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Photoperiodically-induced changes in hypothalamic–pituitary–adrenal axis sensitivity in captive house sparrows (*Passer domesticus*)

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Abstract

We used captive house sparrows (*Passer domesticus*) to identify regulatory mechanisms underlying seasonal (mimicked by changes in photoperiod) and diel differences in corticosterone output. Corticosterone responses were measured during three simulated seasons: short-day and long-day photoperiods and while birds underwent a pre-basic molt. Under all three conditions we tested for adrenal sensitivity by injecting exogenous ACTH, for pituitary sensitivity by injecting corticotropin-releasing factor (CRF) and arginine vasotocin (AVT), and for diel changes by repeating the injections during the day and at night. The daytime adrenal sensitivities were greatest on long days, lower on short days, and lowest during molt. These data suggest that reductions in either adrenal sensitivity to ACTH and/or capacity to secrete corticosterone could explain lowered endogenous corticosterone titers during molt. Furthermore, adrenal sensitivity to ACTH and pituitary sensitivity to AVT appeared to be greatest at night. This suggests that both the adrenal's sensitivity to the ACTH signal and the pituitary's capacity to secrete ACTH might provide a mechanism allowing for diel changes in corticosterone titers. This differs substantially from what is known about diel regulation in rodents. Taken together, these data provide further evidence that there are complex regulatory mechanisms controlling diel and seasonal changes in corticosterone titers in birds.

Keywords: Bird; Circadian; Seasonal rhythms; Glucocorticoids

1. Introduction

Corticosterone concentrations in captive birds vary daily (*e.g.* Dusseau and Meier, 1971; Breuner et al., 1999; Ramenofsky et al., 1999; Romero and Remage-Healey, 2000; Landys et al., 2004) and seasonally (*e.g.* Marra et al., 1995; Kotrschal et al., 1998; Romero and Wingfield, 1999; Piersma et al., 2000) in a variety of species. These variations occur in both the basal (nonstressed) corticosterone concentrations as well as the corticosterone released in response to stress. In general, corticosterone titers are lower during the day and higher at night (*e.g.* Breuner et al., 1999), and higher during breeding and lowest when birds are undergoing a pre-basic molt (reviewed by Romero, 2002). Both daily and seasonal variations indicate that corticosterone release must be differentially regulated, but how that regulation occurs is not fully known.

Corticosterone release is the final step of an endocrine cascade forming the hypothalamic–pituitary–adrenal (HPA) axis that begins in the brain (Romero, 2004). The hypothalamus receives signals from higher brain centers to initiate the release of corticotrophin-releasing factor (CRF) and arginine vasotocin (AVT). CRF and AVT travel to the pituitary via the hypothalamic portal system and elicit pituitary release of adrenocorticotropin (ACTH) (Carsia et al., 1986; Castro et al., 1986). In many avian species AVT appears to provide a more potent secretory signal (*e.g.* Castro et al., 1986; Rich and Romero, 2005). ACTH then travels via the peripheral circulation and stimulates the production and release of corticosterone (Carsia et al., 1987). Consequently, variation in corticosterone release must be regulated at one or more of these steps in the HPA axis.

Daily variation in corticosterone release is well characterized in rodents and is primarily driven by changes in CRF release (reviewed by Dallman et al., 1993), but to our knowledge this has not been verified in birds. Regulation of seasonal variation has been the subject of comparatively few studies, mostly in birds. Available data suggest that seasonal regulation occurs at

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various points of the HPA axis and appears to be species specific. Some species modulate the sensitivity of the adrenal tissue to the ACTH signal (Romero and Wingfield, 1998), others modulate the pituitary's sensitivity to CRF and/or AVT (Romero et al., 1998a), and still others appear to modulate the CRF and/or AVT signal coming from the hypothalamus (Astheimer et al., 1995; Romero et al., 1998b,c). In these last species, regulating the release of CRF and/or AVT may be the physiological correlate of an animal changing the interpretation of whether a stimulus is stressful (Wingfield, 2005).

Recent work indicates that house sparrows (*Passer domesticus*) are another species that show both daily and seasonal variation in corticosterone concentrations. Field studies indicate that house sparrows have higher corticosterone titers during breeding than during molt (Hegner and Wingfield, 1990; Breuner and Orchinik, 2001; Martin et al., 2005; Romero et al., 2006). Regulation of this variation is via a complex mix of changes in adrenal, pituitary, and hypothalamic function (Romero, 2006). The regulatory site of the HPA axis differs depending upon the season and the population under study. Seasonal variation in house sparrows can be mimicked with photoperiod manipulation in the lab (Rich and Romero, 2001), so our first goal was to examine seasonal regulation of the HPA axis under controlled conditions by removing the confounding variables inherent in field studies. Furthermore, captive house sparrows show classic daily variation in corticosterone release with titers higher at night than during the day (Rich and Romero, 2001). Our second goal was to determine whether birds regulate diurnal HPA axis changes in a manner similar to mammals.

2. Materials and methods

Wild house sparrows were captured in Eastern Massachusetts and brought into captivity. Birds were a mixture of males and females, but no distinction was made for sex since earlier work indicated that male and female captive house sparrows do not differ in their responses to stress (Rich and Romero, 2001). Birds were housed in large indoor flight aviaries for at least 2 weeks to acclimate to captivity and subsequently transferred to an experimental room. They were then housed in pairs in separate cages with all cages placed next to each other so that no bird was isolated. The temperature in all rooms was set at 25 °C and birds were provided food and water *ad libitum*. All procedures were performed according to AALAC procedures and approved by the Tufts University Institutional Animal Care and Use Committee.

The experiments were performed under 3 conditions: when the experimental room was held on a short-day photoperiod (an 11L:13D light:dark cycle with lights on at 07:00); when the experimental room was held on a long-day photoperiod (a 19L:5D cycle with lights on at 02:00); and when the experimental room was held on a long-day photoperiod and the house sparrows were undergoing a pre-basic molt. House sparrows usually undergo a pre-basic molt between August and October (Lowther and Cink, 1992), beginning to replace feathers 3 to 4 months after the onset of long days. Birds remained on long days until they began to molt. Laparotomies were not performed, so gonadal status during the long-day photoperiod was unknown. Birds were given a minimum of 2 weeks to acclimate to the new photoperiod before sampling commenced.

Under each condition, blood samples were obtained by puncturing the alar vein and collecting approximately 40 μl of the upwelling blood in heparinized microhematocrit tubes. Each bird was injected with a HPA releasing hormone within 2–3 min of entering the room and immediately placed in an opaque cloth bag for a 30 min period of restraint. The 30 min of restraint was intended to make these data directly comparable to an earlier field study on house sparrows (Romero, 2006). Post-injection samples were collected at the end of the 30 min of restraint. Adrenal sensitivity was tested by injecting 100 or 200 IU/kg body mass of porcine ACTH (Sigma Chemical Co.). Pituitary sensitivity was tested by injecting 3 μg/kg body mass of ovine CRF (Sigma Chemical Co.), 3 μg/kg body mass AVT (Sigma Chemical Co.), or a combination of 3 μg/kg body mass of both CRF and AVT. These doses have been shown to saturate the pituitary response in several avian species (*e.g.* Castro et al., 1986; Westerhof et al., 1992; Romero et al., 1998b) and CRF and AVT were injected in tandem because CRF and AVT can synergize (stimulate more ACTH release when injected together than simply an additive response) in some species (Vale et al., 1983). All injections were in a volume of 10 μl (including the CRF + AVT injection), and were administered intrajugularly. Lactated Ringer's Solution (sodium = 130 meq, chloride = 109 meq, calcium = 3 meq, potassium = 4 meq, lactate = 28 meq) served as the vehicle and 10 μl was injected as the control. After injection birds were immediately placed into opaque cloth bags for a 30 min period of restraint to stimulate a CORT stress response, followed by taking a second blood sample.

Adrenal and pituitary sensitivities were assessed during both the day (10:30–11:00) and night (22:00–22:30) for all 3 experimental conditions. Both the hormone injected and the time (day or night) were selected randomly and administered in a repeated measures design. All injections were randomly spaced over at least a 6-week period and no bird was injected more than twice per week to ensure that the birds had sufficiently replenished their blood volume between bleeds and were thus unstressed from the previous sampling. We also verified that the birds were unaffected by the repeated sampling by ensuring that they lacked any symptoms of long-term, or chronic, stress (such as loss of body weight, lethargy, *etc.* Sapolsky et al., 2000). Samples were collected during the night while using a white light bulb filtered to allow only blue light into the room. This provided sufficient light for sample collection, but does not penetrate the avian skull to stimulate photoreceptors on the pineal as do other wavelengths (Oishi and Lauber, 1973).

We originally intended that all birds would receive all 36 injections (5 hormone injections plus the Ringer's control, both day and night, for 3 experimental conditions). However, lighting malfunctions and a few mortalities over the long time in captivity made this impossible. Consequently, new birds were entered into the experiment at different points. We used the