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EFFECTS OF EARLY EXPERIENCE ON FEAR BEHAVIOUR OF *COTURNIX COTURNIX*

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Moltz (1960) defined 'imprinting' as 'a procedure (which) has been found to evoke close following of the object in such precocial avian species as ducks, geese, coots, moorhens and domestic fowl.' Other investigators (Scott 1962; Sluckin 1965) have not limited the definition of this phenomenon to the topographical characteristic of following behaviour. These authors indicate that many 'attachments' revealed by responses other than following should be classified as 'imprinting'.

The reciprocal relationship between fear behaviours and approach and following responses ('imprinting') in precocial birds has been well documented. The development of fear behaviour is involved in the termination of the sensitive period for 'imprinting' (Collias 1952; Hess & Schaefer 1959; Salzen 1962; Schaller & Emlen 1962; Bateson 1964). Conversely, after following has been established, presence of the stimulus object tends to inhibit fear behaviours while its absence will evoke distress calling and increased activity (Hoffman et al. 1966; Stettner & Tilds 1966).

From these studies it is not clear whether fear behaviours are directly affected by early exposure to a stimulus or whether these effects are dependent on the establishment of approach and following responses. The latter suggests that 'imprinting' is a relatively circumscribed phenomenon, while the former implies that approach and following are only part of an array of behaviours which can be altered during a sensitive period in avian development. In the present study coturnix quail were used to investigate the effects of early exposure to a stimulus on the fear behaviours later elicited by that stimulus. Approach and following behaviours did not occur during testing. Thus, it was possible to demonstrate the existence of a sensitive period during which stimulation will affect fear behaviours independently of the establishment of following, suggesting that 'imprinting' can occur without following.

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Methods

Subjects

The subjects were thirty coturnix quail poults (*Coturnix coturnix*). Eggs were collected daily from the breeding colony in the psychology laboratory at Michigan State University and set within two days after collection. The eggs were incubated at 37·8°C and turned automatically every 3 hr. Within an hour of hatching the young poults were placed in individual isolation cages maintained at 37±1°C where they resided for the entire experiment except during treatment and testing. Food and water were available ad libitum. Each animal was housed in visual but not auditory isolation from the other animals.

Apparatus

Subjects and recording equipment were housed separately from the treatment and testing room which was maintained at 35° to 37°C. The initial treatment and first test were given in a 182 × 76 × 61-cm wooden alley with white sides. The alley floor was covered with green, rough surfaced paper to provide adequate traction for the birds. The top was open and a pulley at each end supported the stimulus which travelled at a rate of approximately 10 cm per s up and down the centre of the alley about 5 cm above the floor.

The second test was conducted in a chamber consisting of two 30 × 46 × 36-cm wooden boxes connected by an alley 20 cm wide and 41 cm long. The inside of this chamber was painted dark grey and the top and bottom were constructed of hardware cloth. The stimulus was positioned in one of the compartments in such a way that it could not be seen from either the alley or the other compartment. This testing chamber was, except for the stimulus, a completely new environment for the poult.

The stimulus was a red-orange translucent cylinder (5·7 cm diameter × 12·7 cm high) with a closed bottom and open top. Within the cylinder was a 6-V bulb controlled by a timer which caused the light to flash during stimulus movement. The auditory stimulus was the click of the timer, which was mounted between the alley and the testing chamber.

Procedure

Upon hatching, each poult was taken from the incubator and placed in an isolation cage. If the poult appeared normal and healthy, it was randomly assigned to one of the five treatment groups listed in Table I ($N = 6$ for each group).

Table I. Treatment Groups

Group	Age (hr)	Treatment
I_1: early-exposed	5–9	Placed in alley for 1 hr with the stimulus alternating each 1/2 min between S–ON (moving, flashing, clicking) and S–OFF (still, light off, quiet)*
I_2: late-exposed	10–14	
NI_1: early-non-exposed	5–9	Placed in alley for 1 hr with the stimulus in S–OFF condition at all times
NI_2: late-non-exposed	10–14	
C: control	-	No initial treatment; remained in isolation cage until testing

*The S–ON and S–OFF conditions alternated in order to provide equal habituation time in the alley under each condition.

After the appropriate treatment each bird was individually given two test sessions. Each test session was 18 min long and consisted of three 3-min trials with the stimulus in the S–ON condition (stimulus moving, light flashing and clicking noise present) and three 3-min trials with the stimulus in the S–OFF condition (stimulus still, light off, and quiet). The S–ON and S–OFF trials were counter-balanced so that each subject received either ON–OFF–ON–OFF–ON–OFF or OFF–ON–OFF–ON–OFF–ON in a test session. The first test session (36-hr test) was 36 to 42 hr after hatching. The bird was placed in the centre of the alley. Within 15 s testing was initiated with the experimenter recording the number of distress calls during each 3-min trial. For twenty-five of the thirty subjects the amount of time that the poult was either making distress calls, running about the perimeter of the alley or jumping against the walls was also recorded during each trial. The total time of these responses was taken as an indicator of total fear behaviour (Schaller & Emlen 1962).

The second test session (48-hr test) was conducted 48 to 52 hr after hatching. In this test the poult was placed in the centre of the testing chamber. The same procedure for presenting the stimulus conditions was used as in the 36-hr test. Distress calls were measured as above. The total amount of time in each trial that the subject spent in the compartment with the imprinting stimulus was also recorded during this test. This measure was included to get some idea of the relative importance of audio and visual properties of the stimulus. Perception of the visual stimulus was restricted to one of the compartments and that of the auditory stimulus was not. If the visual properties were of primary importance, subjects who had formed an 'attachment' to the stimulus might be expected to spend more time in the compartment with the stimulus than subjects in which this attachment had not occurred.

At the termination of the 48-hr test, twenty-six of the thirty subjects were sexed by cloacal examination (Homma et al. 1965). This sample consisted of twelve males and fourteen females (each treatment group split three to three or four to two).

Results

Following Behaviour

Active following of the stimulus object during the 36-hr test was not observed in any of the subjects from any group. Although Schaller & Emlen (1962) report that 31 per cent of their coturnix were observed to do some following and Ozmon (1968) has observed following behaviour, it is clear that under the conditions of this experiment following behaviour does not occur during testing.

Distress Calls, 36-hr Test

The distress call data from the 36-hr test are shown in Figs 1 and 2. In Fig. 1 the trend for each subject within any treatment group can be determined by comparing the position of letters in the S–ON and S–OFF conditions. Figure 1 reveals that in each group, except the late-exposed group (I_2), more distress calls are emitted during the S–OFF condition than during the S–ON condition.

Figure 2 indicates that when the five treatment groups are compared within the S–OFF condition only small differences occur. However, within the S–ON condition, the early-exposed group (I_1) produces fewer distress calls than each of the other groups, and the late-exposed group (I_2) produces more distress calls than each of the other groups.

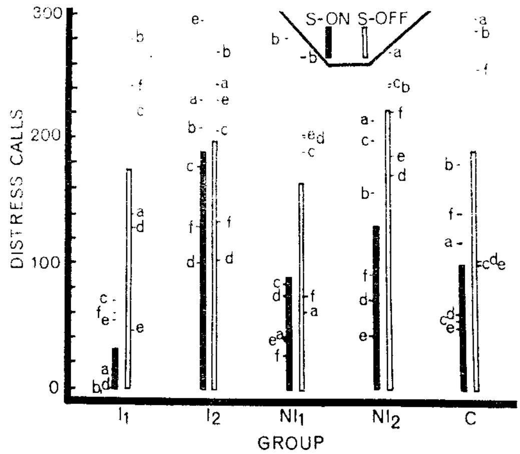

Fig. 1. Distress calls emitted during the 36-hr test. Bars represent the mean number of distress calls in each stimulus condition for each group. The letters (a–f in each group) reveal, for each subject in each stimulus condition, the total number of distress calls made. In each group, except I2, reliably more distress calls are made during the S–OFF condition (Duncan's test, $P<0{\cdot}01$ for each group).

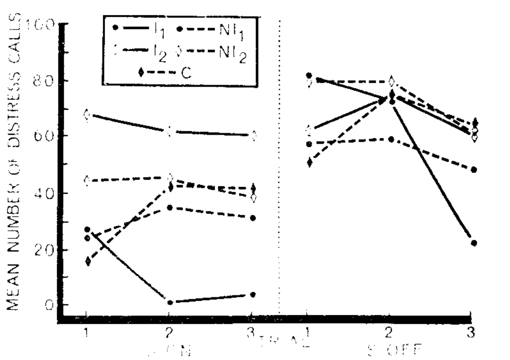

Fig. 2. Mean number of distress calls in 36-hr test for each treatment group as a function of the three trials in each stimulus condition. Differences within S–OFF are not significant. Within S–ON, I_1 subjects make less distress calls than other subjects (Duncan's test, $P<0{\cdot}05$) and I2 birds produce more than others (Duncan's test, $P<0{\cdot}025$).

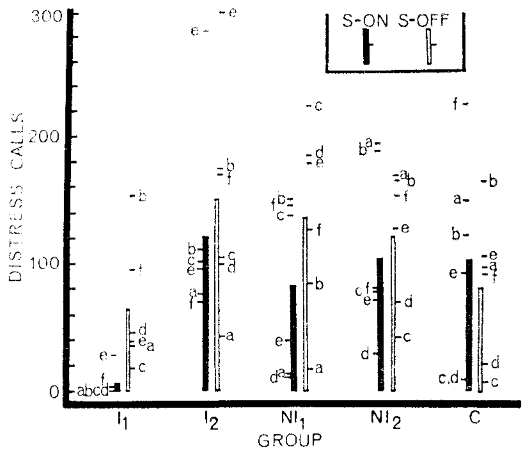

Fig. 3. Distress calls emitted during 48-hr test. The bars represent the mean number of distress calls in each stimulus condition for each group. The letters a–f in each group) reveal, for each subject in each stimulus condition, the total number of distress calls made. Reliably more distress calls are emitted during the S–OFF than S–ON condition in group I_1 (Duncan's test $P<0{\cdot}01$) and in combined other groups (Duncan's test, $P<0{\cdot}05$).

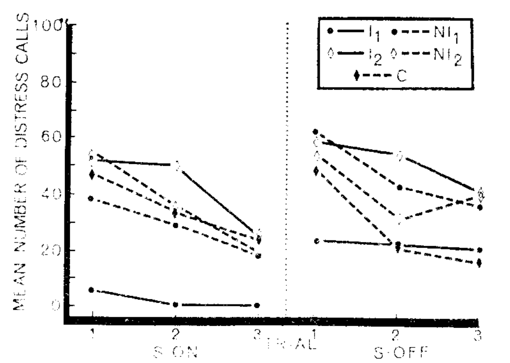

Fig. 4. Mean number of distress calls in 48-hr test for each treatment group as a function of the three trials in each stimulus condition. No reliable differences exist during the S–OFF condition. Within S–ON, I_1 birds emit less calls than subjects of each of the other groups (All Mann–Whitney U tests, $P<0{\cdot}01$), which do not differ amongst themselves.

Distress Calls, 48-hr Test

Figures 3 and 4 are analogous to Figs 1 and 2 respectively, except that they represent the results of the 48-hr test. Figure 3 reveals that within each of the treatment groups, except the unexposed controls (C), more distress calls were emitted during the S–OFF condition than during the S–ON condition, This effect, although statistically significant when the groups are combined, is not nearly as large as in the 36-hr test. Also evident from Figs 3 and 4 is that in the S–ON condition, I_1 poults emit fewer distress calls than subjects of the other groups, which do not differ.

Fear Behaviour, 36-hr Test Only

The measure of the total fear behaviour shown by each animal is the proportion of time in each trial of the 36-hr test that was spent making distress calls, running about the perimeter of the alley or jumping and pecking at the walls. These data were collected on only twenty-five of the thirty subjects. Figure 5 demonstrates that during each trial within the

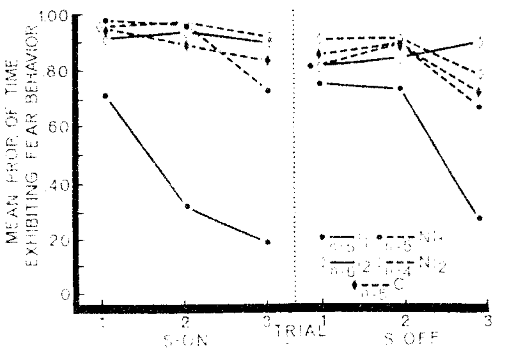

Fig. 5. Mean proportion of trial time (3 min) showing fear behaviour in 36-hr test as a function of treatment group and stimulus condition. I_1 subjects show less fear behaviour than the other subjects during each trial within S–ON (All Mann-Whitney U tests, $P < 0.03$). In S–ON other groups do not differ and within S–OFF no differences are reliable.

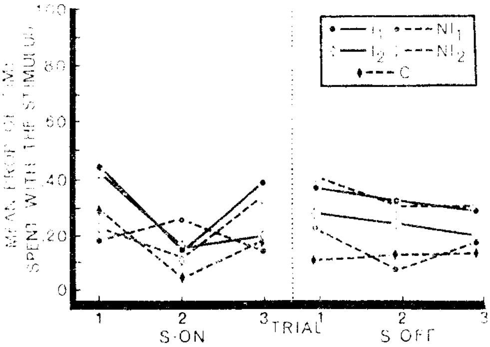

Fig. 7. Mean proportion of trial time (3 min) in 48-hr test that the subjects in each treatment group spent in the compartment with the stimulus in each stimulus condition. No differences are reliable.

S–ON condition, the I_1 (early-exposed group) shows less fear behaviour than each of the other groups. When this group is excluded from the analysis, no significant differences exist suggesting that the other four groups do not differ in total fear behaviour in the S–ON condition.

When the S–ON and S–OFF conditions are compared, Fig. 6 shows that within the early-exposed group (I_1) there is more total fear behaviour during the S–OFF condition than in the S–ON condition. However, in the remaining groups there is less fear behaviour exhibited in the S–OFF condition than the S–ON condition.

Time Spent with Stimulus, 48-hr Test Only

Figure 7 shows the mean proportions of trial time that the subjects in each group spent in the box with the stimulus. As can be seen from the figure, none of the treatment groups differed significantly from each other and the time spent with the stimulus in the two conditions does not differ.

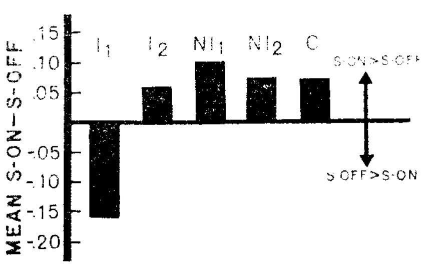

Fig. 6. Mean difference (S–ON minus S–OFF) in the proportion of time exhibiting fear behaviour as a function of treatment group. Positive going bars indicate a greater amount of fear behaviour was shown during S–ON than S–OFF. Negative bars indicate the opposite. I_1 birds show more fear behaviour during S–OFF (Wilcoxon test, $P < 0.01$). Other subjects show more fear behaviour during S–ON (Wilcoxon test, $P < 0.01$).

Discussion

Evidence of Imprinting

Although none of the subjects showed approach and following responses during testing, behavioural differences between the early-exposed (I_1) birds and those of the other four groups were quite striking. During the S–ON condition, subjects of the early-exposed group spent most of the time quietly sitting near the centre of the apparatus in the posture depicted in Fig. 8(a), while during S–OFF they stood up, moved around or emitted distress calls. The birds of the other groups were continually running back and forth along the perimeter of the cage, jumping and pecking at the walls, or stopping only long enough to emit a series of distress calls. These findings are similar to observations on fear behaviour in 14-day-old imprinted ducklings (Stettner & Tilds 1966) and the development of avoidance behaviour in several species of precocial birds including the coturnix quail (Schaller & Emlen 1962).

In both tests the early-exposed group showed considerably fewer distress calls in the presence of the stimulus than each of the other groups. In addition, the amount of total fear behaviour evoked by the stimulus was reliably less in the early-exposed group than in the other groups. The early-exposed group made fewer distress calls and showed less fear behaviour during the S–ON condition than the S–OFF condition, whereas the other groups showed greater fear behaviour while the stimulus was present than in its absence (Fig. 6). The fact that during the

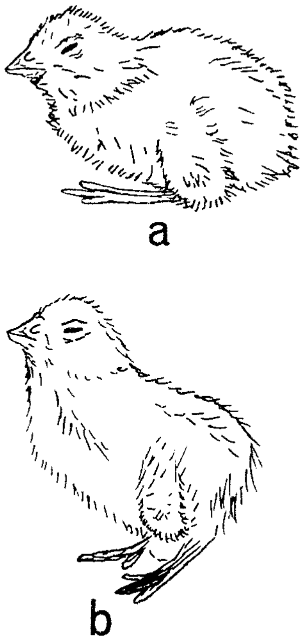

Fig. 8. (a) Posture of coturnix poult while sitting quietly. (b) Posture of coturnix poult while making distress calls.

S–OFF condition there were no reliable differences in distress calls or fear behaviour suggests that in the absence of the stimulus all of the groups are approximately equal in their reaction to the environment. [The sharp decline in distress calls and fear behaviour, seen in the early-exposed group between trials 2 and 3 of the S–OFF condition (Figs 2 and 5), can be explained by the observation that many of the poults from this group appeared to fall asleep during the preceding S–ON trial and continued sleeping throughout most of the final S–OFF trial.] Comparison of the results obtained from the early-exposed and late-exposed (I_2) birds eliminates habituation to the stimulus as an explanation for these data, since both of these groups had the same amount of exposure to the stimulus. Also, the comparison between the early-exposed group and the early-non-exposed (NI_1) group indicates that fewer distress calls and less fear behaviour are due to early exposure to the stimulus and not a general effect of early exposure to the alley.

One other similarity existed between the early-exposed (I_1) subjects of this study and 'imprinted' birds described by other authors: The subjects frequently emitted soft peeping notes while the stimulus was in the S–ON condition. None of the birds from the other groups showed this type of vocalization during the tests. According to Farris (1964) these vocalizations sound much like those that Collias (1952) calls 'contentment notes' emitted by chicks when approaching familiar objects, eating, or moving about undisturbed. Collias & Joos (1953) show spectrographic differences between these calls and the distress calls of the chick to variation in 'stimulus contrast'. Other investigators (Hess 1959; Moltz 1960) have noted that chicks tend to make 'contentment calls' in the presence of an object on which they have imprinted.

This study indicates that early exposure to the stimulus resulted in a diminution of fear behaviours elicited by that stimulus whereas later exposure was ineffectual. These results suggest that the early-exposed subjects may have formed an 'attachment' to the stimulus object analogous to that seen in 'imprinted' chicks or ducklings, but that the topography of the response differed. This interpretation does not imply that following behaviour is absent from the parent–young interactions of coturnix or that it cannot be artificially elicited (Ozmon 1968; Schaller & Emlen 1962). It does suggest that imprinting involves a complex repertory of behaviours including, but not exclusively, approach and following. The particular behavioural responses which occur at any time may depend on many factors such as species, age of treatment, age of testing, and natural ecology of the animal.

Sensitive Period

The results of this experiment indicate that there is a sensitive period for the reduction of fear behaviours in coturnix. This period, however, does not coincide with the sensitive period for approach and following behaviour in the species studied here. Whereas birds exposed to the stimulus 5 to 9 hr after hatching exhibited relatively little fear behaviour during testing, the late-exposed group showed as much total fear behaviour and as many or more distress calls than did the control groups. The disparity between these data and the sensitive period for the establishment of approach and following behaviour in other gallinaceous birds (Sluckin 1965) may be due to the difference in response parameters or to the exceedingly rapid maturational rate of the coturnix quail (Wetherbee 1961).

Relationship Between Fear Behaviour and Distress Calls, An Hypothesis

Two sets of rather unexpected, but interesting, results occurred in the present experiment. First, the control groups (NI_1, NI_2, and C) produced more distress calls in the S–OFF than the S–ON condition in both tests. Secondly, the late-exposed group emitted more distress calls than the other groups during the 36-hr test. These results can be explained if the mutually exclusive nature of the two fear responses exhibited by young coturnix quail is considered. One of the fear responses is escape behaviour. This activity includes running around the perimeter of the enclosure, pecking at the walls, and jumping at the corners. The second fear activity is distress calling. In the present experiment it was observed that the birds never displayed these behaviours simultaneously. The distress call of the young coturnix quail only occurred when the bird was in the posture depicted in Fig. 8 (b), and never while the bird was running.

It has been suggested that fear responses can be considered a systematic sequence of activities where the amount of 'stimulus contrast' or novelty in the environment determines which particular response will occur (Kaufman & Hinde 1961; Salzen 1962; Schaller & Emlen 1962; Andrew 1964; Phillips & Siegel 1966; Ratner 1966; Broom 1969). These reports also suggest that escape responses (i.e. intense locomotion and jumping against the walls of the enclosure) are elicited in situations of greater 'stimulus contrast' or novelty than those situations normally evoking distress calls. That is, escape behaviour appears to be a stronger fear response than distress calling; however, direct experimentation is needed to establish this point. Thus, it is hypothesized here that the respective probabilities for the appearance of the two responses are dependent on the amount of novelty in the total stimulus complex of the bird.

Although this hypothesis is in need of direct experimental testing, several points of evidence can be established for interpreting the behaviour of the control groups and late-exposed group with this model. First, it predicts that presentation of the imprinting stimulus will elicit increased fear behaviour in subjects to whom the stimulus is entirely novel. This prediction suggests that the reason the control groups produced fewer distress calls in the S–ON than during S–OFF is because the intensity of fear in the S–ON condition raised the probability of escape behaviour, thereby inhibiting distress calling. Such an interpretation is further supported by the fact that although distress calls occurred with reliably greater frequency in the S–OFF condition, total fear behaviour (escape activity + distress calls) diminished going from the S–ON to the S–OFF condition (Fig. 6); again suggesting that escape behaviour was markedly increased in the presence of the stimulus. The behaviour of the late-exposed group is explained by the fact that these subjects had been previously exposed to the imprinting stimulus, allowing them to habituate to some extent; thereby lessening the probability of escape behaviour and raising the probability of distress calling. One point of further evidence lends support to this interpretation. Although this group (I_2) showed reliably more distress calls than the other groups in the S–ON condition of the 36-hr test, these subjects did not exceed the other groups in total fear behaviour. Thus, the amount of escape behaviour must have been less than that of the control groups.

Summary

Isolated coturnix quail of two post-hatch ages were individually exposed to a moving, flashing, clicking stimulus on the day of hatching. Two other groups received identical treatment except for exposure to the stimulus, and a fifth group was given no initial treatment. Thirty-six and 48 hr after hatching each bird was tested with the stimulus functionally present and absent. Results indicate that there is a sensitive period in the development of coturnix during which exposure to a stimulus will produce a diminution in fear behaviours later elicited by that stimulus. Following of the stimulus object did not occur during testing. Birds exposed to the stimulus 5 to 9 hr after hatching exhibited fewer distress calls and less fear behaviour when later tested with the stimulus present than each of the other groups including those birds exposed to the stimulus 10 to 14 hr after hatching. It is suggested that the following response may be only one of an array of responses involved in imprinting.

The results of this experiment also suggest that there is a systematic hierarchy of behavioural responses to novel stimuli in this species. A testable hypothesis was presented which suggests a mutually exclusive set of responses with probabilities of occurrence dependent on the amount of novelty in the stimulus complex of the organism. This hypothesis is amenable to direct experimental testing since both stimulus parameters, novelty, and response parameters,

activity and distress calls, can be readily quantified.

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