Seasonal Variation in Corticosterone Receptor Binding in Brain, Hippocampus, and Gonads in House Sparrows (Passer domesticus)

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SEASONAL VARIATION IN CORTICOSTERONE RECEPTOR BINDING IN BRAIN, HIPPOCAMPUS, AND GONADS IN HOUSE SPARROWS (*PASSER DOMESTICUS*)

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Abstract.—Both baseline and stress-induced concentrations of corticosterone (CORT) vary seasonally in a predictable fashion in many wild birds. Hypotheses about why these patterns exist include the "behavior hypothesis," which predicts that animals will down-regulate stress-induced CORT when CORT-induced behaviors are too likely to cause reproductive failure; and the "preparative hypothesis," which posits that baseline and stress-induced CORT will both be high at times of year with a higher incidence of predictable stressors. We tested predictions made by the behavior and predictive hypotheses about the CORT sensitivity of tissues involved in breeding: whole brain, hippocampus, and gonads. We used radioligand binding assays to examine glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) binding in free-living House Sparrows (*Passer domesticus*) at several different life history stages. We found lowest GR binding in whole brain during breeding; this suggests relative insensitivity of brain tissue to CORT at this time of year, which is consistent with predictions made by the behavior hypothesis. We found highest GR binding in whole brain in the pre-egg-laying period, which is consistent with the preparative hypothesis, given that this life stage is associated with a predictable increase in the likelihood of stressful events such as threats to territory and nest sites. However, we found no seasonal changes in GR or MR binding in gonads or hippocampus. Our results suggest that down-regulation of brain GR could be one way birds limit the negative effects of CORT release on breeding behavior, but further studies are necessary to understand the anatomic specificity of these changes. *Received 6 March 2013, accepted 6 June 2013.*

Key words: corticosterone, glucocorticoid receptor, mineralocorticoid receptor, ovary, seasonality, testes.

Variation saisonnière de la liaison aux récepteurs de corticostérone dans le cerveau, l'hippocampe et les gonades chez *Passer domesticus*

Résumé.—Les concentrations de base de corticostérone (CORT) et celles induites par le stress varient d'une saison à l'autre de façon prévisible chez plusieurs oiseaux sauvages. Les hypothèses tentant d'expliquer pourquoi ces patrons existent comprennent « l'hypothèse comportementale », qui prédit que les animaux réduiront la CORT induite par le stress lorsque les comportements causés par la CORT sont trop susceptibles de causer un échec de la reproduction, et « l'hypothèse préparative », qui postule que la CORT de base et celle induite par le stress seront élevées aux moments de l'année ayant une incidence plus élevée de facteurs de stress prévisibles. Nous avons testé les prédictions faites par les hypothèses comportementale et préparative sur la sensibilité à la CORT des tissus impliqués dans la reproduction : l'ensemble du cerveau, l'hippocampe et les gonades. Nous avons utilisé des tests de liaison aux radioligands pour examiner les liaisons aux récepteurs de glucocorticoïdes (GR) et de minéralocorticoïdes (MR) chez des *Passer domesticus* sauvages à différents stades du cycle vital. Nous avons trouvé moins de liaisons aux GR dans l'ensemble du cerveau durant la reproduction; ceci suggère une insensibilité relative des tissus du cerveau à la CORT à ce moment de l'année, ce qui est cohérent avec les prédictions de l'hypothèse comportementale. Nous avons trouvé plus de liaisons GR dans l'ensemble du cerveau durant la période de pré-ponte, ce qui est cohérent avec l'hypothèse préparative, étant donné que ce stade vital est associé à l'augmentation prévisible de la probabilité d'événements stressants tels que les menaces territoriales et au site de nidification. Toutefois, nous n'avons trouvé aucun changement saisonnier dans les liaisons aux GR ou aux MR dans les gonades ou l'hippocampe. Nos résultats suggèrent que la régulation à la baisse des GR du cerveau peut être une façon qu'utilisent les oiseaux pour limiter les effets négatifs de la libération de CORT sur le comportement reproducteur. D'autres études sont nécessaires pour comprendre la spécificité anatomique de ces changements.

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At baseline concentrations, the glucocorticoid hormone corticosterone (CORT) helps wild birds regulate metabolism and activity levels; in response to environmental perturbations, secretion of this hormone increases, and it plays a key role in regulating energy, immune function, and reproduction during the physiological stress response (Sapolsky et al. 2000). Both baseline and stress-induced CORT titers show predictable seasonal patterns in a wide variety of avian species (Romero 2002). For example, most birds show an annual peak in both baseline and stress-induced CORT during breeding, and an annual trough in baseline and stress-induced CORT during molt. Although some studies have clarified the role of particular seasonal patterns in CORT (e.g., low CORT in molting birds may be necessary for the growth of high-quality feathers; DesRochers et al. 2009), it is not well understood why these seasonal patterns exist.

Because CORT has a number of different physiological roles depending on whether it is acting on muscle, immune tissue, brain, or elsewhere (Landys et al. 2006), one way to better understand why CORT titers might be high or low at different times of year is to look "downstream" of the hormone to its receptors in different target tissues. This is especially true given that receptor density in different tissues can show seasonal patterns distinct from circulating baseline and stress-induced CORT (Breuner and Orchinik 2001, Breuner et al. 2003, Lattin et al. 2013). In birds, intracellular receptors that bind CORT and affect gene transcription are practically ubiquitous (Lattin et al. 2012b). At baseline concentrations, CORT's effects are thought to be primarily mediated through binding to a high-affinity receptor similar to mammalian mineralocorticoid receptor (MR), whereas stress-induced CORT concentrations cause increased binding to a lower-affinity receptor similar to mammalian glucocorticoid receptor (GR; de Kloet et al. 1990, Landys et al. 2006). Both GR and MR have recently been characterized in the brain, testes, and ovary of House Sparrows (*Passer domesticus*; Breuner and Orchinik 2001, Lattin et al. 2012b). This allows us to compare seasonal patterns in sensitivity to CORT in these tissues to better understand how CORT's effects on reproductive behavior might vary at different times of year.

Two relevant hypotheses have been proposed to explain seasonal variation in CORT titers (reviewed in Romero 2002). The "behavior hypothesis" posits that seasonal variation in stress-induced CORT can be explained, at least in part, by considering CORT-mediated behaviors (Romero 2002). Stress-induced CORT concentrations suppress nonessential functions like reproduction and enhance the likelihood of behaviors related to survival, such as increased foraging and dispersal from unfavorable environmental conditions (Wingfield et al. 1998, Breuner and Hahn 2003). Although these behaviors may enhance survival, they can result in breeding failure if triggered at the wrong time. Therefore, the behavior hypothesis predicts that seasonally breeding animals will suppress behavioral effects of high CORT concentrations later in the breeding season because of increased investment costs and reduced opportunities for starting a new breeding attempt. It also predicts that animals with limited reproductive opportunities (e.g., birds breeding in the short Arctic summer) or increased reproductive investment (e.g., when only one sex provides parental care) should show increased CORT down-regulation. Several correlative studies and experimental tests support the predictions of this hypothesis (O'Reilly and Wingfield 2001, Wingfield and Romero 2001, Breuner et al. 2003, Lendvai et al. 2007, Lendvai and Chastel 2008, Bókony et al. 2009); however, this hypothesis does not explain patterns in baseline CORT or patterns in CORT secretion outside of the breeding season (Romero 2002).

Because one of CORT's key roles is to prepare animals for future challenges (Sapolsky et al. 2000), the "preparative hypothesis" proposes that CORT titers will be higher at times of year associated with an increased incidence of stressors (Romero 2002). For example, because the early breeding season is associated with a change from flocking to territorial behavior that can increase (1) predation risk (Studd et al. 1983, Gotmark and Post 1996), (2) the need to defend territories and nest sites (Lowther and Cink 2006), and (3) exposure to late-winter storms that can interfere with the start of breeding (Breuner and Hahn 2003), we might expect to see increased baseline and/or stress-induced CORT titers at this time of year. Although the preparative hypothesis has not been tested as extensively as the behavior hypothesis, in part because it requires a detailed understanding of the different risks that animals encounter at different times of year, it provides a potentially powerful framework for understanding patterns of CORT secretion. The behavior and preparative hypotheses are not mutually exclusive, and in fact both may be required to understand why CORT titers vary seasonally as they do.

We used radioligand binding assays to test predictions related to these two hypotheses regarding seasonal variation in plasma CORT. We compared GR and MR binding in House Sparrow whole brain and hippocampus at six life history stages: early and late winter, pre-egg-laying, breeding, late breeding, and molt. We also compared binding in testes and ovary during prelaying, breeding, and late breeding, the only periods in which tissue volume was large enough to compare individuals. Although brain shows a highly heterogeneous distribution of GR and MR (Morimoto et al. 1996), we used a whole-brain assay to be able to compare the results to previous work (Breuner and Orchinik 2001). We also examined CORT receptor binding in the hippocampus, an area involved in CORT negative feedback regulation in mammals (Jacobson and Sapolsky 1991, Jacobson 2005) and in CORT-sensitive memory formation and storage in birds (Pravosudov 2003, 2005; Roth et al. 2012). House Sparrows show seasonal patterns in CORT concentrations similar to those in many other seasonally breeding vertebrates (Romero et al. 2006, Lattin et al. 2012a); furthermore, because their CORT receptors have been fully characterized in both brain (Breuner and Orchinik 2009) and gonads (Lattin et al. 2012b), they are an excellent subject for this type of study.

On the basis of the behavior hypothesis, we expected to see decreased sensitivity to stress-induced CORT (and, therefore, decreased GR binding) in whole brain and gonads during breeding and late breeding (Table 1), when the expression of CORT-mediated behaviors and suppression of reproduction could cause breeding failure (Silverin 1986, Lynn et al. 2010, Ouyang et al. 2012). On the basis of the preparative hypothesis, we expected to see increased whole-brain and gonadal sensitivity to baseline and/or stress-induced CORT (and, therefore, increased GR and/or MR binding) during the pre-egg-laying period (Table 1), which is associated with a number of predictable stressors, as described above. Our predictions for hippocampal binding were based on evidence that avian hippocampus is homologous to mammalian hippocampus (Colombo and Broadbent 2000) and on the results of a recent study that showed seasonal variation in negative feedback regulation in House Sparrows (Lattin et al. 2012a). If avian hippocampus is involved in negative feedback regulation, as it is

Table 1. Summary of predictions made by seasonal CORT hypotheses on glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) concentrations in three tissues related to breeding, and results from the present study.

Tissue	Predictions of the behavior hypothesis	Predictions of the preparative hypothesis	Results
Gonads	Lower GR during breeding and late breeding than during pre-egg-laying	Increased GR and/or MR during pre-egg-laying	No significant seasonal variation
Hippocampus	In keeping with negative feedback findings, increased GR and/or MR during breeding and late winter, decreased GR and/or MR in pre-egg-laying	Increased GR and/or MR during winter	No significant seasonal variation
Whole brain	Lower GR during breeding and late breeding than during pre-egg-laying	Increased GR and/or MR during pre-egg-laying	Lowest GR during breeding, highest during pre-egg-laying

in mammals, we would expect to see increased GR and MR binding in this brain structure during breeding and late winter when negative feedback is highest, and decreased binding in the prelaying period when negative feedback is lowest. However, given the role of the hippocampus in CORT-enhanced spatial memory (Pravosudov 2005), the preparative hypothesis would predict highest GR and MR binding during the winter, when CORT might help House Sparrows remember the locations of reliable food sources such as bird feeders.

Methods

Study subjects and chemical adrenalectomy.—We captured free-living House Sparrows during six life history stages: molt (6–19 September 2010, n = 12), early winter (12–15 December 2010, n = 12), late winter (1–14 February 2011, n =12), pre-egg-laying (31 March–5 April 2011, n = 12), breeding (23–24 May 2011, n = 12), and late breeding (12–18 July 2011, n = 12). Equal numbers of males and females were captured at each stage, and fledglings were excluded from sampling during breeding and late breeding. All molting birds were molting primary feathers (range: P3–P9). We confirmed breeding stage by inspecting cloacal protuberances and beak color (in males) and brood patches (in females), and by inspecting and weighing whole gonads after sacrifice (for additional information, see Lattin et al. 2012a).

House Sparrows were captured at bird feeders in Medford (42.4183°N, 71.1067°W) and Somerville, Massachusetts (42.3875°N, 71.1000°W), using mist nets and Potter traps. Immediately after capture, birds were transferred to the laboratory for 36 h and housed two in a cage under day-length conditions corresponding to their capture date. To reduce endogenous CORT that would interfere with receptor binding assays, House Sparrows received intramuscular injections of mitotane (ortho, para-DDD; 180 mg kg^{-1} body weight; Sigma Aldrich, St. Louis, Missouri) at ~36 h and ~24 h prior to sacrifice (Breuner et al. 2000, Breuner and Orchinik 2001).

Blood sampling and radioimmunoassays.—To measure the success of mitotane treatment, 36 h after the first injection birds were restrained in cloth bags for 30 min and ~30 µL of blood was taken from the alar vein using heparinized capillary tubes. Blood samples were stored on ice until centrifugation; plasma was removed and stored at –20°C. We determined CORT concentrations in each sample using radioimmunoassay following the methods of Wingfield et al. (1992b). Samples were assayed in duplicate using antibody B3-163 (Esoterix, Calabasas Hills, California), and values were corrected for individual recoveries following extraction. Average recovery was 87%, and detectability was 1 ng CORT mL^{-1} plasma. Intra- and inter-assay coefficients of variation were 3% and 22%, respectively. In all cases, mitotane reduced stress-induced CORT; mean (± SD) CORT was 1.7 ± 3.5 ng mL^{-1}, compared with approximately 20–30 ng mL^{-1} for House Sparrows not treated with mitotane (Romero et al. 2006).

Tissue processing.—Birds were deeply anesthetized with intramuscular injections of ketamine (~80 mg kg^{-1} body weight; Fort Dodge Animal Health, Fort Dodge, Iowa) and xylazine (~20 mg kg^{-1} body weight; Akorn, Decatur, Illinois), at appropriate doses for House Sparrows (Muresan et al. 2008). Birds were transcardially perfused with ice-cold heparinized saline, and brain and gonads were removed and flash frozen on dry ice. Frozen brains were cut in half; one hemisphere was randomly assigned for whole-brain assays, and the other for hippocampus assays. Other tissues were taken for additional studies at the same time. Tissues were always taken in the same order, and the time required to take all tissues was recorded for each bird (mean [± SD] time = 13.5 ± 1.3 min). Tissues were stored at –80°C until assay.

Receptor binding assays.—Receptor binding assays were done following Breuner and Orchinik (2001) and have been described in detail elsewhere (Lattin et al. 2012b). We used homogenization techniques, tissue-to-buffer ratios, incubation times, and temperatures optimized for House Sparrow brain, testes, and ovary (Breuner and Orchinik 2001, Lattin et al. 2012b). Briefly, on the day of the assay, tissue was homogenized in ice-cold buffer and spun at 104,000 g for 1 h in an ultracentrifuge to separate soluble proteins (including MR and GR) from nuclear, mitochondrial, and microsomal proteins. We homogenized whole gonads, one brain hemisphere for whole-brain samples, and the whole hippocampus (dissected out of the other hemisphere using forceps) for hippocampus samples.

Cytosol was incubated with 10 nM [^{3}H]CORT (PerkinElmer, Waltham, Massachusetts) and either (1) buffer, to measure total binding; (2) 1 µM unlabeled CORT (Sigma Aldrich), to measure nonspecific binding; or (3) 1 µM RU486 (mifepristone; Tocris Bioscience, Minneapolis, Minnesota), which binds only GR. After subtracting nonspecific binding, MR binding can be calculated directly from test tubes containing RU486; GR binding can be calculated by subtracting MR binding from total binding. Affinity estimates derived from previous equilibrium saturation analyses in this species (Breuner and Orchinik 2001, Lattin et al. 2012b) predicted that 10 nM [^{3}H]CORT should occupy >95% of MR and ~63% of GR.

Incubations were terminated by rapid filtration using a Brandel harvester (model M24; Brandel, Gaithersburg, Maryland). Filter paper was mixed with Ultima Gold scintillation fluid (PerkinElmer) and vortexed; radioactivity was quantified using

a scintillation counter (TriCarb 1600, PerkinElmer). Binding in individual samples was standardized per milligram of protein, determined using Bradford assays. All samples used for analysis contained 1–10 mg protein mL^{-1} buffer, a range shown to produce accurate results for intracellular glucocorticoid receptor binding assays (López Bernal et al. 1984). Each sample was run in triplicate and, for each tissue, all individuals were assayed at the same time to avoid inter-assay variation.

Data analysis.—Statistical analyses were run using JMP, version 9.0 (SAS Institute, Cary, North Carolina). Tissue mass (for gonads) and GR and MR binding were compared among life history stages using analysis of variance (ANOVA). Because females are often more sensitive to environmental cues related to breeding (Ball and Ketterson 2008), which could include stressors, we also looked for sex differences in GR and MR binding in brain and hippocampus. In a full model that included sex, life history stage, and possible interactions, we found no sex effect (all $P \geq 0.11$), which is consistent with previous studies in this species (Breuner and Orchinik 2001). Because of this, sex was excluded from all other analyses.

With equal sample sizes, ANOVA is fairly robust to violations of normality assumptions, but not to violations of homogeneity of variances (Day and Quinn 1989). We used Levene's test to ensure that data from each analysis met the assumption of homogeneity of variances and, in situations where variances among groups were not homogeneous, ran a Welch's ANOVA (Day and Quinn 1989). In cases where we found a significant difference among groups, we ran Tukey's HSD test as a multiple-comparison procedure, as recommended by Quinn and Keough (2002).

Because gonadal mass is low when animals are not breeding, but a minimum protein concentration is necessary for receptor binding assays (1 mg mL^{-1}; see above), we ran analyses only on the three life history stages with high enough gonadal mass to compare individuals during prelaying, breeding, and late breeding. One individual was excluded from brain analysis of low protein concentrations in cytosol (<1 mg mL^{-1}; see above).

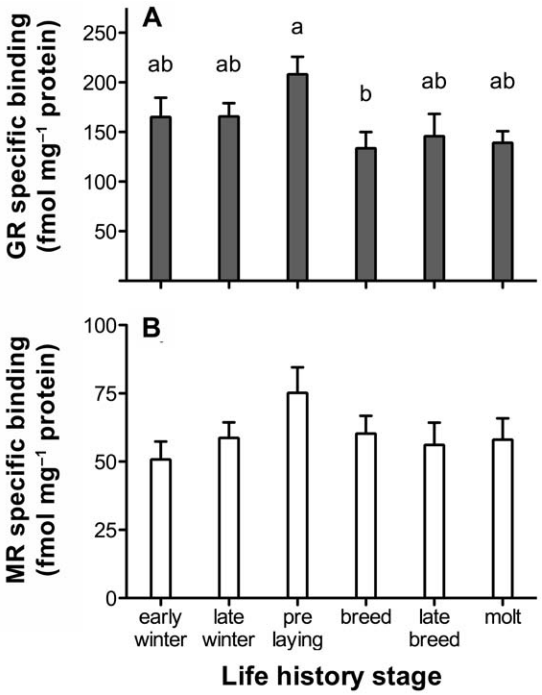

FIG. 1. Point sample analysis of (A) glucocorticoid (GR) and (B) mineralocorticoid (MR) receptors in the homogenized half brains of House Sparrows captured in Massachusetts at six life history stages (n = 6 males and 6 females at each stage, except for 1 male excluded in the prelaying period because of low protein concentrations in cytosol). Data represent means (± SE) of specific binding of 10 nM [^{3}H]CORT to House Sparrow cytosol, standardized by protein concentration. MR receptor capacity was determined by adding 1 μM of the GR-specific antagonist RU486 to tubes. GR receptor capacity was determined by subtracting MR capacity from total specific binding. Different letters represent statistical differences among life history stages as indicated by post hoc tests.

RESULTS

Brain.—GR binding in whole brain varied by life history stage (F = 2.41, df = 5 and 65, P = 0.046; Fig. 1A). Post hoc analysis revealed that GR was higher in the prelaying period than during breeding. There was no seasonal trend in whole-brain MR binding (F = 1.17, df = 5 and 65, P = 0.33; Fig. 1B). In hippocampus alone, neither GR (F = 1.51, df = 5 and 31, P = 0.22; Fig. 2A) nor MR binding (F = 1.52, df = 5 and 66, P = 0.19; Fig. 2B) varied by life history stage.

Gonads.—Mass of testes varied significantly by life history stage (F = 6.48, df = 5 and 18, P = 0.001; Fig. 3A). Post hoc analysis revealed that testes were smaller during molt and in early winter than in late winter, and that late-winter testes were smaller than prelaying, breeding, and late-breeding testes. Among prelaying, breeding, and late-breeding individuals, there was no difference in either GR (F = 1.40, df = 2 and 15, P = 0.28; Fig. 4A) or MR binding (F = 0.12, df = 2 and 15, P = 0.89; Fig. 4A).

Ovary mass also varied by life history stage (F = 12.14, df = 5 and 12, P = 0.0005; Fig. 3B). Post hoc analysis found that ovaries of breeding females were larger than those of females during molt, early winter, and late winter. However, among prelaying, breeding, and late-breeding individuals, there was no difference in GR (F = 0.79, df = 2 and 15, P = 0.47; Fig. 4B) or MR binding (F = 0.53, df = 2 and 9, P = 0.61; Fig. 4B).

DISCUSSION

Stress can act at multiple levels to disrupt breeding, including at the brain, the pituitary, and directly at the level of the gonads (Consten et al. 2002, Michael et al. 2003, Breen and Karsch 2006, Oakley et al. 2009, Schoech et al. 2009). In House Sparrows, we found evidence of seasonal regulation of CORT receptors in whole brain, but not in hippocampus or gonads. The pattern seen in brain, where breeding House Sparrows expressed fewer GR than prelaying House Sparrows, supported some of the predictions of both the behavioral and preparative hypotheses (Table 1). These data suggest that breeding House Sparrows were less sensitive to the behavioral effects of stress-induced CORT. Although stress-induced CORT is high in House Sparrows during breeding, negative feedback is also high (Lattin et al. 2012a), which means that breeding House Sparrows are able to mount a robust stress response but shut it down quickly, before it can interfere with reproduction. Therefore,

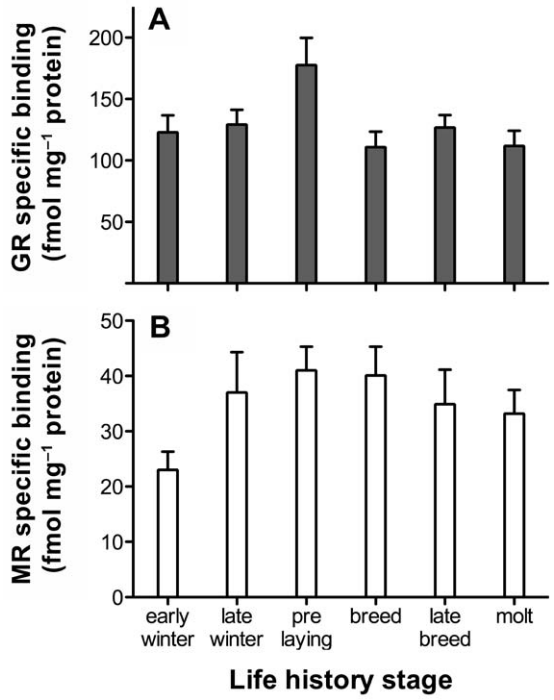

FIG. 2. Point sample analysis of (A) glucocorticoid (GR) and (B) mineralocorticoid (MR) receptors in the hippocampus of House Sparrows captured in Massachusetts at six life history stages (n = 6 males and 6 females at each stage). Data represent means (± SE) of specific binding of 10 nM [^{3}H]CORT to House Sparrow cytosol, standardized by protein concentration. For more information, see Figure 1 caption.

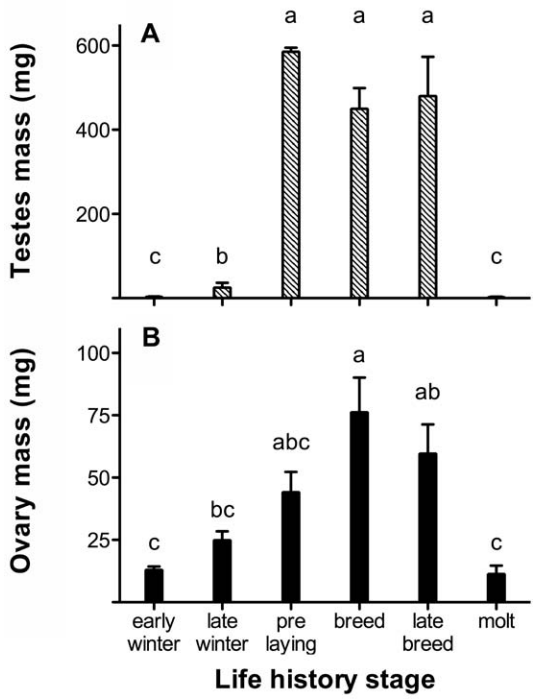

FIG. 3. Mass of paired (A) testes and (B) whole ovary from free-living House Sparrows captured in Massachusetts at six life history stages (n = 6 at each stage). Different letters represent statistical differences as indicated by post hoc tests. All values are presented as means ± SE.

House Sparrows may use both increased-CORT negative feedback and decreased brain sensitivity to CORT to avoid stress-induced reproductive failure.

The receptor binding technique we used provides a robust quantification of changes in receptor numbers, but at the cost of anatomical specificity. This is a tradeoff, because more anatomically specific techniques, such as immunohistochemistry, typically provide only semiquantitative measures of changes in receptor density (de Matos et al. 2010). Consequently, even though we could not identify specific brain areas where GR binding decreased, we are confident that the seasonal changes in binding represent physiologically relevant differences in the number of receptors. Specifically, mean whole-brain GR concentrations dropped by ~35% between pre-egg-laying and breeding, a period of just over 1 month. The present study thus represents a first step, and future studies should determine where in the brain the GR concentrations are changing. There are several regions that could be involved. In rats, GR binding is high in many brain areas, including cerebral cortex, amygdala, dorsal thalamus, hypothalamus, cerebellar cortex, locus coeruleus, and dorsal nucleus raphe (Morimoto et al. 1996).

Contrary to predictions of the behavioral hypothesis (Table 1), whole-brain GR was not lower during late breeding than during pre-breeding, although House Sparrows attenuate adrenal sensitivity and the CORT response to stressors later in the breeding season (Lattin et al. 2012a). Consequently, down-regulation of stress-induced behaviors may occur at the level of hormone secretion

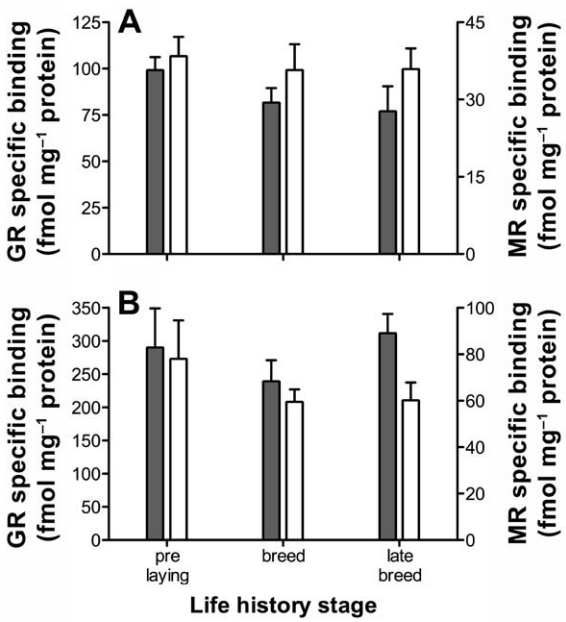

FIG. 4. Point sample analysis of glucocorticoid (GR; gray bars) and mineralocorticoid (MR; white bars) receptors in the (A) testes and (B) ovary of House Sparrows captured in Massachusetts at three life history stages (n = 6 at each stage). Data represent means (± SE) of specific binding of 10 nM [^{3}H]CORT to House Sparrow cytosol, standardized by protein concentration. For more information, see Figure 1 caption.

rather than via brain sensitivity at this time of year. As we predicted on the basis of the preparative hypothesis (Table 1), whole-brain GR binding was highest during the prelaying period, which suggests that House Sparrows are most sensitive to the behavioral effects of CORT at that time of year. This could help House Sparrows respond to predictable stressors that occur during the prelaying stage, such as territory and nest-site defense (Lowther and Cink 2006). Also, although the timing of breeding in birds is primarily regulated by photoperiod, other factors such as temperature, rainfall, and food availability can affect the start of breeding in many species (Ball 1993, Dawson 2008, Schoech et al. 2009), and it has been suggested that CORT could be one hormone involved in integrating these supplemental cues (Wingfield et al. 1992a, Schoech et al. 2009, Goutte et al. 2010). However, baseline (rather than stress-induced) CORT is typically what has been found to correlate with laying date (Schoech et al. 2009, Goutte et al. 2010). The lack of changes in whole-brain MR suggest that onset of laying behavior is regulated via baseline hormone titers, not changes in brain sensitivity to those titers.

The results of our study of House Sparrows from New England contrast slightly with those from a study of House Sparrows in Arizona (Breuner and Orchinik 2001). Although the New England population showed lowest whole-brain GR binding during breeding, the Arizona population showed lower GR and MR in whole brain in winter than during molt and breeding. The breeding season of House Sparrows in the southern United States begins several weeks before that of more northerly populations (Lowther and Cink 2006). Because of increased reproductive opportunities, House Sparrows in Arizona may have a reduced need to down-regulate stress-induced CORT effects on breeding behavior.

Contrary to our predictions, we saw no seasonal modulation of CORT receptor binding in ovary or testes during the prelaying, breeding, and late-breeding stages (Table 1). Although there is significant GR and MR binding in House Sparrow testes and ovary (Lattin et al. 2012b), and CORT can suppress steroidogenesis and other reproductive processes by acting directly on the gonads (Hsueh and Erickson 1978, Sapolsky 1985, Consten et al. 2002), 11β hydroxysteroid dehydrogenase type 2, which converts CORT into an inactive metabolite, may also be present in these tissues, as it is in the gonads of other animals (Michael et al. 1993, Monder et al. 1994, Denari and Ceballos 2005). This may prevent CORT from binding to gonadal GR and MR in situations of short-term stress and could be the reason that the short-term down-regulation of reproduction by stress seems to happen primarily via regulation at the brain and pituitary (Rivier and Rivest 1991, Breen and Karsch 2006). Suppressing reproduction at the level of the gonads may have longer-term effects that would persist beyond the duration of a transitory stressor. Therefore, it is possible that gonadal GR and MR may bind CORT and down-regulate reproductive function only in situations of chronic stress, as suggested by Rivier and Rivest (1991), rather than as part of normal seasonal regulation.

On the basis of the preparative hypothesis, we also predicted that we might see greater GR and/or MR binding in hippocampus in winter (Table 1). However, we did not find any seasonal patterns in CORT receptor binding in House Sparrow hippocampus. Therefore, CORT's role in enhancing spatial memory via the hippocampus may be less important in House Sparrows than in food-caching birds like Mountain Chickadees (*Poecile gambeli*). We also predicted that patterns in GR and/or MR binding in hippocampus would parallel seasonal variation in CORT negative feedback in House Sparrows (Lattin et al. 2012a), because of the important role of the mammalian hippocampus in negative feedback (Jacobson 2005). Although the avian hippocampus appears to be a functional homologue of the mammalian hippocampus (Colombo and Broadbent 2000), it has not been clearly established that the avian hippocampus is involved in negative feedback. For example, Dickens et al. (2011) did not find changes in hippocampal GR or MR expression in translocated Chukar (*Alectoris chukar*), despite finding differences in negative feedback in these animals (Dickens et al. 2009). In birds, it is possible that other brain areas may be more involved in negative feedback regulation (Mogensen and Divac 1982, Herman and Cullinan 1997).

Overall, in whole brain, this study provides mixed support for both the behavior and preparative hypotheses, although we found no seasonal modulation of CORT sensitivity in hippocampus or gonads. Furthermore, these data suggest that down-regulation of brain GR could be one way in which animals limit the negative effects of CORT release on breeding behavior, and, together with increased negative feedback regulation found in a previous study (Lattin et al. 2012a), help solve the apparent paradox that stress-induced CORT is often high at a time when high CORT could have important negative effects on reproductive success. Further studies are needed to identify which brain areas exhibit seasonal variation in GR binding.

Acknowledgments

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