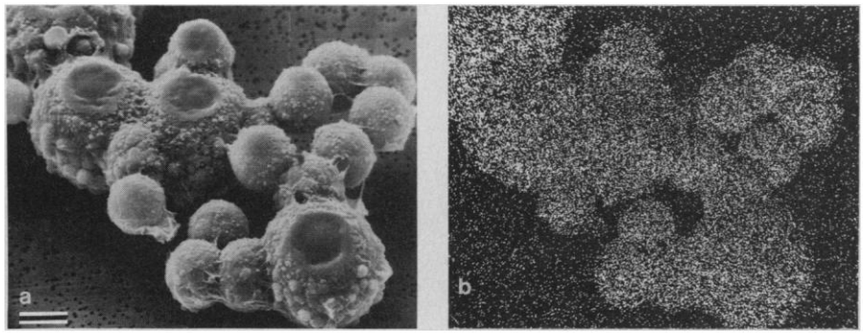

Fig. 1. (a) Scanning electron micrograph of *Chlamydomonas* spores on a Nuclepore filter. (b) X-ray scan (acceleration voltage, 20 kev) in the Mn range of the same area. Scale bar, 10 μm.

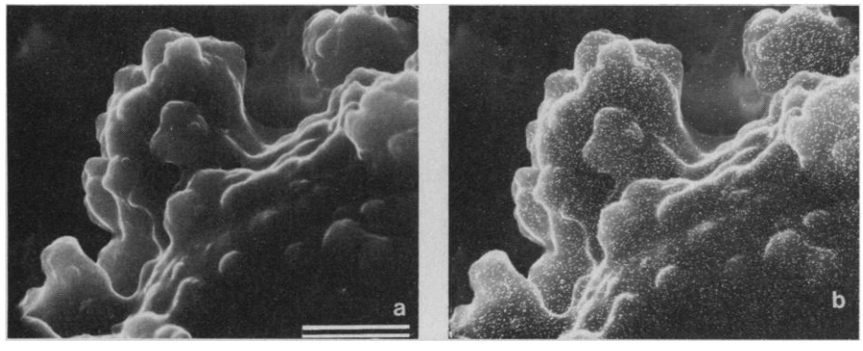

Fig. 2. (a) Scanning electron micrograph of *Chlamydomonas* cell wall encrustation (cell on Nuclepore filter). (b) Same as (a), with superimposed x-ray scan (acceleration voltage, 20 kev) in the Mn range. Scale bar, 5 μm.

mately (within 2 weeks) form a feltlike coat over most or all of the spore surface.

The encrustation is fragile. Pressure between a microscope slide and a cover slip causes the casing to crack into pieces, liberating the orange zygote. The fragility and the dark color of the casing material suggested that it might be composed largely of mineral matter. This was confirmed by physiological experiments, a chemical reaction, and x-ray analysis with a scanning electron microscope (SEM). The cells were cultured in a mineral nutrient medium containing (per liter) KNO_3 (180 mg), $MgSO_4 \cdot 7H_2O$ (50 mg), $CaCl_2$ (16 mg), NaH_2PO_4 (50 mg), vitamins B_1 (0.5 mg) and B_{12} (0.5 μg), and a mixture of trace elements comprising Fe, Mn, and B (0.5 mg) and Cu, Mo, and Zn (0.01 mg). Three or four days after gametes of the two mating types grown in this medium were mixed, brownish black spores formed at the surface of liquid cultures or on 1 percent agar media. On agar prepared with the same medium lacking trace elements, the spores developed normally but their cell walls remained thin and colorless. However, the sole addition of $MnCl_2 \cdot 4H_2O$ (10 mg/liter) to this medium resulted in the formation of black spores as before.

The cells were tested for Mn^{4+} by the benzidine reaction (*2*). The formation of benzidine blue around the mineralized zygospores, but nowhere else, indicated the presence of MnO_2 in the cell casings.

Black spores grown on agar were transferred to a membrane filter (pore size, 1 μm), fixed in 2 percent glutaraldehyde, washed, and desiccated by critical-point drying using Freon 113/13. They were then coated with carbon (for x-ray energy-dispersive analysis) or with gold/palladium (for scanning micrographs) and examined with a SEM (Cambridge model S-4) equipped with an x-ray energy-dispersive unit (Ortec DELPHI). A full x-ray spectrum from 0 to 20 kev (acceleration voltage, 20 kev) yielded two distinct peaks, corresponding to the K_α and K_β lines of Mn. Smaller peaks were identified as K_α lines of Si, P, K, and Ca. Figure 1a is a scanning electron micrograph of a group of zygospores and Fig. 1b, for the same area, is an elemental map for Mn, taken between energy-window limits of 5.73 and 6.04 kev (the Mn K_α line is at 5.89 kev). A small portion of one zygospore wall, shown in a scanning electron micrograph (Fig. 2a), was similarly subjected to an x-ray scan in the Mn range; the resulting image is shown, superimposed on the previous picture, in Fib. 2b. The topological coincidence of the dots indicates the association of Mn with the cell wall surface structures.

Studies of extracellular Mn oxidation and deposition, experimentally demonstrated in cultures of certain bacteria, fungi, and algae (*3*), may ultimately prove of importance in helping to elucidate the problem of Mn nodule formation in the deep sea.

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Double Branched Flicker Fusion Curves from the All-Rod Skate Retina

Abstract. *Electrical responses from the skate retina will only follow flicker up to frequencies of 5 hertz when intensities are below rod saturation. At greater luminances, the eye responds to rates as high as 30 hertz. As a result, a plot of critical flicker fusion as a function of intensity is a double branched curve. It seems that prolonged stimulation of skate rods, at high intensities, permits them to change their temporal response characteristics so that they follow high frequencies much as cones do in the duplex retina.*

Most vertebrates possess a duplex retina that allows them to sense changes in spatial and temporal patterns of light over an enormous range of light intensities. Rod signals mediate low-intensity (scotopic) vision, whereas the cone system subserves the upper (photopic) range of intensities. Consequently, measurements of visual acuity, critical flicker frequency, dark adaptation, and increment threshold as functions of light intensity yield curves which seem always to be divisible into two distinctly different portions. The kink in the curve marks the changeover from pre-

dominantly rod to predominantly cone control of the visual process. Neither receptor mechanism can be studied alone over an extensive range of photic stimuli, hence the search for an all-rod or all-cone retina. Although many animals have been reported to possess pure rod or pure cone retinas (*1*), this has turned out, in nearly every case, to be an oversimplification. A possible exception is the common skate (*Raja erinicea* and *Raja oscellata*), an elasmobranch fish, which appears to have only rods in its retina (*2*).

Several correlative studies of function and histology have provided compelling evidence that the slow but sensitive skate eye performs all of its visual functions with just a rod system (*2, 3*). It was surprising, therefore, to discover that *b*-wave responses of the skate electroretinogram (ERG) to a flickering light follow rates as high as 30 hertz. Moreover, a plot of flicker fusion frequency against the logarithm of stimulus intensity yields a curve having two distinct portions with the characteristic kink which is usually associated with a transition from rod- to cone-mediated activity (Fig. 1A). This double branched function could arise either because there are actually two types of receptor in the retina of the skate or because a single type of receptor, presumably a rod, somehow exhibits a duplex quality in its response.

To decide between these alternatives, we first determined the action spectrum for the low- and high-intensity segments of the curve; a shift in spectral sensitivity would indicate that there is more than one type of photoreceptor. For this purpose, we measured the spectral sensitivity of the *b*-wave with brief monochromatic stimuli, presented first to the dark-adapted and then to the light-adapted retina. Even with adaptation to light intense enough to cause the response to follow high-frequency flicker, the action spectrum was the same as that of the dark-adapted retina, corresponding to the absorption spectrum of rhodopsin. This is in complete agreement with the results of previous studies, which have failed to show a change in spectral sensitivity between dark- and light-adapted skate receptors and horizontal cells (*3*). Clearly, a rhodopsin-filled receptor was mediating the responses of the light-adapted retina to brief flashes, but was it also capable of responding to high-frequency flicker?

We suggest here that skate rods are able to alter their response characteristics under the influence of prolonged illumination, so that they can resolve intermittent stimuli well in the range normally considered to be related to cone function (*4*). To test this, we repeated the critical flicker fusion (CFF) experiment, using scotopically equated monochromatic light at wavelengths of 500 and 600 nm instead of white light. Equated stimuli were necessary because CFF thresholds became stabilized at each successive intensity only after several minutes had elapsed. In fact, if intense stimuli were presented, it took up to 20 minutes for maximal flicker following to occur. By balancing the stimuli, we hoped to maintain the same level of adaptation for the two colored stimuli. Following this procedure, when the lower (scotopic) segments of CFF curves were superimposed, the upper (photopic) branches were also coincident (Fig. 1B). For comparison, an identical experiment was performed on another fish, the sand dab (*Lophopsetta maculata*) which has both rods and cones (*5*), and a clear separation for the two wavelengths at photopic levels was produced (Fig. 1C).

That following high-frequency flicker is not a unique property of the *b*-wave was demonstrated by repeating the CFF experiments using the receptor potential which is isolated by applying sodium aspartate to the retina (*6*) and the intracellularly recorded S-potential (*7*), with essentially the same results as those shown in Fig. 1A for the *b*-wave.

Observation of S-potentials from horizontal cells during flicker stimulation provides some indirect evidence as to how the adaptation to intense intermittent luminances arises. Figure 2A shows horizontal cell responses to short bursts of 1-hertz flickering light at four different luminances. Successive increases in intensity produce deeper hyperpolarizations and clear flicker responses until the cell is saturated. At this point (last trace in Fig. 2A), the membrane potential has been driven to its maximum negative limit and the flicker response is extinguished. Note that the inflection point separating the two branches in Fig. 1A occurs at the same intensity I (approximately 25 quanta μm^{-2} sec^{-1}) that saturates the S-unit. Figure 2B shows that this is unlikely to be coincidental. When the flickering light is left on and the S-unit responses are monitored for several min-

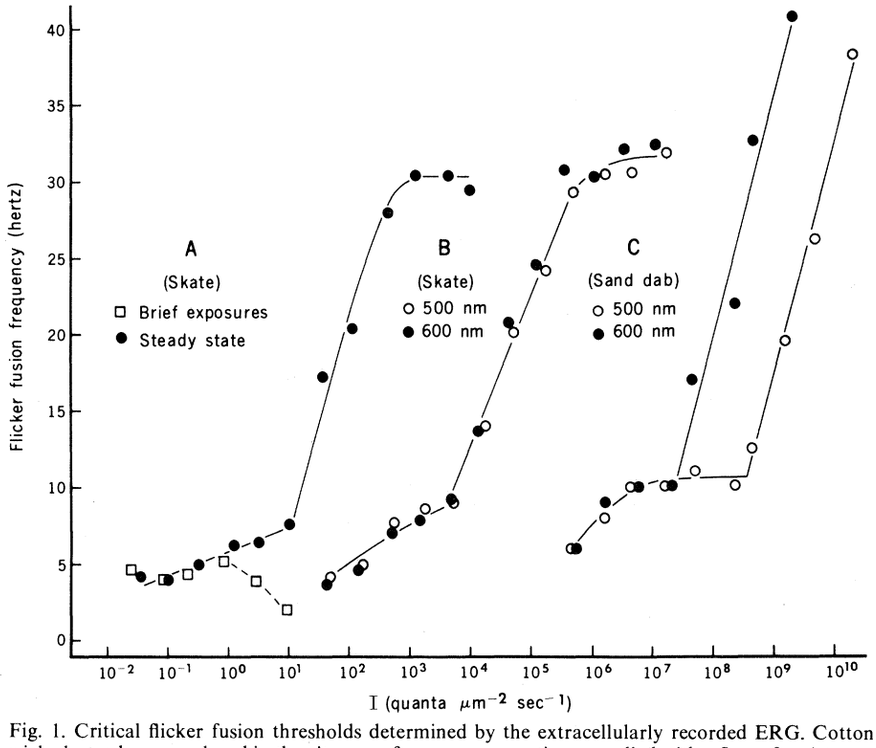

Fig. 1. Critical flicker fusion thresholds determined by the extracellularly recorded ERG. Cotton wick electrodes were placed in the vitreous of eyecup preparations supplied with a flow of moist oxygen. Temperature was maintained at 20°C. A beam of light was interrupted by a 50 : 50 sector disk coupled to a direct-reading tachometer, and brought to a focus size larger than the preparation. Threshold values were obtained by observing the oscilloscope screen for the appearance and disappearance of flicker wavelets (ascending-descending method of limits). Four determinations were made at each intensity, using a 5-μv signal as the threshold criterion. (A) Critical flicker fusion thresholds plotted against mean quantal absorbance with white light (*12*). (□) Values obtained a few seconds after stimulus onset; (●) those obtained after waiting for response equilibration at each luminance. (B) With a different preparation and narrow-band interference filters, CFF thresholds were determined with scotopically equated blue-green and red lights. Curves were shifted to superpose the lower limbs. The entire function is displaced to the right by about 2 log units. (C) Same experiment as (B) but with the cone-rich retina of the sand dab. Note that the sand dab follows considerably higher flicker frequencies than the skate, and that after the scotopic segments of the curves are superposed, the upper limbs are separated. This curve is also displaced along the abscissa.

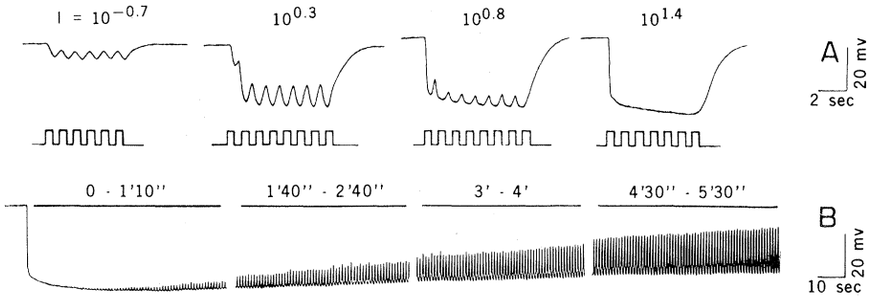

Fig. 2. Intracellular recording of horizontal cells (S-potentials) using glass micropipettes filled with 2*M* KCl. Signals were led through a Bak ELSA-4 negative-capacitance amplifier and written out on a Brush Mark 220 recorder. (A) Responses to short bursts of intermittent light of graded intensity, *I*. Although the more intense stimuli produced a deeper hyperpolarization of the unit, flicker resolution is progressively diminished until, at higher intensities, the membrane potential saturates. (B) Prolonged exposure of the brightest stimulus in the series ($10^{1.4}$ quanta μm^{-2} sec^{-1}), recorded at a paper speed five times slower than in the upper tracings. Note that only minimal flicker following is seen during the first minute. However, as the cell slowly adapts to the light, the membrane repolarizes and flicker responses become larger.

utes, the membrane slowly repolarizes. During this time, small flicker responses are detected, which continue to grow and finally reach maximal amplitude in about 6 minutes. More intense stimuli give rise to higher CFF values, but require longer waiting times for flicker recovery—for example, up to 20 minutes when $I = 10^4$ quanta μm^{-2} sec^{-1}.

The phenomenon we report here is closely related to the results of Dowling and Ripps (*2*), who recorded increment thresholds from the skate retina which, over a response range of 7 log units, followed the Weber-Fechner relation ($\Delta I/I$ = constant) without saturating. Their studies showed that ΔI is a time-dependent variable which, at high background luminances, required waiting periods of 20 to 30 minutes before stable values could be attained. In a later study (*3*), they suggested that this unusual property may reflect an underlying mechanism which exists to prevent saturation of skate photoreceptors. We believe that the same mechanism is responsible for the shifting of the skate photoreceptor to an operating characteristic which enhances its temporal resolution. This very slow process is in contrast to the rapid discrimination achieved by cones in mixed retinas when presented with high rates of intermittent stimulation.

The experimental data reported here seem to establish by conventional tests that all the responses are due to a single photoreceptor type—a rod. However, double branched curves for isolated receptor potentials, intracellular S-potentials, and ERG *b*-waves could also be explained by summation between receptor types. The spectral analysis shows that if this is the case all receptor types have the same photopigments. Final resolution of this point would seem to require intracellular recordings from individual skate photoreceptors. Nonetheless, the anatomical and physiological evidence makes it seem likely that there are no cones in the skate retina.

If skate rods have a dual response, is this property to be found in all rods, or is it unique to the skate rod? Only intracellular recordings of rods in other species (*8*) will satisfactorily answer this question. In any case, one requirement for efficient temporal resolution would be that the decay of the receptor potential change from the slow form normally typical of rods to the brisk off-response normally characteristic of cones (*9*). Such a property of skate rods, if it exists, is a subtle one, since no dramatic changes were noted in preliminary electron microscopic observations (*10*).

Whatever the mechanism, the end result is that the rods are able to resolve high rates of intermittent stimuli and increments against backgrounds far more intense than those required to produce short-term rod saturation (*11*). We suggest that this is possible because skate rods possess the means to alter the basic nature of their response to light and behave in a fashion very similar to that classically ascribed to cones.

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Lysergic Acid Diethylamide: Effect on Histone Acetylation in Rabbit Brain

Abstract. *Lysergic acid diethylamide increased acetylation of histones in rabbit cerebral hemispheres and midbrain 30 minutes after intravenous administration of the drug at doses of 10 and 100 micrograms per kilogram of body weight. Evidence for the stimulation of acetylation in individual histone bands was obtained after separation by electrophoresis on polyacrylamide gels.*

Little is known of the possible effects of psychotropic drugs on gene activity in the brain, particularly when the drug dosage is comparable to levels utilized by man. As covalent modification of chromosomal proteins may be associated with regulation of gene activity (*1*), we analyzed the effect of *d*-lysergic acid diethylamide (LSD) on the acetylation of histones in various regions of the rabbit brain. It has been shown previously that brain nuclei contain histone acetylase and that the activity of this enzyme increases with neural maturation (*2*). We now report the effect of LSD at dosages comparable to concentrations that cause distortion of sensory perception in humans (*3*).

LSD was administered intravenously to