of other substrates. It may be noted that Nielson and Klitgaard (*13*) have suggested the existence of a biological rhythm for succinate oxidation in tissue homogenates from starved rats.

There are no changes in P/O ratios in mitochondria from artificially exercised animals (*3*), so that nocturnal increases in these ratios seem to be part of a rhythm independent of physical activity. The fact that the P/O ratio for succinate alone was unaffected at night implies that the regulation of P/O ratios for the other substrates occurs at the site of phosphorylation associated with NAD. Perhaps adenosine triphosphatase activity, in which an intermediate of oxidative phosphorylation is destroyed, is relatively low at night; ATP production might accelerate. At daybreak large concentrations of ATP or an ATP derivative would either stimulate the enzyme activity directly or inactivate an inhibitor of this enzyme; P/O ratios would fall. By evening, the utilization of excess ATP for other purposes would result in diminished adenosine triphosphatase activity. It should be emphasized that other intracellular feedback systems might regulate P/O ratios. In addition, the hormonal output of animals at night presumably differs from that during the day (*14*), so that there may be many complicating extracellular influences on intracellular rhythms.

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Following and Imprinting: Effects of Light and Social Experience

Abstract. *Ninety-six Vantress broiler chicks were used in two studies of the effects of light and social experience on the "following" response. In addition, the animals used in one study were later tested to determine the amount of imprinting during following. The difference between the behavior of these animals and animals maintained in isolation and in darkness prior to exposure to a model, indicate that variability in treatment can influence the following behavior, and that following in itself cannot be equated with imprinting.*

The variability in techniques and procedures used in the study of imprinting has increased greatly with the large number of experimenters now working on the same subject. This would not be a problem if there were not the tendency to relate the results of one study to those of another without taking these differences in procedure into consideration.

The maintenance of the animals prior to the initial exposure to a model is one of the areas of variability in imprinting procedures. Animals have been kept isolated in the dark (*1–3*), isolated in the light (*4, 5*), and maintained in groups in the light up to the time of imprinting (*6*). The two short studies in this report were designed to show the effects of light and group experience on the "following" response in an effort to indicate the impossibility of making direct comparisons between the results of two individual experimenters when each has used a different procedure. While this report deals only with the variable of how the animals are treated before imprinting, it would be expected that differences in behavior would be found where different procedures have been used regarding the use of sound, speed of model, and species of animal.

In the first study, 56 Vantress broiler chicks hatched in this laboratory were divided into four groups of 12, 14, 14, and 16. Within 2 hours after hatching in the darkroom, all chicks were removed from the incubator and placed individually in cardboard boxes measuring 11.5 by 11.5 by 14 cm. The chicks remained in these boxes until used experimentally.

One group of 12 chicks and another of 14 chicks were exposed to a model at 16 hours after hatching, and one group of 14 and one group of 16 chicks were exposed to the same model at 48 hours. The control groups ($N = 14$) were given no experience prior to imprinting. The animals in the experimental groups ($N = 12$, $N = 16$) were treated in exactly the same way except that they were placed individually in a wooden isolation box, 10 by 25.5 by 30.5 cm, for 2 hours before imprinting, with a 100-watt bulb suspended over the box.

All animals were run in the Hess imprinting apparatus, described in detail elsewhere (*7*). The model used was a blue ball (Ostwaldpa 14) 20 cm in diameter. A speaker inside the ball, connected to a conventional tape recorder, provided a continuous and rhythmic call: "Come-chick-chick-chick." The model moved 30.5 cm in 6 seconds and was stationary for 12 seconds, making one turn around the 3-m runway in 3 minutes.

The experimental animals were returned to their cardboard boxes for transportation to the imprinting room. With the room in darkness the chick was eased onto the runway next to the model. The experimenter took his place behind the control panel where the chicks could be observed through a one-way screen. The lights and sound of the apparatus were turned on. The model remained stationary for 10 minutes, then made four turns around the runway. The control animals were run in the same way. Table 1 shows the mean distance followed by each of the four groups. The effect of the light experience on the chicks at 16 hours was slight. There was also little difference between the groups given light experience at 16 hours and at 48 hours. There was, however, significantly more following by the animals given light experience at 48 hours than by the controls at 48 hours ($p < .05$, Mann-Whitney U-Test) (*8*). There was also significantly more following by the 16-hour-old controls than by the 48-hour-old controls ($p < .05$, Mann-Whitney U-Test).

In the second study, four groups of ten Vantress broiler chicks were used. One control group was imprinted at

16 hours, having been maintained in the dark and in isolation from the time of hatching to the time of imprinting. A second control group was maintained under the same conditions until 36 hours of age and then imprinted. Each chick in the two experimental groups was given 2 hours of experience in a brooder containing ten other chicks and illuminated by a fluorescent bulb immediately before exposure to the model. One group was removed from isolation at 14 hours and imprinted at 16 hours. The second experimental group was removed from isolation at 34 hours and imprinted at 36 hours of age.

The animals were run on the same apparatus and in the same manner as the animals in the first study. However, after imprinting, all animals were returned to their boxes and placed in isolation in the darkroom until testing, which took place on the following day, 20 to 28 hours after the animals had been imprinted.

Testing consisted of returning the animal to the runway of the imprinting apparatus. On one side of the chick, at a distance of 61 cm, was the original imprinting model; at an equal distance on the other side was a group of four chicks in a plastic "corral," 20 cm in diameter. Following the procedure of Ramsay and Hess (*1*), there were four 2-minute test periods: period 1, model stationary, silent; period 2, model stationary, sound; period 3, model moved to center, silent; and period 4, model moved to center, sound. If, at the end of a 2-minute period, the chick was with the model, it was given a score of 2.5 points. In this way, an animal going to the model during the first period, and staying with it during the next three periods, could get the maximum score of 10 points.

The results shown in Table 2 indicate that 2 hours of experience in the light with a group of chicks had a much stronger effect on the following response than did experience in the light alone. At each age, the groups given social experience showed significantly more following than the control groups (16 hours, $p < .05$; 36 hours, $p < .001$). While there was little difference between the means of the two groups that were given social experience, there was a marked difference between the two control groups, significant at the 0.02 level (Mann-Whitney U-Test).

Table 2 also shows the correlations between the individual imprinting and the test scores of the animals in the four groups (Spearman rank correlation coefficient) (*9*). The only group in which the animals with high following scores responded to the model well during testing was the control group aged 16 hours. For the two groups aged 36 hours the behavior during testing was random, with no relationship between following scores and test scores. The behavior of the 16-hour-old group given social experience was exactly opposite to that of the control group, the animals with high following scores responding more to the group of chicks and less to the model during testing.

Table 1. Mean following scores of four groups of chicks.

Group	Distance followed (m)	
	At 16 hours	At 48 hours
Control	6.5	4.0
Light experience	7.5	6.8

Even though the size of the group of animals used in these studies was relatively small for imprinting research, there is a consistency in the results between the two experiments. That the control group at 36 hours followed less than the control group of 48 hours might be due to the group size where individual differences would tend to have a more pronounced effect. Gray (*2*) and Baer and Gray (*9*) had a similar situation when, using the same basic technique in both experiments, they found response peaks on the 2nd and 5th days, respectively.

Experience in the light clearly had an effect on the older animals in the first study, which could explain why some experimenters (*4*) have found animals follow well when past the critical period as delineated by Ramsay and Hess (*1*). Moltz and Stettner (*10*) found increased following up to 72 hours of age in ducklings maintained in diffuse light rather than patterned light, which might indicate even more extensive variability in another species.

Table 2. Correlation between following and test scores with mean rank of following and test scores for four groups of chicks.*

Group	Mean distance followed (m)	Rank	Mean test scores (m)	Rank	Correlation
		At 16 hours			
Control	5.5	3	5.75	1	+0.76
Social	9.3	2	1.00	4	− .78
		At 36 hours			
Control	1.5	4	2.00	2	+ .02
Social	10.0	1	1.50	3	+ .08

* Spearman rank correlation coefficient.

The effect of the social experience on the following response was even more pronounced than the effect of light experience, and was found to influence the behavior of the chicks at 16 hours as well as the behavior of the older animals. It is almost as though the experience had primed the animals to follow, and yet, the results of the second study would indicate that, on the basis of the test scores, the only group that had actually been imprinted with the model was the 16-hour-old control group. The results of the control groups in both experiments support the critical period concept (*1*), and the second experiment is in line with earlier findings, that, insofar as imprinting is concerned, primacy of experience has a greater effect on behavior than recency (*11*).

Guiton (*12*) has stated, "Preliminary observations have indicated that at least some communally reared chicks will continue to respond to a strange moving object by following it if they have been deprived of food and/or water." The bulk of his data on social experience, however, concerned animals that were older than those in our studies, and that were maintained on food and water after hatching. Also, he gave no comparative data for animals in the age range dealt with in our experiments. Gottlieb and Klopfer (*13*) reported that Pekin ducks with social experience follow less than ducks raised in isolation. Hess (*14*) has found an even greater reduction in following in Mallards after social experience, which could be attributed to the generally stronger instinctive behavior in wild birds.

Since the variables of light experience and social experience in the light have been shown to have a definite effect upon the following response, it might be worthwhile to keep two points in mind when doing research on imprinting. First, it is not always possible to equate following with imprinting. In our second study, both groups that were given social experience showed excellent following, but testing showed no evidence of imprinting. The second point is, that while it is difficult to generalize from one person's work to another's, if there are differences in the procedures used, it is impossible to

make comparisons between experiments when completely different phenomena are being studied.

This might help to explain some of the differences which have been reported in the literature on imprinting, and might help to avoid further misunderstandings among experimenters in this area.

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Early Arousal and Imprinting in Chicks

Abstract. *Arousal outside the test situation and prior to response acquisition can affect strength of imprinting, if such treatment occurs early enough in life. Young chicks handled in darkness either at 5 or 9 hours of age and exposed to a moving surrogate a number of hours later were compared with nonhandled controls by means of following tests given at 30 and 54 hours of age. Handling at 5 hours resulted in a significant increment in the later following response. Handling at 9 hours produced no effect.*

It has been suggested by several writers that the attachment formed between a young bird and its mother or some surrogate is dependent for its strength, duration, and time of occurrence on the emotionality of the young animal (*1–3*). Thus, the addition of emotion-arousing stimuli to the imprinting situation may increase the strength of following during (*3*) or after (*2*) the period critical for acquisition of this response. The results of Moltz *et al.* (*2*) and of Pitz and Ross (*3*) indicated that arousal is effective in producing strong imprinting only if it is elicited in close conjunction with the stimulus conditions in which the response is tested. Thus the subjects of Pitz and Ross, stimulated whenever they were not near the surrogate, and the subjects of Moltz *et al.*, shocked outside the imprinting alley, followed poorly in comparison with animals aroused when near the surrogate or shocked inside the imprinting alley. Such data suggest that arousal may be important, not in general, but only as it is concerned with the formation of an associative bond between the stimulus and the response. However, it is also possible that the generality or specificity of the contribution of arousal is age-dependent. Indeed, at the time of arousal, the subjects of Moltz *et al.* and of Pitz and Ross were 7 days old or more and 1 day old or more, respectively. In view of the large amount of work (*3*) showing the later effects on rodent behavior of early manipulations (for example, handling or shocking), it seems likely that nonspecific arousal outside the test situation, if applied early enough, might have considerable effects on imprinting in chicks. Concomitantly, the effects might be expected to decline as the stimulus is applied later in age. Such an interpretation is consistent with the results of Moltz *et al.* and of Pitz and Ross. Thus the experiment we report here was designed to offer a limited test of this hypothesis: that nonspecific arousal imposed on chicks outside the test situation and prior to acquisition of the imprinting response has greater effects on the latter when it is imposed at an early age than when it is imposed at a later age.

Forty-eight commercially hatched Vantress chicks were used. They were transferred to the laboratory in closed boxes within three hours of hatching and were individually housed in wooden cages (22 by 20.5 by 13 cm) with fine mesh nylon screening. Lighting was supplied by the 200-watt ceiling bulb. Cage temperatures were maintained at approximately 31°C.

The testing apparatus consisted of a runway measuring 3 by 0.3 by 0.6 m. Its floor was covered with white cardboard and its walls were painted white with heavy irregular black lines. Illumination was supplied by a single 200-watt bulb hung over the center of the runway. The imprinting object was suspended 5 cm above the floor from a rope belt run between two pulleys located one at each end of the runway, and geared to a variable speed motor. The imprinting object was a cellulose toy duck and the unfamiliar object (used in the "following" test) was a rubber toy man. A semicircular restraining unit of wire mesh, 30 cm high with a diameter of 23 cm, was placed against the middle of one wall of the runway.

The procedure was as follows. Chicks were assigned randomly to one of four groups: H5, handled 5 hours after hatching; C5, control for H5; H9, handled 9 hours after hatching; or C9, control for H9. Arousal was produced by tactile stimulation or handling for a single 10-minute session, either 5 or 9 hours after hatching; the chick was stroked from head to tail at the rate of approximately 15 to 20 strokes per minute. All such stimulation was done in complete darkness to avoid visual effects. Con-

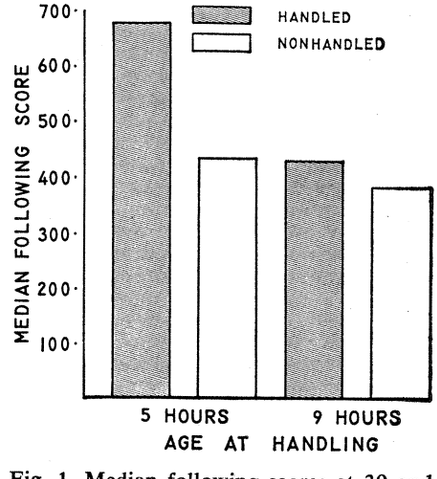

Fig. 1. Median following scores at 30 and 54 hours of control chicks and chicks handled at 5 and 9 hours of age.