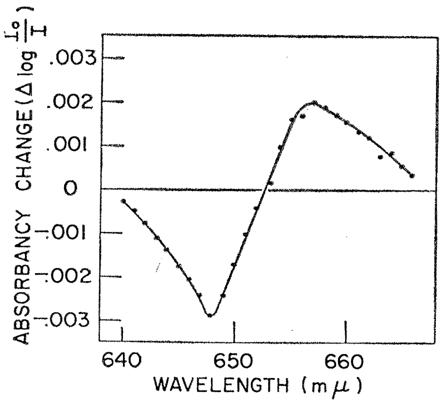

Fig. 1. The change in the absorption spectrum of a *Chlorella* suspension upon irradiation with blue light of high intensity (corrected for fluorescence).

from overloading the photomultiplier.

The changes in fluorescence due to the measuring beam were separated from absorption changes by two methods:

1) A narrow band interference filter, placed in front of the photomultiplier, was used to remove a large fraction of the fluorescence due to the measuring beam. A large fraction of the measuring beam passed this filter, which thus reduced the fluorescence effect compared with the absorption effect. Such an interference filter can be used effectively below 670 mμ; at 680 mμ its effectiveness is sharply reduced, since the fluorescence band itself has a peak at 680 to 690 mμ.

Results obtained by this method clearly show that a fluorescence effect is superimposed upon a positive absorption change at 657, and a negative absorption change at 648 mμ (Fig. 1). When Coleman's difference spectrum obtained in high-intensity light is corrected for the fluorescence effect, it agrees with these conclusions; and so do the observations of Strehler and Lynch (*4*) at low intensities of actinic light.

2) In the second method, we utilized the fact that the fluorescence in *Chlorella* is largely (97 percent) depolarized (*10*), while light transmitted in the forward direction is only slightly depolarized (5 percent). We introduced a polarizer in the measuring beam in front of the suspension, and an analyzer in the same beam between it and the photomultiplier. Measurements were made at a series of wavelengths, including 680 mμ, with the polarizer parallel to the analyzer and with the polarizer at 90° to the analyzer. Absorption changes should disappear (within the noise level of the instrument) on crossing the polarizer and the analyzer, while fluorescence changes should be reduced only by 3 percent. At 680 mμ the observed effect was not reduced markedly upon crossing the polarizers, suggesting that it is due predominantly or entirely to changes in fluorescence intensity. Measurements at shorter wavelengths gave results in agreement with those obtained with interference filters.

These results suggest that the 680-mμ band in the difference spectrum that appears in *Chlorella pyrenoidosa* in strong light, is not due to the absorption changes, but to changes in the yield of fluorescence caused by the measuring beam. This can happen not only in steady state measurements (with readings taken during the illumination), but also in "flash" or "flow" experiments where measurements are made in the dark after the flash, because, as Butler (*11*) has recently shown, the higher yield of fluorescence does not decrease immediately upon reduction of the intensity of exciting light, but decays slowly (for several seconds) until it reaches its steady value.

Experiments by Karapetyan, Litvin, and Krasnovsky (*12*), published while this paper was in preparation, suggest that not only the 680 mμ band in *Chlorella,* but also certain changes in the infrared region observed in the difference spectra of photosynthetic bacteria, are due to fluorescence.

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1 July 1963

Technique for Sustaining Behavior with Conditioned Reinforcement

Abstract. *Pigeons were intermittently reinforced with food for pecking at one key. Concurrent pecking at a second key intermittently produced conditioned reinforcers (the set of stimuli that accompanied food reinforcement, but not the food). Under these conditions, responding on the second key was maintained indefinitely. Rates and patterns of responding on the second key were a function of the schedule of conditioned reinforcement.*

In the present experiment, pigeons could peck at either of two keys which were available concurrently. Pecks on one key intermittently produced food on a variable-interval schedule of reinforcement. Pecks on the second key intermittently produced all of the stimuli associated with food reinforcement on the first key but it was impossible for the birds to eat, because the food magazine was raised for one-half second, so there was not time enough for the birds to reach the food.

More specifically, pigeons, maintained at 80 percent of their free-feeding weight, performed daily in a standard, two-key experimental space (*1*). Each daily session terminated after 50 food reinforcements were obtained. A yellow light and a blue light were projected upon the left key and right key, respectively. Responses on the left key were reinforced on a 3-minute variable-interval schedule with a 4-second access to the food magazine. This was accompanied by (i) the sound of the solenoid-operated magazine, (ii) the illumination of a magazine light, (iii) the absence of the key lights, and (iv) the absence of the two house lights which normally illuminated the experimental space. Responses on the right key were reinforced on an independent 3-minute variable-interval schedule with all of the above stimulus changes occurring for a duration of one-half second.

Figure 1 shows a final performance, a stable state which could be maintained indefinitely, under this procedure, for two birds. The left records are cumulative response records of performance maintained by the variable-interval schedule of food reinforcement. A constant rate of responding appropriate to this schedule of reinforcement was sustained at approximately 1.5 to 2.0 pecks per second.

The right records are cumulative response records of the concurrent performance on the right key. Although the rates of responding were approximately one-tenth of those on the left key, the amount of behavior was substantial and was sustained indefinitely. Other pigeons under similar experimental arrangements have persisted in this behavior for 8 months or longer.

In marked contrast to the above results, when pecking the left key was no longer followed by food reinforcement, pigeons stopped pecking the right key within one or two daily experimental sessions. Furthermore, pecking of the right key returned to full strength almost immediately when food reinforcement was reinstated.

The above results suggest, but do not conclusively demonstrate, that pecking of the right key was maintained by stimuli which were functioning as conditioned reinforcers. I finally demonstrated that this was indeed the case, by programming several multiple schedules on the right key while the concurrent food schedule on the left key remained unchanged. The remaining procedures and results will refer only to the right key. In each of the multiple schedules on the right key, the blue light on the key was alternated with a green light after every fifth food reinforcement on the food key. Different schedules of reinforcement were associated with the blue and green lights. The experimental conditions (specific multiple schedules) are listed in order in Table 1.

In the first multiple schedule (condition I), both stimuli on the right key were associated with the same variable-interval schedule of conditioned reinforcement. Conditions I, III, and V were exactly the same. Conditioned reinforcement did not occur (extinction) in the presence of the green stimulus in condition II, and did not occur in the presence of the blue stimulus in condition IV. Finally, in condition VI, the blue stimulus was associated with a fixed-ratio schedule of conditioned reinforcement. Figure 2 presents cumulative response records of right-key responding for a representative subject taken from the final session on each of experimental conditions I through VI. Each record begins with the third presentation of the blue stimulus in a given session. A downward arrow indicates the onset of the blue stimulus and an upward arrow indicates the onset of the green stimulus. The number of each record corresponds to the number of the represented condition. Records I, III, and V show that the rates of responding in the presence of the two stimuli were approximately equal when both stimuli were associated with the same schedule of conditioned reinforcement. Behavior in the presence of either stimulus was quite similar to behavior obtained in the first concurrent schedule (Fig. 1, right records). When either stimulus was associated with extinction, the response rate in the presence of that stimulus dropped markedly

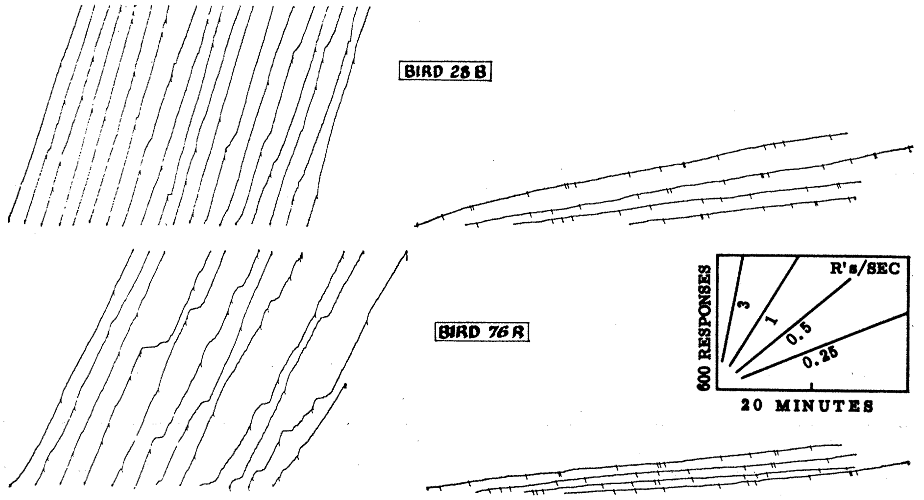

Fig. 1. Cumulative response records for two of the subjects from the final session on the concurrent schedule. Left records represent responding on the left (food) key. Right records represent responding on the right (nonfood) key. Pips indicate reinforcements.

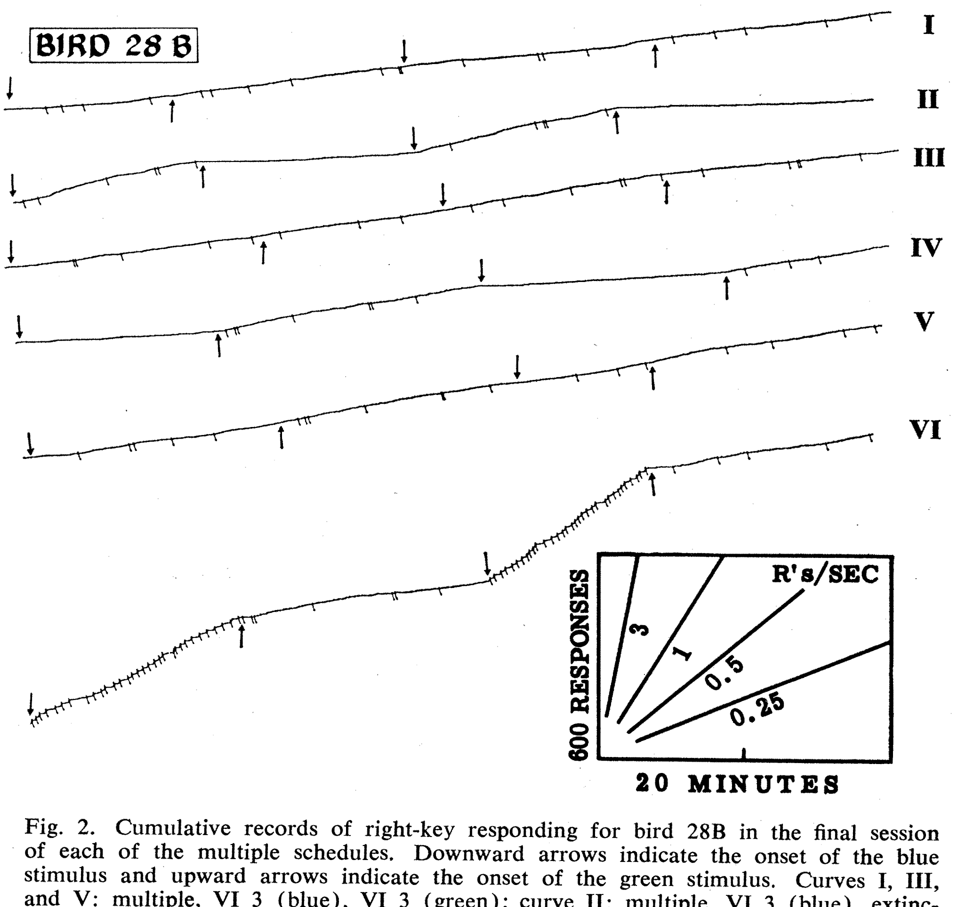

Fig. 2. Cumulative records of right-key responding for bird 28B in the final session of each of the multiple schedules. Downward arrows indicate the onset of the blue stimulus and upward arrows indicate the onset of the green stimulus. Curves I, III, and V: multiple, VI 3 (blue), VI 3 (green); curve II: multiple, VI 3 (blue), extinction (green); curve IV: multiple, extinction (blue), VI 3 (green); curve VI: multiple, FR 10 (blue), VI 3 (green).

Table 1. Right-key multiple schedules.

Condition	Blue schedule	Green schedule
I	VI 3*	VI 3
II	VI 3	Extinction
III	VI 3	VI 3
IV	Extinction	VI 3
V	VI 3	VI 3
VI	FR 10†	VI 3

* Three-minute variable-interval schedule.
† Fixed-ratio schedule of ten responses.

(records II and IV), demonstrating that the occurrence of conditioned reinforcement was necessary to maintain the behavior. Finally, when the two stimuli were associated with two different schedules of conditioned reinforcement (record VI), both the response rates and the patterns of responding were different in the presence of the different stimuli. Responding in the presence of the green stimulus was similar to that observed in any of the earlier variable-interval conditions, while responding in the presence of the blue stimulus was characteristic of behavior generally obtained on fixed-ratio schedules of primary reinforcement. Responding occurred at high steady rates prior to a conditioned reinforcement and pauses of varying durations occurred immediately after a conditioned reinforcement.

These results are qualitatively similar to those obtained with similar multiple schedules of primary reinforcement (*1*). The results obtained from the separate components of the multiple schedules are also similar to results obtained by Kelleher (*2*), who programmed different schedules of conditioned reinforcement during extinction of primary reinforcement.

I have reproduced these results with other subjects in situations that did not utilize multiple schedules but simply examined the various schedules one at a time. I have also demonstrated that these results depend upon the use of stimuli which are paired with primary reinforcement. (The presentation of a set of stimuli which are not associated with primary reinforcement does not maintain behavior).

Stimuli which initially do not influence behavior can acquire the power to reinforce behavior by being associated with primary reinforcement. Since conditioned reinforcement, as a principle, often bears a heavy load in the interpretation of behavioral events, the demonstration and assessment of the conditioned reinforcing function of stimuli is of prime importance to a scientific analysis of behavior. Much of the prior work in this area, however, has involved the examination of conditioned reinforcement after primary reinforcement has been discontinued. A major disadvantage of this approach is that "the effectiveness of the conditioned reinforcing stimulus is being extinguished while it is being evaluated" (*3*). In the present study, it was indeed observed that behavior maintained by conditioned reinforcement rapidly became extinguished after the removal of primary reinforcement.

When concurrent schedules of primary and conditioned reinforcement were in operation in the present study, behavior on the conditioned reinforcement key was virtually indefinitely maintained. This result is of significance both for a theoretical analysis of ongoing animal and human behavior and as a potentially powerful laboratory tool for the assessment of the effects of behavioral variables on both the establishment and power of conditioned reinforcers (*4*).

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Febrile Convulsions in Infant Rats, and Later Behavior

Abstract. *At 3 days of age, rats were (i) subjected to a febrile convulsion, or (ii) were handled, or (iii) were not handled. Evaluation at 30 days of age revealed that animals in the first group were heaviest and most resistant to audiogenic seizure stimuli. No differerences were found in maze-learning ability or activity level.*

Clinical reports (*1*) of the effects of infantile febrile convulsions on seizure susceptibility and other neurological and behavioral disorders in later life suggest that the experience of a febrile convulsion in infancy or childhood may occasionally result in permanent brain damage and a predisposition to recurrent seizures. Other forms of stimulation in infant animals have demonstrated a facilitation of subsequent behavioral performance (*2*). The present investigation was designed to determine the effect of inducing a febrile convulsion in infancy on the later behavior of the rat.

Thirty female albino rats (Sprague-Dawley) were bred in this laboratory and their litters assigned to one of three treatment groups: experimental, control (handled), or reference (not handled). At birth, the pups were removed from the nest (after the mother had first been removed), counted, and either replaced in the nest or discarded, so that there were no more than four males and four females, or a total of eight pups, in each litter.

At 3 days of age, when the rat's threshold for febrile convulsions is lowest (*3*), febrile convulsions were induced in the experimental group by a microwave diathermy generator (Burdick). The method described by Millichap (*3*) was employed with the diathermy unit, providing a stimulus of 2450-Mcy/sec frequency; the unit was operated at 80-watts power output. The animals' rectal temperature was monitored with an inserted thermistor. The control group was handled in an identical manner (at 3 days of age), except that the diathermy machine was not turned on. Since it required approximately 100 seconds for animals treated with the microwave diathermy to reach the convulsive temperature of 40.3°C (basal temperature 32.5°C), rats in the handled, control group had their temperature monitored for a similar time. Their body temperatures showed a drop from a basal temperature of 32.4° to 30.8°C. Animals from each litter nested in cedar shavings until the entire litter completed the treatment, and all were returned to their home cages. Animals in the reference group received no handling except that received at birth. None of the groups received any further handling until weaning at 30 days of age. Each pup was then weighed and evaluated for either learning ability on a Lashley III water maze, activity-wheel performance, or susceptibility to audiogenic seizures. All behavioral measures started when the animals were 30 days old.

The Lashley III maze was modified for use as a water maze with escape from water as the reinforcement. Total time and errors were recorded for the animals to reach the goal box, where they could escape from the water by climbing a wire ramp. Maximum time