ditioned responses observed during the extinction period. Hilgard and Marquis (*6*) have noted that several studies point to negative correlation between conditioning and extinction—that is, that rapid conditioning is associated with slow extinction. In our study it was not possible to measure the conditioned responses in the conditioning period, for the giving of shock for reinforcement always gave a positive psychogalvanic response and the anticipatory response did not occur frequently enough by itself in the 11 conditioning trials to differentiate.

It is of importance to note that only certain orienting or conditioned stimuli offer the opportunity for this type of correlation. Reese, Doss, and Gantt abandoned the auditory conditioned stimulus because they found that the response to it took too many trials to decrease and disappear (*4*). In subsequent experiments they used a light as the conditioned stimulus, to which subjects made fewer orienting responses.

One other aspect of our data is of some importance: the relatively low medians for both types of response. The absence of a normal distribution of these responses suggests that there may be further ways of differentiating subjects.

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Biochemical Responses of Rats to Auditory Stress

Abstract. Prolonged, intense auditory stimulation caused a marked reduction in glutathione levels in the blood of female rats. The frequency of the response was significant statistically and was related inversely to the recovery rate after auditory stimulation. An increase in adrenal weights and ascorbic acid, as well as a decrease in total adrenal cholesterol, were noted.

The present study explores alterations in certain biochemical parameters which were induced in rats subjected to repeated, prolonged, high-intensity auditory stimulation. Specifically, levels of blood glutathione, adrenal ascorbic acid, and total adrenal cholesterol are reported. The work extends the analysis of physiologic changes reported by other workers in the field of audiogenic stress (*1*). Hurder and Sanders (*2*) indicated that animals with larger adrenals were more susceptible to audiogenic seizures than animals with smaller adrenals. However, administration of adrenocorticotropic hormone (ACTH) did not affect seizure susceptibility. Blood glutathione index was employed as a possible indicator of "generalized" stress (*3*), since both cortisone and ACTH cause a transitory drop in the glutathione content in blood of rats and human beings (*4*). Present studies tend to indicate that the glutathione index may be a significant metabolic parameter in the investigation of psychoses (*5*). Both adrenal ascorbic acid and total adrenal cholesterol assays were employed as conventional stress indicators (*6, 7*).

Test and control groups consisted of Wistar female albino rats, weighing 145 to 195 g, paired by weight. The first test group was subjected to daily 1-minute intense auditory stimulation (frequency, 120 cy/sec; level, 100 ± 5 db) on 11 occasions, whereas, the second test group was exposed to daily 5-minute auditory stimulation 15 times. Although the animals were visibly disturbed when subjected to auditory stimulation, a seizure pattern was not induced. Control animals were subjected to the same handling procedures, excluding auditory stimulation. At the conclusion of the experiment all animals were killed with ether and autopsied, and the adrenals were removed immediately for ascorbic acid (*8*) and total cholesterol (*9*) determinations. Blood was obtained directly from the heart and assayed immediately for glutathione content (*10*). Hematocrit levels were determined to calculate the glutathione index.

Blood glutathione levels were reduced markedly in both test groups (Table 1) and t test analyses (*11*) indicated that the blood glutathione reduction in the 1-minute test group approached significance at the 5 percent level of confidence. Although the data for the 1- and 5-minute test groups are treated separately, to determine whether differences in duration of stimulation yielded different consequences, the combined test group data indicate a blood glutathione reduction (Table 1) which approached conventional levels of significance ($P = .07$). From the point of view of general consequences on biochemical indicators, the test data readily could be combined since the two test groups differed from each other only in the duration of the stimulus. Thus the experiment suggests that a drop in glutathione index appears as a result of auditory stimulation. To explore this possibility further, the glutathione data were evaluated on the basis of chi-square distribution (*11*). Even on the basis of the most critical frequency analysis, employing the combined test and control mean (glutathione index 73.5, 26 animals), 79 percent of the auditorily stimulated rats had values smaller than this mean, whereas 75 percent of the control animals had values greater. Despite the failure of t test procedures to yield a 5 percent significance level, chi-square distribution indicated a statistically significant effect ($P = .04$) with regard to the reduction in blood glutathione levels after recurrent auditory stimulation.

Table 1. Blood glutathione and adrenal ascorbic acid levels in female rats after prolonged auditory stimulation. The figures in parentheses indicate the number of values used in determining the mean. The Snedecor t test procedure (*11*) was used to determine the P values.

Blood glutathione index* (Mean ± S.E.)	Adrenal ascorbic acid content† (Mean ± S.E.)
Control	
80 ± 5 (12)	0.532 ± 0.020 (25)
Test‡	
64 ± 5 (6)	0.550 ± 0.038 (15)
$P = .07$	$P = .65$
Test§	
71 ± 5 (8)	0.579 ± 0.024 (12)
$P = .24$	$P = .18$
Combined test	
68 ± 4 (14)	0.563 ± 0.023 (27)
$P = .07$	$P = .37$

* Milligrams of glutathione per 100 ml of red blood cells.
† Milligrams of ascorbic acid per 100 mg of tissue.
‡ Stimulated daily for 1-minute interval, 11 days.
§ Stimulated daily for 5-minute interval, 15 days.

In addition, the blood glutathione data were evaluated and rated on the basis of the amount of locomotor activity exhibited immediately after discontinuance of the auditory stimulus. Preliminary findings suggest that the animals exhibiting the greatest locomotor response upon cessation of auditory stimulation had the lowest glutathione levels. This response relationship was not observed in the adrenal ascorbic acid or total adrenal cholesterol determinations.

Auditorily stressed animals of both groups had higher adrenal weights (*7*) and ascorbic acid values than controls (Table 1). Rats stimulated for 5 minutes had higher adrenal ascorbic acid values (8.8 percent rise) than rats stimulated for 1 minute (3.3 percent rise). This finding is in accord with reactions associated with chronically stressed animals or adaptation to a "continuously applied noxious stimulus," described by Sayers and Sayers (*6*). Adrenal ascorbic acid content would be greater in animals recovering or adapting to a repeated or a prolonged stress.

Total adrenal cholesterol was determined only in the 1-minute test group with suitable controls. The test group exhibited a markedly lower total adrenal cholesterol content (14.9 percent decrease), which is significant statistically at the 5-percent level. This suggests that the adaptation response of adrenal cholesterol lags behind that of ascorbic acid in auditory stress. A recovery differential between ascorbic acid and cholesterol was also noted in the type 4 adrenal response of Sayers and Sayers (*6*).

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Role of Light in the Photoperiodic Responses of Migratory Birds

Abstract. Light-dark cycles with 12-hour and 16-hour photoperiods are known to be effective in 24-hour cycles in inducing gonadal activity and fat deposition in migratory birds. Twenty-four-hour cycles with 16-hour dark periods are not effective. To test the role of the light periods and the dark periods in a given cycle, slate-colored juncos were subjected to light in cycles which combined stimulatory photoperiods (L) and inhibitory dark periods (D) as follows: 12L–16D; 12L–20D; 16L–16D; 16L–22D; 16L–32D. The results indicate that the photoperiod, not the dark period, determined the response.

Two conspicuous changes in physiological state occur in migratory birds each spring—gonadal growth and fat deposition. In the slate-colored junco (*Junco hyemalis*), day length regulates the physiological events which result in the timing of these changes and in two separate phases of the annual cycle—the preparatory phase (previously called the refractory period) and the progressive phase. The preparatory phase occurs in the fall and requires a period of short days, with a daily dark period of 12 hours or more, for its completion. Once the preparatory phase has been completed, the progressive phase begins, in late fall and winter, and results in gonadal growth and fat deposition which reach a maximum in the spring. The rate at which testicular growth occurs and the time when fat deposits first appear are a function of the daily photoperiod. Long days, with 16-hour or 20-hour photoperiods, or continuous light, induce a rapid response. Short days, with 9-hour photoperiods, induce a response but a much slower one; a 12-hour photoperiod induces a moderately rapid response similar to that induced by photoperiods of natural days. The postulate that there are different degrees of response to the photoperiod each day which summate was advanced to explain these results (*1, 2*).

The discovery that interruption of the long night of a short day with a brief period of light results in a rate of response comparable to that of a long day (*3*) has raised the question of the role of the light and dark periods. The experiments reported here are the most recent of an extensive series which was designed to determine the role of light and darkness in both the preparatory and the progressive phases. A brief summary of the results of all of the previous experiments has been presented elsewhere (*1, 2*).

From previous studies it was known that a 12-hour photoperiod, or a daily cycle of 12L–12D, was effective in inducing the progressive phase of the gonadal cycle, but that an 8-hour or 9-hour photoperiod, or daily cycle of 8L–16D, was not (*4*). In the first experiment, therefore, a 12-hour light period, known to be effective, was alternated with a 16-hour dark period, known to be "inhibitory," to give a 28-hour cycle.

The experiment, in which 19 juncos were used, began on 11 Dec. 1956 and ended 27 May 1957. These birds had already completed the preparatory phase under natural day lengths of fall. The gonadal response is given in Tables 1 and 2. As the tables show, 16 out of the 19 individuals responded, and the testicular activity continued for several months. Data are given for each individual, because of the variation in response. This variation is typical of birds exposed to 12-hour photoperiods in a 24-hour cycle. The gonadal response simulated that observed previously in connection with a 12L–12D schedule, not only in variation in rate of response but also in duration. With 16-hour photoperiods,

Table 1. Gonadal response in male juncos to 12L–16D cycles of light and darkness, 11 December to 27 May.

No.	Autopsy date	Vol. of testes* (mm^3)	Wt. of testes* (mg)	Stage†
252	20 Feb.	22.4	14.8	3
253	20 Feb.	1.4	1.3	1
364	20 Feb.	31.9	17.6	3
375	20 Feb.	27.1	9.6	3
264	22 Mar.	47.4	34.2	4
365	22 Mar.	9.7	7.0	3R
367	22 Mar.	142.1	110.8	5
249	15 Apr.	0.7	0.5	1
251	15 Apr.	12.1	7.2	3
267	5 May	146.5	156.6	5
366	27 May	22.3	11.0	3R
376	27 May	226.5	128.6	5
377	27 May	218.5	157.8	5

* For both testes. † Stages of testis indicate state of spermatogenesis. Stage 1 is the minimum and stage 5 is the maximum. Response is positive when stage 3 or higher is reached. The letter R following the stage indicates that regression had begun.

testicular activity ends by March 1. Seventeen of the 19 birds also showed a fat response (*5*). This occurred primarily during the period 10 January–12 February, and here again the timing was similar to that observed previously with a 12L–12D schedule (*1*). Thus, the 12L–16D schedule was found to have the same effect as a 12L–12D schedule and pointed to the duration of the light period as the effective timer of the photoperiodic response.

A weakness in the experiment was the fact that the ratio of the period of light to that of darkness was close to 1, especially if there was a carry-over effect of the photoperiod (*6*). To test further, therefore, the following schedules, with longer dark periods, were selected: 12L–20D; 16L–16D; 16L–22D; and 16L–32D. This experiment was performed in the spring, when fat deposition and gonadal

Table 2. Gonadal response in female juncos to 12L–16D cycles of light and darkness, 11 December to 27 May.

No.	Autopsy date	Wt. of ovary (mg)	Follicles* (mm)	Wt. of oviduct (mg)
268	19 Feb.	3.6		3.0
266	22 Mar.		0.6	5.0
250	15 Apr.	27.6	1.0	6.0
254	27 May	36.2	2.0	11.2
263	27 May	20.4	1.0	10.0
265	27 May	21.0	0.9	5.4

* Differentiated large follicles in the ovary indicate activity; hence, the diameter of the largest follicle is given when differentiated follicles are present.