roots and the pointed flagellar tip are not represented in the diagram.) During the first step in fusion, the membrane of one spermatozoid flagellum coalesces with the membrane of the egg and the fibrillar core of the flagellum is engulfed by the egg protoplast. Subsequent stages of fusion are also represented in Fig. 1 (*B*, *C*, and *D*). The stage represented in *C* might not occur in each fusion although it has been observed frequently. When it does occur, the absorbed flagellum forms a "bridge" with an unfused indentation beneath and often penetrates deep into the zygote protoplast during comparatively late stages of fusion, near the time when the zygote begins to round up.

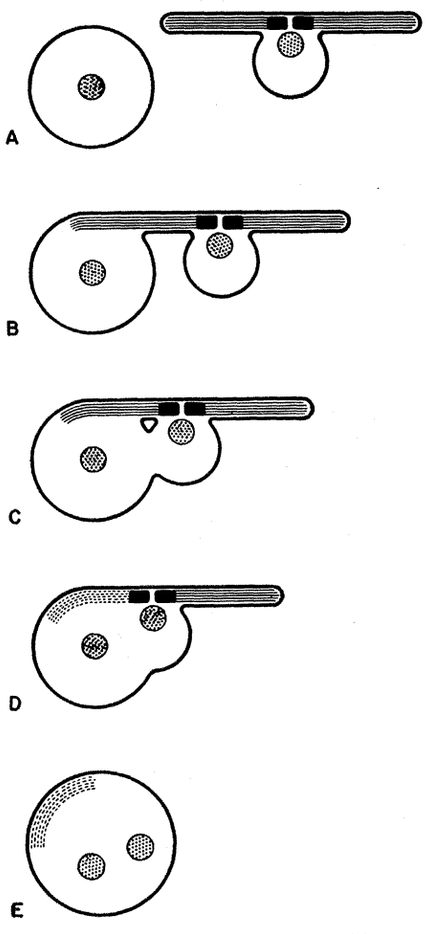

Fig. 1. Plasmatic fusion in the fertilization of the green alga *Prasiola stipitata* Suhr, based on the investigations of Friedmann (*3*) and Manton and Friedmann (*4*). *A*, Egg cell and biflagellate spermatozoid. *B*, Beginning of fusion. Coalescence of the membranes of the egg cell and of the tip of one of the spermatozoid flagella. Engulfing of the fibrillar core of the fusing flagellum by the egg cell protoplast. *C*, Later stage in fusion with a "bridge" and an unfused depression between the gametes. *D*, Motile stage of the zygote. Disintegration of the fibrils of the engulfed flagellum. *E*, Immobile stage of the zygote after "rounding up." Withdrawal of the motile flagellum and disintegration of its fibrils.

The pear-shaped zygote in Fig. 1*D* carries in its protoplasm the engulfed flagellum, which later disintegrates. The second, free flagellum remains motile at this stage but it is withdrawn when the zygote rounds up (Fig. 1*E*). The nucleus is then no longer attached to the flagellum, and the flagellum disintegrates.

There are several points of interest which emerge from the comparison of the fertilization processes in *Prasiola*, *Hydroides*, and the rat. The actual fusion of the protoplasts is preceded and apparently initiated by the coalescence of the male and female cell membranes. The ability to fuse is certainly a very specific state of the cell surface membrane.

In the egg, there is no morphological evidence that a particular area of the cell surface is predetermined to be the point of fusion. On the contrary, in the male gamete, the potentiality of the surface membrane to fuse is restricted to a distinct area. In animal sperms, this area seems to be the membrane of the acrosome (when this organelle is apparent). In *Prasiola*, the distinct area which can fuse is the membrane of the apical region of the flagellum, which thus assumes a function apparently analogous with the function of the acrosome. In spite of their very different physiological behavior, there is no structural difference between the fusing and nonfusing flagellum of the *Prasiola* spermatozoids and there is even some evidence that each of the flagella is potentially capable of functioning in either way (*5*).

In the brown algae (Phaeophyta), however, where the two flagella of the spermatozoid are morphologically different, only one flagellar type seems to participate in fusion (*6*).

The strict comparability of "bridges" and unfused depressions in *Prasiola*, "vesiculation" in *Hydroides* (*1*), or the "fold" which is present during the fertilization in rat (*2*) remains to be clarified by further investigations. The fact that all these structures appear at the area of actual fusion of the male and female cell membranes is suggestive.

There is indication that some analogous biophysical or biochemical mechanisms might have a basic role in both plant and animal fertilization. Some of the elastic properties of the cell membranes of flagellated plant cells were discussed to some extent by Manton and Friedmann (*3*). Similar analysis might also be considered in connection with the fertilization mechanism in animals.

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14 November 1961

Conditioning in Fish: Effects of X-irradiation

Abstract. Fish were subjected to different levels of high intensity x-irradiation to ascertain the effects of x-rays on acquisition and retention of conditioning. Lethal dosages were also determined. Light was the conditioned stimulus, and electric shock the unconditioned stimulus. Responses were forward darting and backward swimming movements accompanied by increased gill movement. Acquisition of conditioned responses was suppressed by higher dose levels.

Conditioned responses in various mammals are affected by x-irradiation. The general effects are transitory or gradual decrements in responses (*1*). However, Furchgott (*2*), in summarizing a number of ionizing radiation experiments, concluded that general learning functions are relatively unaffected by high doses of x-irradiation, even in the lethal ranges. Any decrements in learning were considered to be due to motivational and perceptual factors.

Table 1. Comparison of overall mean and standard deviation (S.D.) of conditioned response frequency of x-irradiated and non-irradiated fish.

Av. x-irradiation (r)	No.	Mean	S.D.
0	14	77.2	8.8
7,200	12	76.7	10.2
10,100	32	66.8	11.6
18,400	10	51.6	18.0

The primary purpose of our study was to observe the effects of large single dosages of x-rays on the rate of acquisition and retention of conditioned responses in a species of fish, and to determine the lethal dosage. Golden shiner minnows, *Notemigonus crysolecus* (Mitchill), were used.

The conditioning apparatus delivered 5 seconds of light (conditioned stimulus) and 1 second of electric shock (unconditioned stimulus) overlapping the last second of light, to a small aquarium (12 by 7¼ by 9 inches). The light stimulus of 520 ft-ca of illumination was provided by a 75-watt bulb directly over the aquarium. The light was diffused from the bottom of the aquarium by a strip of white opaque Plexiglas. The shock was 6 to 8 volts of half-wave direct current produced from a rectified and transformed 110-volt a-c source. The shock was delivered through two brass plates located at each end of the aquarium. Both the light and the shock were timed by cam-operated microswitches.

Before the initial conditioning session each subject was given five preliminary trials with the conditioned stimulus. All subjects were then given 20 consecutive trials each day for five sessions. Every tenth trial was a test trial with the light only. The shock level during each session was adjusted to maintain a vigorous unconditioned response.

The source of x-rays was a 3-Mev Van de Graff electron accelerator. A spectrophotometer was used to determine dosage by analyzing the change in the absorption spectrum of irradiated Frike dosimeter solution by comparing it with a nonirradiated sample (*3*). An absorption spectrum of 304 mμ was used to analyze the dosimeter solution.

The low, medium, and high dosage groups were assigned to 12, 32, and 10 subjects, respectively. The three dosage levels (Table 1) were given in 6, 8, and 12 minutes, respectively, about 7 to 8 hours before the first conditioning session. Fourteen subjects received regular conditioning trials as a nonirradiation control group, and a fifth group of 10 subjects was given trials with the conditioned stimulus only as a stimulus control group.

Two distinct types of responses were observed in response to the conditioned stimulus when paired with the unconditioned stimulus. Approximately 95 percent of the responses recorded were forward darting, and the remainder were backward swimming movements. The first response was counted. The responses were usually accompanied by increased gill movement or respiration. The responses during the 4 seconds of light preceding the shock were counted, except for the test trials in which responses for the entire 5 seconds were counted. The unconditioned responses were always more vigorous than the conditioned responses and were accompanied by flexure of the fins. The few responses during preliminary trials were similar to random intertrial movements.

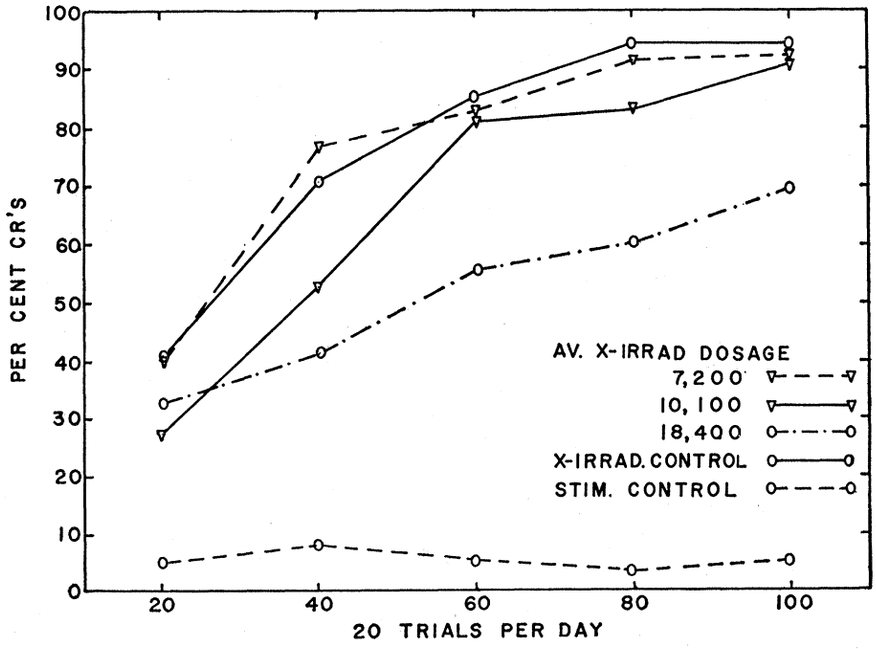

Fig. 1. Comparison of conditioned responses of x-irradiated and nonirradiated fish.

The three irradiated groups were compared statistically with the nonirradiated control group for each point in Fig. 1 (*4*). Significant differences were found between the medium dosage group and the control group on the second 20 trials ($p < .05$); and the high dosage group and the control group for the last four blocks of 20 trials ($p < .01$).

The data in Table 1 indicate that as the dose level increases the mean response level decreases and the variability increases. However, a test for homogeneity of variance (*5*) resulted in a nonsignificant χ^2.

The nonirradiated control group and eight subjects receiving a dosage of 11,000 r (av.) about 8 hours before the beginning of the third conditioning session, were compared to determine the effect of x-rays on further acquisition and retention. A significant difference ($p < .01$) was found for the last 2 days with the irradiated group showing a decrement in the percentage of conditioned responses.

The lethal dose of x-irradiation was determined on 20 subjects. All dosages between 9600 r and 11,800 r were 100 percent lethal within 16 days. Five controls survived identical conditions for 6 weeks. Four subjects, given 66,000 r in five 10-minute sessions, died within 2 minutes after the fifth exposure.

Definite periods of hyperactivity during irradiation were observed in all fish by closed circuit television. The fish generally became very active when the accelerator reached peak output at about 20 to 30 seconds, and they remained active for 60 to 90 seconds. After a period of quiescence of about 30 to 60 seconds the fish became moderately active again. The periods of activity and nonactivity usually continued throughout the irradiation period, with the activity periods becoming shorter and the activity less vigorous. It is not known whether the fish were reacting to the x-rays or to chemical changes produced in the water by the x-rays (*6*).

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Self-Determination of Critical Flicker Frequencies in Monkeys

Abstract. Five rhesus monkeys have been successfully trained by operant conditioning techniques to continuously adjust the rate of flicker of an illuminated target above and below what is presumed to be their fusion threshold.

Critical flicker frequency, defined as the lowest rate of intermittence of a flashing light which gives rise to a sensation of steady illumination, has often been employed as an indicator of the physiological state of human subjects. So many physiological factors have been identified as interacting with the critical flicker frequency that it is difficult to be specific about those which are primary (*1*). Animal experimentation with better control of many of these factors is a natural approach, but the difficulty of establishing the necessary discrimination has limited the number of attempts to obtain behavioral thresholds in animals and has made interpretation of the results difficult.

Recently, procedures for obtaining behavioral thresholds in animals have been described which appear well suited for determination of values for the critical flicker frequency (*2*). The outstanding feature of these procedures (originally worked out in the pigeon for measurement of absolute brightness thresholds) is that the organism directly controls the magnitude of the physical difference which is being discriminated, as contrasted with conventional discrimination techniques in which the experimenter selects these magnitudes for each trial, or each block of trials. It seemed desirable to attempt to use the "Blough procedure" for estimation of the critical flicker frequency in monkeys and to compare the results with those obtained by other methods (*3*).

The first step was to teach hungry monkeys to press one lever (A) if a target appeared flickering, and a second level (B) if the target appeared to be continuously illuminated. Initial training utilized an "easy" discrimination, namely, ten interruptions per second for the flickering condition. For the continuous or fused condition a rate ten times higher was used, rather than a physically steady light, to avoid the difficulty of matching the two target conditions for apparent brightness (*4*). In this early training two responses in the correct order were necessary for the monkey to obtain reward. The first response, on lever A, switched in the 10× multiplier, (*Sx* in Blough's terminology) and the second, on lever B, produced a banana-flavored pellet and extinguished the target for 5 seconds. The target was then reilluminated at the 10 cy/sec rate, and the cycle was repeated. After the monkeys had learned to obtain rewards rapidly this way, the number of responses required on each lever was increased gradually to a maximum of 15, and then varied. Two conditions suggested by Blough were introduced during this phase of the training: (i) responses on the wrong lever counted against the monkey by increasing the number required on the correct lever; (ii) some rewards (about one in five) were followed not by a flickering but by a continuous target, allowing the monkey to begin pressing the B lever for food directly. Responses on lever A never produced food. The result of this training was to encourage the monkeys to maintain a high rate of responding and to pay close attention to the target, since the number of responses could not be anticipated and errors delayed reward. Six of seven adolescent rhesus monkeys (*Macaca mulatta*) mastered this initial training in a range of 26 to 73 daily sessions of approximately 45 minutes duration.

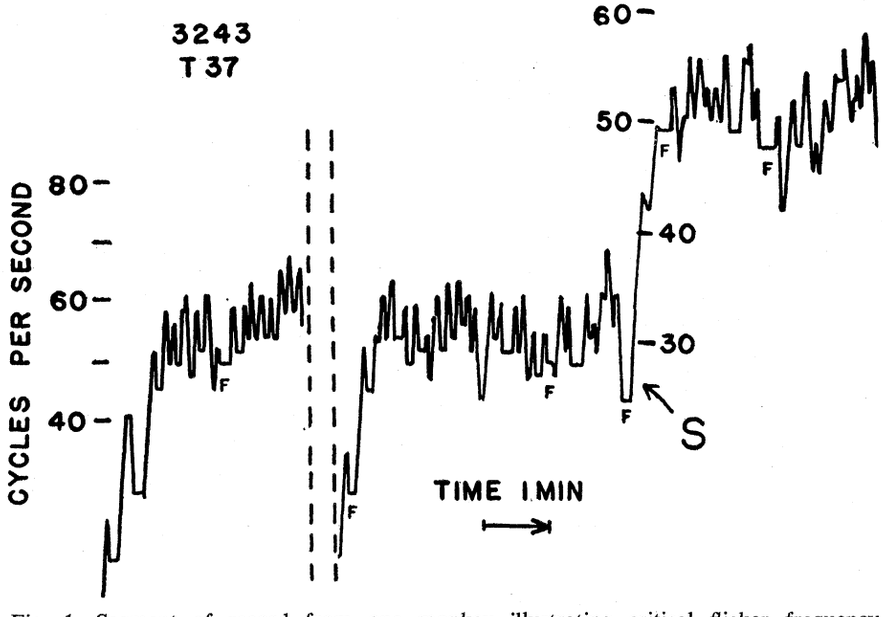

Fig. 1. Segment of record from one monkey illustrating critical flicker frequency threshold determination. At *S* the range of flash rates was changed without interruption of the run. *F*, food pellet.

Critical flicker frequency thresholds were obtained in these trained monkeys by placing the rate of flicker under the control of the animal. This was accomplished by providing 49 equal step changes in rate via a bothway stepping relay, so connected that a press on lever A increased rate one step, and a press on lever B decreased it. Reward procedures were changed at this point. A high rate of responding was maintained by aperiodically providing a continuous target (*Sx*) for an A response, after which a predetermined number of B responses produced the food pellet. During the *Sx* period responses did not affect flash rate, so that the rate existing just before *Sx* reappeared after the food interval. In this way the monkey was free to drive the flash rate up and down through the critical fusion zone with only occasional interruptions for delivery of food rewards. The frequency of this reward procedure varied considerably from monkey to monkey and from time to time, but the most com-