

Differential mechanisms for regulation of the stress response across latitudinal gradients

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Breuner, C. W., M. Orchinik, T. P. Hahn, S. L. Meddle, I. T. Moore, N. T. Owen-Ashley, T. S. Sperry, and J. C. Wingfield. Differential mechanisms for regulation of the stress response across latitudinal gradients. *Am J Physiol Regul Integr Comp Physiol* 285: R594–R600, 2003. First published June 5, 2003; 10.1152/ajpregu.00748.2002.—We examined plasticity of the stress response among three populations of the white-crowned sparrow (*Zonotrichia leucophrys*). These populations breed at different elevations and latitudes and thus have breeding seasons that differ markedly in length. We hypothesize that in populations where birds raise only one or rarely two broods in a season, the fitness costs of abandoning a nest are substantially larger than in closely related populations that raise up to three broods per season. Thus individuals with short breeding seasons should be less responsive to stressors and therefore less likely to abandon their young. In our study, baseline and handling-induced corticosterone levels were similar among populations, but corticosteroid-binding globulins differed, leading to a direct relationship between stress-induced free corticosteroid levels and length of breeding season. There were also population-specific differences in intracellular low-affinity (glucocorticoid-like) receptors in both liver and brain tissue. Although investigations of population-based differences in glucocorticoid secretion are common, this is the first study to demonstrate population-level differences in binding globulins. These differences could lead to dramatically different physiological and behavioral responses to stress.

white-crowned sparrow; corticosterone; corticosteroid-binding globulin; corticosteroid receptor; reproduction

COPING WITH POTENTIALLY STRESSFUL events in the environment is fundamentally important for all organisms. Much is known now about the general mechanisms underlying the “stress response” in vertebrates; it is a many-layered and finely tuned physiological process that can have profound behavioral and physiological consequences. In vertebrates, stressors typically enhance sympathetic outflow, activate the hypothalamic-pituitary-adrenal (HPA) axis to release corticosteroids, and stimulate the concurrent release of neuropeptides

(20). The composition of the particular neuroendocrine response varies with the type, intensity, and duration of the perceived threat (27), but one constant in a stress response is the acute release of corticosteroids (Cort) from adrenal glands (18).

This series of experiments examined plasticity of the stress response among three populations of white-crowned sparrow (*Zonotrichia leucophrys*). The three populations have different distributions that are likely to affect the evolutionary pressures shaping the stress response. In particular, these populations breed at different elevations and latitudes and thus have breeding seasons that differ markedly in length (see Fig. 1). High-latitude birds (from the subspecies *Z. l. gambelii*) were sampled north of the Brooks Range in the Alaskan Arctic, where the short breeding season allows only one clutch per season (10). High-elevation birds (from the subspecies *Z. l. oriantha*) were sampled in subalpine meadows of the Sierra Nevada, where two broods per season are sometimes possible (10, 17). Low-elevation temperate zone birds (from the subspecies *Z. l. pugetensis*) were sampled in western Washington State, where they often raise three broods per season (10, 28).

We hypothesized that the length of breeding season has a strong evolutionary influence on how birds respond to stress, particularly in short-lived taxa such as white-crowned sparrows where reproductive opportunities in subsequent years are relatively unlikely. Specifically, in birds with time to raise only one or rarely two broods in a season, the fitness costs of abandoning a nest may be substantially larger than in birds that can raise up to three broods per season. There is evidence that both environmental and physiological stressors can induce nest abandonment (11, 26, 31) and that these effects may be mediated through increased Cort (Refs. 21 and 31 and O. Love, unpublished data). Thus individuals with short breeding seasons should be less responsive to stressors and therefore less likely to abandon their young.

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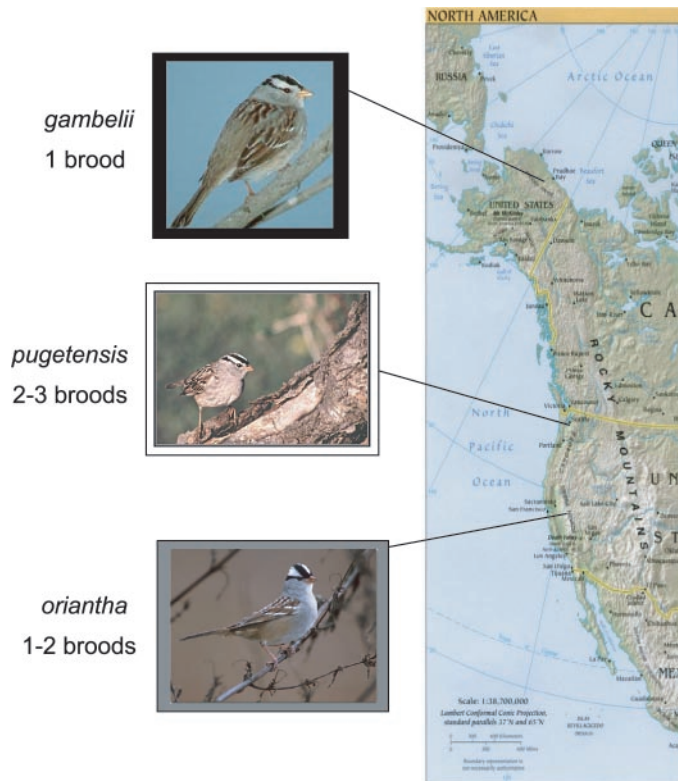


Fig. 1. Location of each population studied, with average no. of clutches raised per season for that subspecies.

Surprisingly, the adrenocortical response to stress (secretion of glucocorticoids from the adrenal gland) does not differ in the two subspecies measured during nesting (*oriantha* and *pugetensis*; Refs. 4 and 30). However, Cort secretion is only one component of a complex pathway from stressor to behavioral and physiological responses. Although it appears that *oriantha* and *pugetensis* do not show the predicted differences in Cort secretion, there could be regulation further downstream in the system, with changes occurring in levels of corticosteroid-binding globulin (affecting free hormone levels available to cells; Ref. 16) as well as tissue-specific changes in the three types of corticosteroid receptors (2 intracellular and 1 membrane bound).

Within this study, we investigated several components of the stress response to test the hypothesis that populations with shorter breeding seasons will be less sensitive to stressors. Toward this end, we caught birds on their territories during the nesting phase of the breeding season, measuring baseline and handling-induced Cort secretion, plasma corticosteroid-binding globulin capacity, and intracellular corticosteroid receptor capacity in liver and brain tissue. Samples were collected from the *oriantha* and *gambelii* during their first clutch and from *pugetensis* during their second to third clutch. Our predictions, based on simple extrapolation from the hypotheses, are 1) although baseline and stress-induced Cort levels may be similar among populations during nesting, corticosteroid-binding globulin levels will be higher in *gambelii* (the popula-

tion with the shortest breeding season), decreasing the amount of stress-induced free Cort; 2) intracellular glucocorticoid-like receptors (the low-affinity receptor that responds to stress-induced levels of Cort) will be present in lower levels in the brain of the *gambelii* population during nesting, potentially resulting in a reduced behavioral sensitivity to stress; and, finally, 3) levels of both intracellular receptor types in the liver will be similar in each population, because metabolic need for glucose in response to stress should not vary geographically.

MATERIALS AND METHODS

Animals

Males at each location were caught between 7:00 and 11:00 AM with the use of mist nets and conspecific playback on their territories during the nesting phase of the breeding season. A baseline blood sample was obtained within 3 min of capture (alar vein puncture with a 26-gauge needle; ~80 μ l of blood collected into heparinized microcapillary tubes). Males were held in a cloth bag until another blood sample was taken at 30 min. Blood samples were kept on ice until plasma was separated by centrifugation and stored at -20°C . For liver and brain tissue collection, males were brought into captivity and immediately given a mitotane injection (0.71 mg/g body wt) to reduce endogenous glucocorticoid production (5). Captive photoperiods were matched to current day length. Approximately 36 h after injection, 3- and 30-min blood samples were taken to assess the efficacy of the mitotane treatment; animals were then anesthetized with Nembutal (0.09 mg/kg) and perfused transcardially with heparinized saline. Brain and liver tissue was removed, snap frozen on dry ice, and stored at -75°C . All procedures complied with university and federal regulations.

Z. l. pugetensis. Eight male *pugetensis* were captured at Charles Lathrop Pack Forest Station (Puget Sound region, south of Seattle, WA; 47° north, 275 m elevation) on July 7 and 8, 2000. After capture, birds were held in environmental chambers at the Univ. of Washington, given mitotane injections, and perfused 36 h later.

Z. l. oriantha. All males were captured at Sonora Pass in the Sierra Nevada, CA (38° north, 2,940 m elevation). Blood samples were collected from eight free-living males captured June 2 and 3, 2000. Eight males were brought into captivity May 25 and 26, 2001 (at the field station near Lee Vining, CA), given mitotane injections, and processed for tissue collection 36 h later.

Z. l. gambelii. All males were captured at the Toolik Field Station, north of the Brooks Range in Arkansas (68° north, 720 m elevation). Eight males were brought to the field station June 8 and 16, 2000, for mitotane injections and tissue collection. Blood samples were collected from eight free-living males June 17–24, 2001.

Corticosterone Assay

Plasma Cort levels were determined following the methods of Wingfield et al. (34). Briefly, samples were allowed to equilibrate overnight with 2,000 counts/min (cpm) of corticosterone for determination of individual recoveries. Each sample was extracted with 4.0 ml of dichloromethane, dried under nitrogen, and resuspended in phosphate-buffered saline with 1% gelatin. Samples were assayed in duplicate, and assay values were corrected for plasma volume and individual recoveries after extraction (recoveries after extraction,

71–100%; standard curve range, 2,000–7 pg; accuracy, 83%; detectability, 7.8 pg/tube). Intra-assay coefficients of variation were 4 and 15% (*oriantha* and *pugetensis* samples were run in one assay, and *gambelii* samples were run in a second assay); interassay coefficient of variation was 15%.

Corticosteroid Receptor Assays

Corticoid receptor assays were performed as per Breuner and Orchinik (8). Temperature, rinse volume, and tissue concentration were optimized for each receptor assay to maximize specific binding, and the time to reach equilibrium was empirically determined for each receptor. All assays contained 50 μ l [3 H]Cort, 50 μ l buffer or unlabeled Cort, and 50 μ l tissue or plasma preparation. Nonspecific binding was determined by use of 1 μ M unlabeled Cort. All samples were run in triplicate. Bound and free radioligands were separated with the use of rapid vacuum filtration over glass fiber filters (Brandel Harvester). After filtration, radioactivity bound to filters was measured by standard liquid scintillation spectroscopy.

Corticosteroid-Binding Globulin

Plasma collected to determine baseline Cort levels was also used to measure corticosteroid-binding globulin (CBG) affinity and capacity. Plasma was stripped of endogenous steroid in a 20-min room-temperature incubation with 2 vol dextran-coated charcoal solution (0.1% dextran, 1% Norit A charcoal in 50 mM Tris). Plasma was maintained at $<4^{\circ}\text{C}$ at all times outside of this stripping process. Plasma samples were assayed at a final dilution of 1:900; assays were performed at 4°C in 50 mM Tris buffer and terminated after 2 h. Glass fiber filters were soaked in 25 mM Tris with 0.3% polyethylenimine for 1 h before filtering. Filters were rapidly rinsed with 9 ml ice-cold 25 mM Tris (3 rinses of 3 ml each). Point sample analysis was run on individual plasma samples, whereas saturation analyses were run on pooled samples. For saturation analysis, pooled plasma from each subspecies was incubated with 0.25–12 nM [3 H]Cort in the presence or absence of unlabeled Cort. CBG capacity in individual birds was estimated by use of either 14.4 nM (2001 *pugetensis* and *oriantha*) or 12.6 nM (2002 *gambelii*) [3 H]Cort. On the basis of affinity estimates derived from equilibrium saturation analysis, this ligand concentration should occupy ~74, 80, and 78% of total binding sites in *pugetensis*, *oriantha*, and *gambelii*, respectively. To account for interassay variation, the *pugetensis* samples were run in both assays (2001 and 2002) and corrected to 100% capacity within each assay, and *gambelii* capacity was corrected for the variation in *pugetensis* samples between assays.

Cytosolic Receptors

Saline-perfused brains were hemisectioned with a midsagittal cut, and one-half of the brain was homogenized in a TEGMD buffer (10 mM Tris, 1 mM EDTA, 10% glycerol, 20 mM molybdic acid, and 5 mM dithiothreitol) and centrifuged at 104,000 g for 1 h at 4°C to produce cytosol with a protein concentration of 4–8 mg/ml (determined using Bradford reagent and a standard curve of BSA). Assays were performed at room temperature in TEGM buffer and terminated after 4 h. Glass fiber filters were soaked in TEM buffer with 0.3% polyethylenimine for 1 h before filtering. Tissue on filters was rapidly rinsed with 9 ml ice-cold TEM buffer (3 rinses of 3 ml each). Competition and saturation experiments were performed with the use of tissue pooled from every individual in the population. For single-point assays, each one-half brain

was processed separately. For saturation binding analysis, pooled cytosol was incubated with [3 H]Cort ranging from 0.05 to 12 nM. The high- and low-affinity cytosolic receptors were distinguished by incubation of cytosol with radioligand and 8 nM RU486, a mammalian glucocorticoid receptor antagonist that occupies lower-affinity Cort receptors, or 1 μ M unlabeled Cort to define nonspecific binding. Therefore, at each [3 H]Cort concentration, we had measures of total specific binding to all cytosolic corticosteroid receptors and specific binding to high-affinity receptors (in presence of 8 nM unlabeled RU486). We calculated binding to lower-affinity receptors as the difference between the total specific binding and specific binding to high-affinity sites. Individual estimations of the concentrations of high- and low-affinity receptors were made by using 10 nM [3 H]Cort in the presence of either buffer (labeling both receptors), 8 nM RU486 (blocking the low-affinity receptor), or 1 μ M Cort (blocking both receptors to determine nonspecific binding). On the basis of affinity estimates derived from our equilibrium saturation analysis, mass action predicts that 10 nM [3 H]Cort should occupy $>95\%$ of high-affinity receptors and $\sim 63\%$ of lower-affinity receptors. To avoid interassay variation, receptor number was determined for all individuals in the same assay.

Statistics

Population-dependent differences in total Cort levels were identified by use of a repeated-measures ANOVA (with time as the repeated factor; StatView 5, SAS Institute, Cary, NC). Free Cort levels were log transformed to correct for heteroscedasticity, and population differences were identified with the use of factorial ANOVA on baseline and stress-induced samples separately, followed post hoc by Fisher's protected least-significant difference test (PLSD). Binding parameter estimates from the saturation analysis were obtained by fitting untransformed data to appropriate equations using iterative, least-squares curve-fitting techniques (GraphPad Prism, San Diego, CA). For analysis, CBG and high- and low-affinity receptor capacity data were brought to 100% and compared by use of factorial ANOVAs followed by Fisher's PLSD. A familywise $\alpha = 0.05$ significance level was used for all tests.

Free Cort titers were estimated from total Cort concentrations and CBG binding parameters by use of the equation of Barsano and Baumann (3)

$$H_{\text{free}} = 0.5 \times \left[H_{\text{total}} - B_{\text{max}} - 1/K_a \pm \sqrt{(B_{\text{max}} - H_{\text{total}} + 1/K_a)^2 - 4(H_{\text{total}}/K_a)} \right]$$

where H_{free} is free hormone, H_{total} is total hormone, B_{max} is total binding capacity of CBG, and $K_a = 1/\text{dissociation constant}$ (K_a) (all values in nM). Baseline Cort, stress-induced Cort, and CBG capacity were measured in each individual (from blood samples taken at capture), allowing free Cort estimations for each individual, and mean \pm SE was calculated for each population.

RESULTS

Total Corticosterone

Thirty minutes of capture and handling significantly elevated total Cort in all three populations (ANOVA: $F_{2,21} = 398.9$, $P < 0.0001$). However, there were no significant differences among populations in either baseline or stress-induced total Cort levels (Fig. 2A; ANOVA: $F_{2,21} = 0.639$, $P = 0.54$).

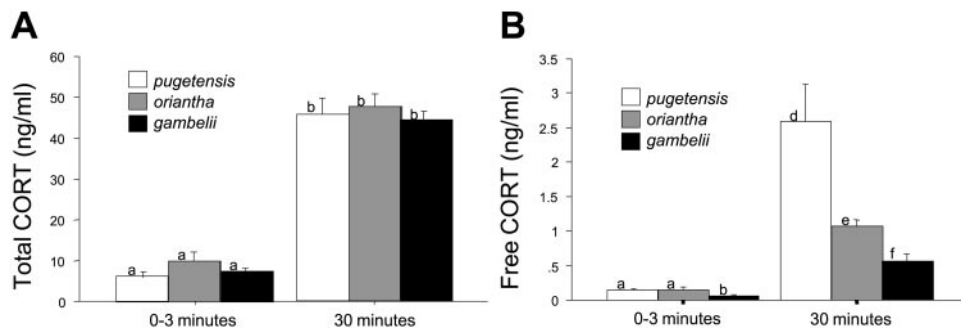


Fig. 2. A: total corticosteroids (Cort) measured within 3 min of capture and after 30 min of capture and handling stress (means \pm SE; $n = 8$ for each subspecies). There is no subspecies difference in either baseline or stress-induced Cort. B: calculated free Cort levels at capture and after 30 min of handling (means \pm SE; $n = 8$). Stress-induced free Cort levels are significantly different in each population. Consecutive letters denote significant differences.

CBG

In white-crowned sparrow plasma, we found a single high-affinity (~ 3 nM) binding site for corticosterone (data not shown), consistent with Lynn et al. (13). CBG affinity for corticosterone and total binding capacity differed among the populations (Fig. 3). CBG from the *gambelii* population had higher capacity than CBG from either the *pugetensis* or the *oriantha* populations (ANOVA: $F_{2,21} = 6.94$, $P < 0.005$), whereas CBG from the *pugetensis* population had lower affinity for corticosterone than CBG from the *oriantha* or *gambelii* populations (ANOVA: $F_{2,21} = 4.99$, $P < 0.02$).

Free Corticosterone

Stress-induced free Cort estimates were lowest in the *gambelii* population, twofold higher in the *oriantha* population, and fivefold higher in the *pugetensis* population (Fig. 2B; ANOVA: $F_{2,21} = 17.32$, $P < 0.0001$). Baseline free Cort estimates were lower in the *gambelii* population than in the *pugetensis* or *oriantha* populations (ANOVA: $F_{2,21} = 4.24$, $P < 0.03$).

Intracellular Receptors

Thirty-six hours of mitotane treatment effectively blocked the adrenocortical response to stress in both *pugetensis* (0- to 3-min sample, 5.09 ± 1.16 ng/ml;

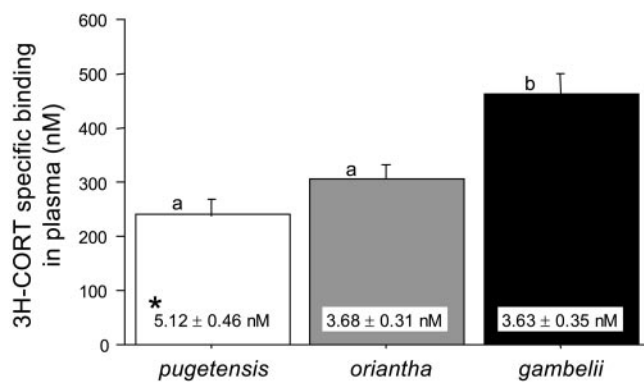


Fig. 3. Corticosteroid-binding globulin (CBG) capacity (bars) and affinity (inset) for *pugetensis*, *oriantha*, and *gambelii* (means \pm SE). CBG capacity is significantly higher in *gambelii* than in *pugetensis* or *oriantha* ($n = 8$; data obtained from point sample analysis; consecutive letters denote significant differences). The K_d (dissociation constant) of Cort for CBG is significantly lower in *pugetensis* (*) than in *oriantha* or *gambelii* (data obtained from saturation analysis of pooled samples).

30-min sample, 6.46 ± 1.80 ng/ml) and *oriantha* (0- to 3-min sample, 12.58 ± 4.19 ng/ml; 30-min sample, 13.51 ± 3.40 ng/ml). In *gambelii*, mitotane treatment was less effective (0- to 3-min sample, 4.50 ± 1.26 ng/ml; 30-min sample, 17.43 ± 3.28 ng/ml). However, estimates of stress-induced free Cort concentrations in mitotane-treated *gambelii* are low (~ 0.76 nM). Mass action predicts that this level of free Cort would occupy only 10% of receptors. As a consequence, our estimate of the total number of low-affinity receptors measured is probably only $\sim 10\%$ low.

In both brain and liver cytosol, there are two specific binding sites for corticosterone: one high-affinity mineralocorticoid receptor (MR)-like site and one lower-affinity glucocorticoid receptor (GR)-like site (Fig. 4). Receptor capacities differed significantly among populations (see Table 1). In liver cytosol, there were no differences in high-affinity receptor capacity, but low-affinity receptor capacity was highest in *oriantha*, 30% lower in *pugetensis*, and 52% lower in *gambelii*. Cytosolic low-affinity receptors from brain tissue showed similar population differences in capacity. In brain cytosol, receptor affinity ranged from 0.08 ± 0.05 to

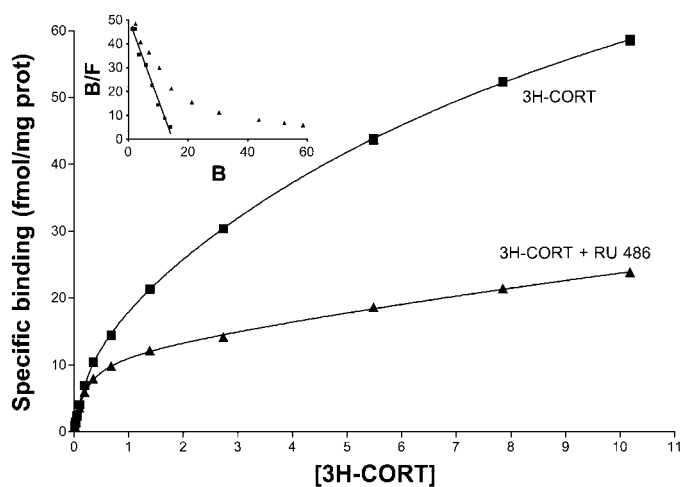


Fig. 4. Intracellular corticosteroid receptors: equilibrium saturation binding of [3 H]Cort to *gambelii* liver tissue. Data are specific binding (means \pm SE at each concentration) of [3 H]Cort in the presence (\blacktriangle) or absence (\blacksquare) of 8 nM RU486. Without RU486, data are best fit by a 2-site model with K_d values of 0.28 ± 0.04 and 11.96 ± 1.43 nM. With RU486, the data are best fit by a 1-site model with $K_d = 0.21 \pm 0.04$ nM. Inset: Scatchard-Rosenthal replot of the data. Analysis of *pugetensis* and *oriantha* tissue gave similar results.

Table 1. Intracellular receptor capacity in each white-crowned sparrow population

	Liver		Brain	
	High affinity	Low affinity	High affinity	Low affinity
<i>pugetensis</i>	14.5 ± 1.3	14.1 ± 2.2	60.8 ± 8.9	81.1 ± 14.1
<i>oriantha</i>	15.2 ± 1.3	20.0 ± 2.0*	54.7 ± 5.4	115.9 ± 4.9*
<i>gambelii</i>	14.0 ± 1.7	9.7 ± 0.7	31.3 ± 4.0*	50.3 ± 12.1
ANOVA	$F = 0.2; P > 0.8$	$F = 8.8; P < 0.002$	$F = 5.9; P < 0.01$	$F = 8.8; P < 0.002$

Values are means ± SE (in nM). *Population is significantly different from other populations within receptor type.

0.32 ± 0.22 nM (high-affinity receptor) and from 5.5 ± 1.4 to 14.2 ± 23.8 nM (low-affinity receptor). In liver cytosol, receptor affinity ranged from 0.21 ± 0.04 to 0.35 ± 0.10 nM (high affinity) and from 12.0 ± 1.4 to 23.2 ± 13.6 nM (low affinity). Within tissue type, however, there were no population-dependent differences in receptor affinities.

DISCUSSION

Glucocorticoid action in vertebrates is highly plastic, allowing for specificity in the response depending on life history, reproductive stage, body condition, or time of day. However, the mechanisms and functional consequences of this plasticity are still relatively unknown. We investigated Cort release, CBG levels, and corticosteroid receptors in three populations of white-crowned sparrows breeding under different temporal constraints. Our data demonstrate that similar to previous studies (4, 31), baseline and stress-induced total Cort levels during nesting were remarkably similar in white-crowned sparrows from three different populations. However, CBG capacity and affinity differed; capacity was highest in *gambelii* and lower in *oriantha* and *pugetensis*. This difference in capacity, combined with lower affinity in *pugetensis*, led to significant differences in estimated free Cort levels. *Pugetensis*, the subspecies with the longest breeding season, had the highest level of free Cort in response to the standardized stressor of capture and handling, suggesting that *pugetensis* would be the most behaviorally and physiologically responsive to environmental perturbation. In contrast, *Gambelii*, with the least amount of free Cort, would be the least sensitive to environmental perturbations and possibly least likely to abandon nests.

In avian cytosol, there are two intracellular corticosteroid receptors. High-affinity receptor capacities in liver tissue were similar among the three populations

but differed in the brain tissue. Within this study, however, we are more interested in concentration of low-affinity glucocorticoid receptors in avian tissue, as this receptor is most likely to play a prominent role in stress-related functions (the low affinity of this receptor indicates it will not be significantly activated until Cort reaches stress-induced levels; Ref. 6). Low-affinity GR-like receptor capacities differed among the three populations in both brain and liver tissue: *oriantha* had the most low-affinity receptors in both liver and brain, whereas *gambelii* had the fewest.

It is hypothesized that in birds breeding in extreme habitats (e.g., *Z. l. gambelii* in the Arctic), the central nervous system is “desensitized” to high corticosterone levels (2, 15, 32) so that harsh environmental conditions do not interrupt breeding. One mechanism to decrease sensitivity to circulating glucocorticoids would be a reduction in neural glucocorticoid receptors; our data indicate that GR-like receptors are lower in *gambelii* than in their temperate conspecifics. With decreased numbers of brain corticosteroid receptors, one would predict reduced behavioral responses in Arctic vs. temperate species, given the same levels of glucocorticoids.

This “Cort insensitivity hypothesis” has been tested on aggressive responses to territorial intrusion; in several temperate species, Cort implants decrease the aggressive response to conspecific playback (29, 33). In Arctic-breeding American tree sparrows (*Spizella arborea*) and Smith’s longspurs (*Calcarius pictus*), Cort implants have no effect on aggressive behavior as predicted by the hypothesis (2, 14). However, Arctic *Z. l. gambelii* do appear to be sensitive to Cort implants (15). To investigate whether there is differential sensitivity to Cort among white-crowned sparrow populations, we need to create similar free Cort levels in each population by Cort implant studies; data presented here indicate that it would be difficult to compare

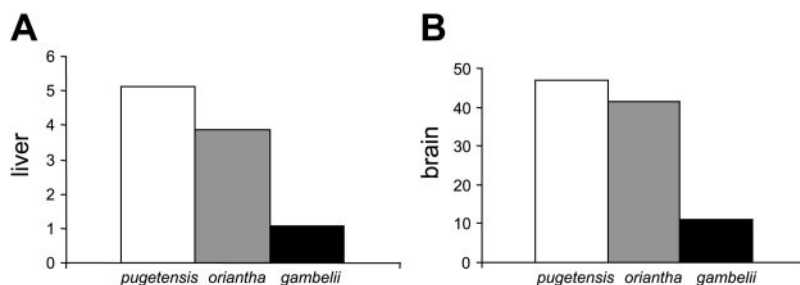


Fig. 5. Model of corticosteroid activity: estimation of low-affinity corticosteroid receptor fractional occupancy after 30 min of capture and handling stress in both liver (A) and brain (B) tissue.

neural sensitivity to increased Cort among populations without accounting for differences in binding globulin parameters. It would also be useful to determine how low-affinity "glucocorticoid" receptor number changes in specific brain regions (this study measured whole brain receptor capacity) and relate this to behavioral effects of exogenous Cort administration.

The data in this study allow us to develop a simple model of corticosteroid activity based on integration of Cort, CBG, and receptor levels in each population. Using mass action, we have estimated the fractional occupancy of the low-affinity receptor at stress-induced free Cort levels in both liver and brain tissue (Fig. 5). This model predicts that the functional output of the stress response varies with the number of broods white-crowned sparrows can typically raise in one season.

What are the origins of differences in stress responses among populations? First, evolutionary pressures are likely to have shaped the stress response to optimize breeding success in particular environments. Shorter breeding seasons should lead to greater fitness costs when nests are abandoned. In this case, selection would favor individuals who are less sensitive to stressors while there are young in the nest and therefore less likely to abandon when environmental conditions deteriorate. The least responsive animals may not survive the breeding effort, leading to stabilizing selection at an intermediate level of sensitivity; however, populations breeding under extreme time constraints should stabilize at a lower sensitivity than those with time to raise several broods per season.

Alternatively, observed differences in stress sensitivity among populations may be related to clutch number at sampling. The stress series were collected from *gambelii* during their first and only clutch and from *oriantha* during their first and (most likely) only clutch. However, stress series from *pugetensis* were collected in early July, when pairs were probably on their second or third clutch of the season. It is possible that the birds become more sensitive to environmental perturbations once they have successfully completed a clutch in one season, resulting in a more responsive HPA axis as they raise their second or third clutch. However, glucocorticoid levels measured in *pugetensis* in this study are similar to those obtained from *pugetensis* in other substages of nesting (Wingfield, unpublished observations). It is possible that CBG capacity changes with clutch order; this has not been tested in any species we are aware of and would present an interesting mechanism for short-term (among-clutch) plasticity in stress responsiveness. Regardless of whether the observed differences reflect inherent differences among groups sampled (phylogenetic or geographic) or a seasonally plastic response that varies with brood number, our results provide novel evidence of within-species variation in several components of stress physiology and suggest numerous productive lines of future investigation.

Perspectives

The most striking result in this study is the difference in CBG among populations. Why increase CBG capacity instead of reducing Cort secretion rate? In general, CBG is thought to regulate bioavailability and metabolic clearance of Cort (16, 19), so CBG-bound Cort are unavailable to enter and activate peripheral tissues. However, higher CBG capacity means a larger pool of Cort is available in the blood if it is needed (9); CBG can be broken down in response to numerous stressors, ranging from social subordination to food deprivation (1, 23, 25) to increased free Cort without a concurrent increase in total Cort. Additionally, binding sites for CBG have been identified in multiple tissues (i.e., Refs. 12, 22, and 24), implicating CBG in site-specific delivery of Cort. Taken together, CBG appears to increase the flexibility and specificity with which animals can respond to various environmental and physiological stressors (7). In the present study, total Cort levels do not vary among populations, but CBG levels do, leading to significant differences in free Cort where none were expected. In house sparrows (*Passer domesticus*), Cort levels vary seasonally but so does CBG capacity, leading to surprisingly static free Cort levels (8). In future studies, consideration must be taken of the role CBG plays in regulating the stress response and how both total and free Cort levels differ among individuals, sexes, populations, and taxa.

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DISCLOSURES

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