Corticosterone manipulations alter morph-specific nestling provisioning behavior in male white-throated sparrows, Zonotrichia albicollis

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A B S T R A C T

In the polymorphic white-throated sparrow (Zonotrichia albicollis), tan-striped males provision nestlings at higher rates than do white-striped males. In a previous study, we found that tan-striped males had lower baseline corticosterone levels than white-striped males during the nestling stage. To determine if this variation in corticosterone influences morph-specific differences in nestling provisioning behavior, we used intraperitoneal osmotic pumps to increase baseline corticosterone levels in tan-striped males (TS CORT) and administer RU486, a glucocorticoid receptor antagonist, in white-striped males (WS RU486). These manipulations essentially reversed morph-specific nestling provisioning behavior in males. TS CORT males fed nestlings at lower rates than TS controls (vehicle-only implant), and at similar rates to WS controls (vehicle-only implant), while WS RU486 males fed nestlings at higher rates than WS controls, and at similar rates to TS controls. These results demonstrate that (1) increases in baseline corticosterone (i.e., below concentrations associated with the adrenocortical response to stress) can directly or indirectly inhibit nestling provisioning behavior, and (2) corticosterone influences morph-specific variation in parental behavior in male white-throated sparrows. This study contributes to the growing evidence that modulating baseline CORT mediates parental care and self-maintenance activities in birds, and thus may serve as a mechanism for balancing current reproductive success with survival.

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Introduction

Physiological processes, including endocrine control mechanisms, regulate the behaviors that characterize different life history strategies (Sinervo and Svensson, 1998; Ketterson and Nolan, 1999; Ricklefs and Wikelski, 2002). Much of the variation in life histories is rooted in trade-offs between the costs and benefits of investing in current reproduction versus investing in survival and future reproduction. The endocrine system may mediate potential conflicts between these two critical fitness components (Ricklefs and Wikelski, 2002).

Corticosterone (CORT), the primary avian glucocorticoid, plays multiple roles in regulating behavioral and physiological adjustments to predictable and unpredictable changes in energy demands associated with survival and with reproduction (Wingfield et al., 1998; Sapolsky et al., 2000; Landys et al., 2006). The ability to rapidly secrete CORT at high concentrations in response to life-threatening perturbation, hereafter referred to as the adrenocortical response, is believed to promote survival (reviewed by Wingfield et al., 1998), but high CORT levels can inhibit reproductive physiology and behavior (reviewed by Wingfield and Sapolsky, 2003). Mounting evidence from breeding birds supports the hypothesis that modulating the adrenocortical response serves as a mechanism for balancing potential trade-offs between current reproductive success and survival (O’Reilly and Wingfield, 2001; Holberton and Wingfield, 2003; Meddle et al., 2003; Wilson and Holberton, 2004; Heidinger et al., 2006).

In contrast to the adrenocortical response, which is a mechanism pertinent only to perturbation events, modulating baseline CORT (i.e., non-stress levels) may be a more practical mechanism for balancing the daily demands of parental care against those of self-maintenance (Kitaysky et al., 2001; Landys et al., 2006; Angelier et al., 2007a). In birds, baseline CORT can vary according to sex and breeding stage, and independent of changes in body condition (Pereyra and Wingfield, 2003; Reneerkens et al., 2002; Meddle et al., 2003; Lorrain et al., 2003; Love et al., 2004; Williams et al., 2008). Because the degree or form of parental care often differs between sexes and across breeding stages, baseline CORT may be modulated according to parental investment. However, disparate patterns among correlative studies make it difficult to determine whether elevations in baseline CORT (i.e., at levels well below those resulting from the adrenocortical response) facilitate or inhibit parental care. Thus, experiments using hormone manipulation are needed to increase our understanding of how changes in baseline CORT during breeding influence parental behavior in birds.

Silverin (1986) found that silastic CORT implants reduced nestling provisioning rates in pied flycatcher (Ficedula hypoleuca) parents. These implants, however, increased plasma CORT concentrations to...
levels observed during a capture-induced adrenocortical response, overshooting seasonal baseline concentrations (Silverin, 1986, 1998; Silverin and Wingfield, 1998). To understand how natural variation in baseline CORT affects parental behavior, circulating hormone levels should be manipulated in such a way as to remain within biologically relevant ranges for the species. Recent experiments that have accomplished this task provide evidence that increased baseline CORT inhibits parental care in large, long-lived species of birds (Kitaysky et al., 2001; Almasi et al., 2008).

Here, we examined the proximate relationship between nesting provisioning behavior and baseline CORT in the white-throated sparrow (Zonotrichia albicollis), a polymorphic passerine that exhibits predictable morph-specific variation in parental behavior. The genetic plumage morphs, tan-stripe (TS) and white-stripe (WS), are distinguished by median crown stripe color, and these color morphs occur in both sexes (Lowther, 1961). These morphs exhibit negative assortative mating such that TS morphs almost always pair with WS morphs, and this mating system is thought to promote stable polymorphism in the population (Falls and Kopachena, 1994). Behavioral differences are associated with color morph. Within each sex, WS morphs sing more and are more aggressive than TS morphs, while TS morphs contribute more to parental care than WS morphs (Falls and Kopachena, 1994). Interestingly, morph differences in behavior may underlie negative assortative mating in this species. For example, both female morphs seem to prefer the more parental TS males, but the more aggressive WS females may outcompete TS females for access to TS males, leaving TS females to pair with WS males (Houtman and Falls, 1994). In turn, the negative assortative mating that results is thought to be favored because it provides an adaptive combination of alternative reproductive strategies (Kopachena, 1992; Falls and Kopachena, 1994). That is, the aggressive WS morphs compensate for the lower territorial behavior of TS morphs, while the more parental TS morphs compensate for the lower offspring care of WS morphs.

Morph-specific differences in parental care are ideal for this study. Specifically, morphs differ in the rate at which they provision nestlings: TS morphs provision nestlings at higher rates than WS morphs (Knapton and Falls, 1983; Kopachena and Falls, 1993; Horton and Holberton, in review). Moreover, we previously found that TS males in this population exhibit lower baseline CORT levels during the nestling provisioning stage than do WS males (Horton and Holberton, in review). To determine if morph-specific differences in baseline CORT influence morph-specific differences in male provisioning behavior, we used intraperitoneal (IP) osmotic pumps to induce biologically relevant increases in baseline CORT, or to block CORT's effects, and recorded nestling provisioning rates following implant treatments. In particular, we increased baseline CORT in parental TS males in such a way as to remain within the natural range of hormone levels previously measured in parental WS males. We implanted parental WS males with pumps filled with RU486, a glucocorticoid receptor (GR) antagonist, to block the effects of elevated baseline CORT during that stage. RU486 has shown promise as an effective tool for use in studies of CORT in birds due to its ability to inhibit CORT's actions through the GR peripherally and in the brain (Koch et al., 2002; Landys et al., 2004a,b; Horton, 2007).

Our assumption was that these birds retain phenotypic plasticity in provisioning rates in spite of their genetic plumage morph. We predicted that, if elevations in baseline CORT act to reduce nestling provisioning behavior, CORT-implanted TS males would behave more like WS males and provision young at lower rates than controls (TS males with vehicle-only implants). Similarly, RU486-implanted WS males, who would normally be able to respond to the elevated CORT experienced during parental provisioning, would behave more like TS males and provision at higher rates than their respective controls (WS males with vehicle-only implants). In addition to male provisioning rates, we measured female provisioning rates because birds often increase their parental effort to compensate for reductions in their mates' parental effort (Martin, 1974; Weatherhead, 1979; Smith et al., 1982; Breitwisch, 1988; Wolf et al., 1990). Therefore, we predicted that females paired with these manipulated males would compensate for any treatment-induced increases or decreases in male provisioning behavior by adjusting their own provisioning rates accordingly.

**Methods**

**Capture, sampling, and implant surgery**

This study was conducted at the Penobscot Experimental Forest in Bradley, Maine (44°52′N, 68°38′W) from late May through early August, 2005 and 2006. Nests were found by either flushing incubating females or by observing the behavior of nesting adults. Once nests were located, we monitored them daily for hatching and deduced the color morph of the male from that of the incubating female. Same-morph pairs were not observed during this study; thus, all WS males were paired with TS females, and all TS males were paired with WS females. Because provisioning rates of white-throated sparrow parents vary with the number of nestlings (Horton and Holberton, in review), we focused only on nests with either three or four nestlings to minimize this source of variation in parental behavior. Nests for each male morph were randomly allocated to one of two treatment groups for that morph (described below).

When nestlings were 1–3 days old, we captured parental males in mist nets using conspecific song recordings (playback) and a white-throated sparrow model, or by strategically placing nets to capture males during feeding visits. Nests were monitored to record the actual time of capture, but at a distance that minimized disturbance. Within 3 min of capture, we collected a small blood sample (~80 μL) in a heparinized capillary tube following puncture of the brachial vein with a 26 gauge needle. Once bleeding was staunched with cotton and manual pressure, we implanted males with intraperitoneal osmotic pumps (see below) using the surgical methods described by Horton et al. (2007). All implant surgeries were conducted in the field under aseptic conditions, and all procedures received prior approval by the University of Maine Institutional Animal Care and Use Committee (Protocol #A2005-04-02). After implant surgery, we recorded tarsus length (±0.1 mm), unflattened wing chord (±0.25 mm), body mass (±0.25 g), and furcular fat score (range: ‘0’ (no fat) to ‘2’ (fat completely filled furcular cavity); from Helms and Drury, 1960). We recorded body mass after implant surgery to account for the additional weight of the osmotic pump in both pre- and post-implant measures. We calculated a body condition index (residuals of a mass X tarsus regression) to control for individual differences in mass due to structural body size. We also scored the shape of the pectoral muscle so that we could document muscle catabolism if it occurred. Muscle scores ranged from 1 to 5, where 1 represented a severely concaved pectoral muscle (i.e., advanced muscle catabolism), and 5 represented a fully convex pectoral muscle (i.e., no observable catabolism). All muscles scores were measured by a single observer (B. Horton). Prior to release on their territory, each male received an aluminum USGS numbered leg band and a unique combination of colored leg bands.

We attempted to recapture each male 4–5 days after implantation (i.e., when nestlings were 6–7 days old) to assess implant effects on body condition and plasma hormone levels. When successful, we collected another blood sample (~80 μL) within 3 min of capture and recorded body mass, pectoral muscle score, and furcular fat score. We also examined each male for changes in pump position, wound healing, or visible complications from implant surgery. Throughout, only blood samples from birds with known capture times were used in analyses of baseline CORT. Blood samples were kept on ice in the

**Results**

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field until centrifuged, usually within 6 h of collection, after which plasma was removed and kept frozen until assayed for CORT by radioimmunoassay in the University of Maine Laboratory of Avian Biology (Wingfield et al., 1992; Cash and Holberton, 1999).

Osmotic pumps and implant treatments

The osmotic pump used in this study, Alzet® model 1007D (length = 1.5 cm, diameter = 0.6 cm, filled mass = ~5 g), was previously tested in captive white-throated sparrow males (Horton et al., 2007). This model has a reservoir volume of 100 μL and a 7-day delivery duration. Using an avian extracellular fluid osmolarity of 9.02 atm (converted from 350 mmol/kg, Goldstein and Rothschild, 1993) and body temperature of 41 °C (Goldstein and Rothschild, 1993), the estimated flow rate of these pumps in vivo is 0.61 μL per hour (Alzet® Calculator, www.alzet.com). We filled pumps under aseptic conditions according to the manufacturer’s instructions (www.alzet.com) and primed them in sterile saline for 12–36 h prior to implantation.

In our study population, tan-striped (TS) males naturally have lower baseline CORT than white-striped (WS) males during the nestling stage (Horton and Holberton, in review; also see below); therefore, manipulations via IP osmotic pumps were intended to elevate baseline plasma CORT in TS males and inhibit the effects of CORT in WS males. To elevate baseline CORT in TS males, we filled pumps with a solution of 3.5 mg CORT (#C-2505, Sigma® Chemical, St. Louis, MO) per mL polyethylene glycol 400 (PEG, Fisher Chemical®, Hampton, NH) vehicle. When delivered via IP osmotic pump in captive white-throated sparrow males, this concentration provided sustained elevations of plasma CORT within the natural range of variation in baseline CORT for free-living white-throated sparrow males (Horton et al., 2007). Furthermore, captive males implanted with IP pumps filled with a 3.5 mg/mL CORT concentration exhibited increased food intake, fat deposition, and muscle catabolism in relation to controls (Horton, 2007), suggesting that this dose is sufficient for inducing changes in behavior and physiology associated with sustained elevations in plasma CORT. Hereafter, TS males implanted with CORT-filled pumps are referred to as TS CORT males. Control TS males in this study received implants filled with PEG vehicle only.

To inhibit CORT’s effects in WS males, we implanted WS males with pumps filled with a solution of 27 mg RU486 (#M8046, mifepristone, Sigma® Chemical, St. Louis, MO) per mL PEG vehicle. When delivered via IP osmotic pump, this same dose of RU486 reduced food intake rates in captive white-throated sparrow males, a behavioral effect opposite to that induced by CORT-filled pumps (Horton, 2007). RU486 has also been shown to inhibit feeding behavior in other avian species (Landys et al., 2004a,b). In this study, samples collected 4–5 days after implantation indicate that RU486 treatment did not affect endogenous CORT secretion in WS males (see below; also, see Horton et al., 2007). Together, these data illustrate that RU486 effectively inhibits the behavioral effects of CORT. Hereafter, WS males implanted with RU486-filled pumps are referred to as WS RU486 males. Control WS males in this study received implants filled with PEG vehicle only.

Parental behavior

We used video cameras (Hi8, Samsung® models, 10× optical zoom) to record the parental behavior of implanted males and their mates. We began recording parental behavior 2 days after implantation and continued until nestlings were 8 days old, or until the nest was depredated. When weather permitted, each nest was filmed for 2 h each day between 05:30 and 11:30 EDT. Cameras were concealed among standing vegetation 3–5 m from the nest. With 10× optical zoom lenses, views of the nest through the camera were clear enough to determine both morph and color band combinations of parents and confirmed that we had implanted the proper male at each nest. Behavior and vocalizations monitored on video suggest that the vast majority of parents were either not disturbed by cameras or adjusted rapidly (<10 min) to them. Video data were not used in analyses if there were any signs (e.g., continued alarm-calling or lack of feeding visits) that the presence of cameras disrupted parental behavior for an extended period of time (i.e., >10 min). On days when capture efforts and video footage coincided (usually day 6 or 7), we collected video data prior to capture attempts to avoid disturbance effects on parental behavior. During video transcription, we recorded the number of feeding trips per hour made by each parent; morphs of the white-throated sparrow have been shown to differ in the rate at which they provision young, but not in the number or size of items they deliver per trip (Knapton and Falls, 1983). Visits to the nest by a parent were considered feeding trips only when food items were actually delivered to nestlings. We also recorded brood time (minutes per hour) for females; according to our video data, males do not typically brood in this population (also see Falls and Kopachen, 1994).

Statistical analyses

To compare pre-implant baseline CORT, body condition indices, furcular fat score, and pectoral muscle score among treatment groups, we used a one-way ANOVA. In addition, we used an unpaired (two sample) t-test to determine if baseline CORT differed between male morphs prior to implantation. For these ‘pre-implant’ analyses, we used data from all implanted males included in the study (see Fig. 1). We conducted two types of ‘post-implant’ analyses, which used only data from males that were recaptured 4–5 days after implantation (see Fig. 2). First, we used a paired t-test to determine, for each treatment group, if post-implant levels of baseline CORT, body condition index, fat score, or muscle score of recaptured males differed from their respective pre-implant levels. Second, we used a one-way ANOVA to compare post-implant measures among treatment groups. All CORT data were log$_{10}$ transformed for use in parametric analyses. To analyze parental behavior, we used a repeated measures ANCOVA, with nestling age included as a covariate, to determine if implant treatment affected male and female provisioning rates and female brooding time. The time of day during which nests were filmed and nestling number were not significant covariates of parental behavior and were excluded from the models. We used a Fisher’s protected least-significant-difference test (PLSD) for post hoc comparisons among treatment groups. All statistical analyses were performed using Systat® 11.

Results

Sample sizes

We implanted a total of 41 males, comprising the following numbers for each treatment: TS CORT ($n=11$), TS control ($n=7$), WS RU486 ($n=13$), and WS control ($n=10$). Unequal sample sizes for each group were the result of either nest depredation just prior to implantation or an inability to capture the target male. Video data used in analyses of parental behavior totaled 228 h of footage, or 114 2-h videos (one video per day per nest), on 33 different nests. These data comprised the following sample sizes: TS CORT (35 videos from 11 nests), TS control (14 videos from 4 nests), WS RU486 (29 videos from 9 nests), and WS control (36 videos from 9 nests). We could not obtain adequate parental data for all nests with implanted males due to either inclement weather or nest depredation shortly after implantation. We were able to recapture (4–5 days after implantation) a portion of those males for which parental data were obtained.
Recapture numbers were the following: TS CORT \((n = 5)\), TS control \((n = 4)\), WS RU486 \((n = 5)\), and WS control \((n = 4)\).

Corticosterone and body condition

Prior to implantation, there were significant differences between implant treatment groups in baseline CORT \((F_{3,37} = 3.68, p = 0.021; \text{Fig. } 1a)\). Pre-implant baseline CORT levels were higher in WS RU486 males than in TS CORT males \((p = 0.015)\) and TS controls \((p = 0.005)\). Within morphs, however, pre-implant baseline CORT levels did not differ between TS CORT males and TS controls \((p = 0.475)\), or between WS RU486 males and WS controls \((p = 0.151)\). When treatment groups were pooled by morph, pre-implant baseline CORT levels were, on average, lower in TS males than in WS males \((t_{39} = -2.93, p = 0.006; \text{Fig. } 1a)\). Pre-implant body condition index \((F_{3,37} = 0.30, p = 0.826; \text{Fig. } 1b)\), furcular fat score \((F_{3,37} = 0.43, p = 0.731)\), or pectoral muscle score \((F_{3,37} = 0.60, p = 0.626)\) did not differ among treatment groups.

Post-implant

Interestingly, TS CORT males \((t_{4} = -3.10, p = 0.036)\), TS controls \((t_{3} = -4.47, p = 0.021)\), and WS controls \((t_{3} = 3.20, p = 0.049)\) all had higher baseline CORT levels 4–5 days after implantation than they did prior to implantation \((\text{Fig. } 2a)\). Post-implant baseline CORT levels did, however, vary among treatment groups \((F_{3,14} = 3.49, p = 0.045; \text{Fig. } 2a)\), such that TS CORT males had post-implant CORT levels that were significantly higher than those of TS controls \((p = 0.037)\) and WS RU486 males \((p = 0.008)\), but not significantly higher than those of WS controls \((p = 0.173)\). When compared to their pre-implant condition, TS CORT males exhibited a decline in body condition \((t_{4} = 12.22, p < 0.001; \text{Fig. } 2b)\) and muscle score \((t_{4} = 5.20, p = 0.014)\), but an increase in fat score \((t_{4} = 4.49, p = 0.011)\) when measured 4–5 days after implantation. In contrast, post-implant body condition indices, muscle scores, and fat scores did not differ from pre-implant levels in any of the other treatment groups \((\text{all } t \leq 2.83, \text{all } p \geq 0.066)\).

Parental behavior

Implant treatments significantly affected male provisioning behavior \((F_{3,109} = 5.44, p = 0.002; \text{Fig. } 3a)\). TS CORT males fed nestlings at lower rates than TS controls \((p = 0.013)\) and at similar rates to WS controls \((p = 0.471)\). WS RU486 males fed young at higher rates than WS controls \((p = 0.005)\), and at similar rates to TS controls \((p = 0.806)\). As expected, TS controls fed young at higher rates than WS controls \((p = 0.034)\).

In addition, there was a significant overall effect of male implant treatment on the provisioning rate \((F_{3,109} = 5.99, p = 0.001; \text{Fig. } 3b)\) and brooding time \((F_{3,109} = 7.43, p < 0.001; \text{Fig. } 3c)\) of their female mates, but female response to male treatment was morph-specific.

Fig. 1. Baseline corticosterone levels (a) and body condition indices (b) for male white-throated sparrows prior to receiving intraperitoneal osmotic pumps. Treatment groups included tan-striped (TS) males implanted with pumps filled with either corticosterone (TS CORT) or vehicle only (TS Control), and white-striped (WS) males implanted with pumps filled with either RU486 (WS RU486) or vehicle only (WS Control). The above figures depict pre-implant data for all males included in this study. Values are means±SE.

Fig. 2. Baseline corticosterone levels (a) and body mass (b) in male white-throated sparrows prior to receiving implants (open bars) and 4–5 days after implantation (filled bars). In contrast to Fig. 1, the above figures depict data only for those males that were recaptured (approximately half of the individuals). Values are means±SE. Asterisks above bars indicate significant changes from pre- to post-implant levels. See Fig. 1 or text for implant treatments.
WS females paired with TS controls fed young more (PLSD, \( p < 0.001 \)) and brooded less (PLSD, \( p < 0.001 \)) than WS males paired with TS CORT males. But among TS females, there was no difference in provisioning rate (PLSD, \( p = 0.658 \)) or brooding time (PLSD, \( p = 0.194 \)) according to WS male implant treatment. Male brooding behavior (shading nestlings with outstretched wings) was observed only twice during this study, and each occurrence was brief (<5 min) and exhibited by a different male.

Fig. 3. Nestling provisioning rates of (a) male white-throated sparrows following implant treatments. Also, (b) nestling provisioning rates and (c) brooding time of female white-throated sparrows paired with males that received implant treatments. Values are LS means±SE from ANCOVA with nestling age as a covariate. See Fig. 1 or text for implant treatments.

Discussion

Nestling provisioning behavior

In the polymorphic white-throated sparrow, TS males naturally provision young at higher rates (Knapton and Falls, 1983; Kopachena and Falls, 1993; Horton and Holberton, in review) and have lower baseline CORT than WS males during the nestling stage (Horton and Holberton, in review; this study). By experimentally elevating baseline CORT in TS males and inhibiting the effects of CORT in WS males, we essentially reversed morph-specific differences in male provisioning behavior. These results provide strong evidence that, while morph-specific differences in nestling provisioning behavior in male white-throated sparrows may be predictable, it can show phenotypic plasticity that is regulated, directly or indirectly, by levels of baseline CORT.

Following implantation, TS controls fed young at higher rates than WS controls, and the provisioning rates of both control groups were similar to those of unmanipulated males from this same population (Horton and Holberton, in review). In addition, neither TS controls nor WS controls showed changes in body mass, fat score, or pectoral muscle score as a result of implantation. These results suggest that IP implantation alone had little, if any, effect on natural levels of male provisioning behavior or on the ability to maintain body condition. However, both TS controls and WS controls showed higher baseline CORT 4–5 days after implantation when compared to pre-implant levels. This pattern may reflect either an effect of the implant on baseline CORT levels (see Horton et al., 2007), or an elevation in baseline CORT in response to the increasing energetic demands of provisioning a growing brood (Landys et al., 2006; Bokony et al., 2009). Nonetheless, TS CORT males had higher post-implant baseline CORT levels compared to TS controls and WS RU486 males, but not compared to WS controls (Fig. 2a). Furthermore, post-implant baseline levels in TS CORT males were within natural range for parental males in this population (range: 1–33 ng/mL), and well below those of unmanipulated TS males exposed to capture and handling stress during the nestling stage (mean: 59 ng/mL±11.2; Horton and Holberton, in review). Thus, the reduced provisioning behavior of TS CORT males occurred with plasma CORT concentrations representing elevated baseline levels rather than levels that arise through the adrenocortical response. To our knowledge, the present study is the first to show that biologically relevant increases in baseline CORT can inhibit nestling provisioning behavior in passerines.

At this time, it is not clear whether our CORT manipulations affected male provisioning behavior through the direct actions of CORT on target tissues in the brain, or indirectly through its interactions with other hormones. The inhibitory effects of increased baseline CORT on the parental behavior of TS CORT males may have resulted through the activation of a larger number of either high-affinity mineralocorticoid receptors (MR) or low-affinity glucocorticoid receptors (GR). Current evidence suggests that the MR is almost fully occupied at low (or basal) plasma CORT concentrations (see Landys et al., 2006). Thus, the reduced provisioning behavior of TS CORT males occurred with plasma CORT concentrations representing elevated baseline levels rather than levels that arise through the adrenocortical response. To our knowledge, the present study is the first to show that biologically relevant increases in baseline CORT can inhibit nestling provisioning behavior in passerines.

Here, we argue that the inhibitory effects of increased baseline CORT on provisioning behavior, as seen in TS CORT males, are mediated through activation of the GR in the brain, because WS males given RU486, a GR antagonist, fed nestlings at higher rates than WS controls. RU486 binds to a GR-like cytosolic receptor in the brain of the closely related white-crowned sparrow (Breuner et al., 2001), and there is increasing evidence that RU486 inhibits behavioral effects of exogenous glucocorticoids in birds (Koch et al., 2002; Landys et al., 2006).
been shown to promote food-searching behavior (e.g., Wing et al., 1988; Landys et al., 2004a). In the present study, however, baseline CORT levels of WS males were not affected by RU486 treatment (also see Landys et al., 2004b; Horton et al., 2007), suggesting that the increased provisioning rates of WS RU486 males did not result from an RU486-induced change in circulating baseline CORT levels. Instead, our results support the hypothesis that, in white-throated sparrows, the inhibitory effects of increased baseline CORT on male provisioning behavior are mediated, at least in part, by activation of the GR.

It is also possible that CORT influences male provisioning behavior indirectly through its effects on other hormones. For example, the hormone prolactin (PRL) is believed to play an integral role in the initiation and maintenance of parental care in birds (Buntin, 1996). Indeed, prolactin has been shown to influence morph-specific differences in the provisioning behavior of male house finches (Carpodacus mexicanus; Badyaev and Duckworth, 2005). Recent evidence suggests that CORT and PRL can interact to regulate parental behavior (e.g., Koch et al., 2004; Criscuolo et al., 2006), and PRL secretion in response to acute stress may counter the inhibitory effects of the adrenocortical response on parental behavior (Chastel et al., 2005; Angelier et al., 2007b). Experimental increases in CORT can decrease plasma PRL concentrations (e.g., Criscuolo et al., 2005, 2006). Thus, our CORT manipulations may have influenced provisioning behavior indirectly by affecting PRL secretion. Further study on PRL's role in white-throated sparrow parental care, and on the relationship between PRL and CORT during the nestling stage, is warranted. It should be noted that RU486 is also a progesterone receptor antagonist (Cadepond et al., 1997). Thus, it is also possible that the effects of RU486 on the provisioning behavior of WS males involved progesterone inhibition.

Parental care versus self-maintenance

Regardless of how CORT regulates the behavior, our results support the hypothesis that elevated baseline CORT during the nestling stage can act to reduce nestling provisioning rates (also see Silverin, 1986; Almasi et al., 2008). This finding may seem incongruous due to the well-known permissive effects of CORT on foraging behavior in birds (Astheimer et al., 1992; Gray et al., 1990; Wingfield et al., 1990; Landys et al., 2006; Lohmus et al., 2006; Holberton et al., 2007). But, in the context of nestling provisioning behavior, it is important to distinguish whether CORT's permissive effects on foraging translate to increased food delivery to offspring. CORT has been shown to promote food-searching behavior (e.g., Wingfield et al., 1990; Landys et al., 2004b), which could help parents find more food for their young. However, CORT is also a potent stimulator of food intake (Wingfield et al., 1990; Dallman et al., 1993; Landys et al., 2004b; La Fleur, 2006; Landys et al., 2006), and this effect could either augment or detract from nestling provisioning efforts. For example, if parents ingest food when foraging and regurgitate to provision their young, then a CORT-induced increase in food intake may indeed facilitate nestling provisioning efforts by promoting parental hyperphagia (e.g., in Streptopelia risoria; Lea et al., 1992; Koch et al., 2002, 2004). On the other hand, for bird species that do not ingest the food they deliver to nestlings, including white-throated sparrows, a CORT-induced increase in food intake during foraging would correspond to an increase in self-maintenance activity. Thus, if resources are limited, elevated baseline CORT during the nestling stage could promote self-maintenance activity at the expense of reduced nestling provisioning in these species (Silverin, 1986; Almasi et al., 2008; Jenni-Eiermann et al., 2008).

Recent work on seabirds suggests that the effects of CORT on self-maintenance and parental behaviors are context dependent, and that food availability, body condition, and parent age can serve as modifying factors with respect to how CORT levels ultimately influence parental care (Kitsysky et al., 2001; Angelier et al., 2006; Angelier et al., 2007a; Doody et al., 2008). During periods of low food availability, for example, elevated baseline CORT may increase foraging efforts in such a way as to allow parents to uphold both self-maintenance and offspring provisioning (Kitsysky et al., 2001; Angelier et al., 2007a; Doody et al., 2008), but this increase in time spent foraging may come at the expense of a reduction in other forms of parental care (e.g., brooding or guarding chicks; Kitsysky et al., 2001; Angelier et al., 2007a). Furthermore, individuals in good body condition may be more likely to maintain brood provisioning in response to elevated CORT levels, while those in poor condition may invest in self-maintenance at the expense of parental care (Angelier et al., 2007a; Doody et al., 2008). Food availability and body condition appear to be important modifying factors in species that ingest offspring provisions (e.g., Rissa tridactyla; Kitsysky et al., 2001; Angelier et al., 2007a), and in species that do not ingest offspring provisions (e.g., Uria aalge; Doody et al., 2008). Additional study is needed to elucidate CORT's role in mediating self-maintenance and parental care under different circumstances.

It is possible that our CORT manipulations altered the allocation of foraging activity (i.e., for self vs. for young) in parental white-throated sparrow males. Although the decline in body mass observed in TS CORT males does not directly support this hypothesis, prolonged CORT manipulations can redirect intermediary metabolism independent of effects on feeding behavior (Holberton, 1999; Dallman et al., 1993; Holberton et al., 2007), which confounds our ability to assess self-foraging activity from changes in body mass. In a previous implant validation study on captive white-throated sparrow males, we used identical implant treatments (i.e., same size, dose, and delivery duration) as were used in this field study. In the captive study, elevated baseline CORT increased food intake while RU486 decreased food intake (Horton, 2007), which supports the hypothesis that baseline CORT levels alter the allocation of foraging activity of parental males. Furthermore, captive CORT-implanted males showed increased fat deposition and pectoral muscle catabolism as did TS CORT males in the present study (Horton, 2007). Indeed, CORT implants induced muscle catabolism in captive males despite increased food intake and ad libitum food supply (Horton, 2007). Thus, the pectoral muscle loss observed in TS CORT males, which explains their decline in body mass, could have occurred despite increases in self-foraging activity.

Though it remains unclear how our CORT manipulations influenced self-maintenance activity, a previous study on white-throated sparrows suggests that females of both morphs invest heavily in parental care at the expense of self-maintenance (Horton and Holberton, in review). These females provision young at high rates and exhibit low baseline CORT throughout the nestling stage despite significant declines in their body condition (Holberton and Holberton, in review). Thus, during the energetically demanding chick-rearing period, white-throated sparrow females may suppress baseline CORT secretion to favor high levels of parental care at the expense of self-maintenance. It is also possible that TS males maintain low baseline CORT in favor of high levels of parental care, but both parental TS males and WS males appear to maintain body condition while provisioning young despite differences in baseline CORT levels during the nestling stage (Horton and Holberton, in review).

Morph-specific behavior

This study generated experimental data to support the hypothesis that variation in CORT secretion influences morph-specific
parental care in white-throated sparrows (Horton and Holberton, in review), which increases our understanding of how endocrine mechanisms can underlie behavioral variation in this, and other, polymorphic species (Knapp, 2004; Maney et al., 2005; Sinervo and Svensson, 1998; Spinney et al., 2006; Pryke et al., 2007; Almasi et al., 2008; Maney, 2008; Lake et al., 2008). Yet, we have much to learn about morph-specific behavior in white-throated sparrows. For example, if male morphs invest similarly in self-maintenance activity, but WS males invest less in nesting provisioning than do TS males, then how do WS males spend their ‘extra’ time during the nestling stage? Re-nesting and double-brooding is common in white-throated sparrows (Falls and Kopachena, 1994; Horton, personal observation); thus, extra-pair mating opportunities often overlap with parental stages. Since WS males have been shown to sire more extra-pair offspring than TS males (Tuttle, 2003), perhaps WS males invest in extra-pair mating effort at the expense of parental care. It is also possible that the more aggressive WS males contribute more to nest defense than do TS males; however, morph differences in nest defense have not been studied. An inclusive study of a suite of male behaviors during the nestling stage is needed to clarify the alternative reproductive strategies of male morphs.

Our study also revealed an unexpected, but curious, morph-specific difference in the behavioral responses of females to the altered provisioning rates of their mates. Contrary to our predictions, neither female morph compensated for a reduction in their mate’s provisioning rate. Indeed, the parental behavior of TS females mated with WS RU486 males (higher provisioning) did not differ from that of TS females mated to WS controls (lower provisioning). Yet, the provisioning behavior of WS females seemed to mirror those of their TS male partners; that is, WS females mated with TS CORT males (lower provisioning) actually provisioned nestlings less than WS females mated with TS controls (higher provisioning). Although the adaptive significance of this differential response by female morphs remains unclear, this finding provides new information for evaluating alternative reproductive strategies in female white-throated sparrows.

Conclusions

Here, we showed that elevations in baseline CORT can inhibit nestling provisioning behavior in passerine birds, and this finding contributes to the growing evidence that baseline CORT mediates parental care and self-maintenance during breeding (Silverin, 1986; Kitaysky et al., 2001; Pereyra and Wingfield, 2003; Angelier et al., 2007a; Muller et al., 2007; Jenni-Eismann et al., 2008; Almasi et al., 2008; Doody et al., 2008). Thus, as with the adrenocortical response, modulating baseline CORT can be an important mechanism for balancing current reproductive success with survival and future reproduction. Recent studies have found positive relationships between baseline CORT and survival (Commendant et al., 2003; Cote et al., 2006; Cabezás et al., 2007; Lancaster et al., 2007), and negative relationships between baseline CORT and current reproductive success (Criscuolo et al., 2005; Angelier et al., 2007c; Bonier et al., 2007; Buck et al., 2007; Ellenberg et al., 2007). However, the interrelationship between baseline CORT, reproductive success, and survival is certainly complex, and a number of intrinsic and extrinsic factors are likely involved. Further research aimed at elucidating the role of CORT, and other hormones, in mediating fitness trade-offs is clearly warranted (see Breuner et al., 2008).

In particular, we encourage future research designs to consider how different ranges of baseline CORT affect parental behavior and self-maintenance activity under different circumstances. Also, because patterns of baseline CORT and the adrenocortical response to perturbation can vary among species according to differences in their life history strategy (Bokony et al., 2009), it is important to consider how CORT’s effects on physiology and behavior may likewise vary according to life history. We believe experimental manipulations that target biologically relevant ranges in free-living birds hold great promise for increasing our understanding of the endocrine system’s role in vertebrate life history variation.

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