ANXIETY AFTER AVOIDANCE LEARNING AS MEASURED BY GASTRIC SECRETIONS

Thesis for the Degree of M. A.
MICHIGAN STATE UNIVERSITY
Ted Tetzlaff
1961

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By

TED TETZLAFF

A THESIS

Submitted to the College of Arts and Sciences of
Michigan State University of Agriculture and
Applied Science in partial fulfillment of
of the requirements for the degree of

MASTER OF ARTS

Department of Psychology

1961

ABSTRACT

The purpose of this study is to test, through an objective physiological measure, Denny's "incubation of anxiety" hypothesis advanced to account for the unusual retention of an incompletely learned avoidance response. Free HCl secretion in the stomach was used as a measure of anxiety. Three experimental groups of rats were given 25 avoidance learning trials in a shuttlebox followed by a second set of 25 relearning trials at intervals of 1 hour, 6 hours, and 24 hours, respectively.

One hour prior to the second set of trials, the surgical operation of pylorus ligation was performed as a means of collecting the gastric secretions, including free HCl. A control group which did not receive any avoidance trials was pylorus ligated and used to establish a basal level of HCl secretions.

The results of the behavioral data were consistent with previous findings which had been obtained under similar conditions. The results of the analysis of free HCl yielded no significant differences among groups. However, the trend of the means for the amount of free HCl was in approximate alignment with the predictions from Denny's "incubation of anxiety" hypothesis.

Approved: Stanley C. Patten Date: Jan. 17, 1961
Major Professor

To Marjie

ACKNOWLEDGMENT

The author wishes to express his sincere thanks and appreciation to the committee chairman, Dr. S. Ratner, whose guidance and encouragement aided so much in the completion of this study.

A special debt of gratitude is also owed to the late Dr. J. F. King for his assistance and advice in the planning and initial execution of this research.

The author wishes to convey his thanks to Dr. Terrence M. Allen, Dr. Charles Hanley, and Dr. M. Ray Denny for their helpful suggestions of the analysis of the data.

The cooperation of Dr. R. K. Ringer of the Department of Poultry Science in providing necessary equipment and the invaluable assistance of the author's wife, Marjorie, in conducting the gastric analysis is also gratefully appreciated.

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INTRODUCTION

Investigating the retention of an incompletely learned avoidance response, Kamin (1957) obtained results which pose some interesting problems of interpretation. In Kamin's procedure hooded rats were given 25 avoidance learning trials in a shuttlebox. Retention of the avoidance response was measured in 25 additional learning trials after delays of 0, .5, 1, 6, 24 hours, and 19 days. Instead of a monotonic decreasing relationship between retention and time, Kamin found a significant curvilinear relationship, with retention declining significantly from 0 to 1 hour, then rising significantly from 1 hour to 19 days. To account for this relationship, two independent processes were postulated. The first segment of the curve extending from 0 to 1 hour was described in terms of a continued dissipation of positive transfer and agrees with the established vernacular concept of forgetting. For the rising segment of the curve extending from 1 hour to 19 days, Kamin suggested an incubation or jelling of the avoidance response which begins to have a facilitory effect on avoidance performance about 1 hour after the initial learning trials.

In a replication of Kamin's study, Denny (1958) obtained similar results but offered a different interpretation. Denny explained the decline in retention of the 0-1 hour segment of the curve by hypothesizing that anxiety builds up

after the initial set of learning trials to the point of interfering with avoidance performance. This incubated anxiety reaches a peak in about 1 hour and then begins to dissipate. The rising segment of the curve, from 1 hour to 19 days, is explained in terms of the dissipation of anxiety. Experimental evidence supporting this hypothesis was derived from Denny's study, in which two conditions designed to counteract the incubation of anxiety during the 1 hour interval yielded results consistent with expectations.

The present study represents an attempt to assess Denny's interpretation of the Kamin effect by using a physiological index of anxiety. More specifically, the amount of free HCl secreted in the stomach will be used as a measure of anxiety.

The relationship between anxiety and free HCl secretion has led to considerable disagreement among investigators in the past. This seems to be due, in part, to a failure to recognize the differential reaction of gastric secretions to chronic anxiety as opposed to acute anxiety. Studies involving chronic anxiety have generally revealed a positive relationship between anxiety and free HCl. Mahl (1949) paired shock and buzzer over a prolonged period of time to develop a state of chronic anxiety in dogs. Significant increases in free HCl accompanied increases in anxiety. More recently, a number of investigators (e.g., Sawrey (1956), Porter (1958), and Sines (1959)) have successfully produced gastric ulcers (primarily as a result of increased free HCl secretion) in

animals under various forms of prolonged anxiety states.

In direct contrast, the majority of studies employing acute anxiety have yielded a negative relationship between free HCl secretion and anxiety. Observations by Beaumont (1834) and Wolf and Wolff (1943) on human subjects with gastric fistulas have revealed inhibiting effects of acute fear and anxiety on free HCl secretions. More recently, Mahl (1952) reported the results of a study in which three monkeys were subjected to two different buzzer-shock conditions designed to produce acute and chronic anxiety. Only in the chronic anxiety situation was free HCl found to increase significantly. A short preliminary study was conducted by the present author to further clarify the relationship of acute anxiety to gastric secretions. Three pylorus operated rats were given 25 avoidance trials in a shuttlebox. Measurement of gastric secretions after 1 hour showed a substantial decrease in the volume of gastric secretions as compared to a control group.

The data from some of the above studies are admittedly based on small numbers of observations. However, the evidence seems to indicate that hypersecretion of free HCl is characteristic of chronic anxiety states, and hyposecretion of free HCl is characteristic of acute anxiety states.

Anxiety aroused during the 1 hour period in which Kamin (1957) and Denny (1958) found decreasing performance will be considered as "acute." In line with Denny's hypothesis, the incubation of acute anxiety during the 1 hour

interval (following the initial set of learning trials) should result in an inhibition of free HCl secretion. Further, the hypothesized dissipation of anxiety following the 1 hour interval should be paralleled by a corresponding increase in free HCl.

METHOD

Subjects

The Ss were 40 experimentally naive albino rats selected from the colony maintained by the Department of Psychology at Michigan State University. Twenty males and twenty females were used; they ranged in age from 90-100 days. Two female Ss died during the course of the experiment and were replaced with similar Ss. The colony was maintained on an *ad lib* feeding schedule and was housed in cages in a room adjoining the experimental room with 4-5 Ss of the same sex in each cage. The Ss were equated for weight, age, and sex, and with these restrictions were assigned at random to one of four groups of 10 Ss each. Groups A, B, and C comprised the experimental groups, group D served as the control to establish a basal level of HCl secretion. Four Ss, one from each group, were run each day and this was continued for 10 consecutive days.

Apparatus

The apparatus was a modified Mower-Miller shuttlebox. The shuttlebox, 36 inches long, 4 inches wide, and 14 inches high, was of wood construction and painted flat black. For observation purposes, the entire front of the box was clear glass. The floor was constructed of 1/8 inch brass rods

spaced approximately 1/4 inch apart. The experimenter could administer the unconditioned stimulus (US), an electric charge through the brass rods, independently to either half of the grid floor. A C. J. Applegate stimulator was used to deliver the continuous 1.7 milliamp charge to the grid. A 70 decibel buzzer attached to one side of the shuttlebox and activated by four dry cells served as the conditioned stimulus (CS). The onset and termination of the CS and US were controlled by the experimenter who manually operated individual switches.

Procedure

The treatments for the various groups followed the design illustrated in Table 1. Avoidance learning of the type used by Kamin (1957) was the experimental task. The experimental groups A, B, and C were given 25 avoidance learning trials in the shuttlebox under the following conditions. The Ss were placed in the apparatus and allowed a one minute adaption period followed by 25 avoidance trials with a one minute intertrial interval. The CS-US interval was 5 seconds and the CS was response terminated. Each trial ended either in escape or avoidance responses. Following the initial set of trials, groups A, B, and C received an additional set of 25 trials at intervals of 1 hour, 6 hours, or 24 hours, respectively. Group D did not receive any avoidance learning trials. During both conditioning phases of the experiment Ss in this group remained in their cages. The experimental

treatments of group D therefore differed from the experimental treatments of groups A, B, and C by the absence of avoidance trials, contact with the apparatus, and handling during the avoidance learning procedure.

For all Ss in the experiment the surgical operation of pylorus ligation, described by Shay (1945), was performed. The operation which required approximately 10 minutes was so timed that its completion preceeded the second set of avoidance trials by one hour. The experimental groups A, B, and C were differentiated in terms of the number of hours which elapsed between the end of the first conditioning session and the beginning of the second conditioning session. In group A, Ss were pylorus ligated immediately after the initial set of avoidance trials. One hour after the completion of the operation Ss in this group were returned to the apparatus for the second set of avoidance trials. The Ss in group B were pylorus ligated 5 hours after the initial set of avoidance trials and were returned to the apparatus one hour later for the second set of avoidance trials. The Ss in group C were pylorus ligated 23 hours after the initial set of avoidance trials and were also returned to the apparatus for the second set of avoidance trials one hour after the completion of the operation. In group D Ss were pylorus ligated shortly after being removed from their cages.

Since the operation of pylorus ligated was performed as a means to retain gastric secretions for analyses, certain

precautions were necessary to eliminate contamination of the fluids. Contamination from undigested food was reduced by maintaining all Ss in the experiment on a 72 hour total food deprivation prior to pylorus ligation. Copraphagy was controlled during the deprivation period by housing the Ss in cages of the same dimensions as their home cages, but without bedding and with raised 1/2 inch wire mesh floors. Post-operatively all Ss were placed in individual cages measuring 1 foot x 1 foot x 1 foot with raised 1/2 inch wire mesh floors.

Immediately after the second set of trials, or in the case of group D, 1 hour and 26 minutes after pylorus ligation, all Ss were sacrificed by over-anesthetistizing with ether. Death from over-anesthetistizing took place within 30-120 seconds. The incision was reopened, and a hemostat was clamped on the esophagus near the diaphram. The stomach was completely excised from the body cavity and its contents drained into a 10 cc. graduated cylinder by releasing the hemostat. The gastric contents of each S were analyzed for determination of total volume, acidity (pH), percentage of free HCl, percentage of combined HCl, and percentage of total HCl.

The one drop sample electrode attachment for the "Beckman Model H" pH meter was used for pH determinations. The test for free HCl was carried out using Topfers Reagent as an indicator and titrating with 0.1 N. NaOH. A 1% alcoholic solution of phenolpethalein was used as an indicator and

titration again carried out with 0.1 N. NaOH in the test for total HCl. Combined HCl was derived by subtracting the calculated free HCl from the calculated total HCl.

RESULTS

Behavioral Data

The mean number of avoidances and standard deviations for the three experimental groups, A, B, and C, on original and relearning avoidance trials are presented in Table 2.

The random assignment of Ss to groups to each of ten levels, equated for weight, age, and sex lent itself to a treatment X levels analysis of variance design with one observation per cell, as described in Linquist (1956). The between groups F for the number of avoidances during the original learning trials was 0.33 (2 and 18 df). This value falling short of significance indicates that initially the experimental groups which had not yet been experimentally differentiated did not differ significantly on original avoidance learning. The between groups F for the number of avoidances during the relearning trials was 0.91 which is also far short of significance (2 and 18 df).

Since the analysis of variance does not take into consideration the correlation between original and relearning scores, a double classification analysis of covariance as described in Edwards (1950) was used as a more precise test. The analysis is summarized in Table 3. A within groups correlation of 0.78 was found between the avoidance scores on original and relearning trials. The between groups F for the

adjusted means relearning scores was 3.93 (2 and 17 df). This value was found to be significant at the .05 level. Table 4 presents both the raw and adjusted mean scores for avoidances during relearning.

Application of individual *t* tests to the three pairs of adjusted means yielded one significant difference. Group A (1 hour interval) made significantly fewer avoidances on the relearning trials than Group C (24 hour interval). ($t = 2.7$ $p < .05$ 17 df)

Physiological Data

Table 4 presents the means and standard deviations of the physiological measure of total volume, free HCl, combined HCl, total HCl, and acidity (pH) for each of the experimental groups and the control group. Free HCl, combined HCl, and total HCl are expressed in degrees which are units of acid concentration.

The measure of free HCl was submitted to a treatments X levels analysis of variance. The between groups *F* was .09 (3 and 27 df) falling short of significance at the .05 level. The total volume of gastric secretion was similarly submitted to a treatments X levels analysis of variance. The between groups *F* was 2.78 (3 and 27 df), also falling short of significance at the .05 level.

Although the primary interest in this study was the quantity of free HCl and the resultant quantity of total

gastric secretions as a measure of anxiety, all of the remaining related physiological measures were analyzed with a treatments X levels analysis of variance. None of the between groups *F*s were found to be significant, and as observed from Table 5, no strong trends among the group means were evident.

DISCUSSION

The results of the behavioral data were consistent with the results from studies performed under similar conditions. Both Kamin (1957) and Denny (1958) found that the one hour interval group (Group A) made significantly fewer avoidance responses than the 24 hour interval group (Group C) on the relearning trials. The only major discrepancy between the data reported by Kamin and the data of the present study is that better performance was obtained on original learning and relearning by the present investigator. Kamin obtained a grand mean number of avoidances during original learning of 5.7 versus 9.3 for the present study; Kamin obtained a grand mean number of avoidances during relearning of 6.6 versus 14.5 for the present study.

The discrepancies in avoidance performance levels may be accounted for in terms of the results of a recent study by Thomas (1960). In this study, Thomas found that avoidance performance on original and relearning trials was considerably depressed in shuttleboxes with low ceiling heights. Since the shuttlebox used by Kamin had a ceiling height of 4-3/4 inches as compared to a ceiling height of 14 inches in the present study, the apparent discrepancies in avoidance performance seems to be resolved.

The fact that the behavioral data yielded no other marked deviations from that of the previous investigations has particular significance in this study.

The Ss in this experiment were subjected to several unique conditions not present in previous avoidance learning studies. In the present study all Ss were maintained on 72 hour food deprivation, all Ss were anesthetized and pylorus ligated, and during the course of the experiment there were two cage changes for all Ss. Although the possibility of isolating the individual effects of each of these variables is not possible in this study, the combined effects of food deprivation, pylorus ligation, and cage changes seems to have had little bearing on the outcome of avoidance learning and relearning behavior as compared with the findings of previous studies.

The results of the analysis of physiological measures of total volume of gastric secretion, free HCl, combined HCl, total HCl, and pH failed to yield any significant differences among groups. However, the means of free HCl and total volume of gastric contents can be seen, from Table 5, to be in approximate alignment with the predictions from Denny's "incubation of anxiety" hypothesis. That is, the one hour interval group (Group A) was considered to be in a state of acute anxiety and, therefore, was expected to have had the lowest output of free HCl. The hypothesized dissipation of anxiety following the one hour interval was predicted to

be paralleled by an increase in free HCl in Group B (6 hour interval) and Group C (24 hour interval). The control group, having had no experience with avoidance learning and the resultant anxiety, was expected to yield the highest output of free HCl. Since the total volume of gastric secretions would be affected by free HCl secretions, the same relationships were expected to hold for the total volume of gastric secretions. As seen from Table 5, the only case in which the observed trends did not bear out these relationships was the finding of slightly more free HCl in Group A (1 hour interval) than in Group B (6 hour interval).

Although the trends of the means of free HCl and total volume of gastric secretions are in support of the "incubation of anxiety" hypothesis, the failure to obtain statistically significant results leads to the suggestion of some possible influencing factors.

A primary factor responsible for the non-significant differences may have been that the anxiety levels of the four groups did not differ sufficiently for a significant difference in free HCl to arise. That is, free HCl as a measure of anxiety may have been too insensitive under the conditions of this experiment. In addition, the effects of the 72 hour food deprivation, anesthetization, pylorus ligation, and cage changes, may have tended to further reduce the group differences in anxiety by bringing about an emotional leveling effect in all Ss.

Finally, the relatively small number of Ss employed may have considerably decreased the required level of precision for this experiment.

SUMMARY

Previous studies on avoidance learning and relearning have reported a decrement in avoidance performance following a one hour interval between avoidance learning trials and relearning trials. Denny hypothesized that the decrement in performance was due to the incubation of anxiety during the one hour interval. Improved avoidance performance following longer intervals between the two sets of trials would therefore indicate a dissipation of anxiety.

This study attempts to test Denny's "incubation of anxiety" hypothesis by employing free HCl secretion in the stomach as a measure of anxiety. The predictions were that the hypothesized incubation of anxiety during the one hour interval would result in an inhibition of HCl secretion. The hypothesized dissipation of anxiety following the one hour interval would be paralleled by an increase in HCl secretion.

Three experimental groups of rats were given 25 avoidance learning trials in a shuttlebox followed by a second set of 25 relearning trials at intervals of 1 hour, 6 hours, and 24 hours, respectively. As a means of collecting gastric secretions, the surgical operation of pylorus ligation was performed on all experimental Ss 1 hour prior to the relearning trials. A control group, which did not receive any

avoidance learning trials, was pylorus ligated for the purpose of establishing a basal level of HCl secretion.

Immediately after the relearning trials or in the case of the control group, 1 hour and 26 minutes after pylorus ligation, all Ss were sacrificed and the gastric secretions were collected for gastric analysis. Determinations of free HCl, combined HCl, total HCl, total volume, and pH were made for each gastric sample.

The results of the behavioral data were found to be consistent with the results of previous studies which were run under similar conditions. The trends of the means of free HCl and total volume of gastric secretion were found to be in approximate alignment with predictions. The results of the statistical analysis, however, yielded no significant differences. Factors such as the insensitivity of free HCl as a measure of anxiety and the influence of certain experimental treatments on the anxiety levels were discussed as possible reasons for the failure to yield significant results.

TABLE 1

Experimental Procedure and Treatments for the Experimental Groups and Control Group

Experimental Treatment	Group A	Group B	Group C	Group C
Initial set of 25 avoidance learning trials	yes	yes	yes	rest
Interval between termination of avoidance learning trials and operation	0 hour	5 hours	23 hours	rest
Pylorus Ligation	yes	yes	yes	yes
Interval between end of operation and onset of 25 relearning trials	1 hour	1 hour	1 hour	1 hour rest
25 relearning avoidance trials	yes	yes	yes	26 minutes rest
Sacrifice with ether	yes	yes	yes	yes

TABLE 2

Means and Standard Deviations (SD) of the Number of Avoidances on Original and Relearning Trials for the Experimental Groups

Group	Measure	Original Learning Trials	Relearning Trials
	Mean	10.2	12.7
A			
	SD	4.52	4.57
	Mean	9.4	15
B			
	SD	5.52	5.83
	Mean	8.2	15.9
C			
	SD	6.23	5.9

TABLE 3

Analysis of Covariance of the Avoidance Scores for the Experimental Groups

Source of Variation	df	Σx^2	Σxy	Σy^2	ss of Errors of Estimate	df	Mean Square	F
Between Groups	2	20.27	-31.1	54.5				
Between Levels	9	261.17	134.44	146.6				
Residual Within Groups	18	548.43	470.36	660.4	257	17	15.11	
Total	29	829.87	573.70	861.5				
Between Groups Plus Error	20	568.70	439.26	714.9	375.62	19		
Adjusted Means					118.62	2	59.31	*3.93

*significant at .05 level

TABLE 4

Observed and Adjusted Mean Scores for the Number of Avoidances on Relearning Trials

Group	Observed Mean	Adjusted Mean
A	12.7	11.89
B	15	14.88
C	15.9	16.81

TABLE 5

Means of the Physiological Measures for the Experimental Groups and Control Group

Group	Volume in cc	Free HCl in degrees	Comb. HCl in degrees	Total HCl in degrees	pH
A	1.36	29.15	49.20	78.35	1.63
B	1.81	28.50	54.80	83.30	2.25
C	1.96	30.85	51.35	82.20	1.76
D	2.35	34.20	48.26	82.46	1.87

TABLE 6

Standard Deviations of the Physiological Measures for the Experimental Groups and the Control Group

Group	Volume in cc	Free HCl in degrees	Comb. HCl in degrees	Total HCl in degrees	pH
A	0.63	26.43	68.21	33.81	0.78
B	1.09	34.15	21.47	23.83	1.03
C	1.92	23.32	12.88	20.04	0.62
D	.88	28.03	12.41	19.62	0.84

APPENDICES

TABLE 7

Number of Avoidances on Original and Relearning Trials for the Experimental Groups

Group A Subjects

	1	2	3	4	5	6	7	8	9	10	Totals
No. of avoidances on original learning trials	10	7	14	14	3	18	13	9	6	8	102
No. of avoidances on relearning trials	9	12	17	12	7	20	17	6	15	12	107

Group B Subjects

	1	2	3	4	5	6	7	8	9	10	Totals
No. of avoidances on original learning trials	8	5	9	14	14	4	19	4	14	3	94
No. of avoidances on relearning trials	12	10	15	17	23	4	22	18	18	11	150

Group C Subjects

	1	2	3	4	5	6	7	8	9	10	Totals
No. of avoidances on original learning trials	8	1	12	15	5	7	0	19	12	3	82
No. of avoidances on relearning trials	15	12	13	20	18	19	4	23	23	12	159

TABLE 8

Total Volume of Gastric Secretions and Free HCl for the Experimental Groups and Control Group

Ss	Group A		Group B		Group C		Group D	
	Volume of secretion in cc	Degrees of HCl	Volume of secretion in cc	Degrees of HCl	Volume of secretion in cc	Degrees of HCl	Volume of secretion in cc	Degrees of HCl
1	1.2	28	1.6	26	1.9	30	3.8	65
2	.8	32.5	.9	21	2.4	70	1.2	0
3	1.8	85	4	100	2.2	30	3	52
4	0	0	1.7	24	1.9	59	3	35
5	2.1	50	1.2	10	3.3	47	3.3	80
6	1.7	0	1.1	0	1.4	12.5	2.2	28
7	1.8	16	2.4	84	1.4	0	2	35
8	.9	12	3.3	20	2.1	24	1.6	0
9	1.6	45	.6	0	1.2	0	1.4	0
10	1.7	23	1.3	0	1.8	36	2	47
Totals	13.6	291.5	18.1	285	19.6	308.5	23.5	342

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