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EFFECTS OF RESERPINE ON THE WHITE-CROWNED SPARROW *(ZONOTRICHIA LEUCOPHRYS GAMBELII)*

BY

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A number of studies have shown an inhibiting effect of reserpine on sexual function and development in birds. Khazan, Sulman & Winnik (1960) found severe atrophy and impaired spermatogenesis in the testes of pigeons receiving reserpine (0.2 mg/kg daily). Hagen & Wallace (1961) found that after 10 weeks of treatment the weight of the testes of young chickens receiving reserpine (injections of 1, 2, or 4 mg/kg per week) was less than 10% of that of the controls. They found that two large doses of reserpine, one month apart, caused atrophy of the testes of adult roosters. Assenmacher, Tixier-Vidal & Baylé (1961) demonstrated reserpine inhibition of the photo-sexual response of the male duck. At 0.125 and 0.250 mg/kg daily injections of reserpine the response of the testes to continuous light was very limited.

Further effects of reserpine in birds are, among others: reduced food consumption (Assenmacher *et al.*, 1961), decreased growth and body weight (Hagen & Wallace, 1961, " tranquillization " (Sturkie, Durfee & Sheahan, 1958) and reduced locomotor activity (Hagen & Wallace, 1961 ; Wahlström, 1964).

The Gambel's White-crowned Sparrow (*Zonotrichia leucophrys gambelii*) is one of the most widely studied North American passerines. Gonadal development can be induced experimentally at appropriate times by increasing the daily photoperiod (Farner & Wilson, 1957). There is concurrent induction of a number of physiological and behavioral changes associated with the migratory habit of this subspecies (Farner, 1960). These changes, including hyperphagia, increased fat deposition and appearance of night locomotor activity (migratory restlessness or *Zugunruhe*) appear to be quite the opposite of those usually produced in birds by reserpine. We investigated the possible inhibitory effect of reserpine on testicular development, on migratory restlessness (*Zugunruhe*) and other components of the complex of changes associated with migration in Gambel's White-crowned Sparrows.

METHODS

The birds used were captured near San Jose, California, from wild, wintering flocks with Japanese mist nets. Sex was determined by laparotomy. Only adult males were used. After acclimatization in flight cages, each bird was transferred to a separate cage (41×22×26 cm) for the duration of the experiment. Each cage had a central activity sensitive perch. A continuous count of perch registrations was accumulated and printed out hourly on remotely located Elmeg impulse counters. These counters allowed recording of locomotor activity greater than 5,000 perch registrations/hr. The hourly data so collected over a series of days or nights distributes normally only when the hourly

perch registrations are converted to their corresponding logarithms. These activity records represent an index of the bird's actual activity and are so highly individualistic that quantitative comparisons between birds are hazardous. Therefore each bird's activity was related to his own median or other base before statistical tests were applied. The individual cages were placed in 122×122×92 cm chambers, 4 cages to a chamber. Mean temperature in the test chambers varied from 18° C with lights on to 16° C with lights out, providing some diurnal variation. The thermostatically controlled heat pump caused a 2° C fluctuation two to three times each hour. During the day, incandescent lights provided 45 ft. candles at the cage level, at night a dim light of 0.04 ft. candles encouraged night locomotor activity. The birds were isolated visually from the room environment except during injections and servicing.

The birds were injected in their pectoral muscles. Injections were given and food cups were weighed and filled during the second light hour of each day; otherwise the birds were not disturbed. In the main experiment, injections were begun 4 days before the photoperiod was increased to 16 hr (from 0800–1600 to 0800–0100). Injections continued during the 30 days on 16 hr; group one received 0.2 mg/kg every other day, and group two received 1 mg/kg reserpine each week. Controls received only the reserpine vehicle without the reserpine. Volume of injection was 0.05 ml. in all cases. For comparison there were also some uninjected controls. Each of the two groups consisted of two experimentals and two controls. In another experiment, also involving two experimentals and two controls, we tested for a difference in response between birds on 16-hr photoperiods and birds on 8-hr photoperiods. During all experiments food consumption was measured daily and body weight weekly. Food (ground Walter Kendalls Burger Bits Dogfood) and water were available *ad libitum*. At the end of 30 days on 16-hr daily photoperiods, the birds of groups one and two were sacrificed and their testes were weighed, measured and examined histologically.

RESULTS

On necropsy the testes of the birds receiving reserpine (0.2 mg/kg every other day or 1 mg/kg weekly) were slightly but significantly smaller ($P<0.01$, Fisher's t, one-tailed test, $t=3.44$, $df=6$) than those of the controls receiving only the vehicle. (Tests: 149, 171, 178, 209 mg—controls: 193, 217, 233, 279 mg, combined weights of both testes of these 8 birds.) The means of these values show that the experimentals had testes weighing about 77% of those of the controls. However, histologically, there was no apparent difference between the experimentals and controls, and complete spermatogenesis was evident in all birds.

In spite of a transitory suppression of appetite by reserpine in these White-crowned Sparrows, they developed hyperphagia, deposited stores of fat and thus gained in body weight. The birds receiving reserpine, 1 mg/kg weekly, ate no food on the injection days but ate more food on the following days, such an effective compensation that their overall food consumptions and body weight increases were not significantly different from those of the controls. The birds receiving reserpine, 0.2 mg/kg on every other day, ate a small amount on the injection days. They too compensated for this loss; they over-ate on the alternate days particularly during that period when the controls' hyperphagia had begun to drop off (Fig. 1). In spite of this they were not able to compensate completely and they reached peak food consumption slightly later than the controls. They were still gaining weight at the end of the experiment when the controls had begun to lose weight. Their continued high food consumption on the alternate days after the controls' food consumption had dropped shows the remarkable adaptive ability of the photoperiodic hyperphagic mechanism to provide adequate energy storage for migratory flights in spite of the great energy demands of long night flights and periodic adversities.

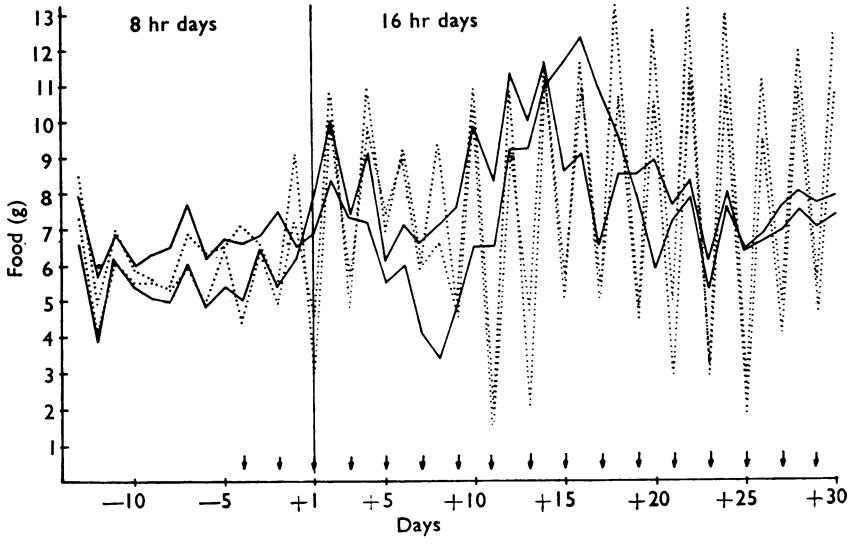

Fig. 1. Daily food consumption of four male White-crowned Sparrows receiving injections every other day of reserpine, 0.2 mg/kg or an equal volume (0.05 ml.) of vehicle. Arrows indicate injection days. ——=vehicle, ...=reserpine.

In spite of the short-term effects of reserpine on food consumption which we have just observed, the reserpine seems not to have significantly inhibited the photoperiodically induced hyperphagia. However, in Fig. 1 the greater oscillation beginning about day 11 suggests that food consumption became much more sensitive to reserpine at this time, also the time when the food consumption of the control birds was in its steepest upward trend. Further evidence for this effect appeared in another experiment designed to show differences in effect of reserpine on birds on 16-hr photoperiods and birds maintained on non-stimulatory 8-hr photoperiods. Reserpine at 0.1 mg/kg daily produced food intake suppression (10 to 50% of vehicle control food consumption) in birds on 16-hr daily photoperiods but not in birds on 8-hr daily photoperiods. It required twice this dosage, 0.2 mg/kg daily, of reserpine to produce equivalent food intake suppression in birds on 8-hr daily photoperiods. The effect was cumulative in both cases and did not appear until day 7 to day 9 of the daily injection regime.

In these birds, reserpine seems not to have interfered with the efficiency of food conversion to body weight. No illnesses or abnormalities seem to have resulted from this long-continued dosage with reserpine. No moult occurred. The birds appeared healthy and normal at all times except in the hours immediately after 1 mg/kg injections. At this time they sat quietly with their feathers fluffed out as though they were cold and were not reactive to normally disturbing stimuli.

Temporally, there is a correlation between the day of highest food consumption and the beginning of night locomotor activity (Fig. 2). Because of the high environmental temperature some birds had night activity preceding the change to 16-hr photoperiods. Most birds lost this with the change ; in those that did not, first significant night activity was taken to be the first night when their activity was twice as high per hour as their maximum on the 8-hr photoperiod. Since the day of highest food consumption was

delayed in the birds receiving reserpine (0.2 mg/kg every other day), the beginning of night locomotor activity was also delayed. Nevertheless, the expected night locomotor activity did develop in all birds. In both experimentals and controls, total (day plus night) activity decreased during the time of greatest body weight increase and then more than doubled itself as night activity appeared.

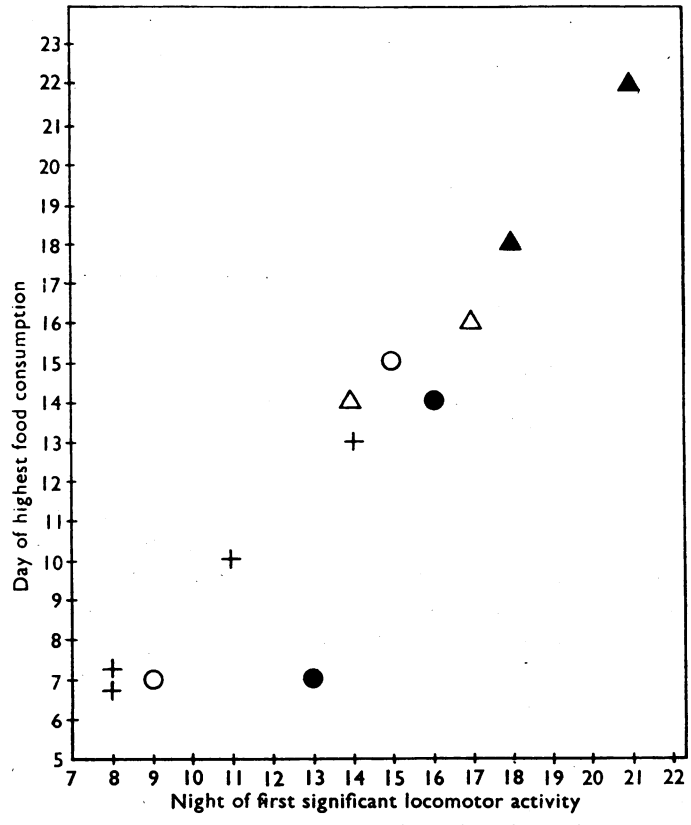

Fig. 2. Correlation of day of highest food consumption with night of first significant locomotor activity in 12 White-crowned Sparrows on 16-hr daily photoperiods. ▲, reserpine 0.2 mg/kg every other day; △, 0.05 ml. of vehicle every other day; ●, reserpine 1 mg/kg weekly; ○, 0.05 ml. of vehicle weekly; +, uninjected controls.

Locomotor activity was, however, affected by reserpine in a complex fashion. The 1 mg/kg injections depressed both day and night locomotor activity in the 24 hr following the injections. Sometimes the birds did not hop on their activity perches for 8 to 10 hr. Usually birds have activity in every hour. By the next day, day activity was back to normal but night activity showed in many cases a rebound effect with extremely high numbers of perch activations. The effects of the 0.2 mg/kg injections were more complex. On the injection days the reserpine birds had hours of quiescence but they also had hours of very high activity. Thus, overall, their day activity appears higher with reserpine. The birds receiving only the vehicle without the reserpine did have clearly depressed activity on the injection days. The vehicle alone did not affect night

activity when given every other day. In the last 10 days on the 16-hr daily photoperiod reserpine in this schedule produced a significant ($P<0.02$) enhancement of the quantity of night activity on the nights following the injections, as compared with the same bird's activity on the alternate nights (Fig. 3).

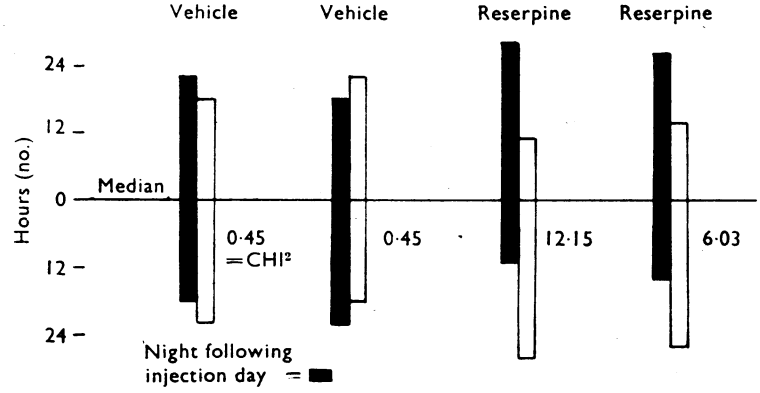

Fig. 3. Locomotor activity of White-crowned Sparrows on nights 21 to 30 on 16-hr daily photoperiods. Distribution of hours of locomotor activity above or below the median of alternate and injection nights combined. Chi squares are for Median Tests (Siegel, 1956); *P* equals 0.01 for chi square greater than or equal to 6.64. Reserpine, 0.2 mg/kg, vehicle, 0.05 ml.

DISCUSSION

Some of the results we have presented here appear to be explainable in the light of present knowledge of the effects of reserpine on birds and its possible modes of action in the nervous system. Testicular suppression in this experiment was much less than that obtained previously in birds (Khazan *et al.*, 1960; Hagen & Wallace, 1961; Assenmacher *et al.*, 1961). This might be accounted for by the somewhat lower dosages used in our experiment. It might also be accounted for by the fact that the birds in our experiment were fed a diet extremely high in essential nutrients, particularly tryptophane. The usual diets fed to birds are apt to be low in tryptophane (for example, Carlson, 1956), the precursor of serotonin (5-hydroxytryptamine) which has been implicated as one of the principal brain substances on which reserpine acts (Pscheidt, 1964). However, there remains a possibility that reserpine action on gonadal developmental mechanism is actually different in these small passerines than in the larger domestic birds previously studied.

The excitatory effect of reserpine which we observed on locomotor activity, while quite unexpected at the time we carried out the experiment, seems possibly explainable in the light of recent studies. The potentiating effect of reserpine on prolactin production in birds is known (Tixier-Vidal & Assenmacher, 1962; Assenmacher & Baylé, 1964). In a recent study it was shown that prolactin injections in *Zonotrichii leucohprys gambelii* can cause fat deposition and can induce night locomotor activity (Meier, Farner & King, 1965). Therefore, it seems likely that the increased night locomotor activity we observed with reserpine might have been caused by reserpine potentiation of release of prolactin and the unique role it plays in these migratory birds.

There are, however, further possibilities which may account for this somewhat perplexing excitatory effect of a tranquillizer. Hunger has been shown to increase day locomotor activity in *Zonotrichia* (Eyster, 1954); since the reserpine caused the birds to eat less, perhaps hunger was a factor in the elevated day activity observed. Moreover, recently, reports of increased locomotor activity due to reserpine in mammals have appeared in the literature (Gluckman, 1964; Magus, Krause & Riedel, 1964), which have not been satisfactorily explained in the light of theories of mechanisms of reserpine sedation. Meier *et al.* (1965) found that adrenocortical hormones potentiate the prolactin effect on night activity. Under long-term treatment with reserpine the adrenocortical system is depleted, at least in mammals (Brodie, Maickel & Westerman, 1961). Likewise, the brain (Pscheidt, 1964) and adrenal (Burack, Weiner & Hagen, 1960) amine stores in birds are depleted by reserpine. We may infer such depletions probably occurred in our reserpine birds. Nevertheless, they managed to have adequate testicular development, hyperphagia, body weight increase, and night activity. The interrelationships between reserpine and brain amines, " tranquillization," excitation, food consumption, pituitary hormones, and gonadal development are undoubtedly quite complex and further explorations at a more fundamental level in both migratory and non-migratory birds should be rewarding. Under our experimental conditions of diet and dosage reserpine altered but certainly did not completely suppress the photoperiodic changes associated with reproduction and migration in *Zonotrichia*.

SUMMARY

1. White-crowned Sparrows were given reserpine injections of 1 mg/kg weekly or 0.2 mg/kg every other day. Injections started 4 days before and continued during a 30-day period of 16-hr daily photoperiods. Controls received comparable injections of vehicle. For comparison there were also some uninjected controls.

2. Reserpine caused a slight suppression of testicular growth but did not affect spermatogenesis.

3. All birds developed hyperphagia, migratory fat and night locomotor activity in spite of food intake suppression by reserpine on the injection days. Peak hyperphagia and the beginning of night locomotor activity were delayed several days by the more frequent injections of reserpine.

4. Although there was some suppression of locomotor activity with reserpine, locomotor activity (particularly night activity) was apparently increased by reserpine in long-continued intermittent doses.

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