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Covariation among glucocorticoid regulatory elements varies seasonally in house sparrows

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ABSTRACT

Glucocorticoids (GCs) help individuals cope with changes throughout life; one such change is the seasonal transition through life-history stages. Previous research shows that many animals exhibit seasonal variation in baseline GCs and GC responses to stressors, but the effects of season on other aspects of GC regulation have been less studied. Moreover, whether elements of GC regulation covary within individuals and whether covariation changes seasonally has been not been investigated. Evolutionarily, strong linkages among GC regulatory elements is predicted to enhance system efficiency and regulation, however may reduce the plasticity necessary to ensure appropriate responses under varying conditions. Here, we measured corticosterone (CORT), the major avian GC, at baseline, after exposure to a restraint stressor, and in response to dexamethasone (to assess negative feedback capacity) in wild house sparrows (*Passer domesticus*) during the breeding and molting seasons. We also measured hippocampal mRNA expression of the two receptors primarily responsible for CORT regulation: the mineralocorticoid and glucocorticoid receptors (MR and GR, respectively). Consistent with previous studies, restraint-induced CORT was lower during molt than breeding, but negative-feedback was not influenced by season. Receptor gene expression was affected by season, however, as during breeding, the ratio of MR to GR expression was significantly lower than during molt. Furthermore, MR expression was negatively correlated with CORT released in response to a stressor, but only during molt. We found that individuals that most strongly up-regulated CORT in response to restraint were also most effective at reducing CORT via negative feedback; although these relationships were independent of season, they were stronger during molt.

1. Introduction

Hormones modulate physiology and behavior in response to the environment [21], and may allow organisms to allocate resources optimally among competing systems (e.g., growth, reproduction, or immunity) [15,17]. During different life-history stages, organisms often regulate certain hormones differently. For instance, many songbirds down-regulate corticosterone (CORT; the main avian glucocorticoid stress hormone) seasonally: both baseline and stress-induced levels are lower during molt than when breeding [25]. Although many organisms down-regulate CORT during molt, it is important to note that not all species do it in the same way: some species regulate binding proteins, whereas others change physiological set points in the adrenal glands, pituitary, or hypothalamus [2,22–24,29]. While molting, it might be adaptive to minimize CORT responses to maximize feather quality [27] and/or because individuals may not physically be able to respond to a stressor when feathers are reduced, such as during molt. During breeding, however, a strong CORT response may increase long-term fitness in the face of a serious stressor (e.g., severe weather or predator attack), even if that means abandonment of a nest.

One way vertebrates regulate their stress response is by the mineralocorticoid and glucocorticoid receptors (MR and GR, respectively). Expression of both receptors can increase or decrease depending on the environment, differ systematically among phenotypes [11], and vary seasonally: MR was higher in short-day golden hamsters (*Mesocricetus auratus*) [14,30], which led to increased receptor binding and more rapid negative feedback of CORT. In house sparrows (*Passer domesticus*), cytosolic CORT receptors were lower during winter than either breeding or molting, but membrane-associated receptors were lowest during breeding [7]. MR primarily regulates basal fluctuations in CORT and plays a significant role in the sensitivity of negative feedback [9], whereas GR predominantly mediates physiological and behavioral changes necessary to restore homeostasis after a stressor [9]. Although GR controls CORT negative feedback in the hypothalamus and pituitary gland, in the hippocampus, both MR and GR work in a coordinated and antagonistic fashion to mediate CORT (i.e., the MR/GR balance hypothesis [9]). MR:GR ratios in the hippocampus are therefore

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predicted to alter the set-points of cellular excitability, neuroendocrine reactivity, and behavioral adaptation [9]; in fact, in rodents, it is this ratio, not the concentration of either receptor alone, that dictates patterns of gene transcription induced by CORT [19].

One little studied, but likely enlightening area of research is covariation among CORT regulation elements. When traits are related functionally, coselection on the regulatory networks that underlie them is expected to produce correlations among those traits [33,34]; such selection should maximize the precision of control over those systems. However, when correlations among traits are strong, the evolution of a specific trait may become constrained and impede the ability to respond to novel selection pressures [17]. Weak correlations may lead to decreased system efficiency, however it might also increase plasticity in certain systems; in changing environments or contexts (such as different life-history stages), enhanced plasticity might facilitate the most appropriate response to each environment.

Although research has addressed individual and seasonal variation in CORT response to stressors, less has evaluated the seasonal variation of CORT negative feed-back or covariation among elements of CORT regulation. Failure to down-regulate CORT after a stressor can lead to persistently elevated levels and allostatic overload [10,26,31,35]. In marine iguanas (*Amblyrhynchus cristatus*), individuals that were most effective at down-regulating CORT after a stress response were also most likely to survive adverse conditions [28]. Here, we characterized seasonal variation in CORT regulatory elements in a wide-spread songbird, the house sparrow, which shows seasonal variation in baseline and stressor-induced levels of CORT and CORT receptor density in the brain [7]. We asked whether CORT differed at baseline, post-restraint, and post negative feedback between breeding and molting seasons. Keeping with other studies, we predicted reduced CORT at baseline and in response to a stressor during molt; additionally, though, we also predicted enhanced negative feedback (i.e., more rapid reduction of CORT) during molt. We also predicted that MR expression in the hippocampus would be higher during molt, thereby increasing the MR to GR ratio and enhancing negative feedback [9]. We were also interested to ascertain whether CORT regulatory elements (i.e., up- and down-regulation of CORT in response to a stressor and negative feedback and CORT receptors) covaried in the same fashion in seasons with distinct life history demands. We predicted that increased CORT release would be associated with reduced MR density in the hippocampus, whereas an increased capacity to down-regulate CORT would be associated with an increased GR density in the hippocampus. Further, we predicted increases in CORT would be correlated with CORT negative feedback to minimize the detrimental effects of chronically elevated CORT and that this covariation would be stronger during molt because of the greater risk CORT imposes on the demanding process of feather growth; as breeding is longer and is comprised of diverse substages, we expected greater flexibility in CORT regulation during this time.

2. Methods

2.1. Dexamethasone (DEX) dose validation

Adult house sparrows were captured using mist nets from the Tampa, FL area in October 2009. Within 3 min of capture, a small blood sample (~25 μl) was taken from the brachial vein using a 26-gauge needle and a heparinized micro-capillary tube. Birds were kept in an opaque, cloth bag for 30 min to elicit a CORT response and bled again (~25 μl). After the second blood sample, birds were injected with 1 μg/g ($n = 7$) dexamethasone (DEX) and returned to the cloth bag for an additional 60 min (most rodent studies inject individuals with between 0.3 and 3 μg/g, with many injecting 1 μg/g; e.g., [20]). After this 60 min period, a final blood sample was taken (~25 μl), and birds were released. All blood was kept on ice (<2 h) and centrifuged (9300*g* at 4 °C) for 10 min; plasma was extracted and frozen at −40 °C until analysis for CORT (described below).

2.2. Seasonal variation

During October 2010 (molting; $n = 11$) and June 2011 (breeding; $n = 19$) adult house sparrows were caught from the Tampa, FL area using mist nets. Molt was confirmed visually by active growth of at least one of the primary or secondary flight or tail feathers, and breeding was confirmed by presence of a brood patch or cloacal protuberance. Within 3 min of capture, a small blood sample (~25 μl) was taken from the brachial vein using a 26-gauge needle. Birds were then kept in a bag for 30 min, and after a second blood sample (~25 μl) was extracted, birds were injected with 1 μg/g DEX (as above). Birds were returned to the bag for 60 min and a final blood sample (~25 μl) was taken. Blood was centrifuged (9300*g* at 4 °C) for 10 min and plasma was extracted and frozen at −40 °C until analysis. Following the final blood sample, individuals were heavily anesthetized and rapidly decapitated; after removal of the skull, using RNase-free tools, we conservatively cut around the anterior perimeter of the hippocampus and removed it from the rest of the forebrain. Hippocampal tissue was frozen (−40 °C in RNALater (Qiagen) until mRNA could be extracted (1–4 weeks). Although the avian hippocampus can be difficult to differentiate, it is located on the posterior region of the dorsomedial surface of the telencephalon, above the cerebellum, with a ventricle directly ventral. By cutting just at the periphery of the most dorsal area, we ensured that we removed only hippocampal tissue; additionally, only one researcher extracted tissues (A.L.), making the extraction methods consistent among all individuals.

2.3. CORT assay

To measure CORT, an EIA kit (Assay Designs, Ann Arbor, MI; cat# 900-097) [6,13] was used according to manufacturer's directions. An equal volume of 10% steroid displacement reagent was added to plasma (5 μl) and 5 min later, assay buffer (240 μl) was added, vortexed, and aliquoted in duplicate (100 μl per well) to provided 96-well plates. Additionally, CORT standards between 200,000 to 32 pg were measured to make a 5-point standard curve. Conjugated CORT and antibody were added to the plate and incubated for 2 h at room temperature while shaking. Wells were emptied and washed and substrate added before incubation for an additional hour at room temperature. Finally, stop solution was added, and the plate was read at 415 nm (corrected at 590 nm to minimize background absorbance). The detection limit of this assay is 27 pg (per company's manual). We estimated cross reactivity to DEX at 7.3% by spiking sparrow plasma with 4 known concentrations of DEX (data not shown). Samples taken to measure DEX dosing were randomly distributed on one plate; samples used to analyze seasonal CORT variation were distributed on an additional two plates; average inter- and intra-plate variation were <10%.

2.4. mRNA extraction and cDNA synthesis

RNA was extracted from ⩽30 mg of hippocampal tissue using a rotor–stator homogenizer and an RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. cDNA was synthesized using the SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen) following manufacturer's instructions using up to 0.5 μg/μl total RNA; RNA and cDNA concentrations were determined using a spectrophotometer.