# THE CONDITIONED LICKING RESPONSE IN RATS AS A FUNCTION OF THE CS-UCS INTERVAL

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# THE CONDITIONED LICKING RESPONSE IN RATS AS A FUNCTION OF THE CS-UCS INTERVAL

By

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ABSTRACT OF A MASTER'S THESIS

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Using Weisman's new method of classically conditioning the licking response in rats, the acquisition of test trial responses and anticipatory CR's was compared over five CS-UCS intervals (0.5", 1", 2", 4", 6"). It was expected that the optimal interval would be longer than one half second because of the nature of the UCR and the type of organism employed. In addition, a comparison between two strains of rats, albino and grey, was made.

The results were as follows: (a) The optimal interval was two seconds with four seconds being nearly as good. (b) The one-half, one, and six second groups were not significantly different from one another. (c) The two optimal intervals were significantly better than each of the other three intervals. (d) Data for CR's and test trials indicated the same trends although the differences were greater

for the CR's. Reasons for that difference were discussed.

(e) There was no genetic difference or interaction over temporal groups.

Approved: M. Ray Denny
Committee Chairman

Date: Aug. 13, 1964

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A THESIS

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# INTRODUCTION

The notion that there is no single optimal CS-UCS interval in classical conditioning dates back to Pavlov (1927) who found a variety of temporal intervals up to five seconds to be equally efficacious. American investigations in the interim, however, have largely neglected using the consummatory response as a UCR. A preponderance of data from eyelid and finger withdrawal conditioning studies has consistently indicated an optimal one-half second interval. Exceptions are evident in procedures utilizing the responses of pupillary dilation and GSR (Jones, 1962). In work with other organisms, an optimal interval of a different length is often found. Table 1 lists a cross section of the various procedures and organisms employed in classical conditioning.

TABLE 1

EXPERIMENTAL STUDIES OF CS-UCS INTERVAL EFFECTS

| INVESTIGATOR | UCR | ORGANISM | OPTIMAL CS-UCS INTERVAL |
|---|---|---|---|
| Kimble (1947) | Eyeblink | Human | .4" |
| Kimble, Mann, and Dufort (1955) | Eyeblink | Human | .5" |
| Schneiderman and Gormezano (1964) | Nictitating Membrane | Rabbit | .25" |
| Cohen (1950) | Flexion | Sheep | 0 |
| Kappauf and Schlosberg (1937) | Respiration | Rat | 1.5" |
| Gerall and Woodward (1958) | Pupillary Dilation | Human | 1.5" |
| Bierbaum (1959) | GSR | Human | 3" |
| Jones (1962) | GSR | Human | 1" |
| Spooner and Kellogg (1947) | Finger Withdrawal | Human | .5" |
| Wolfe (1930) | Finger Withdrawal | Human | .5" |
| Pavlov (1927) | Salivation | Dog | 0" - 5" |
| Goldstein et al. (1964) | Nictitating Membrane | Frog | 2" |

In recent work at this university, R. G. Weisman has developed a new method of classical conditioning in the rat. The UCR is the consummatory response of licking for water. Weisman's doctoral thesis (1964) is based on a concurrent technique which allows comparisons of classical conditioning and instrumental conditioning using the same licking response. His data on successive acquisitions and extinctions, on partial reinforcement effects, and the controls for pseudo-conditioning and superstitious behavior combine to demonstrate evidence for a genuine classical conditioning procedure.

Indications that the licking response in the rat is highly reflexive (a good UCR) can be found in studies by Davis and Keehn (1959) and Keehn and Arnold (1960). They found the mean licking rate in adult rats to be a consistent six or seven licks per second -- over various levels of thirst, for water, sucrose, saccharin, and saline solutions. The question of innateness was answered by Schaffer and Premack (1961) who observed weanling rats to lick in the same range as adults upon first contact with water.

With a new procedure of classical conditioning available and the obvious critics awaiting data (Kimble, 1964), the need for a CS-UCS interval study was present. A standardized CS-UCS study would permit comparisons with other techniques and also establish the most efficient ISI for this method. The apparatus designed by Weisman was

easily modified to allow variance of stimulus intervals and confinement of the S to insure minimal competing responses during the delay of reinforcement period (after Carlton -- in Spence, 1956). In view of the research done with the other consummatory response, salivation, the expectancy of results was only that the optimal interval need not be 0.5 seconds to reflect a true classical conditioning method.

## METHOD

Subjects - The S's were forty experimentally naive rats about four months of age, which were divided equally into two groups of Long-Evans greys and Spartan Colony albinos. Strict conditions of deprivation were imposed on all S's as follows: First, five days of ten minutes of water per day; second, two days of four minutes of water per day; third, on training days, only two minutes of water outside the apparatus. All S's were maintained in individual cages for the twelve day period with *ad lib.* food. Each animal was run at approximately the same time each day, and all training was administered in the evening.

Apparatus - The apparatus used was basically that built by Weisman to test concurrent classical and instrumental conditioning. Two matched systems were incorporated to allow the simultaneous conditioning of two animals. During the experimentation, each licking apparatus was enclosed in a converted refrigerator to insure proper sound insulation. Each refrigerator contained a 60 ft./min. exhaust fan and a white noise speaker for masking apparatus clicks. An idealized representation of one of the "licking boxes" is shown in Figure 1. The wooden insert in the licking box was designed to permit more confinement of the animal that in Weisman's case. The resultant interior dimensions were five inches in length, one and three-quarters inches in

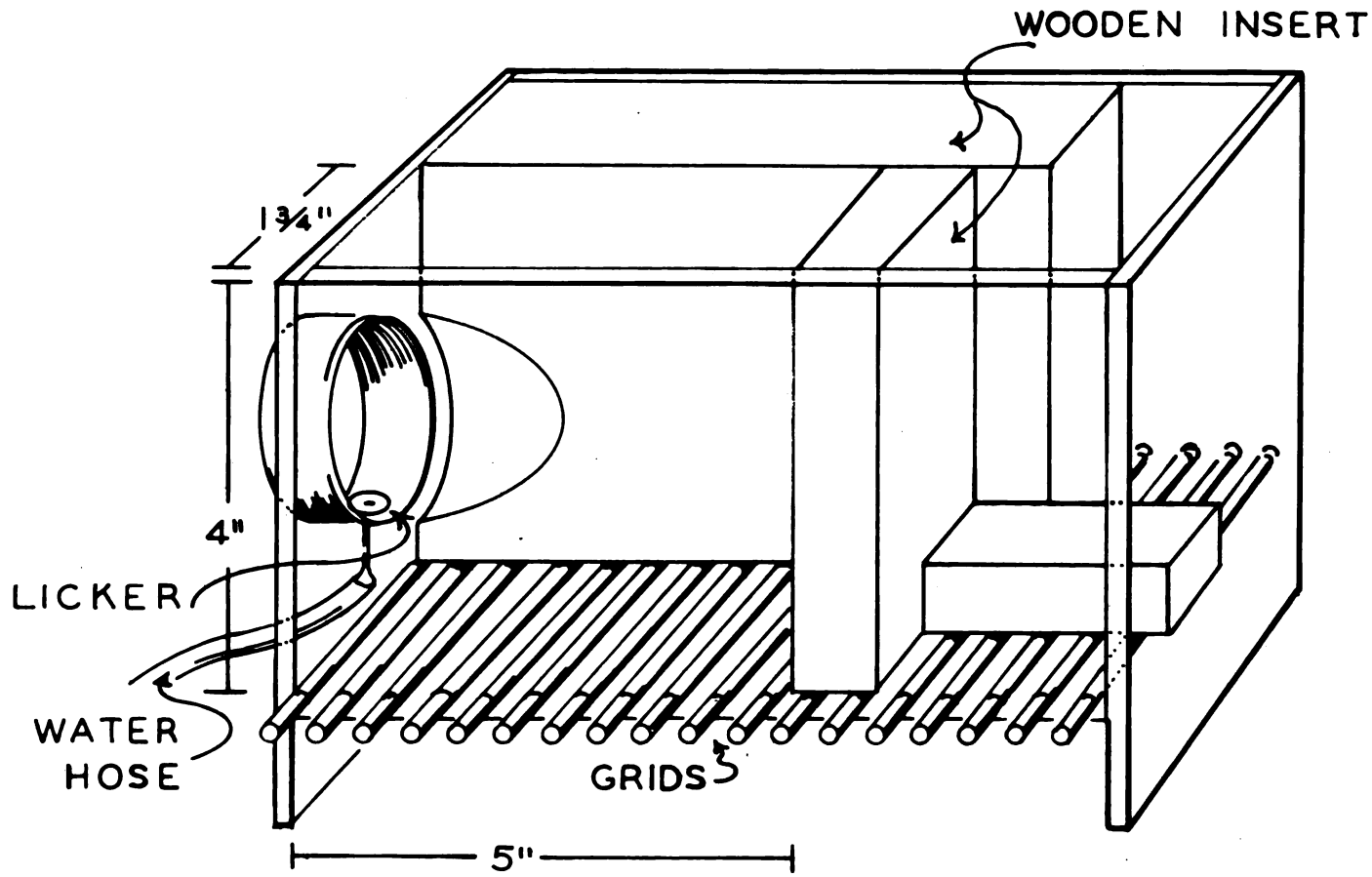



FIGURE 1. THE PLASTIC LICKING BOX

width and four inches in height. The drinking well side and front face were constructed of plexiglass. The floor consisted of a one-eighth inch stainless steel grid.

The drinking well was a fully enclosed one and one-half inch extension on the front face of the box. Water was presented in small quantities, controlled by a solenoid valve, one drop per trial through a #11 needle (ground flat and smooth) which projected through a small opening at the bottom of the well. A small copper ring encircled this opening to prevent gnawing and to measure licking. The CS was a ten watt bulb mounted on the outside of the box next to the well. The general level of illumination in the boxes was 5 ft. c.

Licking was measured by Grason-Stadler drinkometer, Model E 4690A, and recorded with a Gerbrands event recorder. Hunter timers and a Gerbrands punch tape timer provided the temporal intervals. A stepping switch was installed to allow randomized test trials.

Design - The two experimental groups of albinos and greys were randomly divided into five groups of four each, making a total of five interval groups with eight S's each. The five CS-UCS intervals chosen on the basis of pilot work were one-half second, one second, two seconds, four seconds, and six seconds. All CS-UCS presentations were of the delayed type and had a two second overlap.

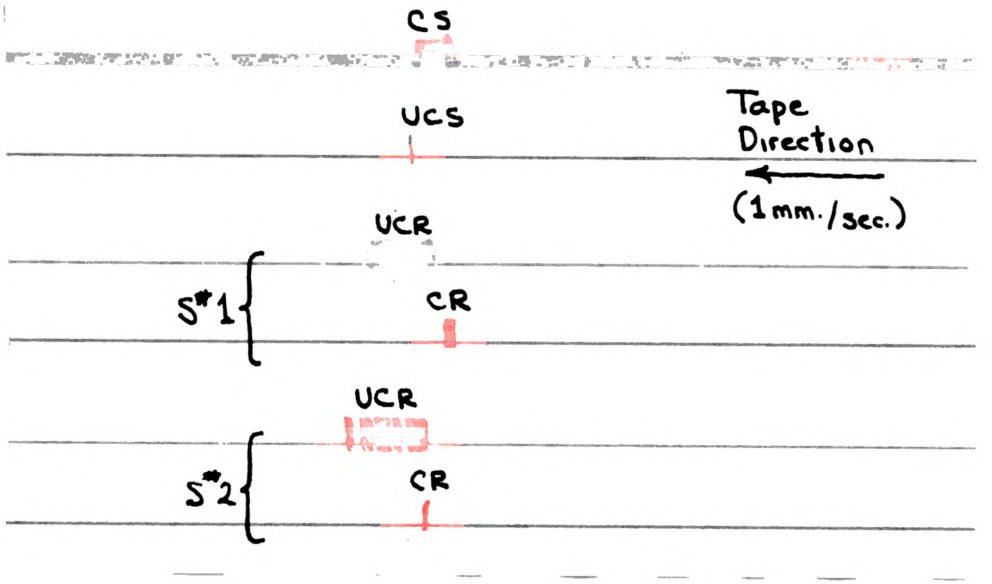
Procedure - The basic procedure consisted of four days of training for all S's. The animals were habituated on the first day in order to familiarize the animal with the apparatus and to insure that the UCS (water) would be a consistent elicitor of licking, once acquisition trials were begun. The criterion of habituation for all S's was fifty UCR's on a one-hundred and twenty second V.I. schedule with no CS present. The three days of conditioning followed immediately, utilizing various ISI intervals as previously indicated.

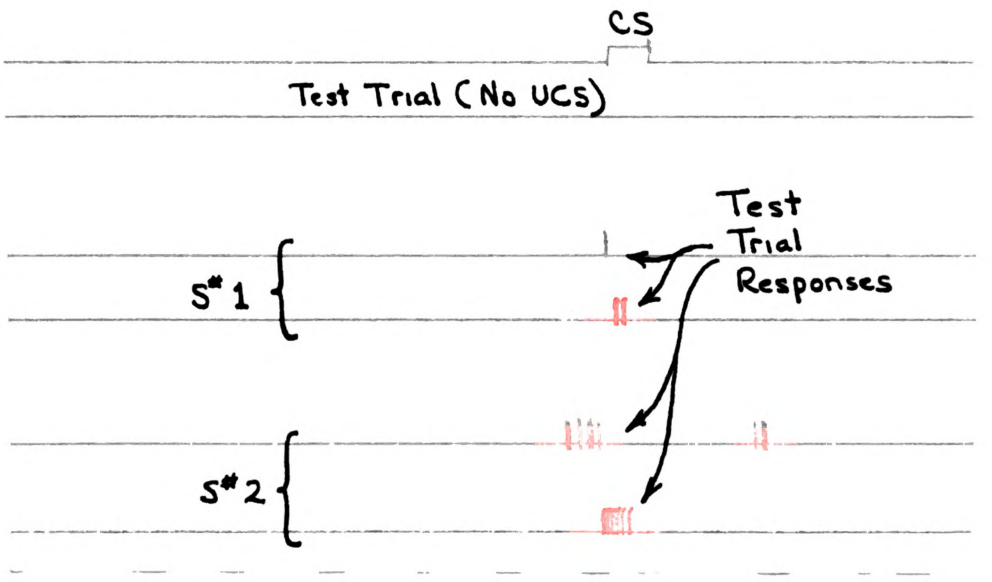
Two pairs of S's were run each evening and the three hour session for each had trials presented on an ITI of one-hundred and twenty seconds, V.I. Test trials were inserted on the average of one in ten trials during the three ninety-trial sessions.

Measures - The two basic measures of conditioned responses were recorded on paper tape by the event recorder. Two samples of that tape appear in Figure 2.

FIGURE 2

RESPONSE TAPES SHOWING A CR AND TEST TRIAL RESPONSE



Anticipatory responses were scored as CR's on the non-test trials if they occurred during the CS-UCS interval and were discriminatory in nature (not part of intertrial responding). To compensate for the obvious disadvantage of measuring anticipatory CR's with the two shortest intervals, test trials were instituted as the primary measure of performance. The criteria for a test trial response were that it also be discriminatory in nature and occur during the presence of the CS. Both measures, CR's and test trials, were used for all groups.

## RESULTS

The results are graphically presented in terms of cumulative acquisition curves (Figure 3), as parametric functions by days (Figure 4), and as total parametric functions (Figure 5). In Figure 5 test trial responses were totaled for all three days of acquisition, whereas CR responses represent only the third day of acquisition.

A visual examination of the three graphs indicates a marked superiority of the two and four second groups in acquisition rates and final levels of conditioning. In fact, two seconds appears to be generally optimal -- although probably not significantly so. The one-half, one, and six second intervals show no important differences at any level. The CR levels for those three intervals are much lower than the corresponding test trial levels in Figure 5. Test trial functions for the three days in Figure 4 seem to maintain the same general relationship among the intervals. In the two and four second groups, the level of anticipatory CR responding on the third day was seventy-four percent. The CR function in Figure 5 does not include the first one hundred and eighty trials because the three less optimal groups showed little conditioning (CR's) during that period.

The design of the experiment was a 2 X 5 factorial with an organismic variable. The same appropriate analysis

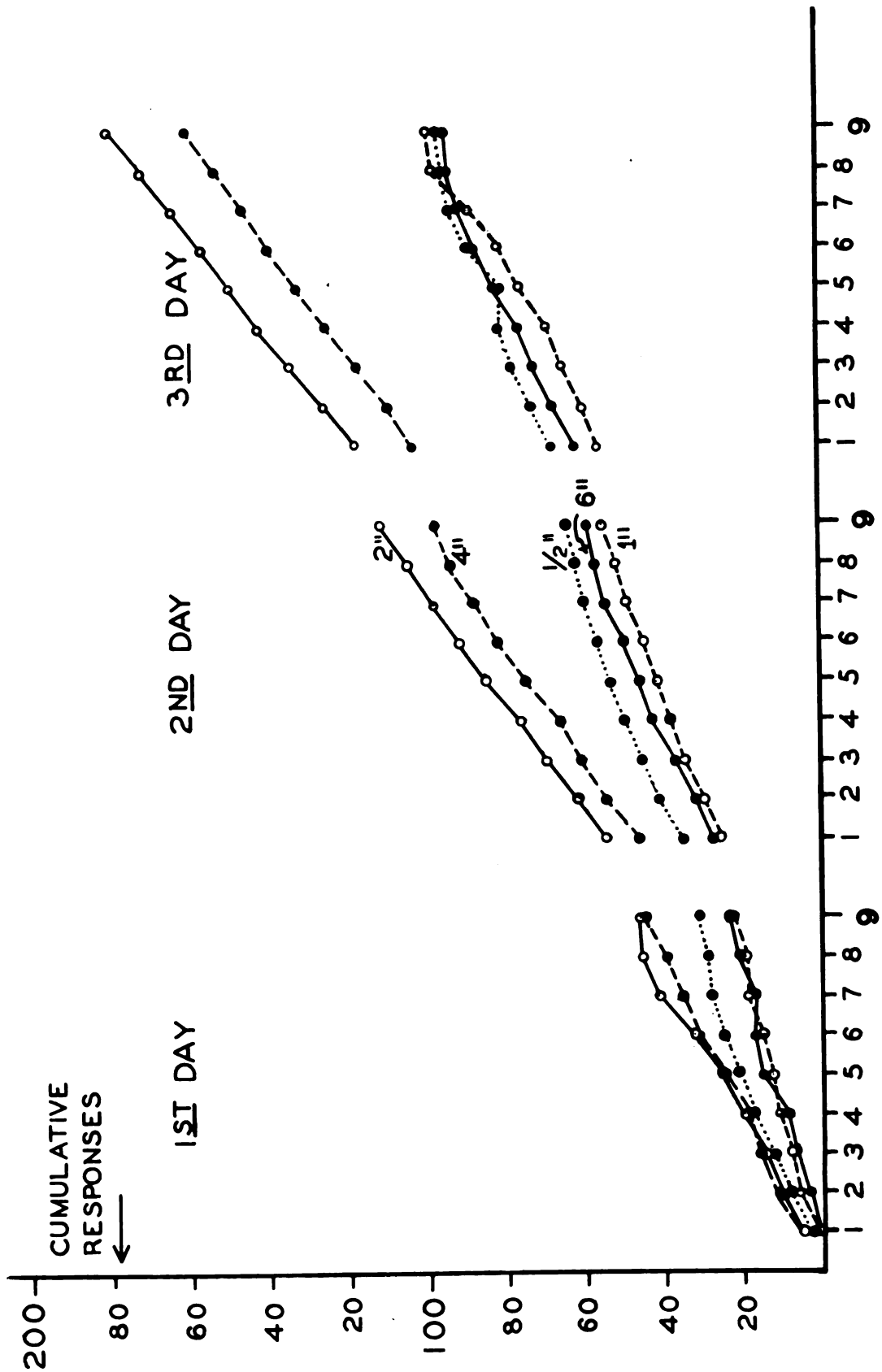



FIGURE 3. CUMULATIVE LEARNING CURVES FOR TEST TRIAL RESPONSES

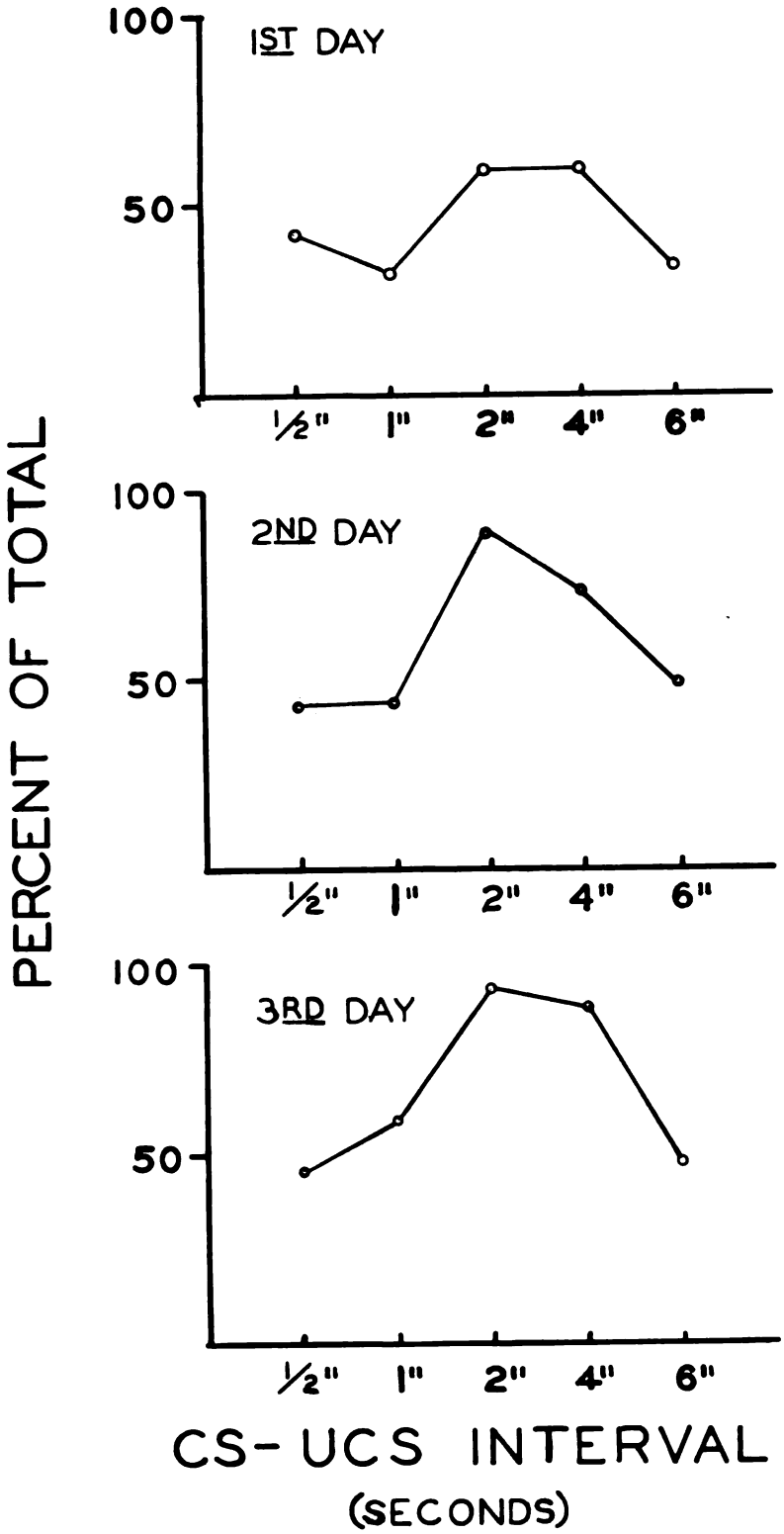



FIGURE 4. PARAMETRIC FUNCTIONS BY DAYS

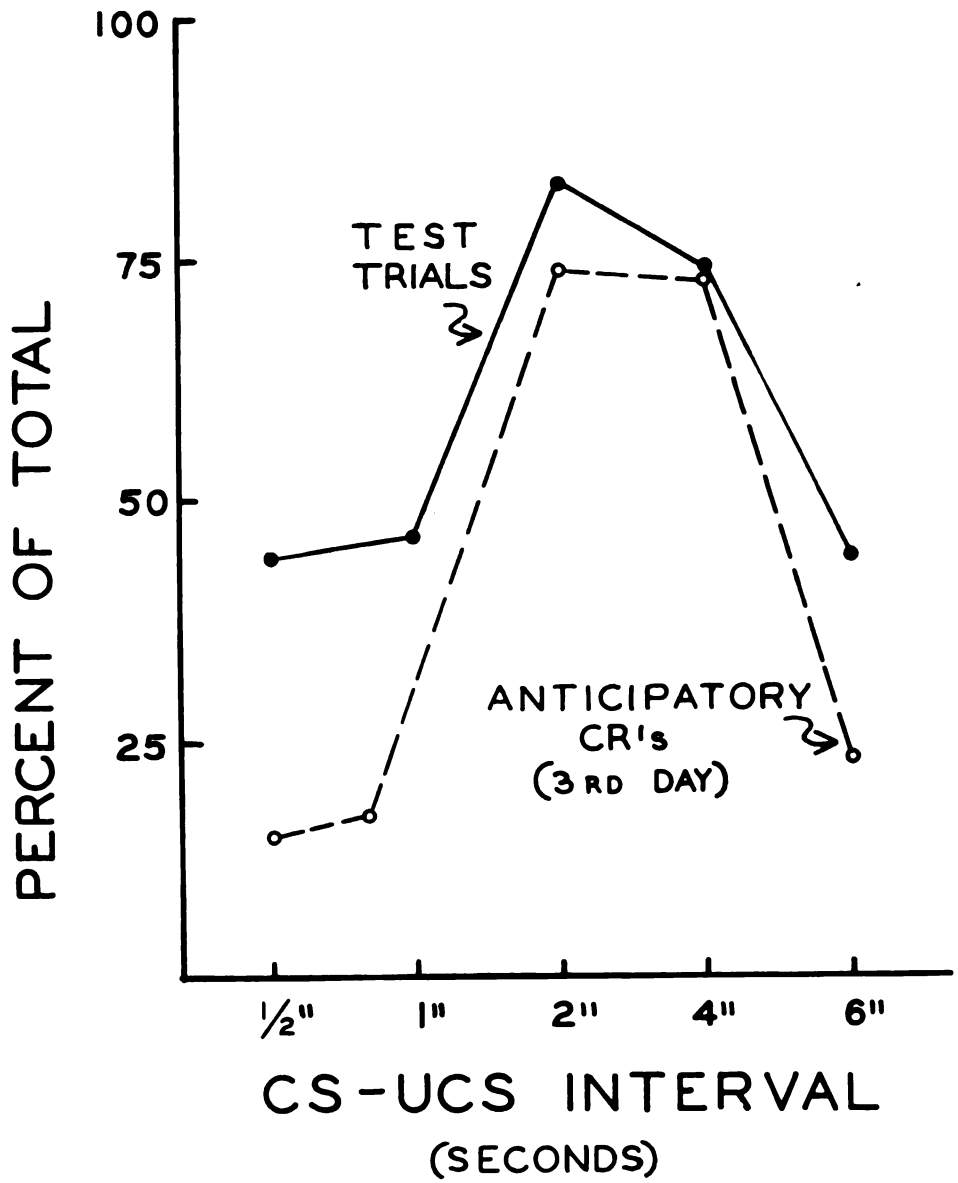



FIGURE 5. TOTAL PARAMETRIC FUNCTIONS

of variance was applied to both sets of data. The summary tables for both analyses appear in Table 2.

TABLE 2

SUMMARY OF ANALYSIS OF VARIANCE FOR TOTAL NUMBER OF TEST TRIAL RESPONSES

| SOURCE OF VARIATION | SS | d.f. | M.S. | F | p |
|---|---|---|---|---|---|
| Between | | | | | |
| Genetic | 18.8 | 1 | 18.8 | | |
| Temporal | 856.1 | 4 | 214.0 | 8.77 | .005 |
| G X T | 63.1 | 4 | 15.8 | | |
| Within | 731 | 30 | 24.4 | | |
| TOTAL | | 39 | | | |

SUMMARY OF ANALYSIS OF VARIANCE FOR THIRD DAY ANTICIPATORY CR'S

| SOURCE OF VARIATION | SS | d.f. | M.S. | F | p |
|---|---|---|---|---|---|
| Between | | | | | |
| Genetic | 14 | 1 | 14 | | |
| Temporal | 19,742 | 4 | 4,936 | 30.85 | .005 |
| G X T | 139 | 4 | 35 | | |
| Within | 4,809 | 30 | 160 | | |
| TOTAL | | 39 | | | |

The organismic variable produced no significant difference between albino and grey rats over temporal groups and no interaction with temporal groups. For both responses the intervals produced significant differences beyond the .005 level.

To determine the differences between the group means, Duncan's Multiple Range Test was administered. The results of the comparisons appear in Table 3.

TABLE 3

SUMMARY OF MULTIPLE COMPARISONS BETWEEN INTERVAL GROUP MEANS FOR TOTAL TEST TRIAL RESPONSES APPLYING DUNCAN'S MULTIPLE RANGE TEST

| Group Means | ½"Group A 11.8 | 1"Group B 12.4 | 2"Group C 22.5 | 4"Group D 20.0 | 6"Group E 11.8 | Shortest Significant Ranges (.01 level) |
|---|---|---|---|---|---|---|
| A 11.8 | | .6 | 10.7 | 8.2 | 0 | 6.8 |
| B 12.4 | | | 10.1 | 7.6 | .6 | 7.1 |
| C 22.5 | | | | 2.5 | 10.7 | 7.3 |
| D 20.0 | | | | | 8.2 | 7.4 |
| E 11.8 | | | | | | |

SUMMARY OF MULTIPLE COMPARISONS BETWEEN
INTERVAL GROUP MEANS FOR THIRD DAY CR'S
APPLYING DUNCAN'S MULTIPLE RANGE TEST

| GROUP MEANS | ½"Group A 13.1 | 1"Group B 12.3 | 2"Group C 62.3 | 4"Group D 58.5 | 6"Group E 18.9 | Shortest Significant Ranges (.01 level) |
|---|---|---|---|---|---|---|
| A 13.1 | | .8 | 49.2 | 45.4 | 5.8 | 17.23 |
| B 12.3 | | | 50.0 | 46.2 | 6.6 | 17.88 |
| C 62.3 | | | | 3.8 | 43.4 | 18.42 |
| D 58.5 | | | | | 39.6 | 18.83 |
| E 18.9 | | | | | | |

As indicated in all previous comparisons, the means for both measures were significantly higher in the two and four second groups. The two optimal intervals were not significantly different according to Duncan's Test.

## DISCUSSION

Effects of CS-UCS interval on test trial response acquisition - Hughes and Schlosberg (1938) found asymptotic levels of conditioning in rats to occur at about seventy-five trials. This investigation used a different modality and technique and the peak of performance was not reached in the two second group until beyond two hundred trials. The three non-optimal intervals reached only about a fifty percent level of conditioned responding during test trials. An interesting characteristic of these three groups was the inconsistency of responding over trials which resembled a sort of inattention on the part of the S. The two and four second groups, on the other hand, displayed almost perfect consistency in responding over all three hour sessions. Gormezano (1964) has mentioned some similar lapses in unconditioned salivary responding.

The effect of introducing some partial reinforcement during acquisition by omitting one tenth of the UCS's was felt to be negligible in view of extensive pilot work where no differences were observed.

Effects of CS-UCS interval on anticipatory CR acquisition - The fact that the shorter interval CR's reached a much lower level than their test trial counterparts was expected because longer temporal intervals allow more random licks and because of the latency of the licking response

when the animal's head is slightly removed from the licker. Base rates for responses randomly falling in the CS-UCS interval during habituation (no CS) appear in Table 4.

TABLE 4

BASE RATES OF RANDOM CR RESPONDING

| INTERVAL | | RATE |
|---|---|---|
| 0.5" | -- | 2 % |
| 1 " | -- | 4 % |
| 2 " | -- | 6 % |
| 4 " | -- | 8 % |
| 6 " | -- | 7 % |

The marked inferiority of CR's as compared to test trials in the six second group was a bit more surprising. The cumulative function for the CR's (not shown) in the longest interval showed a leveling-off to the rate of the shorter intervals. Examination of the latency of response on the paper tapes indicated that a process similar to Pavlov's inhibition of delay might be the cause for the decrement in later trials. Pavlov (1927) mentioned that with intervals over five seconds the onset of the UCR is delayed in a manner proportional to the interval's length. In other words, when the animal learns to wait for the UCS before licking, there will be a decrease in anticipatory CR's.

Effects of genetic strain on conditioning measures - The analyses of variance and a visual inspection of the data indicate a remarkable similarity in performance between the grey and albino rats.

Theoretical implications - As with much previous work which either classically conditioned a consummatory response or used subhuman organisms as S's, an optimal interval between the onset of the CS and UCS of more than one second was found. Pavlov (1927) considered intervals up to five seconds to be almost equally efficacious. This study singled out two and four second temporal intervals as being superior. Kappauf and Schlosberg (1937) found an optimum of over one second in rats.

Some superficial similarity of these results can be noted in instrumental studies where the ISI has been investigated. Bersh (1951), for example, found half-second, one second, and two second intervals to similarly influence the establishment of a secondary reinforcer. Most important in making a comparison to instrumental conditioning is the consideration that almost all delay of reinforcement (ISI) studies in operant techniques show a direct inverse relation of performance to interval in early learning if any competing responses are available (Spence, 1956).

## SUMMARY

Using Weisman's new method of classically conditioning the licking response in rats, the acquisition of test trial responses and anticipatory CR's was compared over five CS-UCS intervals (0.5", 1", 2", 4", 6"). It was expected that the optimal interval would be longer than one half second because of the nature of the UCR and the type of organism employed. In addition, a comparison between two strains of rats, albino and grey, was made.

The results were as follows: (a) The optimal interval was two seconds with four seconds being nearly as good. (b) The one-half, one, and six second groups were not significantly different from one another. (c) The two optimal intervals were significantly better than each of the other three intervals. (d) Data for CR's and test trials indicated the same trends although the differences were greater for the CR's. Reasons for that difference were discussed. (e) There was no genetic difference or interaction over temporal groups.

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