HEART RATE IN BOBWHITE DURING DEFENSIVE (TONIC) IMMOBILITY AND FREEZING

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ABSTRACT

HEART RATE IN BOBWHITE DURING DEFENSIVE (TONIC) IMMOBILITY AND FREEZING

By

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Tonic immobility is characterized by tonic posture and prolonged immobility. The response is elicited in the laboratory by restraint of the animal, usually on its back, for a brief period of time. The response has been observed in a wide variety of species, and some investigators consider it to be a response by prey to capture by a predator (Ratner, 1967). Since Bobwhite quail exhibit the immobility response readily and for long duration, they were studied in this experiment.

The purposes of the present experiment were four. The first was to record a physiological measure, heart rate, throughout the course of testing the tonic immobility response. The second was to assess the effect of testing immobility in the absence of visual stimulation on both duration of immobility and heart rate. The third purpose was to determine the effect of an auditory stimulus, the recorded cry of a Red-shouldered hawk, on both heart rate

and immobility. Finally, heart rate during immobility testing was compared with heart rate during a test for a freezing response.

The first major finding was that the absence of visual stimulation during testing had no effect on either heart rate or tonic immobility. Birds tested under blue light, to which they are insensitive, did not differ from birds tested under white light. The second discovery was that the sample of birds studied was dichotomous in two respects. Half of the birds remained in immobility for short durations, and their heart rate remained above base-line during immobility. The other birds exhibited long durations, and their heart rates decreased to below base-line during immobility. The third result was that the auditory stimulus terminated immobility for most birds and maintained a higher heart rate after termination. Finally, it was shown that heart rate during the freezing test was not as high as during the initial part of immobility testing. Several suggestions for further research are offered.

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INTRODUCTION

The tonic immobility response has attracted the attention of students of animal behavior and others since the seventeenth century (Volgyesi, 1938). The principal characteristics of the response are prolonged immobility, tonic posture, reduced responsiveness to external stimulation, and periods of eye closure. Observers have noted the immobility response in a number of species, including large beetles (Fabre, 1919), guinea pigs (Liberson, 1948), chickens (Gilman, Marcuse, & Moore, 1950; Gallup, Creekmore, & Hill, 1970), pigeons (Ratner, 1967), rhesus macaque (Foley, 1938), Bobwhite quail (Doty, 1969), rabbits (Ratner, 1958), goats (Fraser, 1960), laboratory rats (Ratner, 1975), coyotes (Ratner, 1967), and possibly man (Hoagland, 1928). Recently the Bobwhite quail has been shown to be an excellent preparation for the study of tonic immobility, and in addition it is a wildlife rather than domestic species. Bobwhites exhibit the response readily and for lengthy durations (Eyer & Ratner, in preparation).

The various names which have been used to define the response (animal hypnosis, catalepsy, death feigning, rho, fascination, catatonic trance) indicate the variety of functions or purposes which have been attributed to tonic immobility. Currently, some investigators believe the function of tonic immobility is to reduce the probability and/or vigor of an attack by a predator. Ratner (1975) proposed a defensive distance model of predator-prey relations which includes a role for tonic immobility. Briefly, Ratner's model suggests that the prey makes various responses to a predator and the response that is elicited is a function primarily of the distance between the predator and the prey. As the distance decreases freezing, escape, fighting and tonic immobility can occur. The immobility response will be elicited by tactile stimulation when the defensive distance is reduced to zero and the prey is restrained by the predator. Because the function of immobility appears to be defensive, the name defensive immobility seems appropriate and will be used here.

Evidence produced by other investigators tends to support Ratner's defensive distance model. Tortora and Borchelt (1972) demonstrated that immobility was more readily reduced and lasted longer in Bobwhite quail if escape behavior was elicited prior to manual restraint of the bird. Eyer and Ratner (in preparation) have demonstrated a relationship between conspecific vocalizations used in predator-prey contexts and the duration of defensive immobility in Bobwhite quail. Borchelt and Ratner (1973) studied the development of both freezing and immobility responses in Bobwhites. They found that the two responses emerged at different ages, with freezing developing first. While some questions remain about the distinction between freezing and immobility, especially in adult animals, a further basis for the distinction might be found in a physiological measure such as heart rate.

Gallup and his associates have produced considerable evidence which seems to corroborate the predator-prey explanation of defensive immobility. They have shown that the presence of a stuffed Cooper's hawk (*Accipita cooperri*) significantly prolonged defensive immobility in domestic chicks (Gallup, Nash, Donegan, & McClure, 1971). Exposure to a predator-like stimulus, glass taxidermic eyes, during defensive immobility also significantly increased the duration of the response in domestic chickens (Gallup, Nash, & Ellison, 1971). Other variables which affect the level of fear have also been used to manipulate the duration of the defensive immobility response. The use of electric shock or loud noises prior to restraint increases durations of immobility in chickens (Gallup, Nash, Potter, & Donegan, 1970). Conditioned fear procedures have been used to substantially prolong immobility. Conversely, the effect of a tranquilizer (metoserpate HCl) is to reduce the duration of immobility in chickens (Gallup, Nash, & Brown, 1971).

Quite recently two investigators studied the elicitation of the immobility response (which they termed death feigning) in ducks by red foxes during predation (Sargeant & Eberhardt, 1974). These investigators found that all of the 50 birds studied "death feigned" when seized by the foxes. The authors' description of death feigning would qualify as a good description of defensive immobility as well. Their research provides strong evidence for the role of defensive immobility in predator-prey relationships, especially since the data were gathered under semi-natural conditions.

Gallup, Cummings, and Nash (1972) have shown that the presence of the experimenter is an important variable in testing for defensive immobility. The proximity of the experimenter in relation to the immobile bird (chickens) as well as eye contact between the experimenter and the bird significantly affected the duration of the immobility response. One investigation (Tortora & Borchelt, 1972) made a preliminary attempt to minimize the importance of the experimenter in testing some birds (Bobwhite quail). These birds were tested under blue light to which they are insensitive, and durations of defensive immobility were significantly decreased. The elimination of escape responses prior to induction of immobility probably produced this result, but the effect on defensive immobility of testing under blue light has not been determined.

Physiological correlates of defensive immobility have been studied in mammals, principally by W. R. Klemm (Klemm, 1965; Klemm, 1965a; McBride & Klemm, 1969; and

Klemm, 1971). Klemm maintains that in mammals (rabbits) two stages of immobility occur. The first stage, which he has labeled the light stage, is accompanied by slight slowing of heart rate and respiration from the "awake" state, and the EEG pattern resembles that of an awake animal. Klemm does not report at what point in time the defensive immobility response begins the second or deep stage, but during this stage heart rate and respiration further decrease, and the EEG shows large slow waves typical of sleep. Draper and Klemm (1967), however, have suggested that the animal is capable of receiving and processing information during immobility. They have successfully classically conditioned heart rate in rabbits while the animals were in defensive immobility. Furthermore, Eyer and Ratner (in press) have shown that two conspecific vocalizations affect the duration of immobility differentially in Bobwhite quail. Ratner (1958) has also demonstrated that loud auditory stimulation reliably terminates the immobility response in rabbits, and Thompson and Hatton have shown that a tone of 90 db reliably terminates immobility in domestic chicks.

Physiological studies of immobility in birds are more rare. Silva, Estable, and Segundo (1959) measured EEG in the tero bird during defensive immobility and found, like Klemm with rabbits, an EEG pattern of slow waves typical of sleep. Heart rate was not recorded in their study. Cogger, Otis, and Ringer (1974) measured heart rate in Japanese quail under both freely moving conditions and mechanical restraint. They found heart rate was increased by restraint to approximately 440 beats per minute as compared with a freely moving heart rate averaging 360 beats per minute. Their birds did not, however, appear to be in defensive immobility when restrained (Cogger, personal communication). Heart rate, therefore, has not been measured in birds during defensive immobility.

Statement of the Problem

The present experiment had three goals. The first goal was to compare freezing, defensive immobility, and restraint without defensive immobility using a physiological measure. Since heart rate increases with level of arousal and/or fear, heart rate was measured under those three conditions. Differential heart rates would provide another basis for distinguishing between freezing and defensive immobility. The measurement procedure would also provide information about heart rate during the course of the immobility response.

Second, the effect of testing under blue light with the present procedure was examined. The absence of visual stimulation might be expected to have attenuated the defensive immobility response. The effect of testing under blue light on heart rate was also measured.

The third goal was to test further the effect of an auditory stimulus on defensive immobility. During immobility the cry of a Red-shouldered hawk was presented periodically and its effect on behavior and heart rate was recorded.

The testing procedure minimized the effects of the experimenter's presence and in general limited visual stimulation cues in the testing situation. This allowed specification of the stimulus conditions more precisely than in previous studies, as well as demonstrating a research procedure for future studies of specific visual stimulation during immobility.

METHOD

Subjects

Sixteen adult Bobwhite quail (Colinus virginianus) were obtained from the Poultry Science Research Farm at Michigan State University. The birds, eight males and eight females, were approximately 1 1/2 years old. The quail were housed individually in the cage room at Anthony Hall and maintained on ad lib Quail Breeder (King Milling Company) and water under constant light. The birds were selected on the basis of healthy plumage and the absence of corneal cataracts which would confound the testing of blue and white light. These features were assessed by the visual inspection of each bird by the Poultry Science Husbandryman of Michigan State University.

Apparatus

A white chamber constructed of 1/2-inch plywood measuring 67 cm long, 33 cm wide, and 33 cm high was used for testing during the experiment. One-way glass mounted in the top of the testing chamber permitted observation of the bird. Because another investigator (Gallup, 1972) has demonstrated that mirror images prolong immobility in domestic chickens, the one-way glass on the inside of the testing chamber was covered with 1/4-inch wire mesh recessed 1 cm from the glass. The effect of this precaution was to produce multiple images of the wire mesh on the mirror side of the one-way glass, making any other image very difficult to discern. A small electric fan mounted on one end of the testing chamber provided masking noise, and a speaker on one wall of the chamber was used to present the hawk cry to the bird. The chamber was lighted by two 7.5 watt incandescent white bulbs placed at each end of the testing chamber. Blue light was provided by filtering the chamber lights through Kodak No. 45 Wratten Gelatin filters. A sliding door at one end of the testing chamber provided an entrance through which the bird was introduced to the chamber. Two holes, 12 cm in diameter and covered with slitted rubber dam, served as arm ports through which the experimenter inserted his yellow latex gloved hands into the testing chamber. To contain the bird within reach of the arm ports, a transparent plastic partition on a track was positioned at the center of the chamber prior to testing. A holding box, 20 cm square, also contained a movable partition to allow the experimenter to force gently the bird from the holding box into the testing chamber.

To record heart rate, Justrite 16 mm metal surgical wound clips were permanently fastened to the biceps brachii muscle of the left wing and to the gastroenemius, pars externa muscle of the left leg. The wound clips were affixed prior to the first day of testing. Leads made of light gauge stranded wire were attached to the wound clips by means of an allegator clip immediately prior to each testing session. The leads connected to a Grass Model 5 polygraph which was used to record heart rate on Grass C25-8"-1x5G polygraph paper at a speed of 10 mm per sec.

A Wollensak Model 2520 cassette recorder was used to present the cry of a Red-shouldered hawk through the external speaker mounted in the testing chamber. The hawk cry was recorded from a commercial record (Peterson) and presented at 88 db (A). Sound level of the hawk cry in the testing chamber was measured with a General Radio 15550 Sound Survey meter. Yellow latex gloves were worn by the experimenter during testing, and a stopwatch was used to time intervals and durations. All testing was conducted in the Avian Physiology laboratory, Anthony Hall. The laboratory room was approximately 8 m wide and 10 m long.

Procedure

Birds were tested in four separate squads, each containing two males and two females. On the first day a squad of birds was selected haphazardly using the criteria stated above from the poultry science farm colony and transported to the avian physiology cage room. Wound clips were fastened to the left wing and left leg of each bird before it was housed in its individual cage. Days 2, 3, 4, and 5 were used to habituate the bird to the testing chamber according to the following procedure:

1. The experimenter removed the first bird from its cage, attached the alligator clips to the wound clips, and placed the bird in the holding box.
2. The holding box was quietly transported to the laboratory and placed against the testing chamber door.
3. To test for a clear heart rate record while the bird was in the holding box, the leads were attached to the polygraph, and a sample heart rate record was taken. (On two occasions this step disclosed that an alligator clip had become detached. The birds were then returned to their cages where they remained for two hours before the testing procedure was resumed.)
4. Both sliding doors (holding box and test chamber) were opened, and the experimenter gently forced the bird into the test chamber using the movable partition in the holding box. The test chamber door was then closed.
5. Heart rate was recorded for 30 seconds after 2, 5, 10, 15, and 20 minutes in the testing chamber. The behavior of the bird (movement, posture, plumage, and eye closure) were also observed and recorded in a notebook at these times.

6. At the end of the twenty-first minute, the doors of the testing chamber and holding box were opened and, using the plastic partition in the testing chamber, the bird was gently forced back into the holding box.
7. After disconnecting the leads from the polygraph the holding box was transported to the cage room, alligator clips were disconnected from the wound clips, and the bird was returned to its cage.
8. This procedure was repeated for each of the four birds in each habituation session, yielding a total of 80 minutes of habituation for each bird.

Defensive immobility was tested on Days 6 and 7. The habituation procedure was used until the end of the fifteenth minute, at which time the following procedure was used for birds in the self-paced termination test:

1. The experimenter inserted his gloved hands through the arm ports into the testing chamber and captured the bird.
2. For 30 seconds the experimenter held the bird upright with its feet off the floor.
3. Following this period of upright restraint the experimenter induced the defensive immobility response by inverting the bird and gently restraining it on its back for 30 seconds. The experimenter then withdrew his hands from the testing chamber.

Induction was repeated if the bird did not remain immobile for at least two minutes.

4. Heart rate was recorded continuously from the beginning of the fifteenth minute, through upright restraint and induction, until the second minute of immobility was concluded. At the end of the fifth minute of immobility heart rate was recorded again for 30 seconds, and subsequently heart rate was sampled for 30 second periods at five minute intervals until defensive immobility terminated.
5. Post-termination heart rate was recorded for 30 second periods at the end of 5, 10, and 15 minutes.
6. The same procedure used on habituation days was followed to conclude testing and return the bird to its cage.
7. The entire procedure was repeated to test each of the birds in the squad.

To test the effect of the hawk cry on defensive immobility, the procedure described above was followed until the bird had exhibited the defensive immobility response for 2 minutes. During the third minute of immobility the hawk call was presented. Each presentation consisted of three crys of the hawk in a 3 second period. The interval between the presentations was 10 seconds long, permitting 4 presentations in a test minute. The hawk crys were presented again at the end of 5 minutes of immobility and subsequently at 5 minute intervals until termination of defensive immobility. Heart rate was recorded during the 10 seconds preceding a test minute and during the test minute. Notes on the bird's behavior were recorded during each test. The post-termination procedure for recording heart rate was the same as that used in the self-paced termination test with the exception that the hawk cry continued to be presented at 5 minute intervals. Testing was concluded and the bird returned to its cage using the same procedure as on habituation days. The order of the two immobility tests, self-paced termination and effect of hawk cry, was counterbalanced across squads and light conditions.

On Day 8 a test of heart rate during freezing was conducted. After 15 minutes of the habituation procedure the experimenter inserted his gloved hands into the testing chamber and held them approximately 10 cm from the bird for 30 seconds before withdrawing his hands. Heart rate was recorded while the experimenter's hands were in the testing chamber and then at 5, 10, and 15 minutes. The bird was then returned to its cage.

Testing of the second squad of birds followed the same procedures with the exception that all habituation and testing was conducted using blue light. Squads 3 and 4 were tested using the procedures described for squads 1 and 2, respectively. All testing was conducted during the afternoon and early evening during Winter term, 1974.

Measurement

The polygraph chart record was transformed into quantified heart rate in the following manner. Twenty heart beats were counted, and the distance between the first and the twentieth was measured in tenths of a millimeter. This number was divided into a constant (12,000) which was determined by the chart paper speed to yield heart rate in beats per minute. Measures were taken during the first, middle, and last third of each record and averaged. When the heart rate for each period was determined, the data were analyzed using appropriate statistical tests.

The duration of immobility was measured from the end of induction (experimenter withdrew his hands from the chamber) until the bird got to its feet.

RESULTS

The findings of the experiment will be presented in three major sections. Section I will analyze heart rate and behavior during the habituation sessions. Effects of sex and light will be considered. Section II will examine heart rate and behavior during the two immobility tests. Specifically, Section II A will analyze data relating to testing order and sex; II B will examine light condition; II C will focus on the self-paced termination test; and II D will describe the effect of the hawk call. Section III will summarize the data from the freezing test.

I. Habituation (Days 2-5)

Heart rate during the four habituation sessions is depicted in Figure 1. In general, there was a consistent decrease in heart rate during the course of each session. Heart rates also decreased from Day 2 to Day 3, but increased on Days 4 and 5. The habituation data were tested using a three-way analysis of variance (duration by habituation sessions by time within sessions). The decrease in heart rate within each session was significant

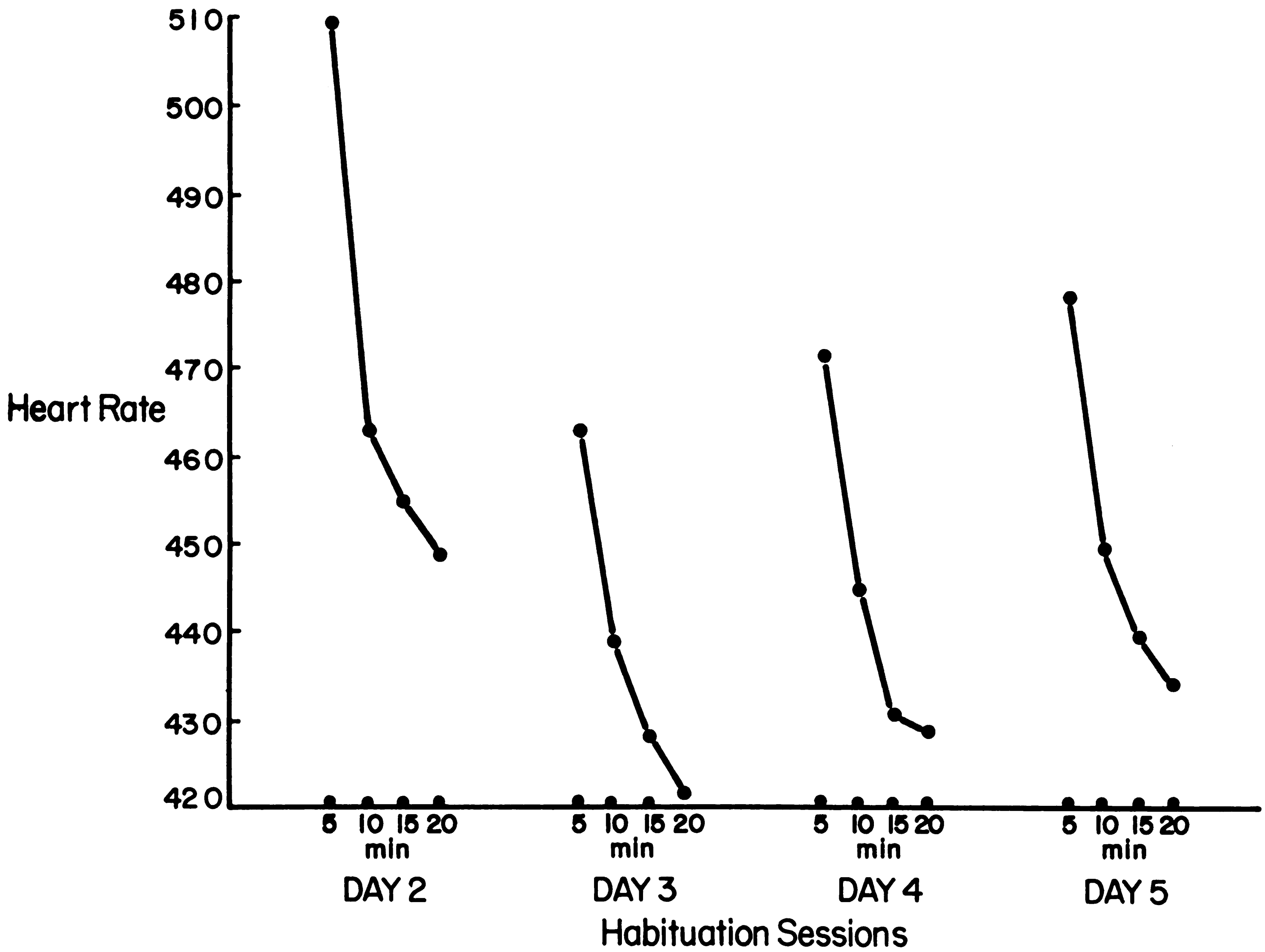

Figure 1. Heart rate during habituation sessions.

($F = 37.37$, df = 3/42, $p < .001$); but change in heart rate across sessions was not significant.

Behavioral descriptions of the birds during habituation were compared between the blue light condition and the white light condition. No differences were evident, and the behavioral sequence diagramed in Figure 2 depicts the general pattern for all birds. When a bird entered the testing chamber, it would stop and stand upright with neck outstretched. Occasionally orienting movements of the head were observed. During this stage the bird's eyes were completely open and the plumage compressed. On the first habituation day, durations of stage 1 ranged between approximately 5 and 15 minutes, while on the later days the pattern lasted between 10 seconds and 5 minutes. The second stage was characterized by squatting with the neck withdrawn, ruffled plumage, and eyes remaining open. The duration of the second stage ranged from 5 to 10 minutes on the first day but usually lasted less than 5 minutes on the later days. Stage 3 differed from stage 2 only in amount of eye closure. During the third stage long, slow blinking and/or periods of eye closure ranging in duration from 5 to 55 seconds were observed. The birds remained in stage 3 until the habituation session was concluded by the experimenter. Although this pattern was typical for all birds during habituation, individuals progressed through the stages at differing rates. It should also be noted that locomotion within the testing chamber during habituation

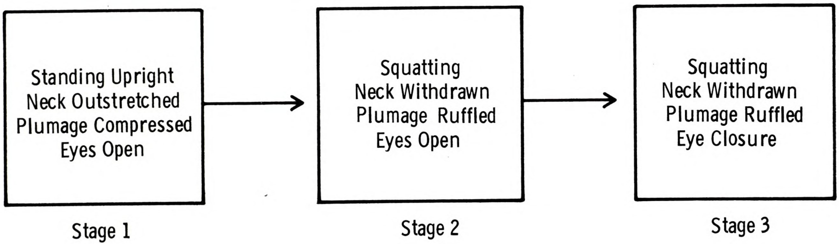

Figure 2. Behavioral sequence during habituation sessions.

occurred on only two occasions. One bird, in stage 3, stood up and ran around the chamber for several seconds and then remained in stage 1 until the end of the session. The dramatic change in behavior corresponded to a loud external auditory stimulus (the slamming of the door to the men's room located across the hall from the laboratory) and occurred during the first habituation session. A second bird, during the third habituation session, changed position by moving approximately 10 cm and then returned to stage 3.

II A. Immobility Tests (Days 6 and 7): Testing Order and Sex

All birds were tested once on day 6 and once on day 7 for defensive immobility. The order of the two tests, self-paced termination test and hawk cry test, was counter-balanced across squads and light conditions. To evaluate testing order effects, the immobility durations of birds which were tested with the self-paced procedure on day 6 were compared with durations of birds which were tested with the self-paced procedure on day 7. There was no significant difference ($t = 1.7$, $df = 14$). To control further for order effects, heart rate immediately prior to testing was compared between day 6 and 7 for all birds. Again the difference was not significant ($t = 0.42$, $df = 15$). These results permitted the collapse of data across days 6 and 7 for further analysis.

Several tests were conducted to examine the effect of sex on defensive immobility. First the self-paced immobility durations of the males and females were compared, and the difference was not significant (t = 0.27, df = 14). The t-test was also used to compare the heart rates of males and females at various points during the self-paced testing procedure. The heart rate recorded immediately prior to the induction of immobility or freezing was established as the baseline for each individual for that test. Thus each bird had a baseline assessed for each test, including self-paced termination, hawk and freezing. In addition, measurement was made of percent of baseline. This was defined by dividing heart rate at each point under consideration (e.g., restraint, induction, etc.) by the baseline heart rate for that test.

Males and females did not differ significantly on baseline heart rate prior to the self-paced termination test (mean for males = 443.9, mean for females = 466.7, t = 0.82, df = 14). Neither did percent of baseline during restraint distinguish males from females (t = 0.22, df = 14). Difference in percent of baseline heart rate during inductions was also not significant when males and females were compared (t = 1.1, df = 14). Finally, the point during immobility at which heart rate was lowest was selected for comparison of the sexes. Percent of baseline heart rate at this point was not significantly different (t = 0.33, df = 14). In light of these results, data were collapsed across sexes for additional statistical analysis.

B. Effect of Blue Light vs. White Light

The durations of immobility during the self-paced termination test were compared between birds tested under white or blue light. Lighting condition did not significantly affect the duration of immobility ($t = 0.39$, $df = 14$). Baseline heart rates under blue or white light were also examined and were found to be not significantly different ($t = 1.14$, $df = 14$). Visual stimulation during induction of immobility (seeing the gloved hands enter the testing chamber) might be expected to affect heart rate. Therefore, percent of baseline heart rate during induction was compared between the two lighting conditions. The difference, however, was not significant ($t = 0.43$, $df = 14$). Similarly, heart rates were not significantly different during restraint ($t = 0.48$, $df = 14$). The two groups were also compared at that point when heart rate was lowest. Percent of baseline did not differ significantly between the groups ($t = 0.0$, $df = 14$). Behavioral descriptions of the birds during the self-paced termination test did not reveal any differences either. Birds in both blue and white light displayed the same behavioral sequence which is described in the next section.

C. Self-Paced Termination Test of Defensive Immobility

Figure 3 depicts the typical behavior sequence during defensive immobility. Stage 1 is characterized by rigidity of the body and plumage compression. The bird

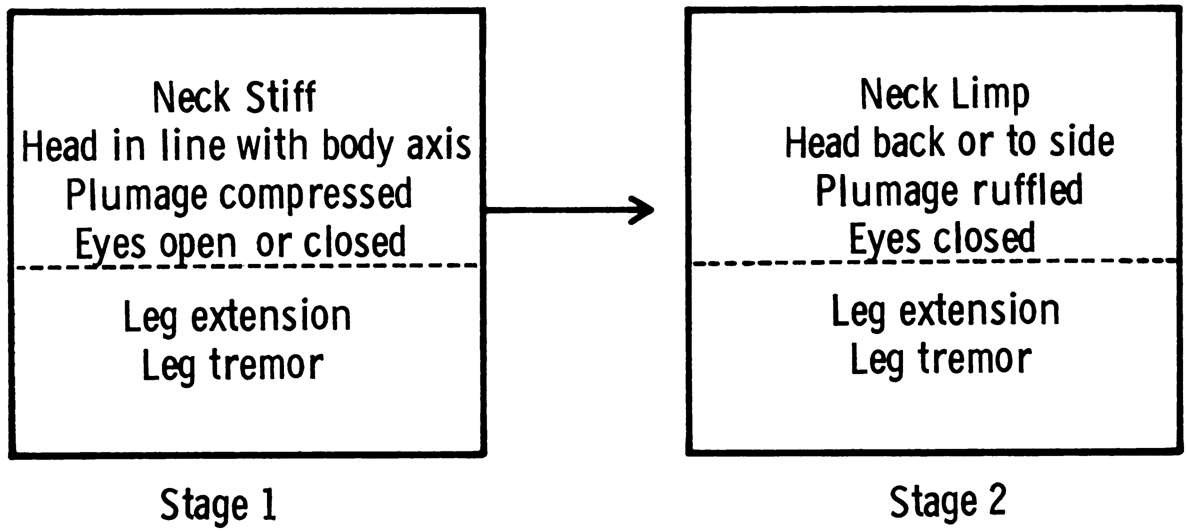

Figure 3. Behavioral sequence during defensive immobility.

held its wings tightly to its sides, its neck stiff, and the beak in line with the body axis. Frequently the eyes were open during stage 1. Within 5 minutes the bird entered stage 2. While stage 1 was one of rigidity, in stage 2 the bird appeared to be limp. Wings fell away from the bird's sides, the head lolled back or to one side, plumage was ruffled, and eyes remained closed. Some birds remained in stage 2 throughout immobility, while others returned briefly to stage 1 and then returned to stage 2. Since 15 of the 16 birds were in stage 2 by 5 minutes of immobility (one entered stage 2 during induction, 12 during the first 2 minutes, two within 5 minutes, and one terminated before entering stage 2), it was not possible to compare heart rate in the two stages.

Heart rate was examined at several points during the course of defensive immobility. First, heart rate during restraint was confirmed to be significantly higher than baseline heart rate ($t = 5.9$, $df = 15$, $p < .001$). Heart rate during induction was also higher than baseline ($t = 10.64$, $df = 15$, $p < .01$). Induction heart rate was also higher than heart rate at the lowest point in immobility, as would be expected ($t = 3.09$, $df = 15$, $p < .01$). Percent of baseline heart rate during the first record (at two minutes) was compared with percent of baseline heart rate during the last record prior to termination. Heart rate prior to termination was lower than during the first part of immobility, and the difference is significant

($t = 3.49$, df = 15, $p < .01$). Finally, Figure 4 (solid line) displays the mean heart rate following termination recorded at 5, 10, and 15 minutes. Heart rate returns to the mean baseline by minute 15. The difference between baseline heart rate and heart rate at 15 minutes post-termination is not significant ($t = 0.55$, df = 15).

In the process of analyzing the data it became apparent that some birds remained in defensive immobility for relatively short durations (4.5 to 28 minutes). The eight birds with short durations included four males and four females, and half the birds had been tested under blue light, half under white light. The remaining eight birds showed durations of immobility ranging from 44 to 240 minutes. If these two groups are compared (short duration birds versus long duration birds), the difference in duration is real and highly significant ($t = 5.32$, df = 14, $p < .001$). Further comparisons between the groups were used to determine whether they differed in terms of heart rate during immobility testing (Figure 5). The groups did not differ significantly in terms of baseline heart rate ($t = 0.33$, df = 14), percent of baseline during restraint ($t = 0.87$, df = 14) or induction ($t = 0.68$, df = 14), and percent of baseline during the first 2 minutes of immobility ($t = 0.05$, df = 14). An examination of the heart rate graphs for individuals did, however, reveal that heart rate for most (7 of 8) birds with short durations remained above baseline during immobility (Figure 5). Conversely, heart

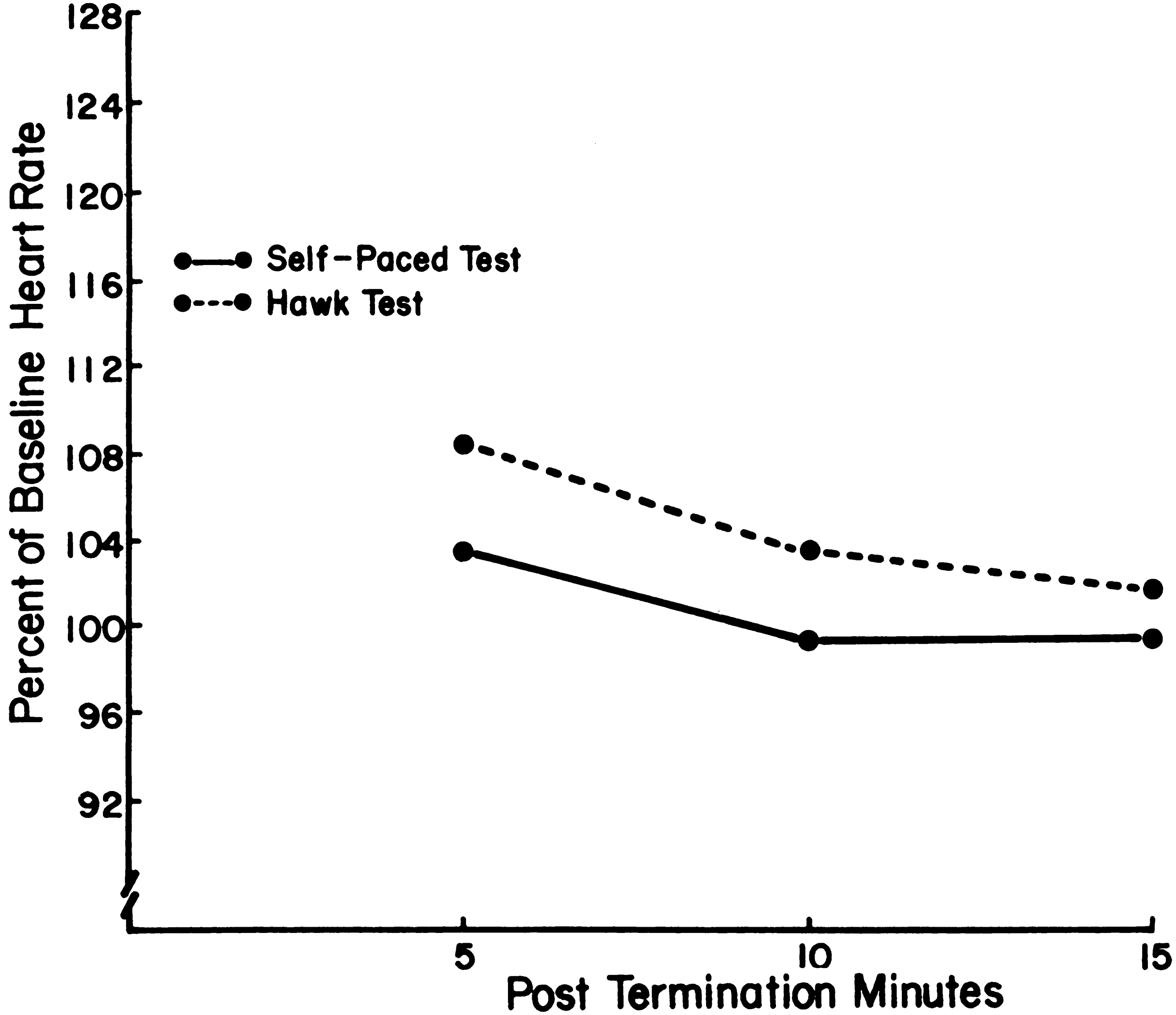

Figure 4. Heart rate following termination of immobility

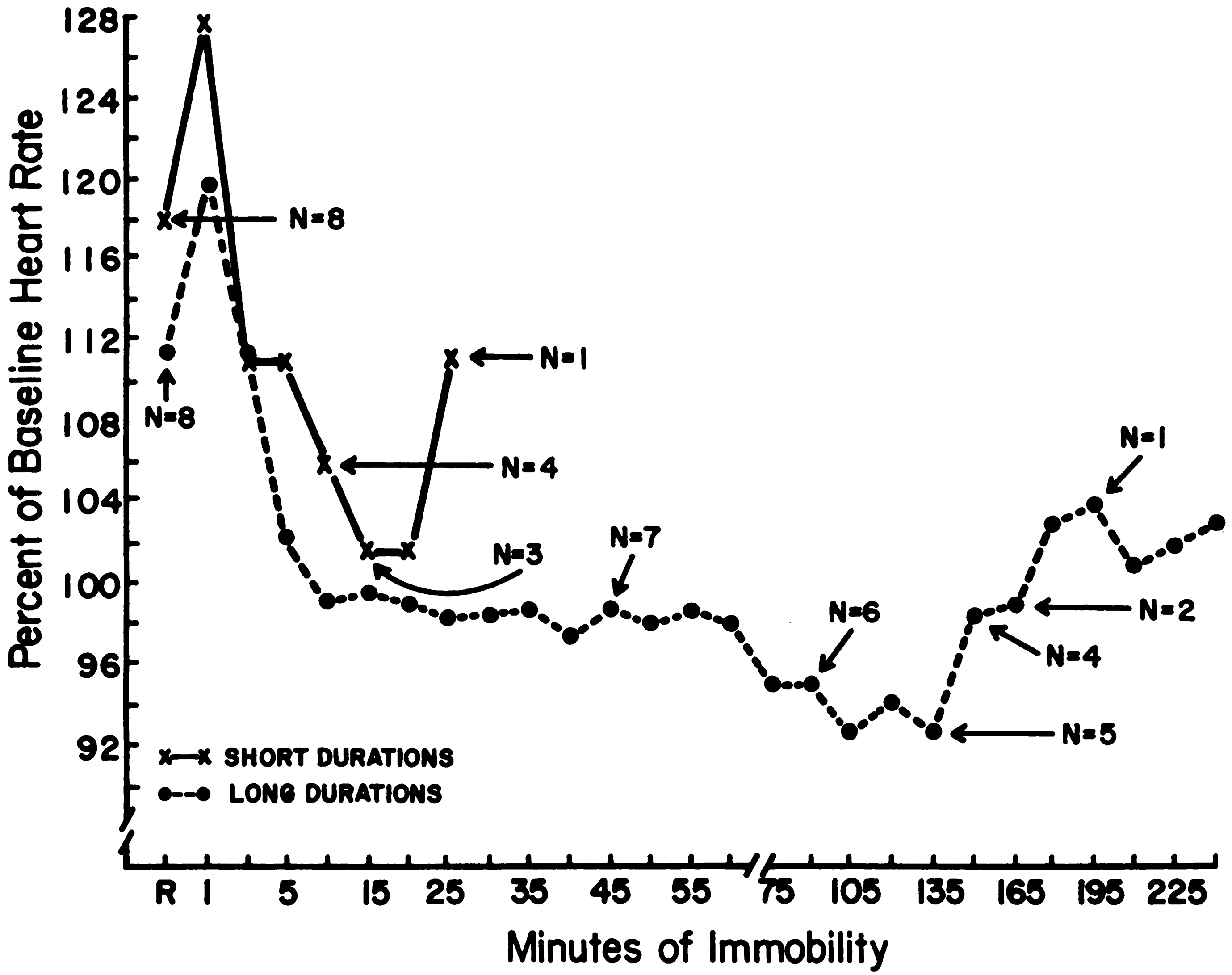

Figure 5. Heart rate during the self-paced termination test comparing birds with long or short durations of immobility.

rate for most birds (7 of 8) with long durations reached or fell below baseline during immobility. The Chi-square test of independence demonstrated this to be a significant difference ($X^2 = 6.36$, df = 1, $p < .02$). Consequently heart rate at the lowest point during immobility was compared between the two groups and was significant ($t = 3.36$, df = 14, $p < .01$). Finally, heart rate tends to decline during immobility with self-paced termination for birds with long durations. The heart rate of long duration birds was compared with that of the short duration birds on the last heart rate record before termination. This difference was also significant ($t = 2.27$, df = 14, $p < .05$).

D. Effect of Hawk Call on Defensive Immobility

The most striking effect of the hawk call on immobility was that most birds (11 of 16) terminated immobility immediately with the first presentation of the hawk cry. Durations of immobility, therefore, were significantly shorter with hawk calls than during self-paced termination ($t = 2.45$, df = 15, $p < .05$). Of the five birds who did not terminate with the first hawk cry, three had exhibited long and two had exhibited short durations during the self-paced termination test. Furthermore, three were female and two male, and three were tested under blue light, two under white light. For these five birds, the self-paced termination durations did not differ significantly

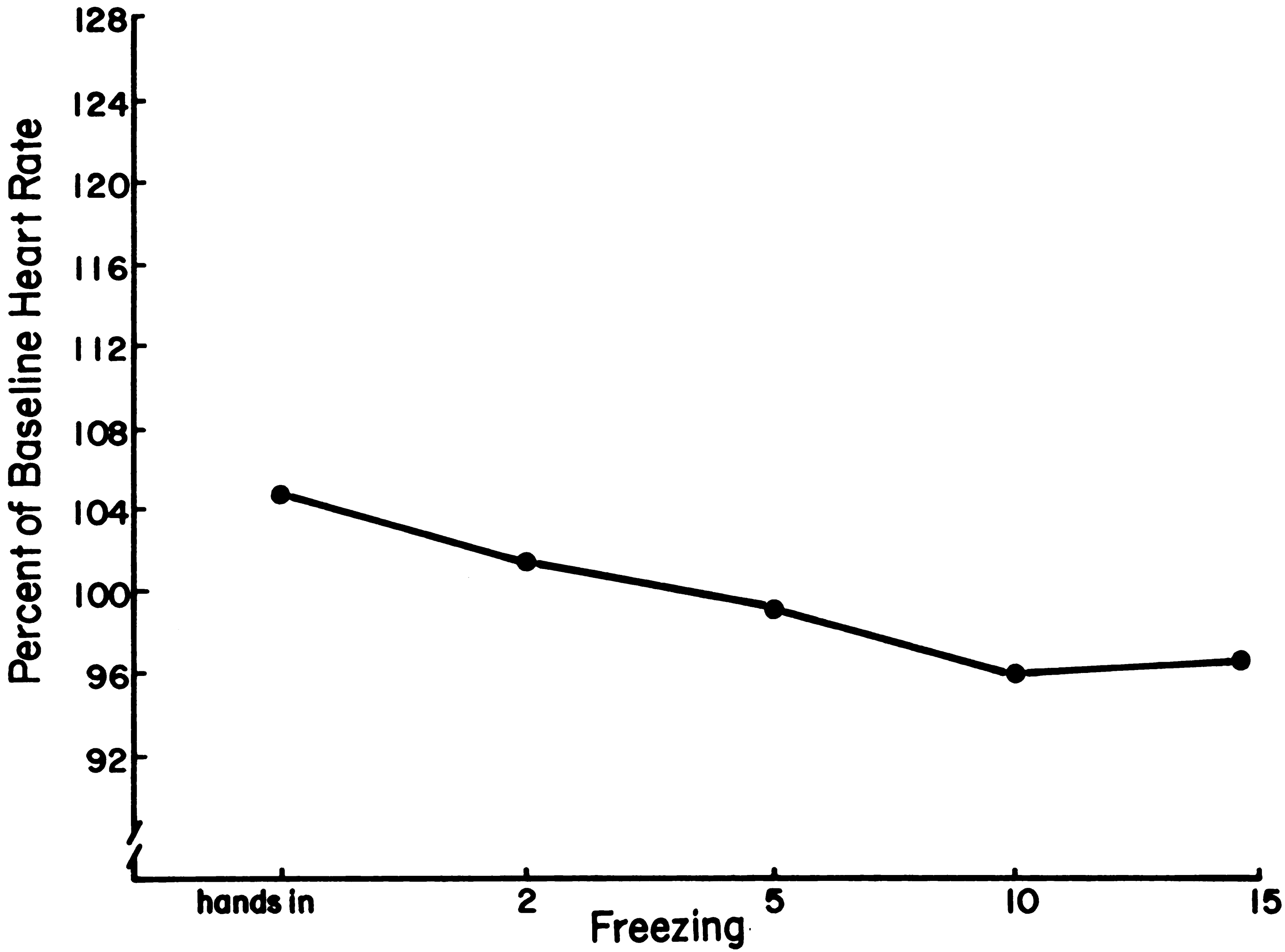

Figure 6. Heart rate during the freezing test.

from durations of immobility during the hawk test ($t = 0.24$, $df = 4$). For three of these birds, termination coincided with a later presentation of the hawk cry.

Because immobility was so brief for most birds, the effect of the hawk cry on heart rate will be based on an analysis of heart rate recorded post-termination. First, heart rates of males and females during the first hawk cry following termination were compared. The difference was not significant ($t = 0.5$, $df = 14$). The same comparison was made using data from the last hawk cry following immobility, and again males did not differ from females. Birds tested under blue light did not differ significantly from birds tested under white light. Their heart rates were compared during the first hawk call presentation ($t = 0.56$, $df = 14$) and the last hawk call presentation ($t = 0.70$, $df = 14$). As a result, data was collapsed across sex and lighting conditions for further analysis.

Figure 4 depicts mean heart rate during post-termination tests. Heart rate was measured immediately prior to the first hawk call presentation during post-termination minutes 5, 10, and 15. Heart rate during hawk presentations was compared with heart rate recorded prior to the first hawk presentation in minutes 5 and 15. The differences were not significant ($t = 0.58$, $df = 15$ and $t = 1.08$, $df = 15$). Heart rate during post-termination minute 15 was compared between the hawk test and the self-paced termination test, and no significant difference was found (t = 0.83, df = 15). Similarly, as can be seen from Figure 4, there was little difference between baseline heart rate and heart rate 15 minutes after termination in the hawk test (t = 0.63, df = 15). However, when heart rate during the hawk presentations is compared between minute 5 and minute 15, the difference is significant (t = 4.06, df = 15, $p < .01$).

After termination, all birds exhibited behavior similar to that described in stage 2 of the habituation behavior (squatting, neck withdrawn, plumage ruffled) with some eye closure. During the hawk call presentations the birds oriented their heads toward the speaker, but did not attempt escape. This behavior pattern continued until the experimenter concluded the test.

III. Freezing Test (Day 8)

Pilot data had suggested that birds would attempt to escape when the gloved hands of the experimenter approached them. Such was not the case in the present study. Prior to insertion of the gloved hands, the birds were all in either stage 2 or stage 3 in comparison with habituation behavior (see Figure 2). Rather than escape, the birds simply shifted position slightly as the gloves approached and remained in stage 2 (Figure 2). Birds in the white light condition oriented their heads toward the gloves, but birds under blue light did not appear to perceive the gloves visually. These birds did hear the

gloves entering the chamber and shifted position toward the wall opposite the arm ports. After the gloved hands were removed, the birds remained in stage 3 (squatting, eye closure, plumage ruffled, neck withdrawn) until the test was concluded by the experimenter.

Figure 6 displays average heart rate during the freezing test. Since heart rates of males and females recorded while the experimenter's hands were in the testing chamber did not differ significantly ($t = 0.76$, $df = 14$), data were collapsed across sex. Heart rate while hands were in was also compared between birds in blue or white light. The difference was not significant ($t = 0.13$, $df = 14$). Data were collapsed across light conditions.

Heart rate while the experimenter's hands were close to the bird was higher than baseline, but the increase was not significant ($t = 1.72$, $df = 15$). Heart rate 15 minutes after the removal of the hands from the chamber was not significantly different from baseline heart rate ($t = 1.86$, $df = 15$). However, the heart rate with hands in was significantly higher than heart rate 15 minutes after the hands were withdrawn.

Heart rate during freezing (i.e., while the experimenter's hands were close to the bird) was compared with heart rate during restraint and induction before the self-paced termination test. Heart rate was substantially higher during restraint ($t = 4.61$, $df = 15$, $p < .01$) and during induction ($t = 8.11$, $df = 15$, $p < .01$) than during freezing. Conversely heart rate at the lowest point during the self-paced termination test did not differ significantly from freezing heart rate.

DISCUSSION

The results of this experiment provide information which is pertinent to four questions under investigation. First, the effect of testing under blue light was considered. Several investigations by Gallup and his colleagues had suggested that the presence of visual stimulation during immobility was an important determinant of the duration of the response (Gallup, 1972; Gallup, et al., 1972; Gallup, et al., 1971). Similarly, Doty (1969) had found that visual stimulation, a revolving nystagmus drum, significantly shortened durations of immobility. Although Tortora and Borchelt (1972) had tested the immobility response for some Bobwhites under blue light, the effect of the relative absence of visual stimulation on defensive immobility and heart rate had not been investigated. The present results suggest that the absence of visual stimulation (testing under blue light) does not produce any reliable effects. No significant differences between the blue and white light conditions were found during habituation, self-paced termination of immobility, the hawk test, or the freezing test. This tends to confirm the report by

Tortora and Borchelt (1972). Further investigations are needed, however, to precisely determine the effect of specific visual stimulation on behavior and heart rate. The testing procedure used in the present experiment could be used to facilitate future studies of this sort.

A second and important question to which the results of the experiment pertain is the pattern of behavior and heart rate throughout the course of immobility testing. Observations during the study confirmed three distinct behavioral stages which occurred in sequence during habituation to the testing situation. Stage 1 is probably identical to the freezing response in the wild, and characterized an alert and aroused bird. Stages 2 and 3 described an increasingly less aroused and alert bird. Decreases in heart rate within the habituation session seems to confirm that the bird's state changes from one of high arousal to lower arousal. Indeed, the experimenter frequently suspected that birds were asleep during stage 3. This hypothesis could be tested in another study by monitoring EEG activity during the three behavioral stages.

Two distinct stages of immobility were also identified behaviorally. Stage 1 was characterized by rigidity of body posture, while stage 2 depicted a rather limp posture. Klemm (1965a) monitored EEG during immobility in rabbits, and Silva, et al. (1959) measured EEG in the Tero bird during immobility. Both investigations revealed two distinct stages of immobility, the first including fast waves typical of an alert organism and the second characterized by waves usually typical of a sleeping organism. Their EEG data and the present behavioral data seem to confirm the existence of two stages of the immobility response.

The pattern of heart rate through the course of the immobility response was investigated. As predicted, manual restraint of the bird dramatically increased heart rate, and heart rate reached the highest level during induction of immobility. This finding lends indirect support to Ratner's defensive distance model of predator-prey behavior. It might be expected that a high level of arousal in prey would accompany capture and manipulation (mouthing, pawing, etc.) by a predator.

The heart rate pattern during the immobility response revealed a most interesting finding. Half of the birds tested exhibited relatively short durations of immobility, and for most of these birds heart rate remained higher than basline throughout the immobility response. The heart rates for these birds, therefore, would support Gallup's fear hypothesis. Furthermore, it would make good (adaptive) sense for birds to remain in a state of arousal and ready to effect an escape from the predator should the opportunity arise. The remaining birds, however, were in immobility for long durations, and for most of the birds, heart rate reached a point below baseline during immobility. This result is difficult to explain in that relaxation (as evidenced by decreased heart rate) and long durations of immobility would not seem to facilitate escape from the predator. Indeed, such a state of incapacitation might invite being devoured by some other predator. The experimenter would like to speculate on two possible reasons for the occurrence of long durations. First, they may be simply an artifact of testing in a quiet laboratory. In the wild many auditory, visual and tactile stimuli are probably present, and these stimuli may terminate immobility to produce short durations. A second reason for long durations might have arisen from breeding in the laboratory. It is possible that birds who remain in immobility for lengthy periods in the wild do not survive. In the laboratory, however, selection pressure against long durations has been removed. A recent study by Klemm (1973) lends support to this idea. Klemm selectively bred Wistar rats for duration of immobility and susceptibility to induction and obtained groups which differed significantly on these measures by the third and fourth generations. Both of these speculations, however, require additional investigations in the form of field studies and further selective breeding experiments.

The effect of the hawk cry, which for most birds was the immediate termination of immobility, confirms the findings of other investigators that loud, auditory stimuli do affect immobility (Ratner, 1958, Thompson & Hatton, 1974). Eyer and Ratner had found that conspecific vocalizations did not usually terminate immobility, but their stimuli were presented at a lower intensity (approximately 70 dbs compared with the 88 db hawk cry). The effect of the hawk cry on heart rate was to maintain heart rate at a higher level during the 15 minutes after termination than it was after self-paced termination (Figure 4). By minute 15, however, heart rate had returned to near baseline in the hawk condition. A further study is needed to compare the hawk call with control stimuli of equal intensity to determine the effect of the hawk call itself.

Heart rate during freezing was significantly lower than during restraint or induction of immobility. While heart rate was significantly higher when the experimenter's hands were in the testing chamber than it was 15 minutes later, at no time did it approach the level of induction heart rate. In this respect heart rate could be used to distinguish between freezing and onset of immobility. The behavior of the birds during the portion of the experiment called the freezing test raises doubt that the birds were really freezing when the behavior was compared with the early habituation sessions. That is, they failed to show plumage compression, response to visual stimulation, and erect posture during the freezing test. Additional tests in which birds are clearly freezing need to be conducted.

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