References and Notes

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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.

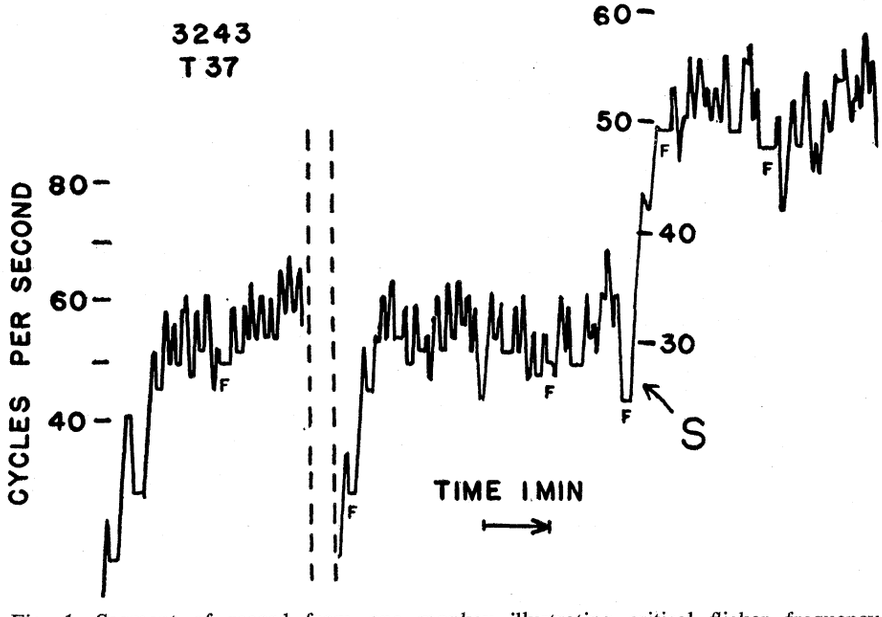

Fig. 1. Segment of record from one monkey illustrating critical flicker frequency threshold determination. At *S* the range of flash rates was changed without interruption of the run. *F*, food pellet.

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-

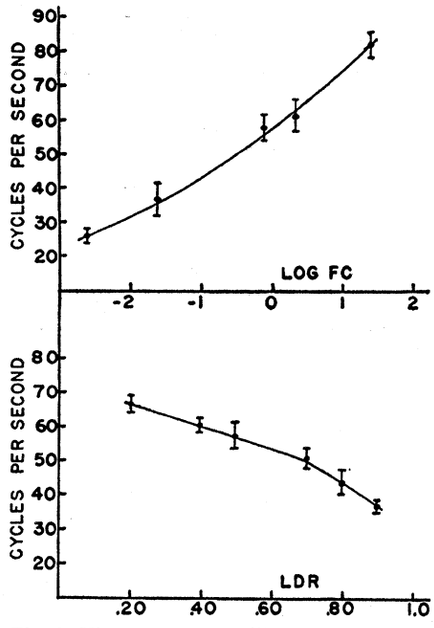

Fig. 2. The upper curve illustrates relationship of target brightness to critical flicker frequency at light-dark ratio (LDR) of .50. The lower curve illustrates relationship of LDR to the critical flicker frequency. Both curves are from the same monkey.

mon values were about 35 responses (both A and B) per reward, and about two rewards per minute.

The stepping relay that controlled flash rate was linked to a graphic recorder so that a continuous record of lever pressing behavior was obtained on a moving chart, calibrated directly in cycles per second for each range employed. Several somewhat arbitrary control procedures were established to determine whether the record being generated represented some form of oscillatory or random behavior or reflected attention to the flash rate by the monkey. These consisted of altering the range of frequencies available and introducing large changes in rate during a food interval. If the monkey produced a new stabilized record in agreement with that produced before the changes, the record was considered to be a reflection of the flicker fusion phenomenon. An example of such a record is seen in Fig. 1. These control procedures led to the rejection of from 20 to 40 percent of threshold records.

In order to compare results of this method with those obtained by different methods it was necessary to designate a critical flicker frequency threshold in cycles per second for a given monkey's performance under a particular set of target parameters. Reasoning that the end of an "A run," or increasing steps of flash rate, corresponded to the point when the target just became equivalent to *Sx* (by definition a fused light), I decided to use only those points for assigning threshold values. Acceptable sections of threshold record were analyzed for the range of those points (eliminating the one highest and lowest), and the midpoint of that range designated the critical flicker frequency, expressed to the nearest whole number of cycles per second.

Despite the rather crude method of assigning frequencies a useful degree of reliability has been obtained. Five of the six monkeys tested showed relatively stable critical flicker frequency thresholds (within 10 cy/sec) which did not change systematically with repeated testing. The most extensively studied animal has shown no improvement over more than 8 months of regular testing. The type of problem for which these procedures appear ideally suited involves the testing of thresholds at a number of values of some stimulus parameter, or following a threshold continuously as a function of some changing physiological variable. Examples of the former are shown in Fig. 2, which illustrates the effects of varying target brightness or light-dark ratio on the critical flicker frequency. Brightness was varied by means of neutral density filters in the light path, and light-dark ratio (more exactly, light to cycle ratio) was varied electronically, a compensated Talbot brightness of 0.8 ft-ca being used for all ratios.

The range of critical flicker frequencies under standard conditions (brightness 2.3 ft-ca, light-dark ratio = .50) observed in this series of monkeys has been wide, extending from just under 30 to about 61 cy/sec. Stability of threshold and wide individual differences have also been observed in a different series of 11 monkeys tested on a conventional "go no-go" type of discrimination (*5*). It appears that values of critical flicker frequency with the same target parameters have tended to be higher than those observed with the self-determination technique, but with a great deal of overlap. Consistent discrimination at very high rates (above 80 cy/sec) has been seen with the "go no-go" method but not with the self-determination method (*6*).

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3. I am indebted to D. S. Blough for many helpful suggestions regarding the development of these procedures.
4. The light source consisted of a Sylvania R113C glow modulator tube driven by a square-wave generator allowing independent adjustment of frequency, duty cycle, and peak current. Light pulses were monitored by a GE 930 photocell and found to be rectangular with extremely brief rise and decay times. I am indebted to John T. Conrad for help in designing the flicker circuits. Light pulses were viewed by the unrestrained monkey in the dark through a 3-inch diameter ground-glass target at distances between 1 and 6 inches.
5. A description of the details of method and results is in preparation. The procedure was a replication in most features of that used by M. Mishkin and L. Weiskrantz [*J. Comp. and Physiol. Psychol.* **52**, 660 (1959)].
6. This research was supported by grant B2681 from the National Institute of Neurological Diseases and Blindness, U.S. Public Health Service. A preliminary report was read at the Eastern Psychological Association meeting in April 1961.

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Fungilytic Activity of a Species of Verticillium

Abstract. A species of *Verticillium* arrested the growth of fungi by extracellular secretions on agar media. The lytic agent in the culture filtrates appeared to be thermolabile and was active in vitro on spores of *Puccinia graminis.* The possibility that the lytic agent is an enzyme has been investigated and purification studies are in progress.

Experiments with species of *Verticillium* have shown that these fungi produce an abundance of enzymes, among which pectin-splitting enzymes are most important (*1*). The discovery of these enzymes has constituted a major step forward in fundamental plant disease research, since it has been shown that the enzymes are responsible for wilt symptoms in susceptible plants.

A study has been made of the ability of *Verticillium* to suppress the growth of fungi, particularly by the production of antifungal enzymes. The most active *Verticillium* strain tested was isolated from a coffee plant disease (*2*).

It was observed that the *Verticillium* completely inhibited fungus growth in marked contrast with the corresponding control cultures without the *Verticillium.*

Direct attack by *Verticillium* sp. on spores and hyphae of *Hemileia vastatrix* has taken place in water. Washed spores of *Hemileia* were suspended in distilled water and incubated with an inoculum