

SOME EFFECTS OF PROLACTIN ON REPRODUCTIVE
BEHAVIOR IN THE BROWN-HEADED COWBIRD
(*MOLOTHRUS ATER*)

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Most birds show some measure of nesting behavior, but a few species are parasitic and deposit their eggs in the nests of other birds, relying on the host to incubate the eggs and care for the young (Weller, 1959). The Brown-headed Cowbird (*Molothrus ater*), an obligate parasite, is one of the most specialized of a series of cowbird species that have nesting habits ranging from nearly normal to fully parasitic (Friedmann, 1929). Field studies on cowbirds have resulted in an adequate description of their general habits, but few studies have been carried out to determine the physiological basis of their aberrant nesting behavior.

Typical nesting behavior may be divided into five categories: construction of the nest, laying of the eggs, incubation of the eggs, feeding of the young, and brooding of the young. Prolactin from the anterior pituitary is involved in the control of three of these five activities: incubation (Breitenbach and Meyer, 1959; Lahr and Riddle, 1938; Riddle, Bates, and Lahr, 1935; Saeki, 1955; Saeki and Tanabe, 1954, 1955): feeding of the young (Lehrman, 1955; Riddle, 1937) and brooding of the young (Nalbandov, 1945; Yamashina, 1952, 1952-1956).

Since all phases of nesting behavior associated with prolactin are lacking in the Brown-headed Cowbird, we thought that investigation of the effects of prolactin on the reproductive behavior of this species might provide information on the physiological basis of parasitic nesting habits and their possible evolutionary development. In a series of four experiments we explored some of these possibilities by comparing the reaction of prolactin-injected cowbirds exposed to nests, eggs, and young with the reactions of uninjected control birds under similar conditions.

METHODS AND MATERIALS

Ten cages measuring two feet by four feet by two feet high ($61 \times 122 \times 61$ cm) were constructed of welded wire of a mesh one-half inch by one inch (1.27×2.54 cm). Two perches were placed across each cage near the top. Food and water dishes were placed in each cage. The cages rested on small tables 2.5 feet high. The only light source was incandescent bulbs. At the onset of each experiment visual shields were placed between the cages and a nest or nest model was placed in each cage. Nests of the Red-winged Blackbird (*Agelaius phoeniceus*) and Yellow-headed Blackbird (*Xanthocephalus xanthocephalus*) set in a dense clump of grass

housed in a no. 10 can were used. The nest model, constructed from clay, was supported by a wooden platform attached to the side of the cage.

One male and one female cowbird were placed in each cage prior to each of the experiments. When captured in mid-August, 1959, these birds were all in nearly full juvenal plumage and probably three months old or younger. The birds in five of the cages were given prolactin dissolved in approximately 0.1 ml of non-pyrogenic saline. This was injected subcutaneously over the pectoral muscles each afternoon during the period indicated in Table 1. The birds in the other five cages served as controls and simultaneously received a similar volume of saline.

For the first two experiments cowbird eggs were used. These were blown and filled with a plastic to give them greater strength and the approximate weight of normal eggs. For the last two experiments plaster of Paris replicas of cowbird eggs were used, being less subject to breakage by the pecking of the birds. In each experiment one egg was added to each nest on each of three consecutive afternoons. Broken or ejected eggs were replaced.

In the course of Experiment 4 one young Red-winged Blackbird between three and seven days old was placed in the nest of each cage on the following occasions: (1) evening of 7 June for one hour, (2) morning of 8 June for one hour, (3) evening of 10 June for one-half hour, and (4) morning of 11 June for five and one-half hours. While the young were in the cage, mealworms were available as a possible source of food for the females to feed the young. During these periods all of the young reacted to any movement by gaping and calling.

Activities related to the nest, nest cup, eggs, nesting material, or to the other cowbird in the cage were recorded.

The males were removed in Experiments 1, 2, and 4 because they interfered with the nest- and egg-oriented activities of the females. This was especially apparent in Experiment 1. When a female attempted to assume the incubation position, the male drove her from the nest.

Experiments were performed with birds in three levels of sexual development. The birds in Experiments 3 and 4 were known to be in breeding condition because song displays by the males were common, copulation was observed, and eggs were laid by several of the females. In Experiment 1 the males showed a moderate state of testicular development as indicated by the moderate level of song displays. The infrequent song displays in Experiment 2 indicate a low level of sexual development during the experiment. No evidence of sexual development was observed in any of the females in Experiments 1 and 2. No molt was observed in any experiments except the postjuvenal molt, in Experiment 1. The general activity level of the birds seemed to parallel their sexual condition. The birds were

comparatively inactive in Experiment 2 and very active in Experiments 3 and 4.

Observations were made by James L. Smith and the authors. Observational procedures and the limits of the various categories used were laid out prior to the experiments to reduce the variability in recording observations. Table 1 gives the details in which the experiments differed from each other.

TABLE 1
COMPARISON OF CONDITIONS FOR EXPERIMENTS

	Hours of light per day	Number of days of controlled light prior to experi- ment	Obser- vation period	Nest type	Egg type	Dates eggs placed	I.U. prolactin per day and injec- tion dates	Males re- moved	Room tempera- ture during experi- ment	Total hours obser- vation each pair
Exp. 1	12	54	15 Oct.— 1 Nov.	blackbird	cowbird	21, 22, 23 October	3.6 17–30 Oct.	22 Oct.	19–23°C	35–37.5
Exp. 2	8	59	19 Jan.— 6 Feb.	clay cup	cowbird	26, 27, 28 January	7.0 22 Jan.— 3 Feb.	28 Jan.	21–25°C	32.5–33
Exp. 3	14	42	25 March– 9 April	clay cup	plaster	1, 2, 3 April	7.0 28 March– 4 April; 20.0 5–9 April**	(not re- moved)	17–27°C	45.5
Exp. 4	14	15*	27 May– 11 June	blackbird	plaster	3, 4, 5 June	7.0 30 May– 7 June; 20.0 8–10 June	5 June	22–30°C	42.5

* Birds were under natural daylight until 12 May.

** Observations on one prolactin-injected male are excluded because of a leg injury on 31 March.

RESULTS AND DISCUSSION

REACTIONS OF THE FEMALES

Incubation and related behavior.—The most outstanding of the several differences between the prolactin-injected birds and the controls is related to incubation behavior. A bird was considered to be in the incubation position if, in the observer's opinion, it lowered itself far enough into the nest to bring its belly into contact with the eggs or nest bottom. Other activity, such as standing in the nest or on the eggs, was not included. However, it is probable that not all of the time recorded as time spent in the incubation position was true incubation behavior (for example, when there were no eggs in the nest).

In Experiment 1 the females that received prolactin were in the incubation position more often and for longer periods of time than were the

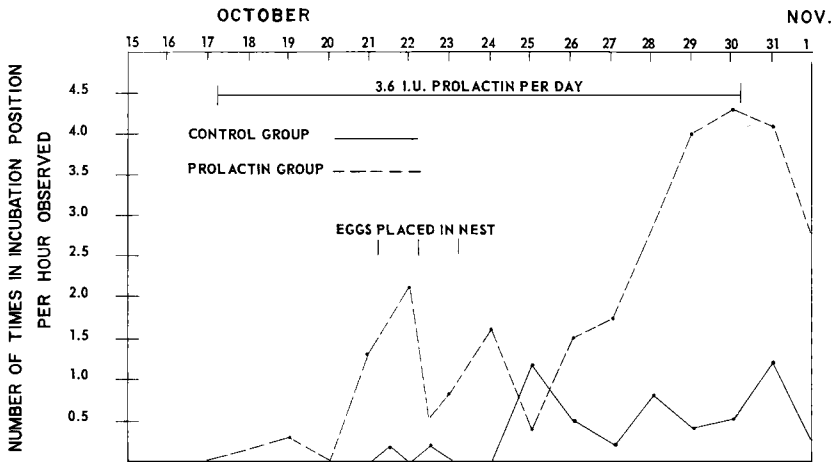


Figure 1. Experiment 1. The average number of times the females assumed the incubation position.

controls (Figures 1 and 2). The greatest increase in this behavior occurred after the first egg was placed in the nest. The average length of time spent sitting in this position for each visit was longer in the prolactin-injected females (35.0 seconds) than in the controls (5.2 seconds). The longest period in the incubation position for each of the prolactin-injected females was 1095, 240+, 205, and 150+ seconds, with average stay for each, ranging from 31.0 to 36.8. The average time in the incubation position for individual control females ranged from 2.8 to 9.1 seconds with the longest stay for each being 42, 40, 20, and 15 seconds. One control female and one prolactin-injected female never sat on the eggs.

The assumption by control birds of the incubation position may have one or two causes. Either some incubation may be caused by confining the birds in cages with nests and eggs and/or some of the time spent in the incubation position may have little to do with true incubation.

In Experiment 3 some of the females of both the prolactin-injected and control groups assumed the incubation position in the nest cup prior to the initiation of injections. On the three days following the first injection the average of the control group reached a very high peak. This was largely the result of a single bird's activities. When the first egg was placed in the nest cup, both groups showed a sharp drop in the time spent in the incubation position in the nest cup. By the time the third egg was added to the nest cup, all the birds had ceased assuming the incubation position in the cup. Therefore, it does not seem that in this instance this activity really represented a drive to incubate.

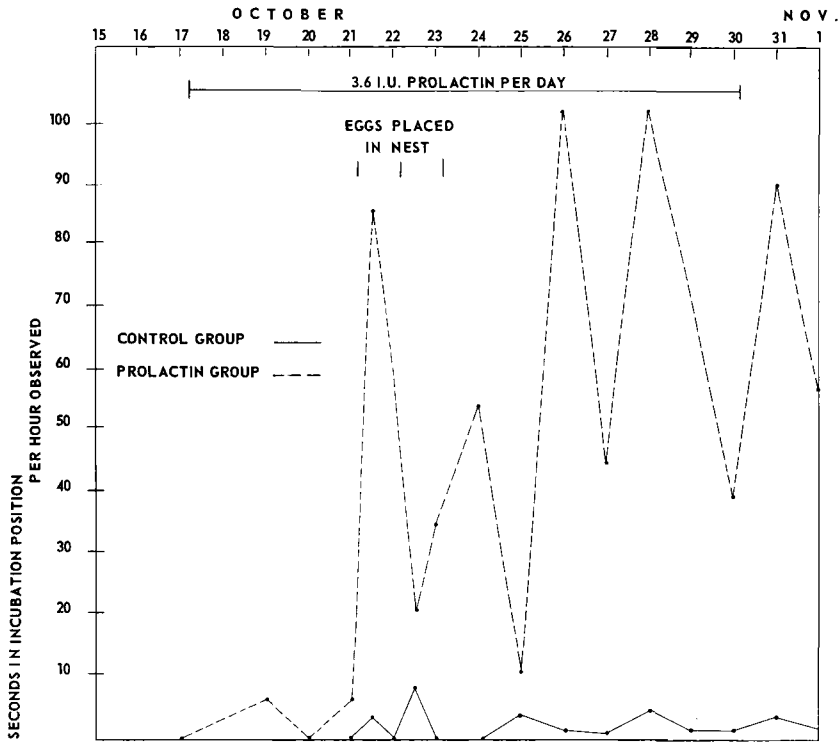


Figure 2. Experiment 1. The average amount of time the females spent in the incubation position.

Following an increase of the daily dosage of prolactin from 7 to 20 I.U. in Experiment 3, two of the prolactin-injected females spent much more time in the incubation position in a depression in the clumps of grass (Figure 3). One female reached a peak in this activity of 27 minutes per hour of observation. This bird was never observed in the incubation position in the clay nest cup. Most of the activity shown on the graph for the control group is that of one bird.

The results of Experiments 2 and 4 show no difference between the control and prolactin-injected females in the incubation behavior. The birds in Experiment 2 showed no incubation behavior while both groups in Experiment 4 showed low levels of incubation similar to that of the controls in Experiment 1 (Figure 1).

On 36 occasions females receiving prolactin abducted the breast and belly feathers slowly while they stood on the rim of the nest or on the perch looking into a nest containing eggs. On these 36 occasions the birds did not assume the incubation position; of these 36, 25 occurred in Ex-

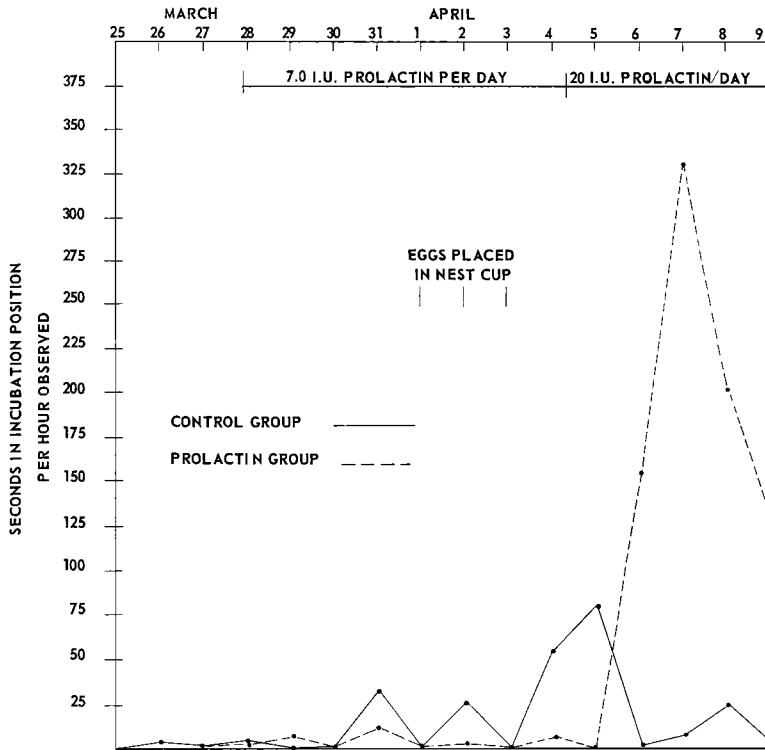


Figure 3. Experiment 3. The average amount of time the females spent in the incubation position.

periment 1, 2 under the 7 I.U. (of prolactin per day) dosage of Experiment 4 and 9 under the 20 I.U. (of prolactin per day) dosage of Experiment 4. This feather abduction was never seen in the control birds. Since this activity was also observed when the prolactin-injected birds were about to assume the incubation position, we think that this abduction of the feathers represents an element of incubation behavior; i.e., exposure of the incubation patch as displayed by normal, incubating birds of other species.

Since marked increase in incubation behavior was obtained in young (approximately 4.5 months old) female cowbirds under 12 hours of light per day with 3.6 I.U. of prolactin per day (Experiment 1) while either a small response or no response with up to 20 I.U. of prolactin per day was obtained in older females (10–12 months) in breeding condition, it seems clear that in Experiments 3 and 4 the birds were much less sensitive to prolactin than the birds in Experiment 1.

This decreased sensitivity to prolactin may be interpreted in one of two ways. The birds may be sensitive to prolactin as young birds and become less sensitive as they mature or they may be insensitive to prolactin only during the breeding season. The works of Friedmann and Bober (*in litt.*) and Selander (1960) lend support to this conclusion. They found that estrogen alone or estrogen and prolactin could not produce an incubation patch in a cowbird that was in breeding condition. Bailey (1952) found that estrogen in the intact bird or prolactin and estrogen in the hypophysectomized bird would bring about formation of an incubation patch in three species of fringillids which show normal nesting. The difference in the results of those experiments would be explained if breeding cowbirds were insensitive to prolactin.

A recent report (Selander and Kuich, 1963) on experiments with prolactin injections in the Brown-headed Cowbird points very strongly to sensitivity only in young birds. In birds as old or older than those used in Experiment 1, Selander and Kuich observed no effect of prolactin on either nest-building or incubation behavior. Our results in Experiments 2, 3, and 4 support their suggestion that there is a selective loss of sensitivity in target organs in the Brown-headed Cowbird.

Selander and Kuich drew attention to similarities between some nest-oriented behavior and egg-laying motions in their experiments. For two reasons the incubation behavior in our experiments cannot be interpreted in this way. The females stayed on the nest for much longer periods of time than did the birds studied by Selander and Kuich. We saw egg-laying motions in the later experiments and these actions were different from the behavior we considered incubation behavior.

Höhn (1959) reported that the pituitary of the breeding Brown-headed Cowbird contains as much prolactin as that of the related Red-winged Blackbird, a species exhibiting normal nesting behavior. Since the cowbird shows no behavioral effects of the prolactin in natural populations, these results would support the conclusion that the adult cowbird is less sensitive to prolactin, at least during the breeding period.

Two objections to Höhn's (1959) methods must be pointed out; in no place does he specify the microscopic criterion that he used in carrying out his assay. Bahn and Bates (1956) have shown that the proliferation of cells in the crop gland of the pigeon is not a response brought about specifically by prolactin, and that diffuse basophilia is the only known criterion specific for this hormone. Other types of basophilia may result from nonspecific agents. Höhn implanted fairly large numbers of whole pituitaries in order to obtain a response. Since the failure to macerate the gland before injection into the pigeon may result in little or no diffusion, from the gland, of any prolactin that may be present, his use of whole

pituitaries may explain the small and spotty response. In view of these two difficulties, caution should be used in applying his conclusions to any work on cowbirds.

It has been shown (Lehrman and Brody, 1961) that prolactin does not *initiate* incubation in the Ring Dove (*Streptopelia risoria*). Several workers have pointed out that any given phase of nesting behavior may be differently controlled in different species (Collias, 1950; Lehrman, 1955, 1958). Our results do not permit us to distinguish between an initiatory and a purely supportive role for prolactin in these experiments.

Responses to young.—When young Red-winged Blackbirds were present in the nest, no clear-cut differences could be seen between the reaction of the control and prolactin-injected females. No attempt by any of the females to feed or brood the young was observed.

Other reactions.—There were small differences in the females' reactions to the eggs (other than incubation), nest, and grass which may indicate increased interest in objects associated with the nest by the prolactin-injected females. However the differences in the data were small and irregular and therefore not reliable.

REACTION OF THE MALES

Song displays.—Males receiving prolactin in Experiment 1 showed a marked decrease in number of song displays compared with control males, although the number of chases (vigorous pursuits of the female) did not decrease (Figure 4). The decrease in song displays may be explained as a result of a decrease in androgen production following injections of prolactin. This decrease in androgen levels has been observed by Nalbandov (1945), Bates, Riddle, and Lahr (1937), and Breneman (1942). The effect of androgens on singing has been noted by Leonard (1939), Shoemaker (1939), and Yamashina (1952).

In Experiments 3 and 4 no decrease in the level of song displays was obtained with 7 I.U. of prolactin per day. Even under 20 I.U. of prolactin per day in Experiment 3 the drop in the level of song displays, that would be expected as a result of prolactin administration, was not observed in two of the four males receiving prolactin. Song displays of one of the males dropped from about 70 per hour of observation to 1.3 per hour of observation. The remaining male receiving prolactin showed some effect; its song displays dropped from about 53 per hour to 24 per hour. As mentioned previously, the difference in the results obtained during Experiments 1, 3, and 4 indicates a decrease in the sensitivity of the birds to prolactin.

Removal of the males from the cages in Experiment 1 seems to be justified by the immediate drop which occurred in the levels of the following activities of the females: inspection of nesting material other

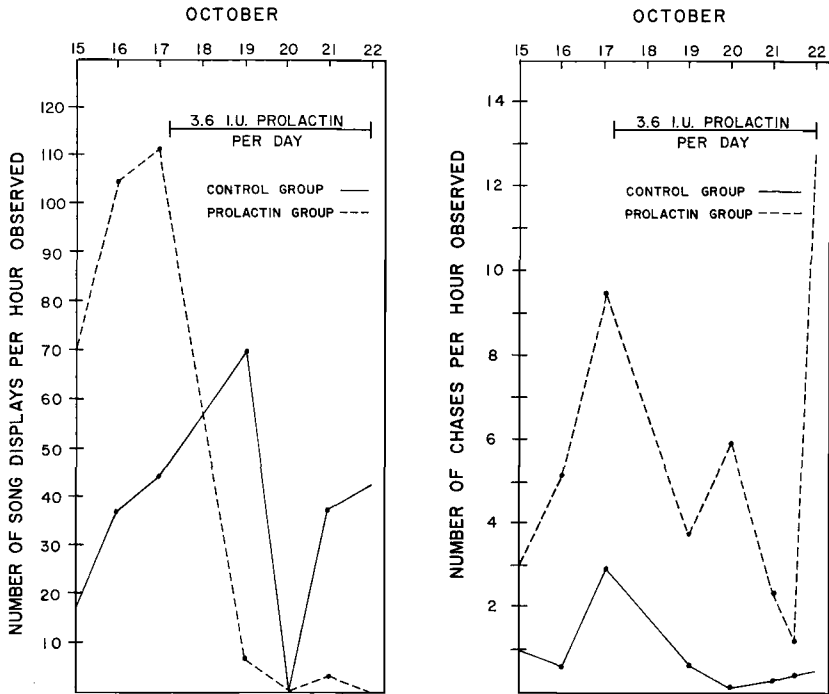


Figure 4. A. Experiment 1. The average number of song displays by the males (left). B. Experiment 1. The average number of vigorous pursuits of the females by the males (right).

than at the nest, and picking at this material (Figure 5). These two categories seem to represent displacement activities engaged in by the female when she was motivated to go to the nest or to sit on the eggs but was chased from the nest by the male. No definite change in the behavior of the females resulted from the removal of the males in later experiments.

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SUMMARY

Four of five young (approximately 4.5 months old) female Brown-headed Cowbirds responded with more than a 30-fold increase in incubation behavior to daily injections of 3.6 I.U. of prolactin. The same level

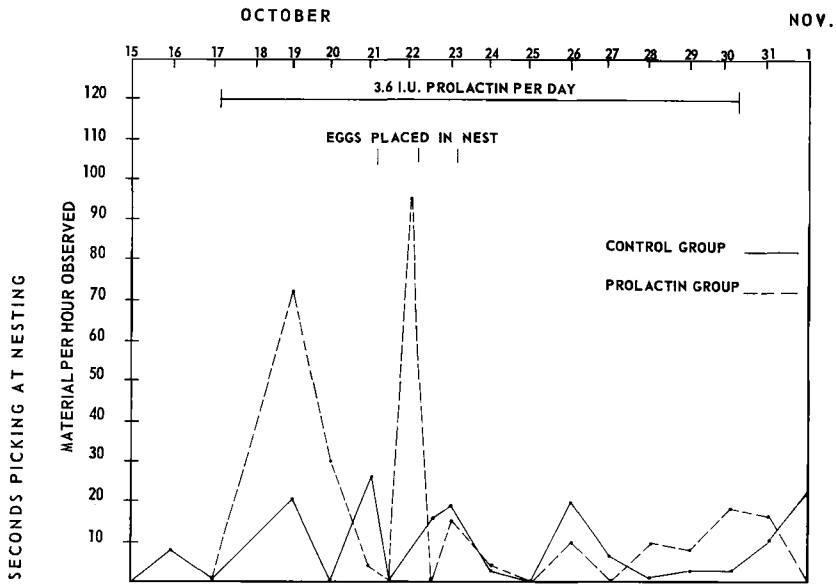


Figure 5. Experiment 1. The average amount of time the females spent picking at nesting materials. The males were removed 22 October.

of prolactin caused a 40-fold reduction in the number of song displays by five young males while the level of singing in control males remained about the same. Even much higher daily doses (20 I.U.) of prolactin did not cause any consistent response in 10–12-month-old male or female cowbirds in breeding condition. These females did not brood or feed nestling birds. No indication of increased nest-building activity in the prolactin-injected birds was observed. Daily injections of 7.0 or 20.0 I.U. of prolactin did not cause female cowbirds in breeding condition to brood or feed nestling birds.

In nature, the absence of incubation behavior in Brown-headed Cowbirds may be the result of a lack of sensitivity to prolactin.

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