Reports

Evidence That Retinal Flicker Is Not a Necessary Condition of Imprinting

Abstract. Thirty-six chicks were exposed to motionless geometric objects during the third, fourth, and fifth days of life to test the contention that retinal flicker is an irreducible condition of imprinting. The results indicated that the only necessary condition for a positive effect is that the model should be prominently displayed in the animal's visual environment.

Not the least disconcerting aspect of the recent revival of interest in imprinting is the various attempts by psychologists to elaborate precise laws from minimal evidence. Thus Hess has proposed a law that the strength of imprinting is equal to the logarithm of the effort expended by the animal in reaching the imprinting model (*1*), although the sizes of the samples used in the experiments from which this law was deduced were not reported (*2*). On the basis of more fully documented data, James has suggested that retinal flicker is not only a critical factor in the phenomenon of imprinting but that it should be considered as an unconditioned stimulus for the following response. In his experiment, chicks were exposed to a novel object in the vicinity of a flickering light source from the third day of age. These animals showed an attachment to the object not paralleled by a control group exposed to the same object in the vicinity of a constant light source. It is to be noted that, with the two light sources opposed, the chicks preferred the flickering light and did not frequent the area of the constant light which, for the control animals, contained the imprinting model (*3*).

In a reformulation of William James and Konrad Lorenz's historic conception of filial attachment, Moltz has raised to the near status of law the conclusion that retinal flicker is an irreducible condition of imprinting (*4*). If true, such a law would clearly be a matter of great theoretical importance. The present experiment testifies that such a law has no basis in fact.

Three groups of White Rock chicks were exposed to motionless black geometric objects for 24-hour periods; half of each group were exposed to a circle 3½ inches in diameter and half to a triangle 4 inches on a side; both objects were ¾ inch thick. The objects were visible through the glass walls of isolation booths (*5*). Each group was composed of 12 subjects, with the first group being exposed on the third day of age, the second group on the fourth day of age, and the third group on the fifth day of age. Each chick was hatched in an isolation compartment and, except for exposure and test periods, remained in visual isolation throughout the experiment. The incandescent light sources were powered by 60 cy/sec current, a frequency which James' data do not suggest is low enough to induce flicker in the chick.

At the end of their respective 24-hour exposure periods, the chicks were tested for their tendency to discriminate the familiar object from the other (unfamiliar) object. The test box was divided into three compartments in its longest dimension; the center compartment held a movable platform, connected by microswitches to electric timers, upon which a subject was placed; one end compartment held a circle model while the other end compartment held a triangle model; the compartments were separated by glass partitions. As in the exposure situation, the models were motionless. The test period was 15 minutes; final scores were expressed as time spent near the familiar model minus time spent near the unfamiliar model.

A statistical assessment of the data with parametric methods yielded the following results. The day-3 group mean of 3.65 minutes near the familiar model approached but did not attain significance at the .05 level with a two-tailed *t*-test against a null hypothesis of zero; the day-4 group mean of 5.77 minutes was significant at less than the .05 level; and the day-5 group mean of 7.83 minutes was significant at less than the .01 level. A between-within analysis of variance gave an *F* slightly less than 1, suggesting that the means were from a homogeneous population. The relative preferences for the familiar over the unfamiliar models are given in Fig. 1.

These results are unequivocal. Chicks will form an attachment to motionless objects even when the objects are not in the vicinity of a flickering light. In this experiment, however, the models were not an unobtrusive part of the environment. Against a background of light gray, the black geometric objects dominated the visual field. Probably anything that will make an object stand out in the chick's visual environment will be a factor in imprinting. Motion would thus be a factor, but it is not an irreducible condition, and neither is retinal flicker; nor is there any obvious merit in calling either motion or flicker an "unconditioned stimulus" for imprinting when the term has a definite meaning in the well-defined phenomenon of classical conditioning but is

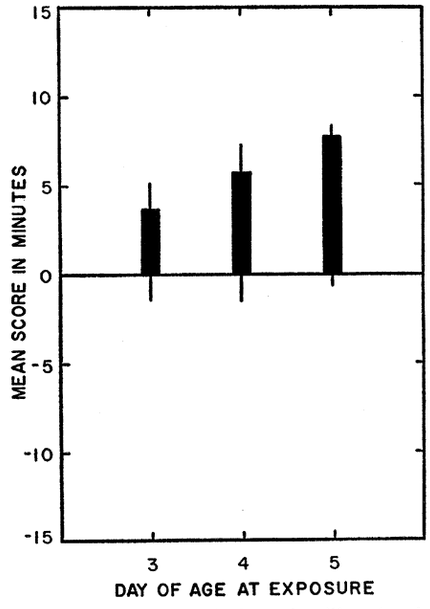

Fig. 1. Time spent before familiar (positive numbers) and unfamiliar (negative numbers) objects as a function of day of exposure. Bars indicate relative mean time and lines indicate absolute mean time either positive or negative. The figure could be thought of as a pictorial representation of the test apparatus platform with zero as the pivotal line.

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper.

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* **125**, 16 (1957)].

merely misleading in reference to imprinting.

It seems likely that James failed to obtain an effect with his control animals simply because the animals did not frequent the area in which the imprinting object was placed (*3*).

PHILIP HOWARD GRAY
Department of Psychology,
Montana State College, Bozeman

References and Notes

1. E. H. Hess, *Science* **130**, 133 (1959).
2. ———, *Ann. N.Y. Acad. Sci.* **67**, 724 (1957).
3. H. James, *Can. J. Psychol.* **13**, 59 (1959).
4. H. Moltz, *Psychol. Bull.* **57**, 291 (1960).
5. This procedure is described at greater length in D. M. Baer and P. H. Gray, *Percept. Mot. Skills* **10**, 171 (1960).

29 August 1960

Ribonuclease of Euglena gracilis

Abstract. An enzyme in extracts of *Euglena gracilis* splits both purine and pyrimidine internucleotide bonds of ribonucleic acid. Its *p*H optimum is at 4.5; it is very heat-labile and is rather insensitive to inhibition by metal ions or sulfhydryl group reagents. A partial purification of the enzyme is described.

Recently, several enzymes of the protistan *Euglena gracilis* were examined in connection with other metabolic studies. Because of the special taxonomic position of this type of organism, it seemed of interest to compare the properties of some of these enzymes with those of similar enzymes from plant and animal sources. This report deals with the isolation, partial purification, and properties of a ribonuclease from *E. gracilis*. Ribonucleases from a wide variety of tissues and organisms have been described in the literature (*1*, *2*).

Mass culture and harvesting of *Euglena,* as well as the extraction of the enzyme, were carried out as previously described (*3*). The ribonuclease assay was a modification of a published spectrophotometric procedure in which the ultraviolet-absorbing material remaining in solution, when the undegraded and partially degraded ribonucleic acid is precipitated from the reaction mixture by uranium acetate at *p*H 3.0, is determined at 260 mμ (*4*). The incubation was carried out at a *p*H of 4.5 in the presence of 0.05*M* acetate buffer for a period of 2 hours with 0.25 ml of the enzyme extracts or corresponding amounts of the purified fractions. The symbol Δ^{260}, which is used to express the enzymatic activity in Fig. 1 and throughout the text, represents the difference between the absorption in the experimental tubes and controls at 260 mμ.

The time course of the degradation of ribonucleic acid by the *Euglena* enzyme is shown in Fig. 1*A*. As shown in Fig. 1*B*, the enzyme has a very sharp optimum of activity at *p*H 4.5. This value is at the lower end of the range of *p*H optima (4.5 to 5.5) reported for a number of plant ribonucleases, while the enzymes of animal origin tend to have a higher optimal *p*H (*2*, *5*). Unlike a number of other ribonucleases, the *Euglena* enzyme was found to be extremely heat-labile. When the enzyme extract was heated to 68°C at a *p*H of 4.6 in the presence of 0.05*M* acetate buffer, all activity was lost within 2 minutes. As in the case of pancreatic ribonuclease (*6*), the activity of the *Euglena* enzyme increased with increasing ionic strength, with an optimum at approximately 0.5*M* sodium chloride (Fig. 1*C*). Metal ions, which have been shown to inhibit pancreatic ribonuclease (*7*), were found to affect the enzyme from *Euglena* at rather high concentrations only (Table 1). The sulfhydryl group reagents *o*-iodosobenzoate and *p*-chloromercuribenzoate, which were tested at concentrations from $10^{-5}M$ $10^{-3}M$, were without effect, except for *p*-chloromercuribenzoate, which had a somewhat inhibiting effect at the highest concentration.

An approximately 15-fold purification of the enzyme was achieved by two ammonium sulfate fractionations; in the first, at *p*H 8.5, the protein precipitating between 38 and 53 percent of saturation in ammonium sulfate was collected; in the second, protein precipitating at *p*H 7.0, between 39 and 51 percent of saturation. These fractionations were followed by the removal of inactive protein by adsorption on calcium phosphate gel (*8*) and a final ammonium sulfate precipitation, at *p*H 7.0, between 39 to 51 percent of saturation, to concentrate the enzyme solution. While the over-all yield in enzymatic activity by this procedure was only of the order of 20 percent, an essentially phosphomonoesterase-free ribonuclease was obtained.

Table 1. Inhibition of *Euglena* ribonuclease by various metal ions at four concentrations. Assay as in text.

Cation	Inhibition (%)			
	$10^{-5}M$	$10^{-4}M$	$10^{-3}M$	$10^{-2}M$
Fe^{+++}	16	20	65	100
Cu^{++}	6	11	40	76
Zn^{++}	19	18	23	96
Pb^{++}	17	43	48	81

The specificity of this enzyme preparation was examined by two methods. In the first, its activity against ribonucleic acid was compared to its activity against the so-called ribonucleic acid "core." This "core" is obtained by exhaustive digestion of ribonucleic acid by pancreatic ribonuclease, and it is resistant to further degradation by this enzyme (*9*). It has been shown to consist of a mixture of oligonucleotides containing mainly purine bases (*9*). When *Euglena* ribonuclease was allowed to act on this "core" and on undegraded ribonucleic acid at *p*H 4.5, values of 0.720 and 2.040, respectively, were ob-

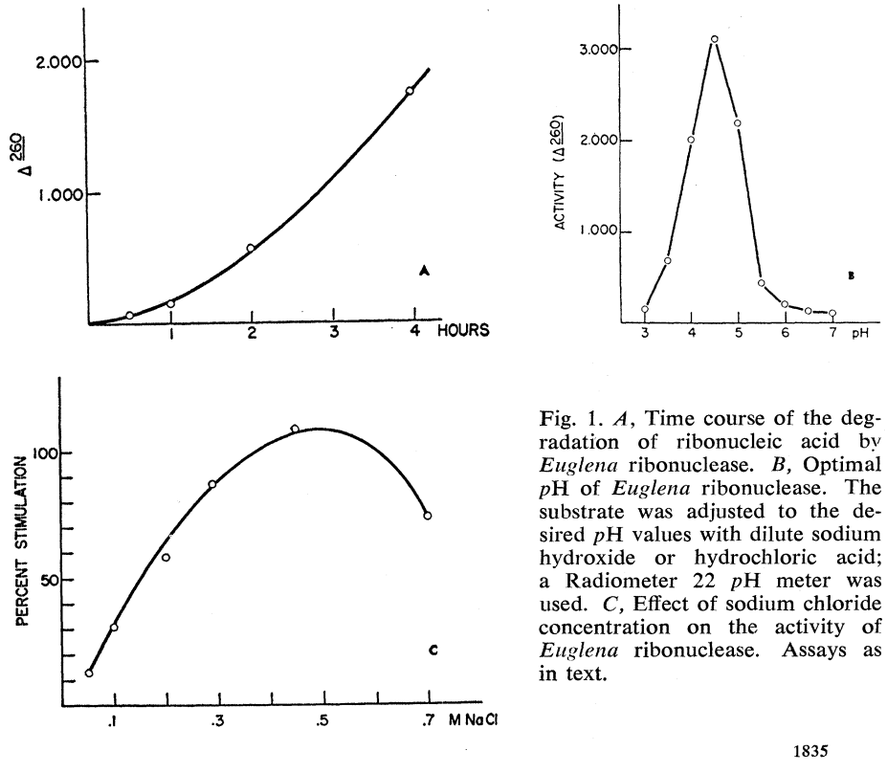

Fig. 1. *A*, Time course of the degradation of ribonucleic acid by *Euglena* ribonuclease. *B*, Optimal *p*H of *Euglena* ribonuclease. The substrate was adjusted to the desired *p*H values with dilute sodium hydroxide or hydrochloric acid; a Radiometer 22 *p*H meter was used. *C*, Effect of sodium chloride concentration on the activity of *Euglena* ribonuclease. Assays as in text.