PSYCHOLOGY

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Effect of Non-Optimally High Incubation Temperatures on T-Maze Learning in the Chick[1]

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Introduction

Considerable research effort has been expended in efforts to assess the effects of various types of environmental stress on behavior (11). Much of this work has involved exposing organisms post-natally to stress-producing situations. Several investigators have sought a correlation between the incidence of mental deficiency in humans and time (season) of conception (7, 8). Their reports indicate some statistical evidence of a higher number of mentally deficient babies conceived during the hot summer months. Gunther (3) and Gunther, et al (5), have pointed up the desirability of extending this type of investigation to a study of the effects of stressful agents operative during the course of embryonic development and have reported qualitative observations of the effects of non-optimal incubation temperatures on the behavior of chicks. Such effects as structural anomalies of various parts of the body, inability to ingest food or water, heightened aggressive and fearful behavior, and hyperexcitability have been observed to result from such temperature insults. More recently, Gunther and Jones (4) have also found lowered mean weights and reduced rates of weight gain in animals which were hatched from eggs incubated at non-optimally high temperatures for varying numbers of days.

The present investigation is concerned with the effect of non-optimally high incubation temperatures on the chick's ability to learn an alternation pattern and a visual discrimination in the T-maze.

Materials and Methods

The T-maze employed in all experiments reported below has been described in detail elsewhere (5). The maze is constructed of wood and is painted flat black inside and out. It is 6″ wide throughout and has a 6″ x 6″ start box with sliding panel door, a 17″ runway, and 24″ arms. Illumination, in addition to normal daylight, is provided by fluorescent lamps mounted over the arms of the maze. The food receptacles used were colored plastic trays, 4″ x 4″ x 1½″. For the temporal maze habit described below, two identical yellow trays were used; for the discrimination task, a red and a green tray were employed. In order to specify the approximate hue, reflectance, and saturation values of the trays used in the discrimination task, the colors of these trays were matched with the dull sides of color chips from the Ostwald Color Harmony Manual (1).

1. This research was supported by grant B-2128, Council on Neurological Diseases and Blindness, National Institutes of Health, United States Public Health Service.

These matches were accomplished independently by three different individuals who unanimously agreed that the colors most closely approximated the following in the Ostwald system: red, 7na; and green, 181a. These two colors have reflectance values of 17 and 30, respectively.

The animal subjects in all experiments were White Leghorn chicks of the DeKalb strain. No crippled animals were used. Control animals were hatched from eggs incubated at the optimal temperature of 37.5° (all temperatures herein are reported as centigrade). Experimental animals were hatched from eggs incubated at 41° for varying numbers of days during either the initial or the terminal phases of the incumbation period and at the optimal temperature for the remainder of the period. Identification of the various experimental groups is in terms of both time and length of exposure to the 41° temperature. Thus, 1-day and 2-day experimental groups were composed of animals hatched from eggs incubated at 41° for the first 24 hours and for the first 48 hours, respectively, of the incubation period; during the remainder of the period, the incubation temperature was optimal. Sixteen-day, 17-day, 18-day, 19-day, and 20-day experimental groups were composed of animals hatched from eggs which were incubated at the optimal temperature for the first 360, 384, 408, 432, and 456 hours, respectively, of the incubation period; during the remainder of the period, the incubation temperature was 41°. Although it was hoped to have available 10 experimental groups (5 comprised of animals hatched from eggs subjected to the 41° temperature during the initial phases of the incubation period and 5 consisting of animals hatched from eggs exposed to the higher temperature terminally in the incubation period), insufficient numbers of chicks hatched to compose 3-day, 4-day, and 5-day groups. All animals received 4 days of pre-training, beginning at 4 days of age. The pre-training on each day consisted of placing the animals individually in the T-maze for 10 minutes with food available in both arms. Prior to each pre-training and test session, the animals were subjected to overnight deprivation of food but not of water.

Since preliminary investigation revealed that individual chicks often display consistent position and/or stimulus preferences in the T-maze and also that the specific nature of these frequently varies among the animals within a particular group, it was deemed desirable to obtain checks on these preferences prior to running the animals on the learning tasks. Consequently, 15-trial position and color preference series were run on all groups before the learning series were begun. The data of these preference tests were analyzed by means of chi-square to identify significant preferences which might be shown by entire groups, and also to detect significant between-group differences in terms of these preferences. The criterion of significance employed was the .05 level of confidence. In addition, preferences shown by individual animals were examined, and these were regarded as significant if a particular animal displayed a consistent preference on 80% or more of the trials. On the basis of the results of these tests, attempts were made to equate as closely as possible those groups performing the same task.

Since several hours were required to run all groups through a particular learning task, animals were selected at random from among the various control and experimental groups in order to establish control for

between-group differences in motivational level due to differential temporal effects of deprivation. Extension of any part of an animal's body into an arm of the maze in the direction of the negative food tray was regarded as an error. Upon reaching the positive food tray, the animal was allowed to peck at the food (Purina Starteena) 2 or 3 times before being returned to the start box for the next trial. As the animals grew larger, it was necessary to elevate the food trays so that the food in the positive tray could not be seen. The criterion of learning employed in all tests was 13 correct trials out of a total trial sequence of 15 ($P = .004$).

Results

Temporal Maze Habit

Ten control animals, ten 1-day animals, and ten 2-day animals were run on a simple alternation pattern in the T-maze. Position preference tests were run when the animals were 8 days old. The results indicated that no significant differences were in evidence among the groups in terms of strength of displayed preference. The data for individual animals revealed that 7 control animals, seven 1-day animals, and eight 2-day animals manifested consistent position preferences on 80% or more of the 15 trials. None displayed spontaneous alternation habits.

The learning situation series was begun when the animals were 9 days old, and consisted of learning an alternate pattern of reinforcement, i. e., the position of the positive food tray was alternated on successive trials. Thus the task involved learning a R L R L R L, etc., pattern. The initial position of the positive tray was randomized left and right within animals on all 15-trial sequences. A non-correction procedure was employed, and a total of 645 trials was run.

Eight control animals, five 1-day animals, and no 2-day animals attained the criterion. The Fisher exact probability test (9) was used to compare the number of control animals reaching the criterion with the number of experimental animals in both the 1-day and 2-day groups reaching the criterion. The results of this test indicated the difference between the two numbers to be significant at the .02 level of confidence. Since different numbers of animals in the control and 1-day groups achieved the criterion, it was not possible to compare meaningfully the mean number of trials required to achieve the criterion by these 2 groups. However, a t-test was used to compare the mean score of the 5 animals of the 1-day group which attained the criterion with the mean score of the first 5 control animals to attain the criterion. The mean for the five 1-day animals was 404.40 and that of the 5 control animals 353.80. This test elicited a non-significant value of t of .540.

Although limitations of space rendered it impossible to retain the animals long enough to run retention tests on all those which reached the criterion, tests were re-run on the 3 control and three 1-day animals which attained the criterion earliest 2 weeks from the date on which the criterion was attained by each animal. In these tests of retention, 2 of the 3 control animals achieved the criterion within 15 trials and the third in 30 trials. Of the three 1-day chicks, one ran to criterion in 30 trials, a second required 60 trials, and the third had not attained the criterion after having run 135 trials.

Discrimination Learning

In an exploratory investigation in which position and stimulus preferences were uncontrolled, significant differences were obtained between mean criterion scores of a control group, a 1-day group, and a 2-day group on a red-green discrimination task ($n = 8$ in each group). The animals employed in this study were not the same as those used in the temporal maze task reported above. The mean criterion scores were the following: control, 40.25; 1-day, 60.75; 2-day, 70.37. Tests of mean differences indicated the control vs. 2-day and control vs. 1-day mean differences to be significant ($P<.01$ and $P<.05$, respectively), while the 1-day vs. 2-day mean difference was non-significant.

In view of these findings, further exploration of the discrimination habit under conditions permitting control over preference behavior seemed desirable. Accordingly, the following groups incubated initially at the optimal temperature were given a color preference series when the animals were 16 days old: control ($n =6$); 20-day ($n = 6$); 19-day ($n = 6$); 18-day ($n = 6$); 17-day ($n = 5$); 16-day ($n = 6$). The results disclosed that no group manifested a significant preference for either of the 2 colored trays, although all groups showed a non-significant tendency to prefer the green stimulus over the red. No significant differences in stimulus preference behavior were in evidence between the groups. The data for individual chicks revealed that one animal in each of the control, 20-day, 19-day, 18-day, and 16-day groups displayed a consistent preference for the green tray over the red on 80% or more of the trials, while no such preference was shown by any of the animals in the 17-day group.

A position preference series was run when the animals were 17 days old. The results indicated that of the 6 groups a significant position preference (right) was displayed by only the 17-day group. The data for individual animals disclosed that 3 animals in the control group, 4 in the 20-day group, 4 in the 19-day group, none in the 18-day group, 2 in the 17-day group, and 2 in the 16-day group displayed consistent position preferences on 80% or more of the 15 trials.

The discrimination task was begun when the animals were 18 days old. A correction procedure was employed with the green tray as the positive stimulus. Immediately after an animal attained the criterion, a discrimination reversal sequence was run. Presentation of stimuli for both the discrimination and the reversal series was in accordance with randomly selected Gellerman sequences (2).

An analysis of variance of the criterion scores for the initial discrimination task elicited a non-significant F of .886. In Table 1 are shown

Table 1

Mean criterion scores of the six groups on discrimination task

Group:	Control	20-day	18-day	19-day	16-day	17-day
Mean:	39.00	39.33	43.83	46.33	53.00	58.40

the mean criterion scores of the 6 groups on the discrimination task. It may be seen that, although none of the mean differences is significant, the order of magnitude of these differences is generally such that animals

hatched from eggs exposed to the temperature insult for longer periods of time tended to require greater numbers of trials to attain the criterion.

Table 2 is the summary of an analysis of variance of the discrimination reversal scores. The F of 2.932 is significant at beyond the .05 level

Table 2

Summary of analysis of variance of criterion scores on discrimination reversal task

Source	df	SS	MS	F
Between Groups	5	2908.65	581.73	2.932*
Within Groups	29	5753.52	198.40	
Total	34	8662.17		

*P <.05

of confidence, indicating differences in performance between the 6 groups. A Bartlett test indicated error variances to be homogeneous. Table 3 contains the mean criterion scores of the 6 groups for the discrimination reversal series and the results of tests of mean differences. The "least significant difference" method, described by Steel and Torrie (10), was

Table 3

Results of tests of mean differences of criterion scores on discrimination reversal task ("least significant difference" method)

Group:	Control	18-day	20-day	19-day	16-day	17-day
n:	6	6	6	6	6	5
Mean:	71.33	76.67	81.33	86.50	94.17	97.60

Means underscored by the same line do not differ significantly.
Means not underscored by the same line differ significantly at or beyond the .05 level of confidence.

employed to assess the reliability of group mean differences at the .05 level of confidence. In Table 3 it is seen that the following 6 mean differences are significant: control vs. 16-day; control vs. 17-day; control vs. 19-day; 20-day vs. 17-day; 16-day vs. 18-day; and 17-day vs. 18-day.

Discussion

Despite the fact that the number of animals employed in this study was quite small, there would appear to be little doubt that exposure of eggs to incubation temperatures which were higher than optimal for the periods mentioned above had a depressive effect on the ability of the experimental animals to perform the two types of learning tasks. Generally the effect was found to be greater the longer the exposure to the higher temperature. Although perhaps somewhat premature, it is interesting to speculate regarding the nature and locus of this effect. Since accuracy rather than speed of performance was stressed by the response measure (number of trials to criterion), it seems unlikely that the effect on learning is related to inter-group differences in quality of motor functioning.

The temporal maze habit would appear to be a task which is near the ceiling of the chick's capacity to learn. Hunter (6) has suggested that the salient cues in the learning of this type of pattern are kinesthetic in nature. If this is assumed to be the case, the impaired learning ability of the experimental animals may have been a function of disturbance of kinesthetic feedback or of a reduced ability to utilize kinesthetic cues.

Although the group differences obtained on the initial discrimination series failed to attain statistical significance, the order of the group means was generally that which would be expected if the higher incubation temperature produced a reduction in ability to learn this task. For the discrimination reversal series this order was maintained, and significant group mean differences were found. Since the colored food trays used for this task differed in hue, saturation, and reflectance, further research will be necessary in order to determine the stimulus dimension or combination of dimensions which the animals utilized in making the discrimination. In the case of this task, hypotheses concerning the nature and locus of the effect of the temperature insult would vary, depending on the particular properties of the stimuli which were critical for the accomplishment of the discriminative response.

Gunther and Jones (4) have found lower mean weights and reduced rates of weight gain in chicks hatched from eggs incubated for various periods of time at non-optimally high temperatures. In considering possible underlying mechanisms which might have mediated these findings, the most plausible appeared to be protein denaturization of one or several enzymes which may have resulted in reversible or irreversible injury to the enzyme systems involved. On this hypothesis, the poorer performance of the experimental animals in the present study may have been related to a generalized pathological effect of the temperature insult on several or possibly all types of tissue. It is interesting to note that, as in the case of reduced weights, deleterious effects on learning ability were evident when the animals were incubated both initially and terminally at non-optimally high temperatures.

In view of the demonstrated deleterious effect of non-optimally high incubation temperatures on weight and rate of weight gain, it is also conceivable that the higher temperature may have more or less permanently altered the motivational level of the experimental animals in such a manner that identical periods of food deprivation may have produced differences in strength of hunger drive between control and experimental animals.

Obviously further study of these phenomena, both at the behavioral level and subsequently at more molecular levels, is indicated. Such research is currently in progress in our laboratory.

Summary

Chicks hatched from eggs incubated at the optimal temperature of 37.5° (control group) and animals hatched from eggs incubated at 41° for varying numbers of days during the initial phases of the incubation period and at the optimal temperature thereafter (experimental groups) were given a simple alternation task in the T-maze. A significantly greater number of control animals attained the criterion of learning than did experimental animals.

In a second experiment a control group and animals hatched from eggs incubated at 41° for varying numbers of days terminally in the incubation period (experimental groups) were run on a color discrimination task followed by a discrimination reversal series in the T-maze. The order of magnitude of the mean criterion scores of the various groups was such that animals hatched from eggs which were exposed to the non-optimally high temperature for longer periods of time tended to require greater numbers of trials to attain the criterion on both the discrimination task and the reversal series. Significant differences were found between the mean criterion scores of control and experimental groups for the reversal series, while group mean differences for the initial discrimination task were non-significant. Possible underlying mechanisms of the observed effects were considered.

Literature Cited

1. Color Harmony Manual, 3d Edition. 1948. Container Corporation of America, Chicago.
2. GELLERMAN, L. W. 1933. Chance orders of alternating stimuli in visual discrimination experiments. J. Genet. Psychol. 42 : 207-208.
3. GUNTHER, W. C. 1958. Effect of abnormal incubating temperature on chick behavior. Proc. Ind. Acad. Sci. 68 : 363-366.
4. GUNTHER, W. C. and R. K. JONES. 1962. Effect of environmental stress on chick weight. Proc. Ind. Acad. Sci. 71 : 385-398.
5. GUNTHER, W. C., ROBERT K. JONES, and PAUL MANSKE. 1961. The effect of high and low incubating temperatures on chick behavior. Proc. Ind. Acad. Sci. 70 :285-292.
6. HUNTER, W. S. 1928. The behavior of raccoons in a double alternation temporal maze. J. Genet. Psychol. 35 : 374-388.
7. KNOBLOCH, H., and B. PASAMANICK. 1958. Seasonal variation in the births of the mentally deficient. Am. J. Pub. Health 48 : 1201-1208.
8. PASAMANICK, BENJAMIN, SIMON DINITZ, and HILDA KNOBLOCH. 1959. Geographic and seasonal variations in births. Public Health Reports 74(4) : 285-288.
9. SIEGEL, SIDNEY. 1956. Nonparametric Statistics. McGraw-Hill Book Company, Inc., New York.
10. STEEL, ROBERT G. D., and JAMES H. TORRIE. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Company, Inc., New York.
11. THOMPSON, W. R. 1960. Research trends in comparative psychology. In Waters, R. H., D. A. Rethlingshafer, and W. E. Caldwell (Eds.), Principles of Comparative Psychology. McGraw-Hill Book Company, Inc., New York.