Effect of Nonoptimally High Incubation Temperature on Frequency of Pecking and on Color Preferences in the Chick[1]

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Introduction

In summarizing a series of studies concerned with the relationship between time of conception and mental deficiency, Pasamanick, Dinitz, and Knobloch (9) report a higher incidence of mental deficiency among children who were conceived during the late spring and early summer. These investigators suggest that the stress of summer heat during the critical embryonic period for central nervous system development may have been an etiological factor in these cases.

Gunther (2) has pointed up the feasibility of evaluating a possible relationship between behavioral anomalies and temperature stress during embryonic development through the use of the chicken in animal analogue investigations. By subjecting chicken eggs to nonoptimally high incubation temperatures during both the initial and terminal phases of the incubation period, it has been found that these higher temperatures produce various types of structural and behavioral abnormalities in the subsequently hatched animals (6) and also depress performance on certain learning tasks (4). In addition, Gunther and Jones (3) have found that nonoptimally high incubation temperatures result in lower body weight at hatching and also in reduced rates of posthatching weight gain.

The present study represents an attempt to assess the effects of nonoptimally high incubation temperature on pecking frequency and on color preference in the chick.

Materials

Subjects

All control and experimental animals used in the study were Cornish White Rock chicks. Control animals were hatched from eggs incubated at the optimal temperature of 37.5° C. for the entire incubation period. Experimental animals were hatched from eggs incubated at the nonoptimally high temperature of 41° C. for the first 72 hours of the incubation period and at the optimal temperature for the remainder of the period. Previous investigation suggested that maximally deleterious behavioral effects are achieved at this nonoptimally high temperature. With incubation temperatures higher than 41° C., or with temperatures between 37.5° C. and 41° C. employed over periods longer than the first three days of incubation, the mortality rate during incubation is inordinately high. The pre-experimental histories of the animal subjects are described in detail in appropriate sections below.

1. Supported by research grant B-2128, U. S. Public Health Service, Council on Neurological Diseases and Blindness.

Apparatus

Pecking apparatus. The apparatus employed was similar to one described by Hess (8). It consists of a large octagonal wooden box, the interior of which is painted a neutral gray. The floor of the box is covered with a wire-mesh screen which can be raised or lowered to accommodate chicks of different sizes. The apparatus is supported from the floor by legs, 24 inches high. Illumination of the interior of the box is provided by a 200-watt incandescent light bulb suspended 32 inches above the floor. To prevent the animals from escaping from the apparatus, the top is covered with a removable chicken-wire screen during testing. A removable wooden partition renders it possible to divide the interior of the apparatus into two sections of equal size. To eliminate extraneous visual stimuli during testing, the apparatus is surrounded by a thin, gray percale curtain.

On each of the eight side panels of the box is a metal receptacle containing two round holes through which the stimuli are presented at the ends of clear plastic cylinders which protrude through the holes. The metal receptacles are fitted flush with the side panels and are painted a neutral gray to render them homogeneous with the remainder of the interior of the box. Since each receptacle displays two stimuli, the entire apparatus permits the simultaneous presentation of 16 different stimuli. The stimuli are presented by attaching them to the insides of the clear plastic ends of the plastic cylinders which are then fitted into the round holes in the side panels. The other end of each plastic cylinder is attached by means of a screw to the lever of a microswitch at the back of the panel. Each switch (No. Bz- 2RW 8435- A2, Microswitch Company) is wired individually to an automatic counter. Thus, each stimulus cylinder, switch, and counter functions as a unit. When an animal pecks at a stimulus, the plastic cylinder and switch lever are moved backward, closing the switch and causing the counter corresponding to that switch to move ahead one integer. In this way, automatic recording of responses is accomplished.

The following are the dimensions of the pecking apparatus: diameter of inscribed circle, 43.5 inches; area of floor space, 10.9 square feet; wall height of box, 15 inches; length of each of the eight panels, 18 inches; size of faces of metal receptacles, 2.75 inches high by 7.37 inches wide; size of holes in receptacle into which plastic cylinders are fitted, 1.06 inches; distance separating holes in each receptacle, 3 inches. The temperature inside the apparatus was maintained at 28° ± 2° C.

Stimuli. Two types of stimuli, designed to simulate food objects, were employed in the tests of pecking frequency. A circular disc, ¼ inch in diameter, was cut from black construction paper and fastened to the center of the inside of the plastic end of each cylinder by means of transparent tape. The second type of target used to test pecking frequency was constructed by filling each clear plastic cylinder with Purina Starter Mash, the only food with which the animals had experience.

The stimuli employed in testing color preference consisted of ¼ inch discs, cut from 16 of the colored chips from the Ostwald Color

Harmony Manual (1). The 16 colors used were the same as those employed in a study of color preferences in chicks and ducklings by Hess (8). In this study, Hess selected these particular 16 colors in order to explore as much as possible of the chick's visible spectrum. The finest gradations among the colored stimuli, in terms of wave length, appear in regions where greatest sensitivity is thought to exist. The stimulus discs were cut from the following fully saturated Ostwald chips: 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 13, 14, 16, 19, 22, and 24. Each disc was attached at the center of the inside of the clear plastic end of each cylinder in such a manner that its dull side was presented to the animals in the apparatus. The colorimetric properties of the 16 chips

TABLE 1
Colorimetric specifications of Ostwald color chips under Illuminant C

Chip Number	Wavelength	Reflectance (%)	Purity (%)
1	572.4	68.8	80.8
2	577.8	63.0	85.2
3	581.9	51.7	85.6
4	588.0	39.5	83.0
5	592.7	32.4	81.0
6	601.2	21.7	75.0
7	617.0	13.5	59.6
8	494.1c	12.7	40.5
10	523.0c	10.0	44.2
12	565.9c	8.8	38.6
13	469.0	10.1	69.4
14	477.0	13.2	59.5
16	484.2	16.0	47.2
19	491.7	16.7	37.0
22	527.0	22.8	28.0
24	562.7	48.7	70.0

c = non-spectral color, wavelength given is that of complementary

under illuminant C are given in Table 1. All of the hues are spectral colors, except chips 8, 10, and 12; the wave length given for each of these three stimuli is the wave length of the complementary color.

Procedure and Results

Tests of Pecking Frequency

Chicks from 3 different lots were used in these tests. Each lot consisted of one control group and one experimental group of animals. Lot A was hatched in June, Lot B in July, and Lot C in August. The animals in all three lots were tested during the months of July and August and were subjected to approximately 14-hour deprivations of food but not water before each test session. Prior to being tested, the animals in all 3 lots had been run on learning tasks which required their being handled daily. All chicks within a given lot had been subjected to the same learning situations and had equal amounts of experience in these situations. Chicks in Lots A, B, and C were tested with the black disc targets, while only chicks in Lots B and C were used in tests in which the pecking targets consisted of food in the form of mash visible through the ends of the transparent plastic cylinders.

During each test session the wooden partition was used to divide the interior of the apparatus into two compartments of equal size, each with a total of 8 pecking targets. The test periods varied in length both within and among the lots. However, during each test period, control and experimental groups composing a particular lot were tested simultaneously in the apparatus and were placed in each of the 2 compartments for equal periods of time.

Although tests were run almost daily throughout the months of July and August, only the data for 6 representative test sessions are reported. Table 2 indicates for both types of pecking targets the total responses delivered by control and experimental groups within each lot, the ages of the animals at testing, the number of animals in each group, the number and length of trial periods for each lot, and the values of chi-square based upon the differential response frequencies of experimental and control groups of each lot.

It is seen that each value of chi-square is significant at beyond the .001 level of confidence, the control animals delivering the greater number of responses in each case. There is a tendency for these values to decrease with increasing age of the chicks, but they remain highly significant when the birds are 52 days of age, in the case of the black disc targets, and when the birds are 53 days of age, in the case of the targets consisting of mash in the plastic cylinders. In a previous investigation (3), it was found that chicks hatched from eggs which had been subjected to nonoptimally high incubation temperatures showed significantly lower posthatching body weight and reduced rates of weight gain when compared with controls. In view of the findings of the current study, it was felt that experimental aniamls may possibly ingest smaller quantities of food than do control animals, and that this might be a factor related to the reduced weights and rates of weight gain observed earlier. Accordingly, the amount of food ingested by the animals of Lots B and C was measured during several free-feeding periods when the animals were between 30 and 45 days of age. Tests of significance of difference between mean amounts of food ingested by experimental and control groups in these 2 lots elicited only very low non-significant values.

Color Preference Test

Forty-five control and 45 experimental aniamls were used in this test. These chicks composed Lot D and were 9 days old when tested. The animals were not experimentally naive, since at 5 and 6 days of age they had been introduced to the 16 color stimuli in tests designed to determine the effects of nonoptimally high incubation temperature on proclivity for preferred versus non-preferred colors. All animals were deprived of food but not water for about 13 hours prior to testing.

TABLE 2

Conditions and Results of Tests of Pecking Frequency on Experimental and Control Groups of Lots A, B, and C
Targets: Black Discs

Lot	Group	Total Responses	Age (days)	n	Chi-square (1 df)	Conditions
A	Control	12,604	12	41	10,199.46*	Two 1-hour trials
	Experimental	877	12	41		
B	Control	5,074	19	24	1,543.14*	Two 2¾-hour trials
	Experimental	1,813	19	24		
C	Control	5,854	52	11	1,271.08*	Two 4½-hour trials
	Experimental	2,579	52	11		
		Targets: Starteena Mash in Transparent Plastic Cylinders				
B	Control	26,748	22	24	9,361.96*	Four 1½-hour trials
	Experimental	7,557	22	24		
B	Control	3,422	33	24	72.96*	Two 1½-hour trials
	Experimental	2,753	33	24		
C	Control	3,883	53	11	503.16*	Two 1½-hour trials
	Experimental	2,141	53	11		

* Significant at beyond .001 level of confidence.

All 16 color stimuli were employed, a different color being situated at each of the 16 target locations. A total of 16 half-hour trial periods was administered. During each half-hour period, experimental and control groups were each exposed to the color stimuli for 15 minutes. At the end of each half-hour period, the positions of the stimuli were randomized among the 16 target locations. The testing was done in August, and the total time required to administer the 16 periods was approximately 14 hours.

The total numbers of responses delivered to each of the 16 stimuli by control and experimental groups are given in Table 3. As was found

TABLE 3

Total Responses Delivered by Control and Experimental Groups of Lot D

Chip number	Responses by control group	Responses by experimental group
1	3,776	24
2	8,022	44
3	5,686	50
4	1,742	26
5	2,821	26
6	1,627	31
7	1,083	25
8	988	23
10	964	20
12	738	22
13	785	47
14	1,504	77
16	540	50
19	479	26
22	1,225	25
24	1,904	39
Total	33,884	555

to be the case in tests of pecking frequency, the total numbers of responses delivered by control and experimental groups differ markedly, and this difference is highly significant statistically. Fig. 1 is a frequency polygon showing the relative percentage of total responses plotted against the decreasing wave length values of the stimuli. It is seen that the highest percentages of responses by the control group were delivered to stimuli in the orange and yellow regions of the spectrum, while the highest percentages for the experimental group occur in the green-blue region. An overall chi-square (10) based on the differential frequencies of response between control and experimental groups for all 16 stimuli elicited a value of 534.55, significant at beyond the .001 level of confidence. The results of chi-square tests, based on

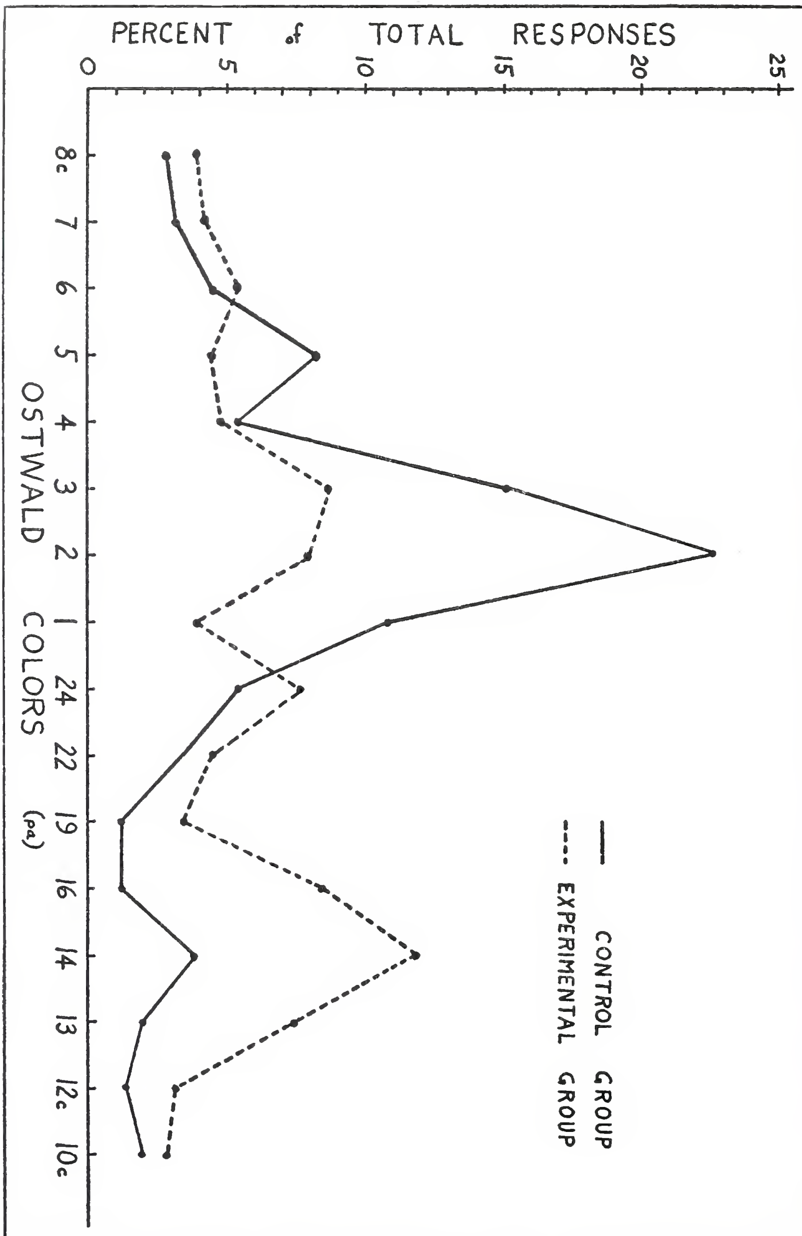

Figure 1 Percentage of total responses delivered by control and experimental groups of Lot D.

percentage differences in response between control and experimental groups for each color stimulus, indicated that the group percentage differences for the following stimuli are significant at or beyond the .01 level of confidence: 1, 2, 3, 5, 12c, 13, 14, 16, and 19. The results of these tests indicate that the group differences in relative preference for stimuli in the two spectral regions noted above are significant.

Discussion

The finding that groups of experimental animals delivered consistently lower numbers of responses than controls in tests of pecking frequency suggests several hypotheses concerning the nature of the effect of the temperature insult on the experimental chicks. In an earlier study (4) in which nonoptimally high incubation temperature was found to depress the chick's ability to learn under food deprivation an alternation pattern in the T-maze, it was considered possible that the higher incubation temperature may have more or less permanently reduced the motivational level of the temperature-stressed animals. The finding in the present study of ingestion of equal amounts of food by control and experimental animals during free-feeding would appear to cast doubt on the tenability of any hypothesis of reduced hunger drive in the experimental animals.

Gunther and Jones (3) have found lowered mean weights and reduced rates of weight gain in chicks hatched from eggs incubated for various periods of time at nonoptimally 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 reduced pecking rates 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.

Still a third possibility is suggested by the results of the color preference test in which experimental animals manifested altered responsiveness to color stimuli. It seems conceivable that the higher incubation temperature may have altered the peripheral and/or central visual apparatus in such a manner that acuity for visual stimuli was generally reduced in the experimental animals, resulting in reduced response rates on the part of these animals.

In the tests of pecking frequency, a tendency was noted toward reduction of the significance levels of the results of statistical tests with increasing age of the animals. This suggests the possibility that the effects of the temperature insult might be overcome at a more advanced age. It is intended to evaluate this contingency in future research.

In a study in which the experimental apparatus and procedure were closely similar to those employed in the color preference test of the present investigation, Hess (8) found no evidence to suggest that the purity or reflectance of color stimuli was effective in determining preferences of chicks for objects of different colors. The results of the present study appear to be in accord with those of Hess in that a comparison of the purity and reflectance values in Table 1 with the percentages of total responses delivered by control and experimental groups to the series of color stimuli (Fig. 1) does not disclose any greater effectiveness of the dimensions of purity and reflectance over color in either group.

It was found that control animals demonstrated a greater preference for stimuli in the yellow and orange regions of the spectrum, while experimental animals preferred pecking at green and blue stimuli. However, because of the relatively small response total of the experimental group, the reliability of these differences may be questioned. Assuming the differences to be reliable, speculation concerning underlying mechanisms which may have mediated the finding is appropriate.

Since the animals in both groups had been exposed to the color stimuli prior to testing, it is possible that experimental and control groups had been differentially imprinted on the color stimuli. In addition to the absence of an explanation of such differential imprinting, several other considerations would appear to detract from the plausibility of this hypothesis. In previous unpublished studies, the authors have found it difficult to imprint White Rock chicks on color stimuli under optimal imprinting conditions. In this connection Hess (7) had reported that the susceptibility of White Rocks to imprinting on various types of objects is considerably lower than that of other races of chickens. In addition, the pre-test exposure to the color stimuli in the present study occurred when the animals were at an age considerably beyond the optimal or "critical" period for imprinting in most fowl.

It is conceivable that the group differences in response to the color stimuli are related to structural changes which may have been produced in the peripheral visual apparatus by the temperature insult. For example, the differences may reflect an effect of the higher incubation temperature on retinal cones which mediate response to different spectral regions. The cones of the chicken retina contain red, orange, and yellow oil-droplets which are regarded as being responsible for the partial blindness for blue stimuli which is normal in the chicken. According to Walls (11), this partial blindness for blue increases with growth, presumably because of the deepening of pigmentations of the oil-droplets. In view of these considerations, a possible effect of the temperature stress on the experimental animals of the present study may have been to reduce the rate of deepening of oil-droplet pigmentation and/or to produce a reduction in the growth rate of these animals. In either or both of these cases, it might be expected that the experimental animals would show relatively greater sensitivity to stimuli in the blue region of the spectrum than would animals in the control group.

It is also conceivable that the differential response to color stimuli observed between control and experimental groups may have been a function of effects of the higher incubation temperature on more central visual mechanisms, or possibly on a combination of central and peripheral elements. For example, the undetermined neural bases of color preference behavior may have been involved.

At any rate, these results extend the number of different behavioral aberrations previously reported. Since temperature is the only variable (as far as is known at this time), it appears that further studies on human mental deficiency, such as those reported by Pasamanik, et al.

(9), based on temperature data may prove very fruitful. As more research is directed toward an experimental approach to mental illnesses, it becomes increasingly evident that the basic chemistry of brain metabolism is involved (5).

Summary

Experimental groups of chicks were hatched from eggs incubated at the nonoptimally high temperature of 41° C. for the first three days of the incubation period and at the optimal temperature of 37.5° C. for the remainder of the period. Control groups of chicks were hatched from eggs incubated at the optimal temperature for the entire incubation period. Comparisons between experimental and control groups were made by means of an apparatus which permitted automatic recording of responses delivered to pecking targets of different types.

With chicks of different ages and with pecking targets which resembled food objects, the spontaneous pecking frequencies for control groups were significantly greater than those for experimental groups at all ages tested. With chicks of the same age and with pecking targets consisting of objects of different colors, the experimental group delivered significantly higher percentages of responses to targets in the green-blue region of the spectrum than did the control group; the control group delivered significantly greater percentages of responses to targets in the yellow-orange region of the spectrum than did the experimental group. Underlying mechanisms which may have mediated these results were discussed.

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