then vibrated by a tuning fork. When the spider had accepted this artificial prey the latter was again weighed to determine the amount of the drug taken.

The present investigations were designed to explore the possibility of direct dosage without injury in this small