# Effects of Embryonic Temperature Stress on Handedness and Variability In Chicks<sup>1</sup>

# W. C. GUNTHER,<sup>2</sup> Valparaiso University

## Introduction

The response of an organism to its environment is certainly partially innate and partially learned. The degree to which an animal is committed to act because of instinct varies considerably. In chickens (and in other fowl, and even in higher forms) innate behavior may be modified by so-called imprinting, or imprinting is part of innate behavior or of learning (1, 2, 8). The relationship still is not clear. Handedness (perhaps laterality is closely related) appears to be another instinctive behavioral characteristic which can be modified and which can be symmetrical under some conditions but not under others (9).

In our laboratories it seemed desirable to learn whether chicks had an innate tendency to turn right or left—in other words, to ascertain whether the birds were right-handed, left-handed, or neither. If they were strongly inclined in either direction, there might be cases of handedness which could obscure results in testing situations in a maze or similar apparatus. The number of times an animal varied its direction from trial to trial might also be important and might follow from the degree of strength of handedness. Should strong position preferences be shown by the chicks, it might be necessary to equate as closely as possible those groups performing the same task in any situation involving direction change. As a matter of fact, we have done this routinely in our laboratories but without any real knowledge of its value (5).

The work described in this paper is an attempt to ascertain handedness in chicks, and to learn whether stereotypic behavior might also be a factor to consider when analyzing results of position preference or discrimination.

As detailed herein, the control chicks are those hatched from eggs incubated during the entire period under optimal temperature conditions. Experimental chicks are those hatched from eggs incubated under various conditions of nonoptimally high temperature. The assessment of the difference between these two groups is of paramount importance, since it has become obvious that temperature control of the incubating egg within very narrow limits is quite essential in producing chicks whose behavior and mental capabilities might be considered normal (4). We here use the term normal as characteristic of the behavior and mental ability of chicks hatched from eggs incubated under optimal conditions.

### Materials and Methods

A total of 576 White Rock eggs was incubated. Two hundred eightyeight eggs were placed in the normal temperature  $(37.5^{\circ} \text{ C}.)$  incubator

<sup>1.</sup> This research was supported by a grant from the Grass Foundation, whose assistance for the third year is gratefully acknowledged.

<sup>2.</sup> Temporary address: Bureau of Institutional Research, University of Minnesota, Minneapolis, Minnesota.

#### ZOOLOGY

for 15 days. On days 16 through 20, lots of four dozen eggs each were placed successively in a nonoptimally high temperature (42° C.) incubator until hatching. The remaining four dozen eggs (controls) were permitted to continue incubation at the optimal temperature. Those chicks hatched from eggs placed in the high temperature incubator are called 20-day, 19-day, 18-day, 17-day, and 16-day experimentals. These designations refer to the number of days the eggs were in the normal temperature incubator before being placed in the nonoptimally high temperature incubator. Thus, a 20-day experimental is a chick which was hatched from an egg incubated under optimal temperature for 20 days and placed under nonoptimally high temperature for the last day of incubation until hatching.

The remaining 288 eggs were separated into lots of 4 dozen each. One lot (controls) was placed in a normal temperature incubator at  $37.5^{\circ}$  C. The others were placed in a  $41^{\circ}$  C. incubator and were removed one lot per day for five days, each being placed immediately upon removal into the normal temperature incubator. All eggs were then permitted to continue incubation under optimal conditions until hatching. Thus, a 3-day experimental chick is one hatched from an egg incubated initially for 3 days under the nonoptimally high temperature, after which the egg was returned to the optimal temperature and permitted to hatch.

Four days after hatching, testing in a T-maze was begun. The end of each arm of the T-maze contained a tray in which food was placed so as to be visible from the choice point. The T-maze has been described in detail elsewhere (6). 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 is provided by fluorescent lamps mounted over the arms of the maze, and the amount of light on each food receptacle is regulated by rheostats and is equated by means of a light meter. The food trays are red plastic; reflectance, hue, and saturation values of each were checked and matched with the dull side of color chips from the Ostwald Color Harmony Manual (3).

Each chick during testing was placed in the start box of the maze, the gate was lifted, and the chick was permitted to search for the food. The chick had to walk the length of the runway, the end of which is the choice point. There the bird had to make a choice of turning right or left to get to the food in the respective arm of the maze. The first turn the chick made was scored, the chick was permitted to peck once or twice at the food, and was then returned to the start box. The chicks were deprived of food, but not water, for six hours prior to each testing session. The whole procedure was repeated until twenty trials had been recorded in one session. The chick was then returned to the brooder and used in the same way the following day. All chicks were tested according to this schedule.

Eight control chicks and eight 20-day experimentals were used from the group of chicks in which the temperature-stress had been at the end of the incubation period (too few chicks hatched in the other 4 lots to use in this experiment). The number of trials for this group was 3120.

The chicks which were hatched from eggs incubated at the nonoptimally high temperature during the initial period of incubation were run 180 trials each. In each lot there were 4 chicks: controls; and 1-day, 2-day, and 3-day experimentals—a total of 16 chicks (no chicks hatched from the 4- and 5-day lots). The number of trials was 2880.

Data from these position preference tests were analyzed for significance by means of chi-square.

Variability was likewise recorded, and chi-square analysis was made of these data. A sequence such that the animal turned R L R R L, etc., gave an index of variability of 3, etc.

#### Results

Table 1 indicates the number of right and left turns made by the group hatched from eggs incubated at the nonoptimally high temperature during the last days of the incubation period. Comparing the controls with the 20-day birds, the chi-square analysis yields results on rights versus lefts which indicate that the control birds made a significantly greater number of left turns, whereas the 20-day chicks made a significantly greater number of turns to the right. Comparing the number of right turns made by the controls with the number of right turns

# TABLE 1

Right and left turns in T-maze by chicks hatched from eggs subjected to nonoptimally high temperature at end of incubation period

Group	Number of Turns			
	Right	Left	Total	Chi-square (1 df)
All animals	1517	1603	3120	2.37 NS
Controls	517	1043	1560	$177.35^{**}$
20-day	1000	560	1560	124.10**

NS = not significant\*\* = P < .001

# TABLE 2

Right and left turns in T-maze by chicks hatched from eggs subjected to nonoptimally high temperature during initial period of incubation

	Number of Turns			
Group	Right	Left	Total	Chi-square (1 df)
All animals	1929	951	2880	332.11**
Controls	369	351	720	0.45 NS
1-day	518	202	720	138.69**
2-day	387	333	720	4.05*
3-day	655	65	720	483.47**

NS = not significant

$$* = P < .05$$

$$^{**} = P < .001$$

made by the experimentals, the latter turned right a significantly greater number of times (chi-square = 153.04). Readily apparent from inspection is the fact that the controls turned significantly more numbers of times to the left than did the experimentals. When all data are combined, no significant difference appears between these two groups.

From an analysis of Table 2 it is seen that the same kinds of differences were present in the chicks hatched from eggs subjected to the nonoptimally high temperature during the initial stages of incubation. While the control birds did not make a significantly greater number of turns to the left, each of the experimental groups turned right more frequently than to the left. Chi-square values are all significant. If all experimentals are grouped and the average compared with the controls, the number of right turns made by the experimentals is significantly greater than the number of right turns made by the controls (chi-square = 25.64). The converse is likewise true. When all data are combined, it is seen that a significantly greater number of right turns over left were made. This simply may reflect the fact that the control chicks did not make a significantly greater number of turns to the left.

Chi-square analysis of variability (number of times chicks switched from right to left and left to right) indicates that among the groups listed in Table 1 the experimentals are significantly more variable than the controls (chi-square = 6.55). In other words, stereotypy is typical of the controls. However, this is true only of the chicks hatched from eggs undergoing the heat-stress at the end of the incubation period. Chicks hatched from eggs heat-stressed at the beginning of the incubation period show within lot differences, but the averages are not significantly different (chi-square = 1.03). However, unpublished data on later experiments with other types of position preference tend to support the view that the control chick is more stereotyped as far as this variable is concerned.

### Discussion

These results are in agreement with other experiments and indicate that heat stress during development has an influence on the behavior of the hatched chick (4, 5, 6). It would be unwise to say that according to these findings control chicks (that is, essentially normal chicks as we interpret normalcy) appear in a population as either strongly righthanded or strongly left-handed. One would at the same time question that the chicks in these lots, selected completely at random, had been grouped by chance so that the controls exhibit one or no direction preference while the experimentals all strongly favor turning to the right (or at least favoring strongly one position over another). The conclusion is that the heat treatment influenced the developing brain centers, even during late stages of incubation, so that the levels involved were damaged to some extent, and this in turn favored a shift of direction or position to the right. One might go even further and generalize that direction emphasis is one of the results of short periods of nonoptimally high incubation temperatures during incubation. It appears clear that groups should be equated for position preference before testing.

Interpretation of stereotypy is more difficult. It seems that the heatstressed birds are more unpredictable in their position preference from trial to trial, while the controls exhibit stereotypy in this kind of behavior. However, our results do not indicate this strongly, and more research is required. If chicks are matched for handedness, variability may be a factor equated at the same time.

The basic cause of behavior is relatively or completely unknown. The present experiments suggest an innate pattern capable of being modified during development by external sources. The view that development proceeds as the result of interaction between gene and environment is no less tenable from an analysis of these results.

Stereotyped behavior is also relatively unexplored. Hernstein (7), in a completely different experiment with pigeons comes to the conclusion that "a significant but unexplored principle may be involved." This appears to be a suitable note on which to end this paper.

## Summary

Chicken eggs in each of two groups were subjected to nonoptimally high incubating temperatures. The temperature stress was imposed in one group during the first three days and in the other group during the last three days of incubation. In each case the embryos which survived completed development and were permitted to hatch. After hatching, the chicks were tested in a T-maze for right and left turns; variability in turning right or left was also recorded. It was found that the heat-stressed chicks made a significantly greater number of turns to the right than did the chicks hatched from eggs incubated under normal temperatures. Variability, that is the tendency to alter patterns of turns more frequently, was shown in one group of heat-stressed chicks to be significantly greater than in the control birds. Thus, normal chicks (those hatched from eggs not subjected to the heat treatment) tend to show a more stereotyped kind of behavior with respect to this variable. Handedness and stereotypy were discussed further in connection with innate behavior.

### Literature Cited

- 1. Brown, Larry T. 1964. A critical period in the learning of motionless stimulus properties in chicks. Animal Behavior **12**: 353-361.
- Collins, Thomas B. 1965. Strength of the following response in the chick in relation to degree of "parent" contact. Jour. Comp. & Physiol. Psych. 60: 192-195.
- 3. Color Harmony Manual, 3d Edition. 1948. Container Corporation of America, Chicago.
- 4. Gunther, W. C. 1965. Further observations on effect of nonoptimally high incubation temperature on frequency of pecking and color preference in the chick. Proc. Ind. Acad. Sci. **74**: 362-366.
- Gunther, W. C. and Robert K. Jones. 1961. Effect of nonoptimally high incubation temperatures on T-maze learning in the chick. Proc. Ind. Acad. Sci. 71: 327-333.
- 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.
- Hernstein, R. J. 1961. Stereotypy and intermitten reinforcement. Science 133: 2067-2069.
- 8. Hess, E. H. 1964. Imprinting in birds. Science 146: 1128-1139.
- Miller, Marilyn E. 1966. Right-response preference in probability learning and reversal. Jour. Exp. Psych. 71: 776-778.