CARDIAC AND RESPIRATORY CONDITIONING, DIFFERENTIATION, AND EXTINCTION IN THE PIGEON¹

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Paired light and foot shock in 12 pigeons rapidly developed acceleratory heart and respiratory conditioned responses. Sensitization and stimulus control birds did not condition. When a different colored stimulus light that was not reinforced was mixed in a random series with the reinforced light, rapid differentiation of both heart and respiratory responses occurred. In most cases neither heart nor respiratory conditioned responses could be extinguished by 100 non-reinforced presentations of the conditioned stimulus.

Although there have been reports of classical appetitive conditioning in various avian species (Longo, Klempay, and Bitterman, 1964; Voronin, 1962), defensive conditioning paradigms have not often been applied to the bird. Loucks (1961, Ch. 13) failed to condition leg flexion to an exteroceptive stimulus in the chicken, and Longo, Milstein, and Bitterman (1962) conditioned changes in gross activity level of the pigeon. Cardiac and respiratory defensive conditioning studies have been conducted in Tuge's laboratory as a part of his general comparative approach (Tuge and Shima, 1959; 1960; Tuge, Shima, and Koga, 1957; Tuge and Yueh, 1962). However, in Tuge's studies the lack of control birds and the lack of quantitative conditioning measures make it difficult to assess conclusions regarding the cardiac and respiratory rates and levels of conditioning.

In addition to investigating the performance of the pigeon in defensive conditioning, differentiation, and extinction tasks, the present study also sought to develop a conditioning

paradigm that would be easily applicable to pigeons and would provide a standardized situation for evaluating the effects of lesions on learning.

METHOD

Subjects

Sixteen naive, White Carneaux pigeons (*Columba livia*), obtained from the Palmetto Pigeon Plant, Sumter, South Carolina, ranged in age from 2½ to 6 months at the start of the experiment. All were individually housed and permitted free access to food and water.

Apparatus

Conditioning chamber. This was a modified aluminum cooler chest 22 by 13 by 16 in., light proof and moderately sound attenuated. Two Dialco 3-w indicator lights were mounted on one wall approximately 8 in. above the floor. One was fitted with a red lens, the other with a green lens. The two lights were not equated for brightness. A shield of translucent plastic mounted in front of the lights diffused the beams. The chamber also contained a hammock on which the pigeon was mounted and a series of junction boxes that received lead wires from the various recording electrodes.

Stimulus control. The mounted lights delivered the conditioned stimuli, their duration being controlled by a Lafayette Interval-Timer which was connected in sequence with a second timer that controlled duration of the unconditioned stimulus, a 60-cycle ac shock (3 to 7 v) delivered to the foot.

Response measures. For recording EKG two

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needle electrodes were implanted at the start of each session, one in the right wing base and the other in the left foot. This is customarily denoted as lead II in avian electrocardiography. The signal was amplified by a Grass 5P6 EKG preamplifier.

For recording respiration a pneumograph was fixed around the pectoral region and its output connected to a Grass PT 5A Volumetric Pressure Transducer. The output of the transducer was amplified with a Grass 5P1 Low-Level dc preamplifier.

The remaining channels of the polygraph were used to record EEG and slow potential changes from chronically implanted telencephalic electrodes.

To obtain accurate records of the onset and offset of the conditioned and unconditioned stimuli the Lafayette Timers were used to trigger the signal marker of the polygraph.

Procedure

Pre-experimental treatment. Five to 10 days before conditioning sessions, each bird was chronically implanted with recording electrodes in various hemispheric nuclei. The details of the implantation procedure and the changes in EEG and slow potential activity during conditioning will be described elsewhere.

Preparation of the bird for conditioning. Before the start of any training session each bird was prepared as follows: the pneumograph was fixed in place and the pigeon was wrapped in an elastic bandage that permitted movement of legs, neck, and head only. The bird was then mounted on the hammock and stabilized by wrapping another elastic bandage around both the animal and the hammock. The shock electrodes were then attached. One electrode was a needle inserted in the foot pad; the other was a flexible wire wrapped snugly around the metatarsal region of the same foot. To insure good contact of the metatarsal electrode and to protect the foot from injury by the wire the region was first liberally covered with electrode paste and a ¼-in. band of multiple-ply aluminum foil was wrapped around the area. The wire was then wrapped around the foil. Following this procedure the EKG electrodes were placed and the EEG electrode leads connected. The door of the chamber was then closed and the conditioning chamber remained dark except

for the stimulus lights which appeared during trials.

Training sequence. Conditioning consisted of five consecutive days of 20 light-shock pairings, a total of 100 trials. Conditioning and differentiation sessions were separated by a weekend of no training. On the first differentiation session each bird was given 20 presentations of a nonreinforced light different in color from the conditioned stimulus. On the remaining four days the bird received, in randomized order, 10 light-shock trials and 10 trials of the nonreinforced light. Differentiation and extinction sessions were also separated by a weekend of no training. During extinction each bird was run for five days with 20 trials per day of the conditioned stimulus but with the shock withdrawn. The same intertrial interval schedule was used in all sessions. The mean interval of this schedule was 7.4 min with a range of 5 to 10 min.

Stimuli. Within each group, birds were counterbalanced with respect to whether the conditioned stimulus was a red or green light. If during differentiation a given bird's conditioned stimulus was a red light, then the nonreinforced stimulus was a green light. Thus, the groups were also counterbalanced with respect to this nonreinforced stimulus during differentiation. In all cases, duration of the light stimuli was 6.5 sec. The foot shock was 0.5 sec in duration and coincided with the last 0.5 sec of the light. At the start of each session, including differentiation, extinction, and control sessions, a single test shock was given to help determine the intensity that would produce a sharp increase in heart and respiratory rates. This was followed by a 15-min adaptation period. The shock levels throughout were adjusted to maintain the unconditioned response magnitudes at the most constant level possible. Preconditioning control sessions in which only the light was presented were not given since the goal was to maximize conditioning; Lubow (1965) demonstrated that in defensive conditioning nonreinforced pre-exposures of the to-be-conditioned stimulus produce a performance decrement in the acquisition of the conditioned response.

Groups

The experimental group consisted of 12 birds, each of which received conditioning, differentiation, and extinction sessions. Since

the group was counterbalanced with respect to the color of the conditioned stimulus, half received the green light and the other half the red light as the conditioned stimulus.

Two birds were run as stimulus controls, one with a red light, the other with green. The running of these birds was identical to that of the experimental animals with the exception that shock never followed the light.

Two additional birds were run as sensitization controls. They too were counterbalanced with respect to the stimulus light. They received daily 20 presentations of the light and 20 of the shock in randomized order. The mean interval between light presentations was 7.4 min. The shock presentations were randomly assigned with respect to the light with any two presentations separated by at least 1 min.

Data analysis

On each trial the number of heart beats and respirations were counted in the 6-sec period just before stimulus onset. A similar count was made for the first 6 sec of the stimulus period, and the change in heart and respiratory rates was calculated. These counts did not include the final 0.5 sec of the light period since this coincided with the foot shock and the shock artifact obscured the record. When beats were missed (second degree A-V block) a beat was counted in the interval for the purposes of determining rate. Respiratory rate was obtained by counting peaks in the polygraph record. For any given period this counting gave only a gross estimate, but reasonably accurate values were obtained when averaged over a number of trials.

RESULTS

The conditional response was an acceleration of both heart and respiratory rates (see Fig. 1). The form of the cardiac conditioned response varied greatly, even within individual birds. There were two predominant forms of cardiac acceleration and both occurred in all birds showing clear evidence of conditioning. The first was a monotonic increase that had both variable latency and slope. The second was a biphasic response characterized by an acceleration followed by a deceleration; in all cases the decelerative component was at least equal to and in most cases greater than

the basal rate. No relationship was observed between the type of cardiac response and stage of conditioning. The response to the shock was invariably an increase in heart and respiratory rates, and although the conditioned responses were qualitatively similar to the unconditioned responses they were of lower magnitude (see Fig. 1). The somatic response to the shock had little uniformity and included head and neck movements and/or movement of one or both legs.

Conditioning

An analysis of group means indicated that the cardiac and respiratory responses of the conditioning group were significantly greater than the responses of the stimulus control group for each of the five training sessions, all *t* values being significant beyond the .005 level. This demonstrates that even within the initial training session some birds showed substantial conditioned increases in heart and respiratory rates (for example, see bird 10R in Fig. 1). Representative records for three conditioning birds and a sensitization control bird for the first and last conditioning sessions are presented in Fig. 1. In addition to showing the growth of the conditioned responses in the first training sessions, this illustration also provides some idea of the variability within and between experimental animals. To describe more completely individual differences and variability from session to session, the daily means for each bird for heart and respiratory responses are presented in Tables 1 and 2 respectively. It is evident that large individual differences exist in the magnitudes of the conditioned heart and respiratory responses. This wide spectrum of responsiveness includes birds (8R and 20R) showing responses close to control values. Failure of some birds to show conditioning of heart and respiratory rates has been reported by Tuge *et al.* (1957). It will also be noted in Tables 1 and 2 that the control values exclude the possibility that sensitization (pseudo-conditioning) occurred.

Although no attempt was made to measure movement quantitatively, artifact in the EKG, EEG, and pneumographic records was used as an indicator of gross body movement. Four of the 12 experimental animals showed clear evidence of gross somatic responses to the conditioned stimulus, usually in the last two days of conditioning. To investigate somatic

Fig. 1. Representative EKG and pneumographic records of three conditioning birds and a sensitization control bird (19R) for selected trials on the first and last days of conditioning. The second line of each record is the signal marker with markings at 1 per sec. The longer vertical mark near the beginning of this line indicates the start of the control period. The end of the control period and start of the light presentation is indicated by the dark bar above the signal marker line. The period of foot shock is indicated by the short dark bar extending below the line. (This marking is frequently obscured by shock artifact.) Since the records are curvilinear, small vertical marks are included on the heart and respiratory records to indicate the start of the control and light periods. The numbers on each record are the heart and respiratory rates in beats per minute and respirations per minute for the control and light periods.

Table 1
Cardiac Conditioning
Daily Means for Rate Changes (Beats/Min)

<i>Light and Shock: Paired</i>	<i>1</i>	<i>2</i>	<i>Day 3</i>	<i>4</i>	<i>5</i>	<i>Mean</i>
24R	48.0	47.5	59.0	54.0	67.0	55.1
10R	22.5	34.0	39.5	29.0	32.0	31.4
18R	31.5	25.0	30.0	31.0	31.0	29.7
17R	16.5	25.5	34.0	28.0	44.5	29.7
9R	18.5	22.5	30.0	27.0	27.0	25.0
5R	23.5	19.5	14.5	23.5	19.5	20.1
1B	18.5	21.0	15.0	20.5	18.0	18.6
23R	12.5	18.0	21.5	16.0	21.0	17.8
11R	13.5	16.5	11.0	11.0	19.0	14.2
6R	2.5	9.5	12.0	15.0	15.5	10.9
8R	6.5	8.0	18.5	3.0	8.5	8.9
20R	0.0	-3.5	7.5	12.5	3.5	4.0
Mean	17.8	20.3	24.4	22.5	25.5	22.1
<i>Light and Shock: Unpaired</i>						
19R	6.5	4.0	6.0	4.0	-1.0	3.9
25R	-4.5	1.0	1.5	3.0	1.5	0.5
<i>Light Only</i>						
6B	-3.5	1.5	3.5	2.0	4.5	1.6
21R	4.0	5.0	8.0	7.5	8.5	6.6

Table 2
Respiratory Conditioning
Daily Means for Rate Changes (Respirations/Min)

<i>Light and Shock: Paired</i>	<i>1</i>	<i>2</i>	<i>Day 3</i>	<i>4</i>	<i>5</i>	<i>Mean</i>
24R	18.0	14.5	17.0	18.5	20.5	17.7
10R	7.9	9.0	7.0	8.0	8.0	8.0
18R	9.0	8.5	11.0	11.0	10.5	10.0
17R	4.0	6.5	7.5	4.5	8.0	6.1
9R	12.5	7.5	11.0	12.5	12.5	11.2
5R	18.5	8.5	5.5	12.5	11.0	11.2
1B	6.0	5.5	5.0	6.5	3.5	5.3
23R	5.0	2.0	8.0	4.0	9.0	5.6
11R	6.0	10.0	6.5	4.0	9.0	7.1
6R	7.6	6.5	7.4	8.0	8.6	7.6
8R	0.0	1.0	3.0	1.5	1.0	1.3
20R	-3.0	0.0	1.0	4.5	1.0	0.7
Mean	7.6	6.6	7.5	8.0	8.6	7.7
<i>Light and Shock: Unpaired</i>						
19R	2.0	-0.5	-1.5	2.0	0.5	0.5
25R	-0.5	-2.0	0.0	1.5	-1.5	-0.5
<i>Light Only</i>						
6B	-2.0	-2.5	0.5	0.5	0.5	-1.7
21R	0.5	1.5	0.5	0.0	1.0	0.7

conditioning more systematically, electromyographic conditioning studies are presently being conducted.

Three of the birds developed cardiac ar-

rhythmia during conditioning. In the records of these birds dropped beats were quite evident in the control periods and either decreased substantially or were completely abol-

ished during the conditioned stimulus (for example, see 8R and 23R in Fig. 1).

Differentiation

On the first day of the differentiation week each bird received 20 trials of the nonreinforced stimulus only. This provided a measure

of generalization from the reinforced color to the nonreinforced. The group means for that day were increases of 16.0 beats per min and 4.1 respirations per min. These mean changes include both generalization from the red to green light and from the green to red light, since the group was counterbalanced.

Table 3
Cardiac Differentiation
Daily Mean Difference Scores for Rate Changes*

Bird	Day				CS Mean	CS ^a Mean
	1	2	3	4		
24R	7	37	36	16	96.75	72.75
10R	11	16	21	13	40.25	25.00
18R	-3	1	5	12	25.50	21.75
17R	3	16	6	14	29.00	19.25
9R	5	10	11	20	25.25	13.75
5R	8	1	1	6	12.00	8.00
1B	5	8	9	2	15.50	9.50
23R	2	5	8	7	20.25	14.75
11R	6	4	0	-3	28.00	26.25
6R	7	6	14	16	22.00	10.75
8R	4	10	2	7	36.00	30.00
20R	0	-2	2	4	12.50	11.50
CS Mean	29.50	33.67	31.67	26.17	30.25	-----
CS ^a Mean	24.92	24.33	22.00	16.50	-----	21.94

*The mean difference score entries for each animal on each day of differentiation were derived as follows: the mean rate increase for the nonreinforced trials (CS^a) was subtracted from the mean rate increase for the reinforced trials (CS) on each day. The CS means given on the righthand side of the table are the mean rate increases to the reinforced stimulus for all differentiation days combined. Similarly, the CS^a means on the right are the mean rate increases to the nonreinforced light for all days combined. The CS and CS^a means at the bottom of the table are the daily group means for all birds.

Table 4
Respiratory Differentiation
Daily Mean Difference Scores for Rate Changes*

Bird	Day				CS Mean	CS ^a Mean
	1	2	3	4		
24R	8	20	14	11	28.00	14.75
10R	8	6	14	1	13.75	6.50
18R	2	10	9	-3	10.25	5.75
17R	6	10	11	7	11.50	3.00
9R	9	12	10	12	11.25	0.50
5R	7	2	7	2	6.75	2.25
1B	2	-1	9	0	6.25	3.75
23R	3	9	1	1	9.25	5.75
11R	7	4	5	6	12.00	6.50
6R	11	8	15	2	16.25	7.25
8R	0	8	5	6	7.25	2.50
20R	1	-5	4	2	5.00	4.50
CS Mean	10.25	12.50	12.75	10.33	11.46	-----
CS ^a Mean	4.92	5.58	4.08	6.42	-----	5.25

*The mean difference score entries for each animal on each day of differentiation were derived as follows: the mean rate increase for the nonreinforced trials (CS^a) was subtracted from the mean rate increase for the reinforced trials (CS) on each day. The CS means given on the righthand side of the table are the mean rate increases to the reinforced stimulus for all differentiation days combined. Similarly, the CS^a means on the right are the mean rate increases to the nonreinforced light for all days combined. The CS and CS^a means at the bottom of the table are the daily group means for all birds.

Both cardiac and respiratory measures showed a high level of differentiation on all four days of training. Daily *t* tests of the group difference scores were all significant beyond the .01 level for both heart and respiration. Thus, a high level of differentiation obtained within the first day where both light-shock trials and trials of the nonreinforced light were mixed.

The daily mean difference scores for each animal are presented in Tables 3 and 4 for heart and respiration respectively. Included are the daily group means for the responses to the nonreinforced stimulus. If the generalization values are compared with these means it is clear that the response levels to the nonreinforced light did not diminish during differentiation. The tables also include the daily group means for responses to the reinforced stimulus. A comparison of these means with the daily conditioning means indicates a higher level of responding to the reinforced stimulus during differentiation training. Consequently, the differentiation training did not produce extinction to the nonreinforced stimulus, but the procedure was sufficient to maintain a high level of differential responding.

Extinction

The daily group means during the five extinction sessions were increases of 21.8, 24.4, 21.4, 20.5, and 19.4 beats per min and 8.3, 7.1, 6.6, 7.0, and 7.0 respirations per min. There appear to be substantial differences between the magnitudes of the conditioned responses in differentiation and extinction sessions. This may not be an appropriate comparison since the nonreinforced stimulus presentations during differentiation training may affect the level of the conditioned response.

Trend tests of the daily extinction means were not significant for either heart or respiration. Furthermore, the conditioned response levels during extinction are similar to the response levels obtained during conditioning and indicate that despite 100 nonreinforced presentations of the conditioned stimulus there are still substantial response levels. Two of the birds were run for an additional 100 extinction trials, and although there was some decrease from the first to the second 100 presentations, a high response level was still observed in both heart and respiration. Thus, the extinction procedure was ineffective for

most birds. However, one bird (23R) did extinguish on both heart and respiration.

Baseline Rates

A trial-by-trial correlation analysis of the relationship between basal rate and conditioned response magnitude for both heart and respiration indicated significant inverse relationships. The mean correlation between heart base rate and rate increase was $-.469$, and for respiration the mean correlation was $-.473$. For both heart and respiration 10 of the 12 birds showed significant individual negative correlations. This analysis indicated that conditioned response magnitudes were affected by baseline rates. However, this does not affect the cardiac conditioning results since basal heart rate, approximately 130 beats per min, did not change significantly from day to day during either conditioning or extinction. Similarly, basal respiratory rate during extinction, approximately 34 respirations per min, did not change significantly from day to day. However, during conditioning mean basal respiratory rate dropped from 41 respirations per min on the first day to 32 respirations per min on the last day, possibly accounting for some of the day-to-day changes in respiratory conditioning.

DISCUSSION

The finding that cardiac and respiratory changes condition within a few trials in the pigeon substantiates the reports of Tuge and his collaborators. Rapid cardiac conditioning has also been observed in a number of mammalian species including the dog (Church and Black, 1958) and the monkey (Smith and Stebbins, 1965). Regarding the relative rates of conditioning of the heart and respiratory changes, Tuge *et al.* (1957) have claimed that the conditional cardiac reflex appears somewhat earlier than the conditional respiratory response. Since both conditional responses develop so rapidly it is difficult to evaluate this conclusion.

Heart and respiratory differentiation also occurred rapidly, within the first differentiation session, corroborating the findings of Tuge and Shima (1959). It is well established that pigeons easily learn to make hue and brightness discriminations in an operant situation. The present results extend the gener-

ality of this conclusion by including discrimination within a classical conditioning context.

Regarding extinction, for all but one bird the present findings indicated no extinction during 100 nonreinforced trials and only a slight response decrease during an additional 100 extinction trials. These results are consistent with those of Gantt (1960) who reports that it is impossible to extinguish defensive heart and respiratory conditioned responses in some dogs, and in all dogs these responses are more difficult to extinguish than somatic and salivary conditioned responses. However, Tuge *et al.* (1957) obtained extinction of the cardiac and respiratory responses in the pigeon.

One goal of the present study was to evaluate the usefulness of the described method as a conditioning procedure for the pigeon. It has a number of advantages. Partially restraining the bird readily permits recording of electrophysiological measures and other physiological variables such as respiratory and cardiovascular parameters. This enables one to investigate conditioning in a large number of systems simultaneously. Also, with very little modification the procedure is easily adaptable to a large number of species, rendering it an efficient and standardized procedure for use in comparative studies. The present results indicate that this defensive conditioning method is an efficient paradigm for obtaining classical conditioning data for the bird and will provide a useful tool for studying the effects of lesions on learning.

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