

Diel Rhythms of Basal and Stress-Induced Corticosterone in a Wild, Seasonal Vertebrate, Gambel's White-Crowned Sparrow

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ABSTRACT Glucocorticoids have a wide array of actions in vertebrates. Daily fluctuations in basal levels of glucocorticoids are thought to regulate homeostatic mechanisms. In contrast, elevated levels secreted in response to stress stimulate changes in physiology and behavior. These changes are thought to aid an animal in avoiding chronic stress or death. Twenty-four-hour rhythms in basal and stress-induced glucocorticoids have been detected in laboratory mammals, but studies in wild, seasonal vertebrates are rare. Identification of plasticity in hormone secretion in wild vertebrates is critical to understanding the effects of hormones on physiology and behavior, and therefore the success of an animal in its natural environment. In the present study, we characterized diel patterns of basal and stress-induced corticosterone (the avian glucocorticoid) under two photoperiods in Gambel's white-crowned sparrow (*Zonotrichia leucophrys gambelii*). In contrast to previous findings in the white-crowned sparrow, we demonstrated a robust rhythm in basal corticosterone secretion, in which corticosterone reaches peak levels at the end of the inactive period, and has returned to trough levels just after the active period has begun. We also demonstrated a diel rhythm in secretion of corticosterone in response to a stressor, showing the greatest response at the beginning of the active period. Patterns of CORT secretion were similar under both photoperiods. These patterns show interesting similarities and differences to classical mammalian rhythms. *J. Exp. Zool.* 284:334–342, 1999. © 1999 Wiley-Liss, Inc.

In wild vertebrates, environmental perturbations rapidly increase circulating glucocorticoids. We believe the adrenocortical response to stress alters physiology and behavior to help an animal avoid chronic stress (Wingfield, '94; Wingfield et al., '97, '98). Different levels of corticosterone (CORT; the principal glucocorticoid of most terrestrial vertebrates) may induce very different behavioral responses. For example, in captive white-crowned sparrows (*Zonotrichia leucophrys gambelii*), intermediate levels of CORT stimulate locomotor activity, whereas high levels of CORT do not (Breuner et al., '98). At different times during the 24-hour cycle, one behavioral response may be more adaptive than another. Diel variation in sensitivity of the hypothalamic-pituitary-adrenal (HPA) axis may regulate behavioral and physiological responses to stressors over the 24-hour cycle, and consequently affect the survival of a free-living animal in its natural habitat. Although seasonal changes in the adrenocortical response to stress have been well documented in free-living passerines (Wingfield et al., '92, '95), diel changes in these parameters have not been established.

Although the adrenocortical response to stress has been well characterized in several vertebrate classes, the function of daily fluctuations in basal CORT remains poorly understood. In mammals, this rhythm is thought to regulate overall metabolism (Widmaier, '92; Atkinson and Waddell, '95); more specifically, CORT works in concert with insulin to regulate energy acquisition, deposition, and mobilization (Dallman, '93; Santana et al., '95). Some birds (e.g., pigeons and chickens) display CORT rhythms that match the typical mammalian rhythm (Joseph and Meier, '73; Lauber et al., '87; Westerhof et al., '94), but relatively few studies have evaluated CORT rhythms in passerines (the perching birds). Marra et al. ('95) found no diel rhythm in basal CORT secretion in white-crowned sparrows (*Z. l. leucophrys*)—however,

Grant sponsor: NSF; Grant numbers: OPP-9530826 and BIR-9406842.

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Received 22 October 1998; Accepted 15 December 1998.

their sampling methods may have affected circulating CORT levels. Discovery and evaluation of a novel pattern of basal CORT secretion could facilitate comparative studies deciphering the general role of the CORT rhythm.

In passerines, adrenocortical responses to stress and resulting behavioral and physiological changes have been well studied (Buttemer et al., '91; Astheimer et al., '92; Wingfield, '94; Wingfield et al., '95; Romero et al., '97; Breuner et al., '98). Diel changes in basal levels of CORT and/or the sensitivity to stress, however, may affect the interpretation of these experiments. It is possible to adrenalectomize mammals prior to experiments, and thus remove the primary source of variation. In passerines the adrenal gland is partially embedded in the cardinal vein and is difficult to remove. Hence, variation in endogenous CORT over the 24-hour cycle cannot be avoided. A description of diel rhythms of basal CORT secretion would allow researchers to remove that source of variation by planning experiments at the appropriate time of day. An evaluation of the adrenocortical response to stress would determine how the sensitivity of the HPA axis varies over the 24-hour period, and how study results may change depending on the time of day when the experiment is completed.

In this paper, we characterize the diel rhythm of both basal CORT and the adrenocortical response to stress in a wild passerine, Gambel's white-crowned sparrow (*Z. l. gambelii*). Gambel's white-crowned sparrows are long-distance migrants, which breed in Alaska, and winter in the southwestern United States. Thus, during a normal annual cycle, they experience very different photoperiods. Much of the previous work in white-crowned sparrows demonstrates variation in the adrenocortical response to stress depending on the stage in the annual cycle (Astheimer et al., '94; Romero et al., '97). To determine how changing photoperiod may affect diel rhythms in basal and stress induced CORT, we performed these experiments in both photosensitive (short-day) and photostimulated (long-day) sparrows. The results suggest several hypotheses regarding the adaptive value of these rhythms in free-living, seasonal animals.

MATERIALS AND METHODS

Animals

Gambel's white-crowned sparrows were captured in September 1995, during their southern migra-

tion through eastern Washington. Animals were housed in outdoor aviaries until the experiment began in January 1996. Experiments were conducted in environmental chambers: 10 × 6 × 8 ft rooms with air, temperature (20°C), and light control. Birds were housed in individual cages, with four birds per chamber (two males and two females). Bird chow, wild bird seed mix, and water were available ad libitum. Sparrows were kept on short days (8L:16D) for the first 5 weeks of the experiment, then switched to long days (20L:4D) for the remaining 6 weeks. These two photoperiods mimic the natural photoperiods experienced by the sparrows on their wintering and breeding grounds, respectively. Sampling was suspended for 2 weeks after switching to long days to allow birds to acclimate to the new photoperiod.

Sampling

Basal samples were obtained for every third hour of the 24-hr cycle. Stress samples were obtained for every sixth hour of the 24-hr cycle. This schedule was completed on both short and long days (Fig. 1). The sampling order (time of day) was randomized within both short and long days, but all 16 birds were sampled in the same order.

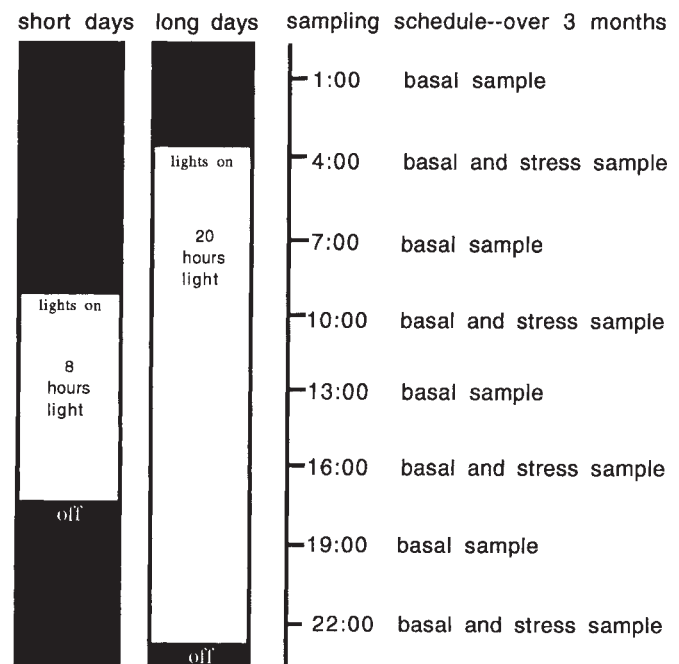


Fig. 1. Experimental design. Basal CORT samples were taken every 3 hr; stress series were taken every 6 hr. Only one time point was sampled per day, and there were only two sampling days per week. Sixteen sparrows were tested on short days, then moved to long days and tested again.

Basal CORT samples

Multiple precautions were taken to ensure that CORT levels sampled represented basal levels. Sparrows were allowed to acclimate to cages for 2 weeks before the experiment began (Wingfield et al., '82). Only one time point was sampled per day, and sparrows were bled only twice weekly, never on consecutive days. On sampling days, sparrows were not disturbed until we entered the chamber to collect blood. We collected samples from all four birds in the chamber within 3 min of entering the chamber, before CORT levels could increase in response to our presence (Wingfield et al., '82). Forty to sixty microliters of blood was collected from the wing vein into heparinized micro-capillary tubes. Plasma was removed and stored at -20°C until analyzed.

Stress-induced CORT samples

To determine the responsivity of the hypothalamic-pituitary-adrenal axis at different times of day, we measured the increase in circulating CORT in response to a standard protocol of handling and restraint. This procedure provides an equivalently stressful stimulus that allows us to compare the adrenocortical response to stress between different individuals under different conditions. The initial blood sample was taken within 3 min of entering the chamber, and thus represented basal CORT levels. We then placed the bird in a small cloth bag for the next 45 min, removing the bird to take blood samples at 15, 30, and 45 min. Blood was collected as previously described.

A 15-watt blue bulb illuminated the chamber for sampling during lights-off. Blue was used (as opposed to red) because blue light does not penetrate the skull as well as red light, and is therefore less likely to activate extra-retinal photoreceptors in the brain (Oishi and Lauber, '73). Only two lux of light reached the bird nearest the light, which is not enough to induce photostimulation (Oishi and Lauber, '73). At the end of the short-day period, the four birds housed closest to the 15-watt bulbs were laparotomized to ensure that the light present during the night bleeds did not induce gonadal recrudescence.

Hormone Assay

We determined plasma levels of CORT by direct radioimmunoassay (RIA; Wingfield et al., '92). CORT was extracted from 15–20 μl plasma with 4 ml redistilled dichloromethane, which was then evaporated under nitrogen. Steroid was reconsti-

tuted in phosphate buffer, and run through the standard RIA in duplicate. Bound steroid was separated from free using dextran-coated charcoal. Percent recovery was determined by adding 2000 cpm tritiated CORT to each sample before extraction. Recoveries ranged between 60–95%, and final CORT concentration was adjusted accordingly for each sample. Interassay variation was less than 14%.

Data analysis

Basal CORT samples

We compared all eight time points taken in one photoperiod with repeated measures ANOVA followed by Tukey's post hoc analysis. We analyzed each photoperiod separately. We also compared the highest basal CORT values from short and long days with a repeated-measures ANOVA. *P*-values were adjusted using a Bonferroni correction for multiple comparisons. Data from the long-day sample taken at 22:00 were not included in the analysis because experimenter error reduced the sample size to eight. Average basal CORT level over the 24-hr cycle were calculated for both short and long days, and the means compared with paired *t*-tests.

Stress-induced CORT samples

We performed two separate analyses on the stress data. (1) We determined the maximal level of CORT experienced by each bird during the 45 min of restraint (which may have occurred at 15, 30, or 45 min, depending on the individual). This provides a measure of the maximum level of CORT experienced by the animal during our testing periods (CORT levels could very well have been higher at some point between each 15 min bleed time). (2) To estimate the total CORT increase over the 45 min of restraint, we subtracted basal values (0 time point) from all four time points (0, 15, 30, and 45), and integrated the data (i.e., calculated the area under the resulting curve). This provides a measure of CORT increase integrated over time, encompassing the rate of both CORT increase and clearance over 45 min of handling and restraint. Both sets of data were analyzed separately using repeated measures ANOVA and Fisher's PLSD post-hoc analysis. Within each analysis, we first analyzed long and short days together to get a measure of photoperiod effects, and then separately to look at time of day effects within a photoperiod. Due to experimenter error, only 8 of the 16 sparrows were included in the stress series taken at 22:00

TABLE 1. Increase in CORT in response to handling and restraint at four times throughout the 24-hr cycle¹

Time of day	Short day (8L:16D)			
	0-3 min	15 min	30 min	45 min
4.00	14.55 ± 2.07	24.41 ± 3.81	24.50 ± 4.33	27.27 ± 4.51
10.00	8.54 ± 1.59	19.94 ± 2.22	33.59 ± 3.76	42.51 ± 4.34
16.00	7.28 ± 0.54	19.94 ± 1.45	26.63 ± 2.24	27.71 ± 2.80
22.00	8.06 ± 0.63	23.57 ± 3.45	26.69 ± 3.40	31.71 ± 4.16
Time of day	Long day (20L:4D)			
	0-3 min	15 min	30 min	45 min
4.00	7.91 ± 0.92	26.65 ± 3.49	39.48 ± 3.72	42.36 ± 4.63
10.00	7.57 ± 0.98	20.05 ± 1.97	31.12 ± 3.75	36.19 ± 4.99
16.00	8.65 ± 0.78	22.69 ± 1.94	29.81 ± 4.02	27.22 ± 2.21
22.00	8.16 ± 1.12	27.39 ± 3.50	24.60 ± 4.18	37.72 ± 4.36

¹Means ± S.E.; CORT levels reported as ng/ml; N = 16, except LD 22:00: N = 8.

on long days. Given the nature of the repeated measures ANOVA, only those 8 birds were included in the analysis of long days. Data from all 16 birds is presented in Table 1, except for long-day 22:00. All statistical tests except for the Tukey's post hoc analysis was completed using StatView 5.0 (SAS Incorporated). Tukey's post hoc analysis (Zar, '84) was completed in Microsoft Excel 5.0.

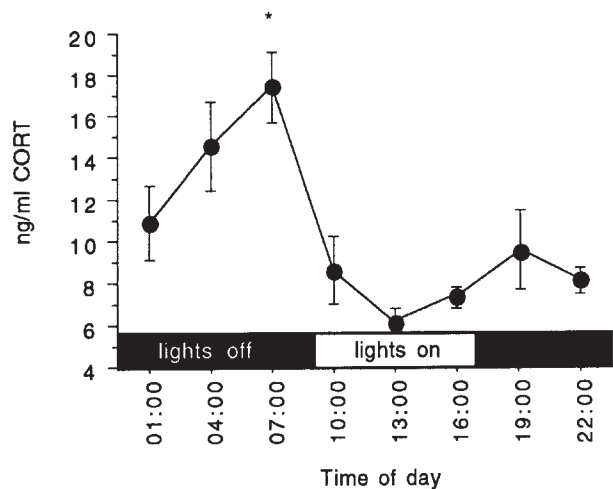
RESULTS

Basal CORT

On both long- and short-day photoperiods, white-crowned sparrows showed 24-hr rhythm of CORT secretion (Fig. 2A). On short days (8L:16D), basal levels remained low throughout the light (active) period, and increased slowly over the dark (inactive) period; the highest level measured was in the last sample taken 2 hr before lights came on. Basal CORT levels obtained during the photophase (the light period) were statistically similar. The maximal basal CORT level during the scotophase (the dark period) was significantly elevated over all basal samples taken during the photophase (ANOVA: $F = 6.733$, $P < 0.0001$ (Bonferroni critical value = 0.025), $q_{(0.05,105,8)} = 4.73$).

On long days (20L:4D) the pattern was similar: all photophase CORT levels were statistically similar, and the only sample taken during the scotophase was significantly elevated over all

A) short day photoperiod



B) long day photoperiod

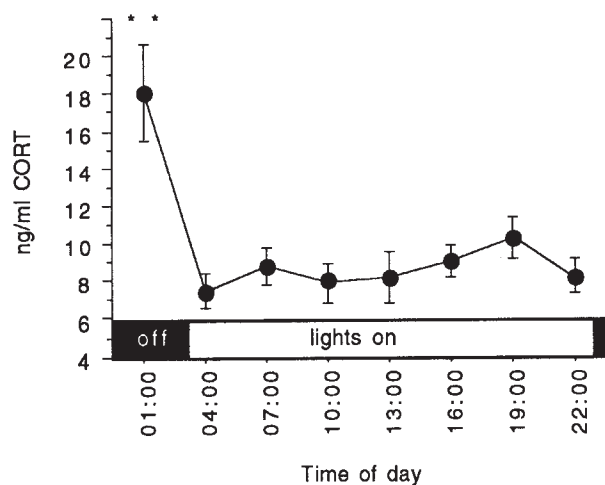


Fig. 2. (A) Basal CORT in short-day sparrows. Means ± S.E., $n = 16$. * $P < 0.0001$, 07:00 is different from all lights on values. (B) Basal CORT in long-day sparrows. Means ± S.E., $n = 16$, except for 22:00, where $n = 8$, and 13:00, where $n = 14$. ** $P < 0.0001$, 01:00 is different from all lights on values.

samples taken during the photophase (Fig. 2B; ANOVA: $F = 7.306$, $P < 0.0001$ (Bonferroni critical value = 0.025), $q_{(0.05,91,8)} = 4.75$). Basal CORT levels peaked at the same level just before lights-on in both long and short days (ANOVA: $F = 0.004$, $P = 0.952$). Levels of basal CORT did not differ between males and females in either photoperiod (data not shown).

The average CORT level over the 24-hr period did not differ between short and long day photoperiods (10.68 ± 0.62 and 9.66 ± 0.527 , respectively; paired t -test: $t = 1.459$, $P = 0.147$).

Stress-induced CORT

A summary of CORT levels measured in response to handling and restraint is presented in Table 1. For analysis, we determined both the maximal level of CORT experienced by each bird during the 45 min of restraint (Fig. 3A), and the integrated values of stress-induced CORT over the 45 min of restraint (Fig. 3B). Effects of photoperiod and time of day were identified with repeated-measures ANOVA and Fishers PLSD post hoc analysis. There is no effect of photoperiod on CORT in either analysis ($P = 0.112$, and $P = 0.082$, respectively), although the integrated CORT values suggest a trend toward higher CORT under long-day photoperiods.

The level of CORT secreted in response to handling and restraint changes depending on the time of day; the HPA axis is most sensitive to handling and restraint just after lights come on under both long- and short-day photoperiods (Fisher's PLSD, $P < 0.05$).

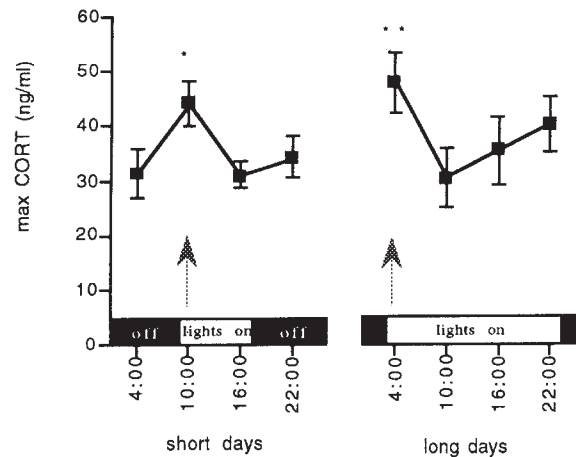
Maximal CORT

On short days, maximal CORT from the series taken just after lights came on (10:00) is significantly elevated over maximal CORT from both the 16:00 series and the 04:00 series (ANOVA: $F = 5.66$, $P = 0.0023$). On long days, maximal CORT levels just after lights came on (04:00) are significantly elevated over maximal CORT levels at 16:00 (ANOVA: $F = 3.31$, $P = 0.04$).

Integrated CORT

On short days, integrated CORT from the series taken just after lights came on (10:00) is significantly elevated over integrated CORT from the 04:00 series (ANOVA: $F = 9.76$, $P < 0.0001$). On long days, integrated CORT levels just after lights came on (04:00) are significantly elevated over integrated CORT levels at 10:00 and 16:00 (ANOVA: $F = 3.88$, $P = 0.024$).

A) Maximal CORT levels



B) Integrated CORT levels

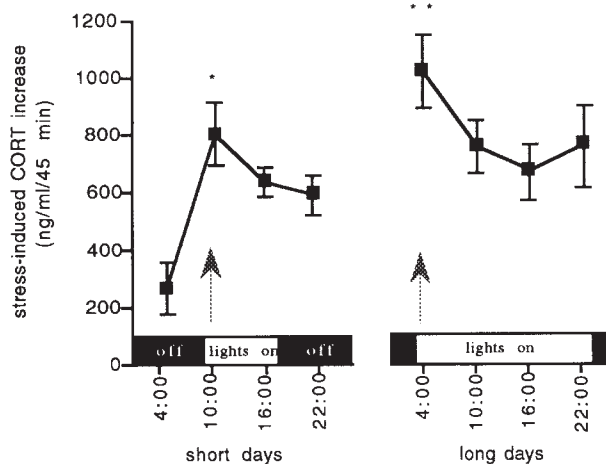


Fig. 3. Stress-induced increase in CORT under short- and long-day photoperiods. Arrows point to CORT levels just after lights on in both long and short days. Means \pm SE, $n = 16$ for short days, $n = 8$ for long days. (A) Maximal level of CORT experienced by each bird during the 45 min of restraint (which may have occurred at 15, 30, or 45 min, depending on the individual). * $P = 0.0023$, different from 16:00 and 4:00 on short days, ** $P = 0.04$, different from 10:00 on long days. (B) An integrated measure of stress-induced CORT. To estimate the total CORT increase over the 45 min of restraint, we subtracted basal values (0 time point) from all four time points (0, 15, 30, and 45), and integrated the data (i.e., calculated the area under the resulting curve). * $P < 0.0001$, different from 4:00 on short days, ** $P = 0.024$, different from 10:00 and 16:00 on long days.

DISCUSSION

Basal CORT

Contrary to previous findings in the white-crowned sparrow (Marra et al., '95), we detected a diel rhythm in basal CORT secretion. Although basal CORT remained consistently low through-

out the active period (photophase), CORT increased over the inactive period (scotophase), reaching the most elevated level in the last sample taken 2 hr before lights came on (the "pre-active peak" in basal CORT). This pattern is similar in both long- and short-day birds. Interestingly, long-day birds reach the same pre-active peak as short-day birds. Given that basal levels of CORT do not begin to increase until the lights are off, the rate of CORT increase must be much more rapid in long-day sparrows: basal CORT levels double in a maximum of 3 hr, whereas in short-day sparrows, it takes about 9 hr. Unlike many mammals, sparrows do not eat during the inactive period (L. Astheimer, personal communication), so the change in rate of CORT increase cannot be attributed to differential food intake while lights are off. These data suggest that it is not the rate of CORT increase that is important, but the pre-active peak. The basal CORT rhythm is thought to regulate energy acquisition, mobilization, and deposition (Dallman et al., '93). The pre-active peak in CORT may be necessary to prepare an appropriate physiological state to meet energetic demands as the active period begins.

The typical mammalian rhythm shows the same peak in basal CORT just before the active period, but in contrast to our data, basal CORT levels do not reach the trough (the lowest level of basal CORT) until the beginning of the inactive period (Engeland et al., '77; Dallman, '93). The rate of decrease in circulating CORT in mammals is much slower than in sparrows. Consequently, mammals have high levels of basal CORT during the most active period of their "day," whereas sparrows do not. A number of hypotheses may explain this difference. It is possible that CORT levels in sparrows decrease more rapidly than in mammals because of the sparrow's high mass-specific metabolic rate. This, however, is probably not the case, given that smaller mammals with high mass-specific metabolic rates (mice, for example) also show the slow decrease of the basal rhythm of CORT once the active period has begun (Nichols and Chevins, '81; Weinert et al., '94). Another possible explanation for the rapid decrease in basal CORT is that passerines have very low levels of circulating CORT as compared to rats and mice, i.e., they have much less CORT to clear from the blood. Mouse and rat basal CORT levels are 5–10 times higher than the levels we measured in sparrows (Nichols and Chevins, '81; Dallman, '93; Weinert et al., '94). The garden warbler, *Sylvia borin*, which also has low circulating levels of CORT,

shows a similar rapid decrease in basal CORT at the beginning of the active period (Schwabl et al., '91). Basal CORT levels may reach the trough of the cycle sooner in passerines solely because of the lower levels present in the blood. Given that CORT secretion and clearance is tied to food intake (Woodward et al., '91; Dallman et al., '94), experiments investigating the changes in CORT secretion and CORT clearance under varied feeding cycles may help to establish the cause of the rapid decline.

In most species examined, basal CORT rhythms are circadian in nature (Aschoff, '79; Krieger, '79; Weinert et al., '94). Whether the diel rhythm in basal CORT in sparrows is regulated by an endogenous pacemaker is not yet known. Experiments evaluating the basal CORT rhythm under constant dim light would be useful. To our knowledge, avian circadian rhythms in basal CORT have been demonstrated only in the quail (Bayle et al., '71).

The diel rhythm in basal CORT secretion has two interesting characteristics that warrant further study. First, under two different photoperiods, CORT reached similar levels in the last sample taken before the active period, indicating the potential importance of this peak in driving behavioral or physiological processes. Second, unlike mammals, basal CORT levels in sparrows are low at the beginning of the active period, suggesting different mechanisms of regulation in sparrows and mammals.

Stress-induced CORT

In the white-crowned sparrow, time of day effects the sensitivity of the HPA axis to stress. This study provides the first evidence of a diel rhythm in the adrenocortical response to stress in an avian species. The HPA axis is most sensitive to stress just after lights-on, in both long and short days. We analyzed both the maximal CORT reached in response to handling and restraint, and the integrated CORT response. Although maximal CORT measures the peak CORT an animal will experience, it only represents one point in time. The integrated measure of CORT represents a broader measure of the CORT response, including rate of CORT increase and clearance. The two analyses gave similar results.

Mammalian studies also demonstrate diurnal variation in the hormonal response to stress, with the greatest sensitivity to stress also occurring when basal CORT levels reach the trough of the cycle (Engeland et al., '77; Kant et al., '86; Bradbury et al., '91). The difference between spar-

rows and mammals is that basal CORT in sparrows reaches the trough of the cycle at the beginning of the active period, whereas in mammals, the trough is not reached until the beginning of the inactive period. One may hypothesize that the HPA axis is most sensitive to stress at this point because basal levels of CORT have been elevated in recent hours, possibly downregulating glucocorticoid receptors and lowering the intensity of negative feedback. However, Bradbury et al. ('91) demonstrated a diel rhythm in the ACTH response to stress (upstream of CORT) in the absence of basal CORT rhythms. This indicates that different neural mechanisms regulate the sensitivity of the HPA axis, independent of negative feedback. It is interesting to note that in laboratory mammals, to study the HPA axis at its most sensitive state, one must complete experiments at the beginning of the inactive period. If one assumed the same pattern for passerines, experiments would occur exactly 12 hr from the most sensitive period.

Diel variation in the adrenocortical response to stress may greatly affect the behavioral and physiological responses to stressors in free-living animals. Low and high physiological levels of CORT have differing effects on locomotor activity in captive sparrows (Breuner et al., '98). These variations in response may be beneficial for the animal in the wild. For example, if a free-living sparrow encounters inclement weather in the morning, when the HPA axis is most sensitive, there may be a great increase in circulating CORT in response to the inclement weather. That animal is then prepared to initiate facultative behavioral changes, such as seeking out more appropriate habitat, at a time of day when there are many hours of daylight left (Wingfield et al., '98). Conversely, if the stressor occurs late in the day, it may be maladaptive to respond with little daylight left, but best to wait out the night and judge conditions in the morning. A less sensitive HPA axis will secrete less CORT in response to the inclement weather, and the behavioral changes may be less extreme.

When studying captive animals, results must be interpreted with caution. Although initial predictive factors, such as photoperiod, are necessary to initiate the breeding cycle in white-crowned sparrows, they are not sufficient to completely induce breeding. Compared to their free-living conspecifics, captive sparrows show a narrower range of hormone levels. This is probably due to the lack of supplementary factors and social cues in the labo-

ratory (Wingfield and Farner, '80; Wingfield, '83). As a result, testosterone and estrogen levels are much lower in captive sparrows than in free-living sparrows (Wingfield and Farner, '78; Wingfield and Moore, '87). Corticosterone also follows this pattern. In free-living, breeding white-crowned sparrows, baseline CORT levels are ~20 ng/ml during the day; in response to capture and handling, similar to handling and restraint in this study, CORT levels increase to ~80 ng/ml (Romero et al., '97). These values are highly elevated over levels detected in captive birds in this study. Although free-living, breeding white-crowned sparrows have much higher CORT levels than captive, photostimulated sparrows, this trend reverses in winter. Baseline and stress-induced CORT levels in free-living, wintering white-crowned sparrows (~3 and 30 ng/ml, respectively; [Romero et al., '97]) are lower than CORT levels detected in captive, photosensitive sparrows. There are also sex differences in baseline and stress-induced CORT in free-living sparrows (Astheimer et al., '94), that are not evident in our laboratory study. This may be related to the lower level of circulating reproductive hormones, or it may be a direct effect of the lack of supplementary factors and social cues. Unfortunately, true basal levels of CORT cannot be detected in the field, as high levels of activity can increase circulating CORT. Therefore, basal levels must be determined in the laboratory, and information regarding free-living birds extrapolated from these results. Given that levels detected in the laboratory are less extreme than levels detected in the field, diel patterns that we detect in this study probably are more pronounced in free-living sparrows.

In summary, captive white-crowned sparrows show a diel rhythm in basal and stress-induced CORT; this rhythm is maintained in both winter and summer photoperiods. The significance of these results extends to two different fields. First, in planning further experiments characterizing behavioral or physiological effects of CORT in white-crowned sparrows, one can experimentally elevate circulating levels of CORT during the daylight hours without confounding fluctuations in endogenous CORT. For experiments investigating the adrenocortical response to stress, it may be most beneficial to complete the experiments early in the day (at the beginning of the active period), when the HPA axis is most sensitive. Second, these data display a diel plasticity in the adrenocortical response to stress that has not yet been demonstrated in a wild animal. Previously,

it was known that wild, seasonal vertebrates can modulate their adrenocortical response to stress depending on season, level of parental care, and body condition (Bradshaw, '75; Macdonald et al., '88; Paolucci et al., '90; Wingfield et al., '92; Wingfield, '94). The results presented here show that the sensitivity of the HPA axis is altered on a diel basis as well, potentially altering the behavioral and physiological responses to stress as the day progresses.

ACKNOWLEDGMENTS

The authors thank Ela Hau, Martin Wikelski, and Tony Tramontin for assistance with the collection of blood samples. Susan Akana and Art Woods provided valuable comments on the manuscript. This work was supported by NSF grants OPP-9530826 to J.C.W., and BIR-9406842 to L.M.R.

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