

PERFORMANCE IN EYELID CONDITIONING AS A FUNCTION OF
REINFORCEMENT SCHEDULES AND CHANGES IN THEM*

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From the viewpoint of the *empirical construct type* of theory espoused by Hull^{1, 2} and the senior author,³⁻⁵ experimental studies of simple classical and instrumental conditioning are the source of the abstractive kind of theoretical concepts, i.e., intervening variables, that this theory employs. That is, in this type of approach the initial specification of the quantitative properties and interrelations of the intervening variables, both to each other and to the dependent (response) and independent (environmental) variables, is based on empirical laws obtained in these simple types of experiments.

The most highly developed portions of this theory have been concerned with the learning (*H*), incentive (*K*), and motivational (*D*) variables. Much less satisfactory is the part of the theory that deals with such nonreinforcement variables as delay of reward in instrumental conditioning and failure of occurrence of the reinforcing event in both classical and instrumental types of conditioning. Especially neglected have been the phenomena exhibited during learning under partial schedules of reinforcement in which different proportions of nonreinforcements are introduced intermittently during the acquisition period.

This latter area presents an especially formidable challenge to any attempt at theoretical integration, for the experimental findings as to the effects of introducing such nonreinforced trials during the acquisition of CRs have been highly conflicting, differing both within a situation and from situation to situation. In instrumental reward conditioning involving a chain of responses (e.g., runway), the recent studies of Goodrich,⁶ Haggard,⁷ and Weinstock⁸ have found that the effect of such intermittent nonreinforced trials differs not only with the stage of learning but also with the point in the response chain at which the measures are made. Thus, it has been found that partially reinforced *Ss* actually start and run faster *at the limit of learning* in the early portion of the response chain than *Ss* who have been reinforced on all trials. On the other hand, in the early stages of learning, this relation tends to be absent or the reverse. Finally, at the goal end of the chain, the running rate of *Ss* given partial reinforcement is consistently depressed throughout learning. A similar decrement in performance under partial reinforcement as compared with 100 per cent reinforcement has consistently been found in recent studies of classical aversive conditioning involving the human eyelid.⁹⁻¹³

Evidence as to the course of the development of the inhibitory effect under partial reinforcement is provided by comparing the performance curves of partial groups with that of a continuously reinforced group. The available data indicate that the decremental effect (i.e., inhibition) develops in a cumulative fashion and is a direct function of the proportion of unreinforced trials. The findings of the studies conducted in the Iowa laboratory also suggest that the inhibitory effect attains its maximum in considerably fewer trials than are required to reach the acquisition asymptote. Whereas the latter is typically attained in about 80-100 reinforced

trials, inhibition appears to reach its maximum somewhere between 20 to 40 non-reinforced trials, at least for a group on a 50 per cent reinforcement schedule.^{12, 14}

A further source of information concerning the properties of the inhibitory factor resulting from the intermittent nonreinforced trials during acquisition has been provided by shifting the schedule from a partial one (e.g., 50 per cent) to continuous reinforcement. Ross¹² shifted a group of *Ss* from 50 per cent to continuous reinforcement after a total of 40 trials (20 nonreinforced). Following the switch, the percentage CR curve accelerated markedly, and, after 80 continuously reinforced trials, closely approached the level of performance attained by a group continuously reinforced for the same number of trials (i.e., 120). This finding suggested the possibility that the inhibitory factor dissipates completely. However, more information is needed as to the role of the number and proportion of nonreinforcements before more general inferences concerning the dissipation properties of inhibition can safely be made.

The purpose of the present experiment was, in part, concerned with the extension of our empirical knowledge concerning the characteristics of the inhibitory effects of nonreinforcements administered during the acquisition of a classical aversive CR. From the point of view of our empirical approach to theorizing, such lawful knowledge, when attained, will provide the basis for specifying the properties of the intervening variable, inhibition (I_n). A second purpose of the study was that of ascertaining to what extent the data obtained in the experiment would or would not accord with the implications of statistical theories of learning. While there are difficulties in making decisions as to whether data from classical conditioning, with its particular type of reinforcement, are relevant to these theories, it was felt that it might be worthwhile considering their possible implications for this type of theory.

Method.—Subject: The *Ss* were 77 women from an introductory course in psychology. Of these, 12 were discarded because they met the criteria defining a voluntary responder^{15, 16} and 5 for the reason that they gave two CRs in the initial test trials to the CS alone. The remaining 60 *Ss* were assigned at random to the three groups of 20 *Ss* each.

Apparatus: The equipment for recording the response and controlling the presentation of the stimuli was the same as that used in previous studies from the Iowa laboratory.¹⁷ In the present instance, the UCS was a 2.0-psi air puff of 50 msec duration delivered to the right eye through an orifice 0.062 inch in diameter. The CS consisted in an increase in brightness of a 6-cm circular milkglass disk from a level less than 0.004 to 2.95 mL. The word "ready" preceded each presentation of the CS by 2, 3, or 4 sec according to a prearranged schedule.

Procedure: Each *S* was instructed to blink once to the ready signal and then to look at the disk on the wall and remain as relaxed as possible until the light went out. Following the reading of the instructions, three presentations of the CS alone were given in order to check for any existing CR tendency to the light. One presentation of the UCS alone was then given to acquaint *S* with the nature of the air puff.

Two different kinds of trials occurred during the course of training: (a) reinforcing trials (N+) in which the CS-UCS interval was 500 msec; (b) nonreinforcing trials (N-) in which the UCS followed the onset of the CS by 2,400 msec. This latter interval has been shown to produce little or no conditioning of the eyelid

response and to result in its extinction when introduced after establishing a CR at a shorter, optimal interval.^{18, 19} On both types of trials, the CS extended 50 msec beyond the onset of the UCS, both stimuli terminating together.

Two different reinforcement schedules, one partial (50%) and one continuous (100%), were employed. In the partial schedule, the sequence of reinforced and nonreinforced trials was such that the prescribed percentage of reinforced trials occurred within each block of 10 trials, with the additional restriction that no more than two trials of the same kind (N+ or N-) occur in succession. Inter-trial intervals of 15, 20, and 25 sec, averaging 20 sec and arranged by a fixed schedule, were used.

A CR was identified as a deflection of the pen of 1 mm or more that occurred in the interval 200–500 msec following the onset of the CS.

Experimental design: The conditions of reinforcement obtaining on the training trials given each *S* are summarized in Table 1. As may be seen, this design affords

TABLE 1
REINFORCEMENT CONDITIONS IN DIFFERENT TRIAL BLOCKS FOR THE THREE GROUPS

Group	Trials		
	1-100	101-200	201-250
I	50%	100%	50%
II	50%	50%	100%
III	100%	50%	100%

a comparison of performance under continuous reinforcement following two different amounts of training on 50% partial reinforcement (Groups I and II) as well as following extended periods of both continuous and partial reinforcement (Group III). This design also provides an opportunity to check the rather startling finding of Ross¹² that *Ss* shifted from continuous reinforcement to a 50% reinforcement schedule dropped immediately, i.e., after two or three nonreinforcements, to a level of performance characteristic of the 50% schedule.

Results and Discussion.—Decremental effects of partial reinforcement: Acquisition curves, in terms of percentage of CRs as a function of the number of reinforced trials, are presented in Figure 1 for Groups I and II, that started with 50 per cent reinforcement, and for the continuously reinforced Group III. The difference between the two partial groups over the first block of 50 trials was apparently due to sampling, for they received identical treatment during this period, and this difference was not significant (Mann-Whitney *U* test, $P > 0.20$). As may be seen, the performance curves of the partial groups fall below that of the 100 per cent group. The rate of development of this decrement in performance for the partially reinforced groups was negatively accelerated, and appeared to reach maximum between 30 and 40 nonreinforcements, a value similar to that obtained by Ross¹² for a similar comparison. Whether this value is dependent on the proportion of nonreinforcements, 50 per cent in the present experiment, is not known.

In order to provide further information on the nature of the decrement under partial reinforcement, the data of Group II were analyzed in terms of the conditional probability of a CR on the trial following a reinforced trial and following a non-reinforced trial. Acquisition curves plotted separately for these two conditional probabilities started at approximately the same performance level at the beginning of training and then gradually diverged, with the probability of a CR following a

reinforced trial rising to a significantly higher asymptote than the probability of a CR following a nonreinforced trial ($P < 0.01$). Over the last 20 reinforced and 20 nonreinforced trials, the mean number of CRs following a reinforced trial was 9.25 and following a nonreinforcement 6.85.

It should be noted, however, that the performance of the partial group following a reinforced trial is still significantly below that reached by Group III after an equivalent number of reinforced trials under continuous reinforcement ($P < 0.01$, Mann-Whitney U test). This means, of course, that the overall performance decrement under partial reinforcement is not mediated solely by a decrease in the probability of a CR on trials following a nonreinforcement. Response probability following a reinforced trial is also decreased, and in the present case the difference between the performance of Group III on continuous reinforcement and the performance of Group II following a reinforced trial is approximately five times as large as the difference between performance following a reinforced

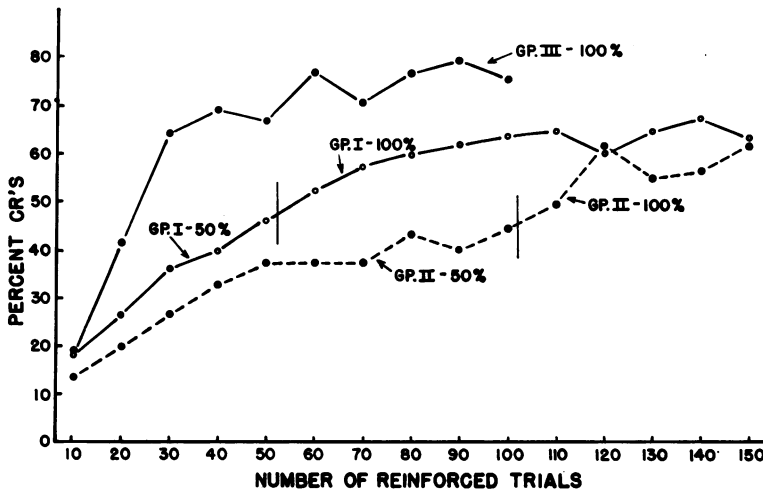


FIG. 1.—Performance as a function of number of reinforced trials. Note that only the first 100 trials for Group III are represented here.

trial and performance following a nonreinforced trial for Group II. Thus, it would appear that the most important factor determining the decrement under partial reinforcement is the cumulative effect of a number of interspersed nonreinforced trials rather than an effect dependent only on whether the immediately preceding trials were reinforced or not.

Shift from partial to continuous reinforcement: The performances of the partial groups following a shift to 100 per cent reinforcement are revealed by the curves for Groups I and II that extend to the right of the two vertical lines in Figure 1. In Group I, which had 100 trials on the original 50 per cent schedule, it may be seen that the curve rises to a level of about 62.5 per cent in the first 50 trials. Group II, which was on the 50 per cent schedule for 200 trials before the shift, also shows an increase to a level slightly but not significantly less than that of Group I. When tested by the Wilcoxon T test, the increase in percentage CRs from reinforced

trials 41-50 to 91-100 for Group I and from reinforced trials 91-100 to 141-150 for Group II are both significant beyond the 0.01 level.

Although not pictured in Figure 1, Group III also provides an instance of a shift from partial to continuous reinforcement. Group III received 100 trials on 100 per cent followed by 100 trials on 50 per cent reinforcement before being shifted back to 100 per cent reinforcement for 50 trials. In this last shift, Group III performance increased from the level characteristic of partial reinforcement to almost exactly the level reached by Groups I and II following their shift to continuous from partial reinforcement. This fact is shown in Figure 2 in which these three partial-to-continuous shifts have been superimposed on the same set of coordinates. It can be seen that regardless of whether the shift is preceded by 100 partial trials (Group I), 200 partial trials (Group II), or 100 continuous and 100

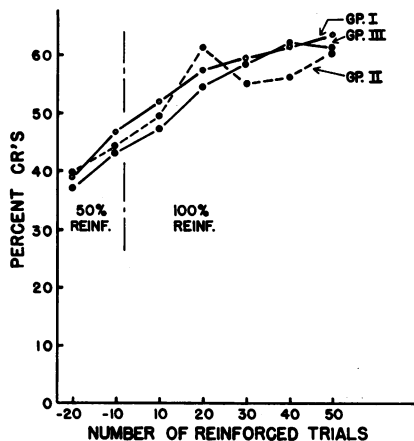


FIG. 2.—Three instances of increases in performance following shifts from 50 per cent to 100 per cent reinforcement.

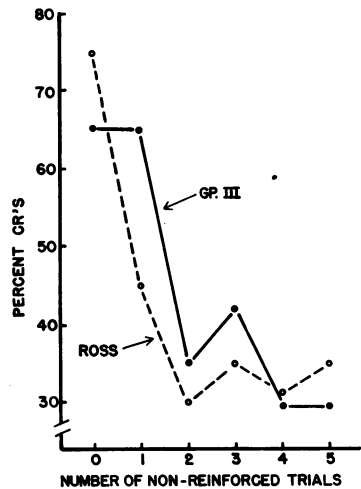


FIG. 3.—Two instances of decreases in performance following shifts from 100 per cent to 50 per cent reinforcement, plotted as a function of individual trials. See text for explanation of the curve labeled "Ross."

partial trials (Group III), all three curves begin at approximately the same point and increase at the same rate to very similar final performance levels. This final level, it should also be noted, is considerably below the asymptotic performance reached by Group III in its initial period of continuous reinforcement. The decrement was significant in the case of Groups II ($P < 0.01$) and III ($P < 0.02$) but failed to reach statistical significance in the case of Group I.

These data, then, indicate that a series of 100 trials under 50 per cent partial reinforcement introduces a decremental or "inhibitory" factor that later serves to decrease performance under continuous reinforcement.

Shift from continuous to partial reinforcement: The shifting of Group III from continuous to partial reinforcement after 100 trials provides a replication of the conditions employed by Ross,¹² and the findings are very similar, as may be seen in Figure 3. Only two nonreinforced trials were necessary to reduce performance

from about 70 per cent to about 30 per cent CRs. This stands in sharp contrast to the minimum of 50 reinforced trials necessary for the complete shift in performance when the change is from partial to continuous reinforcement.

As Ross¹² has pointed out, this abrupt change in performance is not in line with a theory espousing a gradual growth of inhibition. He suggested that the effect may be the result of some as yet little understood "set" variable which comes into play when a disruption is introduced into a previously regular and highly predictable sequence of events. Some support for this is provided by the Ross data in which a much more gradual decrease in performance was obtained when the shift to partial reinforcement was preceded by only 20 continuously reinforced trials than when it was preceded by 100 continuously reinforced trials. The combination of such a "set" effect and the gradual growth of inhibition with nonreinforced trials could have produced the observed pattern of results.

Relation to statistical theories of learning: Inasmuch as Estes has recently reaffirmed the applicability of his statistical learning theory to at least the gross aspects of classical conditioning,²⁰ it is interesting to compare the results of this experiment with the predictions of the theory. According to the theory, the parameter (π) that represents the predicted asymptotic response probability should be identical with the proportion of trials that are reinforced. Thus, groups receiving 50 per cent partial reinforcement should approach 50 per cent CRs as an asymptote, and continuously reinforced groups should approach an asymptote of 100 per cent CRs. However, as Figure 1 shows, Group II clearly levels off at an asymptote closer to 40 per cent than to the predicted 50 per cent value, and Group III, in accord with most previous eyelid conditioning data with continuous reinforcement, does not even approach the predicted asymptote of 100 per cent CRs.

From the point of view of making precise predictions about the response asymptote in eyelid conditioning, there are probably two confounding factors that must be considered. First, although we have as yet been unsuccessful in developing a criterion for their elimination, anyone who has worked with human eyelid conditioning is aware that there are *Ss* who could be labeled "inhibitors"; i.e., *Ss* who voluntarily or involuntarily inhibit the CR on some proportion of the trials. Second, there is the problem of "voluntary" responders. Some success has been achieved¹⁶ in developing a criterion for the elimination of *Ss* who respond with a high incidence of voluntary responses. This criterion, however, has only been concerned with the elimination of *S's* entire data from consideration in the results of an experiment, and as yet there are no procedures for the elimination of *individual responses* of the voluntary form that are made by *Ss* who do not give them frequently enough to be excluded entirely from consideration. Thus, it is quite likely that some percentage of the responses in any eyelid experiment, even though the presently available voluntary criterion is employed, are actually made voluntarily and hence confound the results. The relative frequency of these two opposing and confounding factors probably varies with a large number of experimental conditions, the net effect being sometimes to increase and sometimes to decrease average performance. In the absence of a criterion for clearing the data of these confounding effects, another approach seems *a priori* reasonable. Since inhibitors would most likely be *Ss* with very low conditioning scores, and voluntary responders *Ss* with very high scores, some middle range of *Ss* might be rela-

tively free from those confounding effects. Accordingly, we arbitrarily chose the middle 40 per cent of the *Ss* from each group and plotted their average conditioning curves in Figure 4. Also plotted is the curve for the middle 40 per cent from Ross' partial reinforcement control group.¹² With this procedure, the curves of both partial reinforcement groups, it will be noted, level off very close to the predicted 50 per cent point, and the curve for the 100 per cent group comes much closer to the predicted 100 per cent performance.

A further implication of statistical learning theory is that the parameter representing the rate of change in performance (θ) should be independent of the percentage of reinforced responses or the direction of the change in the reinforcement schedule. Thus, the original acquisition curves for both the partial reinforcement and the continuous reinforcement groups of this experiment, as well as the curves

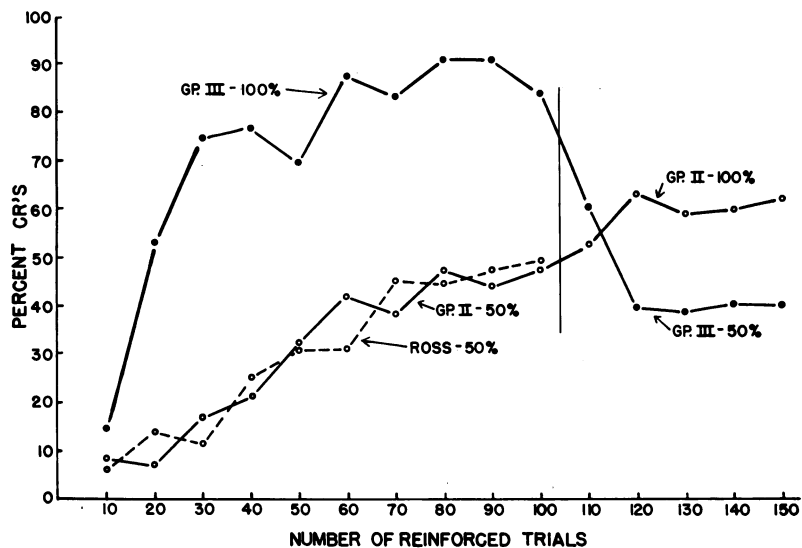


FIG. 4.—Performance on 50 per cent and 100 per cent reinforcement and performance following shifts from one to the other, as a function of the number of reinforced trials. See text for explanation of the curve labeled "Ross."

describing performance changes with shifts in the reinforcement schedule from continuous to partial or vice versa, should all have the same rate parameter.

For changes in reinforcement percentage, this is certainly not the case in this experiment. The drop in performance following a shift from 100 per cent to 50 per cent reinforcement clearly is much faster than the increase in performance following the shifts from 50 per cent to 100 per cent. Moreover, though less strikingly so, the rate of acquisition appears to be slower under 50 per cent reinforcement than under 100 per cent reinforcement.

Finally, we earlier observed that groups switched to continuous reinforcement after a series of trials on partial reinforcement never reached the performance level of a group conditioned under continuous reinforcement from the beginning of training. This also is not in accord with prediction from a model which assumes that performance asymptotes are determined solely by the parameter

π . According to this assumption, all groups on continuous reinforcement, regardless of their prior experience, should eventually reach the same asymptotic response level. At the very least, this finding suggests that a model capable of handling eyelid conditioning data will have to make the asymptote parameter a function of the reinforcement history of the *S* as well as of the immediate reinforcement conditions.

Summary.—An experiment was conducted on partial reinforcement in human eyelid conditioning. Group I received 100 trials of 100 per cent reinforcement, followed by 100 trials of 50 per cent reinforcement, and then 50 more trials of 100 per cent reinforcement. Group II received 100 trials of 50 per cent reinforcement followed by 100 trials of 100 per cent reinforcement, and then 50 more trials of 50 per cent reinforcement. Group III received 200 trials of 50 per cent reinforcement followed by 50 trials of 100 per cent reinforcement. In all groups, a reinforced trial involved a 500 msec CS-UCS interval and a nonreinforced trial of 2,500 msec CS-UCS interval.

The major findings of this experiment were: (1) Partial (50 per cent) reinforcement led to a significantly lower asymptotic level of per cent CRs than 100 per cent reinforcement. (2) A significantly lower level of per cent CRs occurred on trials immediately following a nonreinforced trial than following a reinforced trial, but this difference was not large enough to account for the entire performance difference between 50 per cent and 100 per cent reinforcement. (3) When reinforcement was shifted from 50 per cent to 100 per cent, the per cent of CRs showed a gradual increase to a higher asymptotic level, but one which was significantly lower than that reached by *Ss* conditioned with 100 per cent reinforcement from the beginning. (4) Performance changes following shifts in reinforcement from 50 per cent to 100 per cent followed the same course regardless of whether the shift was preceded by 100 trials of 50 per cent reinforcement, 200 trials of 50 per cent reinforcement, or 100 trials of 100 per cent and 100 trials of 50 per cent reinforcement. (5) When reinforcement was shifted from 100 per cent to 50 per cent, only about two nonreinforcements were necessary to cause performance to drop to approximately the level reached by *Ss* given 50 per cent reinforcement from the beginning.

These results are discussed in relation to those obtained in previous investigations, in relation to possible sources of performance decrement under partial reinforcement, and in relation to predictions made by statistical learning theory.

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*CELLULAR DIFFERENTIATION IN COLONIES DERIVED FROM
SINGLE CELL PLATINGS OF FRESHLY ISOLATED CHICK EMBRYO
MUSCLE CELLS*

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The loss of cell-type specific characteristics which frequently accompanies growth *in vitro* was recognized almost from the inception of the tissue culture technique.¹ This phenomenon even more acutely attends the application of the newer methods of cell culture in which trypsin-dispersed cells are serially cultivated on glass substrata as rapidly proliferating cell monolayers. The properties of cell strains so cultivated, with remarkably few exceptions, do not resemble those of the predominant cell type of the tissue of origin. The precise nature of this loss of identifiable characteristics remains obscure. Alterations in members of the culture population² as well as selection of a rapidly growing contaminant or a variant arising in culture³ could very well be responsible for the observed loss. Alternatively, it has recently been suggested by Sato and his co-workers^{4, 5} that only a small fraction of the cells present in normal tissues can propagate under the conditions of cell and tissue culture techniques. Further, it is assumed that this small fraction represents a class of cells present in all tissues which can be cultivated and which does not share the properties of the parenchymal cells of these various tissues.

Analysis of the problem of the loss of differentiative character is complicated by the disparity between cell culture and the culture techniques generally employed to promote cellular differentiation. Indeed, cultivation of dispersed cells rather than a compact aggregate would be precisely the condition judged to promote such loss. In a previous publication, it was demonstrated that a high degree of differentiation was attained by embryonic chick skeletal muscle cells grown in monolayer cultures.⁶ These cultures were initiated with 10⁶ cells, and the first indices of differentiation coincided with the attainment of confluency. The present report extends this finding to cultures initiated with small numbers of cells (200