Are Receptor Concentrations Correlated Across Tissues Within Individuals? A Case Study Examining Glucocorticoid and Mineralocorticoid Receptor Binding

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Hormone receptors are a necessary (although not sufficient) part of the process through which hormones like corticosterone create physiological responses. However, it is currently unknown to what extent receptor concentrations across different target tissues may be correlated within individual animals. In this study, we examined this question using a large dataset of radioligand binding data for glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs) in 13 different tissues in the house sparrow (*Passer domesticus*) (n = 72). Our data revealed that individual house sparrows tended to exhibit higher or lower receptor binding across all tissues, which could be part of what creates the physiological and behavioral syndromes associated with different hormonal profiles. However, although statistically significant, the correlations between tissues were very weak. Thus, when each tissue was independently regressed on receptor concentrations in the other tissues, multivariate analysis revealed significant relationships only for sc fat (for GR) and whole brain, hippocampus, kidney, omental fat, and sc fat (for MR). We also found significant pairwise correlations only between receptor concentrations in brain and hippocampus, and brain and kidney (both for MR). This research reveals that although there are generalized individual consistencies in GR and MR concentrations, possibly due to such factors as hormonal regulation and genetic effects, the ability of 2 different tissues to respond to the same hormonal signal appears to be affected by additional factors that remain to be identified. **(*Endocrinology* 156: 1354–1361, 2015)**

Hormones play critical roles in transmuting environmental signals into physiological actions, acting across many different tissues to coordinate function and increase fitness (1). The hypothalamus-pituitary-adrenal axis, which secretes glucocorticoid hormones (cortisol and/or corticosterone, depending on the species, hereafter CORT), affects nearly every tissue in the body (2–4) and shows remarkable conservation across different vertebrate lineages (5). At baseline concentrations, CORT helps regulate metabolism, cognition, and immune function (6–8); at higher concentrations, it is a key mediator of the physiological stress response (9). In both mammals and birds, CORT acts primarily by binding to 2 populations of cytosolic receptors, the higher-affinity mineralocorticoid receptor (MR) and lower-affinity glucocorticoid receptor (GR) (3, 10–13). Upon ligand binding, these receptors change conformation, dissociate from their heat shock proteins, form dimers, and enter the cell nucleus (12, 14). The activated receptors then bind to specific response elements in DNA and affect expression at hundreds of gene loci, causing changes in a wide variety of physiological and behavioral traits (15).

Receptor number is highly correlated with the magnitude of the CORT-mediated response on downstream gene expression (16–18). Because of the approximately 10-fold affinity difference between MR and GR, MR is thought to mediate many of the baseline effects of CORT, whereas at higher hormone concentrations, GR binding becomes more important (19, 20). MR's distribution is

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Abbreviations: CORT, corticosterone/cortisol; GR, glucocorticoid receptor; MR, mineralocorticoid receptor.

also more limited than is the ubiquitous GR. For example, in mammalian and avian brain, MR is mostly confined to hippocampus, whereas GR is found throughout the brain (21, 22). In some tissues, MR and/or GR may also bind to other steroid ligands, such as aldosterone and progesterone (23, 24).

It is currently poorly known to what extent responses to circulating hormone are coordinated at the receptor level across the many tissues of individual animals. There are several reasons we might expect to see across-tissue correlations in GR and MR density within an individual. First of all, different tissues within an individual all have the same genetic background, and several studies have found a genetic influence on corticosteroid responsiveness and regulation (25, 26) as well as on the magnitude of expression of particular proteins (27). Secondly, GR and MR concentrations in different target tissues are at least partly regulated by circulating CORT titers. Many studies have shown that as CORT concentrations increase, receptor concentrations decrease, and vice versa (28–33). For example, both the administration of repeated stressors and exogenous CORT to rats caused decreased receptor concentrations in the hippocampus and amygdala (34). This CORT-induced regulation of receptors can occur at transcriptional, posttranscriptional, and posttranslational levels (14, 35).

In contrast, there are also reasons we might not expect to see particularly strong correlations in GR and MR density across different tissues. On closer examination, many of the studies mentioned above paint a more complicated picture than just simple regulation of receptors by ligand. In several rodent studies where exposure to high circulating CORT caused down-regulation of receptors in brain areas such as hippocampus and amygdala, researchers found no effect on receptor concentrations in tissues such as the hypothalamus or pituitary gland (28, 32, 34). GR and MR may also respond to adrenalectomy differently, suggesting that GR may be more subject to auto-regulation than is MR (36). In some cases, GR expression can actually increase in response to ligand binding, which may be important in initiating CORT-induced apoptosis (14). All of this is evidence for a certain amount of independence in the regulation of receptor concentrations in different tissues.

Most studies examining GR and MR in multiple tissue types have only examined different regions of the brain, the pituitary, and perhaps 1 or 2 peripheral tissues. However, as mentioned previously, these receptors are found throughout the body (2, 3), and it is possible that the subset of tissues that have been examined is not representative of receptor regulation. Furthermore, most of these studies were conducted in laboratory rats, and artificial selection during the process of domestication can alter endocrine physiology (26, 37).

In this study, we used a dataset of receptor binding in 72 house sparrows (*Passer domesticus*) to examine individual correlations in GR and MR concentrations across 13 different tissues. Our first objective was to determine whether animals tended to have high or low GR or MR density across all tissue types (Figure 1). We predicted that we would see significant differences between individuals in receptor density and consistency within individuals across tissue types. To explore the between-tissue relationships further, our second objective was to determine whether suites of tissues with related functions (such as the metabolic tissues we examined, liver, fat, muscle, and kidney) would have significantly correlated receptor densities. Consequently, we examined all pairwise correlations among receptor concentrations in different tissues and used multivariate regression to predict receptor density in each tissue type based on an animal's other tissues. We predicted that we would see significant pairwise correlations among tissues of the same function (eg, metabolic, immune, etc) and type (eg, pectoralis and gastrocnemius muscle), but that otherwise, receptor concentrations would not be highly consistent from one tissue type to another.

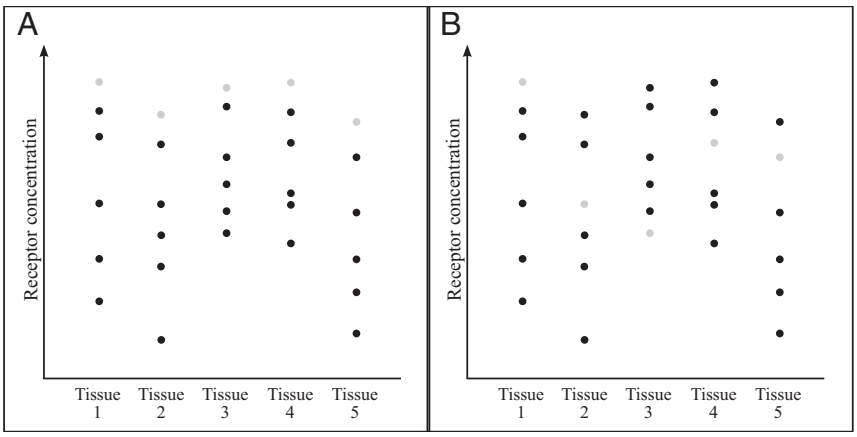

Figure 1. A, In a hypothetical group of 6 individuals (represented as dots), if receptor concentrations were correlated across all tissues within an individual, we would expect that an individual with high receptor density in one tissue would also have high receptor density across 4 other tissues (gray dots). B, If receptor concentrations were completely independent from tissue type to tissue type, we would expect that an individual with high receptor density in 1 tissue would have low or average receptor density in other tissues (gray dots).

Materials and Methods

Study subjects

All procedures involving birds were performed according to Association for

Assessment and Accreditation of Laboratory Animal Care guidelines of humane animal care and approved by the Tufts University Institutional Animal Care and Use Committee. We caught 72 wild house sparrows, 12 each at 6 different times of year: during molt (September 6–19, 2010), early winter (December 12–15, 2010), late winter (February 1–14, 2011), pre-egg-laying (March 31 to April 5, 2011), breeding (May 23–24, 2011), and late breeding (July 12–18, 2011). Equal numbers of males and females were used at each stage. For additional information on study subjects, please see Ref. 38 and Supplemental Methods.

Tissue processing and receptor binding assays

We used radioligand binding assays to quantify GR and MR binding in 13 different house sparrow tissues: whole brain, hippocampus, liver, pectoralis muscle, gastrocnemius muscle, sc fat from the furcula, omental fat from the abdomen, spleen, kidneys, testes or ovary (depending on sex), belly skin, and bib skin. Receptor assays have been fully described and validated for each tissue type (see Supplemental Methods, Supplemental Tables 1–3, and Supplemental Figures 1 and 2 for more details). These data were originally collected to test different hypotheses for seasonal regulation of CORT by examining receptor concentrations (Refs. 39–42 and see also Supplemental Figures 3–14).

Statistical methods

All analyses were run using Stata version 13.1 (StataCorp). We used the "mixed" command with restricted maximum likelihood to estimate a linear mixed model of receptor concentration with fixed effects at the tissue level, random effects at the bird level, and residual variance allowed to differ across tissues (43–46). We included sex and life history stage as fixed effects in the model, because these have both been shown to affect receptor density in house sparrows (see the Supplemental Methods for more details) (39–41). This type of model assumes that individual-specific effects are orthogonal to the other covariates of the model and that errors are normally distributed (43–46), and these assumptions were satisfied by the data.

We analyzed our dataset in 4 different ways. First of all, to determine whether there was a significant individual bird effect on receptor concentrations in all 13 tissues, we carried out a likelihood ratio test (43–45) comparing the log-likelihood of our mixed model with bird-level random effects against that of the nested model without bird-level fixed effects. A significant result here would tell us that receptor concentrations in all tissues were significantly affected by which bird those tissues came from. Secondly, when we found significant individual effects on receptor concentrations, we ran ordinary least-squares regressions of GR and MR concentrations on fixed effects at the bird level (47, 48). This analysis provided simple and easily interpretable r^2 measures of the contribution of bird-level variation to receptor concentrations (ie, the strength of significant individual effects on receptor concentrations). Third, we explored which individual tissues had receptor concentrations that were the most and least correlated with the others using separate multivariate regressions of receptor concentrations in 1 tissue with receptor concentrations in all of the same bird's other tissues and examining the resulting r^2 values and F tests of joint significance (48, 49). This analysis elucidated which individual tissues were the most (and least) correlated with all other tissues in terms of receptor density. Finally, we also calculated pairwise correlations using Spearman's rho for all tissue pairs for both GR and MR concentrations (48, 49). The final 2 analyses were only done using data from the 11 tissues for which we had samples from all 72 birds (sample size was only 18 individuals each for testes and ovary, see the Supplemental Methods for more information). For the latter analysis, we used the Benjamini-Hochberg procedure to control the false discovery rate from multiple comparisons (50).

Results

The likelihood ratio test of the random effects model rejected the null hypothesis of no bird-level variation in GR ($\chi^2_1 = 7.26$, $P = .007$) (Supplemental Table 4) and MR ($\chi^2_1 = 14.43$, $P = .0001$) (Supplemental Table 4) across all tissues. The data thus provide strong evidence for an individual bird effect (ie, differences among birds) in both GR and MR concentrations when all 13 tissues are in the model. However, a regression of the raw receptor data on bird-level fixed-effects indicated that the amount of variation in overall GR or MR density explained by this individual effect was small ($r^2 = 0.062$ for GR, $r^2 = 0.083$

Table 1. Results of Separate Multivariate Regressions and r^2 and F Tests of Joint Significance Examining the Degree to Which Receptor Concentrations in Individual Tissues of House Sparrows (*P. domesticus*; n = 72) Could Be Explained by Receptor Concentrations in All Other Tissues

Dependent Variable	R^2	F Statistic	*P* Value
GR			
Belly skin	0.15	0.90	.54
Bib skin	0.15	0.85	.59
Brain	0.23	1.50	.17
Gastrocnemius	0.13	0.74	.69
Hippocampus	0.18	1.10	.38
Omental fat	0.15	0.86	.57
Kidney	0.19	1.16	.34
Liver	0.27	1.85	.076
Pectoralis	0.19	1.16	.34
Spleen	0.25	1.68	.11
Subcutaneous fat	0.29	2.04	.048[a]
MR			
Belly skin	0.18	1.12	.36
Bib skin	0.18	1.06	.41
Brain	0.34	2.57	.014[a]
Gastrocnemius	0.19	1.20	.31
Hippocampus	0.50	4.96	<.0001[c]
Omental fat	0.29	2.03	.0499[a]
Kidney	0.42	3.59	.0012[b]
Liver	0.29	2.02	.051
Pectoralis	0.28	1.98	.056
Spleen	0.25	1.62	.13
Subcutaneous fat	0.37	2.95	.0055[b]

[a] $P < .05$.

[b] $P < .01$.

[c] $P < .001$.

for MR). Even when we rescaled GR and MR data to remove tissue-level mean and variance differences (51), the amount of variation explained by these individual effects was still relatively low ($r^2 = 0.16$ for GR; $r^2 = 0.20$ for MR). Consequently, we supported our prediction that significant individual differences would be found, but these differences were small.

Using separate multivariate regressions and r^2 and F tests of joint significance for each tissue independently (except for ovary and testes), we found that MR in 5 of the tissues (whole brain, hippocampus, kidney, omental fat, and sc fat) could be significantly predicted by the MR concentrations in the other 10 tissues (Table 1). Liver and pectoralis MR were marginally significant ($P < .06$), and belly skin and bib skin MR receptor concentrations were consistently independent of receptor density in other tissues. For GR, only sc fat receptor density was significantly predicted by receptor concentrations in the other 10 tissues (Table 1). Belly skin, bib skin, gastrocnemius muscle, and omental fat GR receptor concentrations were consistently independent of receptor density in other tissues.

Examining pairwise correlations using Spearman's rho, we found no significant correlations between GR concentrations in pairs of tissues after multiple comparisons corrections (Table 2 and Figure 2). However, we did find significant pairwise correlations between MR concentrations in hippocampus and whole brain and between whole brain and kidney (Table 3 and Figure 3).

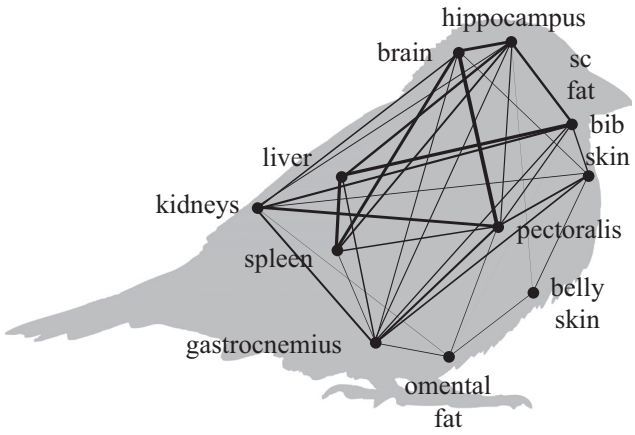

Figure 2. A graph depicting all the positive pairwise correlations between glucocorticoid receptors in pairs of tissues, with the tissues located in their approximate locations, as depicted by dots. The relative thickness of the lines represents the relative strength of the positive correlations, although it is important to note that after multiple comparisons corrections, no pairs of tissues were significantly correlated. Table 2 gives numerical values for all correlations (positive and negative) between glucocorticoid receptors in all pairs of tissues. House sparrow silhouette created by User 4028mdk09 from an original photo by Andreas Plank and made available from Wikimedia Commons under the Creative Commons Attribution-ShareAlike license (CC-BY-SA).

Discussion

A mixed-model analysis of our extensive dataset of radioligand binding in 13 different tissues from 72 individuals revealed that individual house sparrows did tend to exhibit higher or lower GR and MR density across all tissues, although subsequent regression analyses and pairwise comparisons of receptor density in all tissue pairs indicated that this overall effect was very weak. Hormonal mediators, including CORT titers, often show enormous intraspecific variation (52–54). House sparrows caught at the same times and places as the sparrows used in the present study had baseline CORT titers that commonly varied as much as 100-fold among individuals caught during the same life history stages (eg, 0.02 ng/mL compared with 2 ng/mL) and stress-induced CORT titers that commonly varied as much as 6-fold (eg, 12 ng/mL compared with 69 ng/mL) (38). Because GR and MR concentrations

Table 2. Matrix of All the Pairwise Correlations Between Glucocorticoid Receptor Concentrations in Pairs of Tissues From House Sparrows (*P. domesticus*, n = 72)

	Belly Skin	Bib Skin	Brain	Gastroc	Hippo	Oment Fat	Kidney	Liver	Pect	Spleen	Subcutaneous Fat
Belly skin	1										
Bib skin	0.073	1									
Brain	−0.13	0.063	1								
Gastroc	−0.004	0.153	0.096	1							
Hippo	0.014	−0.079	0.27	0.10	1						
Oment fat	0.058	−0.005	−0.068	0.08	−0.028	1					
Kidney	−0.076	0.079	0.14	0.18	0.091	0.026	1				
Liver	−0.072	−0.13	−0.060	0.13	0.23	−0.22	−0.15	1			
Pect	−0.11	0.17	0.36	0.19	0.11	0.062	0.26	−0.070	1		
Spleen	−0.14	0.13	0.28	0.06	0.18	−0.19	−0.041	0.29	0.14	1	
Subcutaneous fat	−0.30	−0.022	−0.093	0.12	0.22	0.002	0.20	0.30	0.12	−0.13	1

Belly skin, bib skin, brain, gastrocnemius (Gastroc), hippocampus (Hippo), omental fat (Oment fat), kidney, liver, pectoralis (Pect), spleen, and sc fat were determined using Spearman's rho. After multiple comparisons corrections, receptor concentrations were not correlated in any tissue pairs.

Table 3. Matrix of All the Pairwise Correlations Between Mineralocorticoid Receptor Concentrations in Pairs of Tissues From House Sparrows (*P. domesticus*, n = 72)

	Belly Skin	Bib Skin	Brain	Gastroc	Hippo	Oment Fat	Kidney	Liver	Pect	Spleen	Subcutaneous Fat
Belly skin	1										
Bib skin	0.16	1									
Brain	−0.16	0.15	1								
Gastroc	0.34	0.22	−0.075	1							
Hippo	0.036	0.19	**0.54**[a]	0.20	1						
Oment fat	0.14	−0.027	0.064	−0.058	−0.021	1					
Kidney	0.060	0.20	**0.45**[a]	0.17	0.38	−0.055	1				
Liver	0.16	0.082	−0.037	−0.11	−0.15	−0.19	0.11	1			
Pect	0.039	0.022	0.19	0.064	0.39	−0.099	0.25	−0.075	1		
Spleen	−0.20	−0.094	0.32	0.010	0.35	0.034	0.19	0.023	0.17	1	
Subcutaneous fat	0.018	−0.036	0.17	0.062	−0.11	0.31	0.11	0.18	−0.14	0.11	1

Belly skin, bib skin, brain, gastrocnemius (Gastroc), hippocampus (Hippo), omental fat (Oment fat), kidney, liver, pectoralis (Pect), spleen, and sc fat were determined using Spearman's rho. Numbers in bold with superscript letter a indicate significance at the $P < .05$ level after multiple-comparisons corrections.

are regulated at least in part by circulating CORT (14, 35), we would expect these large interindividual differences in CORT titers to impact the body's overall receptor concentrations. In fact, having relatively higher or lower CORT titers, and relatively fewer or more receptors throughout the body, may be part of what creates the physiological and behavioral syndromes associated with different hormonal profiles (55–57).

However, individual effects explained only a small percentage of the overall variation in receptor concentrations. Because this overall effect was so weak, when we took away the statistical power provided by 816 samples for each receptor type (GR and MR), and looked for relationships between individual tissue pairs (like brain and liver, where there were only a combined 144 samples to be compared, which magnified both the variation between individual animals and the inherent variability associated with the radioligand binding assays), there were very few significant pairwise correlations. This indicates that knowing the density of GR or MR in only one tissue had little predictive value for other tissue types (Table 1). Thus, although overall there were significant individual effects on GR and MR density, receptor concentrations in any one tissue were not strongly correlated with receptor concentrations in other tissues, suggesting that CORT titers are not the only (or even the main) driver of MR and GR concentrations.

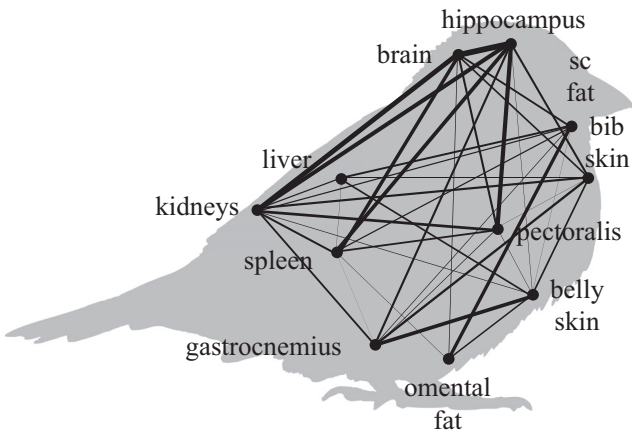

Figure 3. A graph depicting all the positive pairwise correlations between mineralocorticoid receptors in pairs of tissues, with the tissues located in their approximate locations, as depicted by dots. The relative thickness of the lines represents the relative strength of the positive correlations, although it is important to note that after multiple comparisons corrections, the only significant pairwise correlations are the ones between whole brain and hippocampus and brain and kidney. Table 3 gives numerical values for all correlations (positive and negative) between mineralocorticoid receptors in all pairs of tissues. House sparrow silhouette created by User 4028mdk09 from an original photo by Andreas Plank and made available from Wikimedia Commons under the Creative Commons Attribution-ShareAlike license (CC-BY-SA).

Generally, our data suggest more similarity in MR concentrations across different tissue types than in GR concentrations. Only in sc fat was GR density significantly correlated with other tissues, in contrast to whole brain, hippocampus, kidney, omental fat, and sc fat for MR. In our pairwise analysis, no pairs of tissues showed significant correlations in GR receptor density, although hippocampus and whole brain, and whole brain and kidney, showed significant positive correlations in MR receptor density. In mammals and birds, much of the MR in the brain is found in the hippocampus (21, 22), so finding a correlation between MR density in hippocampus from one hemisphere of the brain and in whole brain from the other hemisphere is not a surprise; in fact, the higher the percentage of total brain MR that are in the hippocampus, the stronger this correlation will be (assuming right-left symmetry).

Different tissues with the same function (eg, metabolic tissues), and even regions of the same tissue type (eg, pectoralis vs gastrocnemius muscle), did not show significant

pairwise correlations in GR or MR densities, in contrast to our predictions that they would. These results are consistent, however, with past studies revealing remarkable heterogeneity in the response of different tissues and regions of the same tissue type to changes in CORT (32, 58, 59). For example, in a previous study examining changes in CORT receptor density, adrenalectomized rats increased CORT receptors in all 8 tissues examined, but the magnitude of this increase varied 30-fold among tissues and was unrelated to initial receptor density (60). Overall, our data suggest that although GR and MR densities may be generally higher or lower across all tissues within an individual, this is not a homogeneous effect, and different tissues, and even different regions of the same tissue, can vary greatly in receptor densities.

Aside from receptor concentrations, many other important factors can also influence CORT's actions. These include diurnal variation in CORT release (61, 62), circulating concentrations of plasma binding proteins such as corticosterone binding globulin and albumin (63, 64), local production of CORT (65), the presence of 11β-hydroxysteroid dehydrogenase enzymes that convert CORT to an inactive metabolite or inactive metabolites to CORT (66–69), and tissue-specific variation in nuclear receptor coactivators and corepressors (70–72). This means that although our study contributes to our understanding of the differential effects of CORT across different tissues, it is only a start, and future studies are necessary to clarify additional details of the complex process through which CORT creates physiological responses. Binding capacity can be a useful measure of receptor density, but we did not measure expression changes in any of the many genes regulated by CORT, or any other measure of CORT's effect on different tissues. And although this study has the advantage of reflecting the natural interindividual variation found in a wild animal species, a future study using adrenalectomized laboratory rats given various replacement doses of CORT could lead to a much clearer picture of hormone and receptor coregulation.

In general, data from comparative studies suggest that evolutionary change occurs more often via alterations to the target tissue (changes in receptors, enzymes, etc) than by altering the hormone signal (73–79). These kinds of studies help elucidate the complex nature of hormonal systems. Although hormone systems act across multiple levels (eg, hormone, receptor, gene, etc) to produce a phenotype, the different components of these systems may also be somewhat independently regulated to allow animals to mount flexible and diverse responses to their changing environments. The fact that different components of hormonal systems may be regulated somewhat independently also emphasizes the importance of examining factors other than plasma hormone titers in understanding hormonal actions, whether an investigator is interested in a behavioral output or a physiological one. Overall, this study suggests that the idea that hormone titers and receptor concentrations will be inversely proportional is overly simplistic and reveals that although there are individual effects on receptor concentrations due perhaps to such factors as hormonal regulation and genetic effects, the ability of 2 different tissues to respond to the same CORT signal may be heavily affected by additional factors that remain to be elucidated.

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