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The low-affinity glucocorticoid receptor regulates feeding and lipid breakdown in the migratory Gambel's white-crowned sparrow *Zonotrichia leucophrys gambelii*

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Summary

Plasma corticosterone increases during spring migration in a variety of bird species, including the Gambel's white-crowned sparrow *Zonotrichia leucophrys gambelii*. Corticosterone is elevated specifically in association with migratory flight, suggesting that corticosterone may promote processes such as energy mobilization and/or migratory activity. General effects of glucocorticoids support such a prediction. Because glucocorticoids exert permissive effects on food intake, corticosterone may also participate in the regulation of migratory hyperphagia. To examine the role of corticosterone during migration, we induced Gambel's white-crowned sparrows to enter the migratory condition and compared food intake and locomotor activity between controls and birds injected with RU486 – an antagonist to the low-affinity glucocorticoid receptor (GR). In addition, we investigated effects of RU486 in birds that were subjected to a short-term fast. Results indicate that RU486 did not affect locomotor activity. However, consistent with its effects in mammals, RU486 suppressed food intake. Thus, hyperphagia and migratory restlessness, the two behaviors that characterize migration, may be regulated by different mechanisms. Lastly, RU486 antagonized fasting-induced lipid mobilization, as evidenced by decreased plasma free fatty acids. Thus, data on spring migrants suggest that endogenous corticosterone levels act through the GR to support hyperphagia and that the GR promotes availability of lipid fuel substrates in association with periods of energetic demand, e.g. during migratory flight.

Key words: corticosterone, glucocorticoid (type II) receptor, food intake, hyperphagia, locomotor activity, migratory restlessness, lipolysis, metabolite.

Introduction

Elevated plasma levels of corticosterone have been identified during the course of spring migration in many avian species (e.g. Holberton, 1999; Landys-Ciannelli et al., 2002; Piersma et al., 2000). For example, in comparison to wintering individuals, migrating Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*) show elevated plasma corticosterone (Romero et al., 1997). A recent study suggests that, as in other migrants (e.g. Landys-Ciannelli et al., 2002; Piersma et al., 2000), plasma corticosterone in the white-crowned sparrow increases specifically in association with migratory flight (Landys, 2003). Thus, corticosterone may promote flight-associated processes during the period of migration, e.g. energy mobilization and/or migratory restlessness – the intense and persistent movements that typify birds in the migratory condition. General effects of glucocorticoids support such a prediction: past research has demonstrated that glucocorticoids regulate locomotor activity (e.g. Astheimer et al., 1992; Bruener et al., 1998; Challet et al., 1995) and also the mobilization of lipid energy substrates (e.g. Dallman et al., 1993; Santana et al., 1995).

Research on migratory birds is consistent with the prediction that corticosterone may regulate migratory restlessness. Dolnik and Blyumental (1967) found that injections of cortisol increased daytime activity of pre-migratory chaffinches (*Fringilla coelebs*) to levels seen in migrating conspecifics, and Meier and Martin (1971) found that injections of corticosterone increased daytime and nocturnal activity of photosensitive white-throated sparrows (*Zonotrichia albicollis*) placed on long days. However, results from these experiments may be confounded because administration of glucocorticoids typically elevates plasma concentrations to maximal or even pharmacological levels (e.g. Astheimer et al., 1992; Gray et al., 1990) that are not representative of the migratory condition; corticosterone levels in migratory birds generally circulate at intermediate concentrations (e.g. Jenni et al., 2000; Landys-

Ciannelli et al., 2002; Romero et al., 1997; Schwabl et al., 1991). Thus, we suggest that inhibiting glucocorticoid receptors for corticosterone may be a more salient technique when investigating the role of seasonal corticosterone levels. This approach addresses effects of endogenous elevations that are appropriate to the period of migration.

As already described in mammals, birds possess two intracellular receptors for glucocorticoids: a low-affinity glucocorticoid receptor (GR) and a high-affinity mineralocorticoid receptor (MR; Breuner et al., 2001). The compound mifepristone is widely used as an antagonist to the GR and appears to effectively bind to and inhibit the GR in birds. For example, RU486 binds a GR-like receptor in tissue preparations of the Gambel's white-crowned sparrow (Breuner et al., 2003), and administration of RU486 inhibits behavioral effects induced by dexamethasone – a GR agonist – in the ring dove *Streptopelia risoria* (Koch et al., 2002). To verify the efficacy of RU486 in the Gambel's white-crowned sparrow, we conducted a validation study in which we compared the effects of corticosterone in the presence and absence of RU486.

Given that past and current results suggest an antagonistic role for RU486 with respect to the GR, we used RU486 to determine how endogenous corticosterone levels act through the GR to regulate migratory behavior in Gambel's white-crowned sparrows. We treated spring migrants with RU486 or with vehicle and tested for effects on locomotor activity and food intake. We then compared effects of RU486 treatment between spring migrants and wintering birds to determine whether the role of corticosterone varies with life-history stage, i.e. according to the distinct phenotypic states that maximize fitness in a predictably oscillating environment (Jacobs, 1996).

Based on general effects of glucocorticoids, we hypothesized that RU486 treatment would suppress migratory restlessness during spring migration, especially because captive white-crowned sparrows exhibit distinct elevations in plasma corticosterone in association with migratory activity: 7.9±1.2 ng ml^{-1}, as compared with 2.6±0.2 ng ml^{-1} in wintering birds (Landys, 2003). Also, because corticosterone has been shown to play a permissive role in feeding (Dallman et al., 1993; King, 1987), we predicted that RU486 would suppress migratory hyperphagia.

We also tested the effects of elevated corticosterone levels in relation to an energetic challenge. We administered a second dose of RU486 to the same migratory or wintering birds, removed food from both groups and monitored resulting locomotor activity and plasma metabolites. We predicted that RU486 would suppress the fasting-associated increase in escape behavior (Lynn et al., 2003). Because glucocorticoids have been shown to stimulate glucose production (e.g. Davison et al., 1983; Santana et al., 1995) and lipid mobilization (e.g. Dallman et al., 1993; Mukherjee and Mukherjee, 1973), we also predicted that RU486 treatment would inhibit the fasting-associated mobilization of energy reserves.

Materials and methods

Animals

Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*; Zink and Blackwell, 1996) were captured during fall migration in Sunnyside, Washington in September and October 2001–2002. After capture, sparrows were housed in outdoor flight aviaries at the University of Washington, Seattle. At least two weeks prior to the initiation of experiments, birds were placed in indoor environmental chambers. The temperature in the chambers was set to vary between 20°C during the day and 12°C during the night. Birds were provided with *ad libitum* bird chow and wild bird seed mix at all times other than during controlled fasting periods. A light (<1 lux) was provided during the dark period to simulate starlight/moonlight, without which night migrants such as the white-crowned sparrow typically do not express migratory activity (M. Ramenofsky, unpublished observation). All experiments were performed under the approval of the Institutional Animal Care and Use Committee at the University of Washington (protocol number: 2212-29).

RU486 validation study

First, we tested the efficacy of RU486 as a corticosterone antagonist in the Gambel's white-crowned sparrow. We compared daily food intake and fat depots among three groups of birds. (1) Controls: birds with empty implants and vehicle (peanut oil; Hain Celestial Group, Inc., Uniondale, NY, USA) injections (control group), (2) B group: birds with implants of corticosterone and vehicle injections and (3) B+RU486 group: birds with implants of corticosterone and injections of RU486. Nine animals were included in each treatment group. We administered 1.4 mg of RU486 (Sigma, St Louis, MO, USA) *via* subcutaneous injection to each bird, i.e. at a dose of ~50 mg kg^{-1}. RU486 was suspended in vehicle *via* sonication. Implants were made from 12 mm sections of Silastic laboratory tubing (i.d. 1.47 mm, o.d. 1.96 mm) that were sealed with silicone at both ends. A small hole was cut at one end immediately before implantation to promote corticosterone delivery. We implanted birds subcutaneously one day after the first injection and monitored birds for another seven days, injecting birds with RU486 or with vehicle every other day.

The birds investigated in the validation study were placed into environmental chambers in April 2002. Chambers were kept on a late spring photoperiod (14 h:10 h L:D). Treatment-induced changes in fat deposits and daily food intake were recorded. We scored the deposits of fat in the furcular fossa and in the abdominal cavity on an arbitrary scale ranging from 0 to 5 (Wingfield and Farner, 1978). A score of 0 represents no fat, and a score of 5 indicates the presence of bulging fat bodies. Changes in fat score were compared among treatment groups with a Kruskal–Wallis one-way analysis of variance (ANOVA) on ranks. Changes in daily food intake were investigated with a parametric one-way ANOVA.

Blood samples for the determination of plasma corticosterone were collected in the early afternoon (6 h after lights-on) one day after implantation procedures. We

punctured the alar wing vein with a sterile 26-gauge needle and drew pooling blood droplets into heparinized micro-hematocrit capillary tubes. Blood samples were collected within 3 min of entry into chambers. Corticosterone levels determined from these samples reflect undisturbed baseline concentrations (Wingfield et al., 1982). Plasma corticosterone in blood samples was determined by radioimmunoassay (RIA), as described below. Effects of treatment on corticosterone levels were investigated with a one-way repeated measures ANOVA. Tukey tests were used for *post-hoc* comparisons. All corticosterone data were $\log_{10}$-transformed for use in statistical analyses.

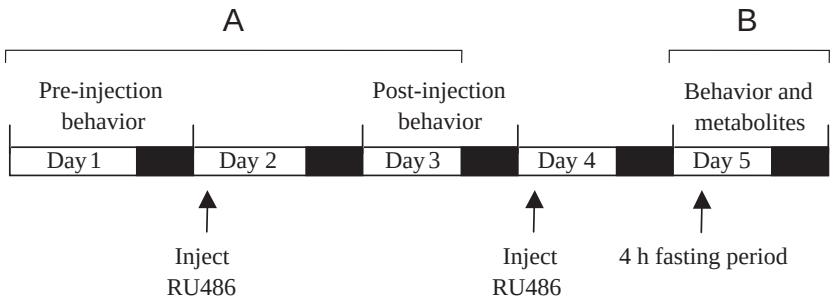

Fig. 1. Pictorial representation of the experimental design used to evaluate effects of RU486 treatment in the Gambel's white-crowned sparrow. Spring migrants and wintering birds were held on 16 h:8 h L:D and 8 h:16 h L:D, respectively. (A) Experimental protocol designed to investigate the behavioral responses of birds to RU486 treatment under *ad libitum* food conditions. Treatment effects were tested by comparing birds one day before and one day after RU486 injections. Controls received vehicle injections. Birds were monitored for differences in locomotor activity (as determined by motion detectors in registration cages) and daily food intake. (B) Experimental protocol designed to investigate the behavioral and metabolic responses of birds to RU486 treatment during a food manipulation trial. On the morning following the second RU486 injection, food in chambers was removed for four hours. Birds in a second chamber continued to experience *ad libitum* food conditions. Locomotor activity and plasma metabolites were monitored during the trial. After food was replaced, locomotor activity and food intake were measured to determine how RU486 affects recovery. Controls continued to receive vehicle injections.

Effects of RU486 treatment during migration and wintering

In January–February 2002 and January 2003, birds in the wintering condition were placed into environmental chambers kept on short days (8 h:16 h L:D). Four weeks before the initiation of experiments, half of the birds were switched to a long-day photoperiod (16 h:8 h L:D) to induce entry into the migratory state (King and Farner, 1963; Ramenofsky et al., 2003).

We administered birds with RU486 at a dose of ~50 mg kg^{-1}. Increasing evidence suggests that, as in mammals, RU486 administration in birds antagonizes the GR (Breuner and Orchinik, 2001; Breuner et al., 2003; Koch et al., 2002). Because RU486 also blocks the progesterone receptor, differences between RU486-treated birds and controls should be interpreted with some caution. Nevertheless, because we measured behavioral and physiological responses that are classically attributed to glucocorticoids rather than to progesterone (Hadley, 1999; Nelson, 2000), we maintain that the effects of RU486 in this study are most likely to be representative of action at the level of the GR.

Effects of RU486 on activity and food intake in ad libitum food conditions

Injections of RU486 or vehicle were administered in the morning, within 4 h of lights turning on. We tested for treatment effects by comparing birds one day before and one day after injections (see Fig. 1A). Birds were monitored for locomotor activity (as determined by motion detectors in registration cages) and food intake.

Effects of RU486 administration on locomotor activity and food intake were analyzed with two-way repeated measures ANOVA tests. Sampling day (pre-injection or post-injection) was included as a repeated factor. RU486 administration was considered to have an effect on measured responses if the interaction between treatment group and sampling day was significant, i.e. if treatment caused a different pattern of change in RU486-treated birds than in controls.

To verify that the long-day photoperiod used here (16 h:8 h L:D) was sufficient to induce birds to enter the migratory condition (characterized by night-time restlessness and increased feeding; Ramenofsky et al., 2003), we compared night-time locomotor activity and daily food intake between spring migrants and wintering birds. Only data collected before the initiation of treatment were considered. Differences were statistically examined with Mann–Whitney rank sum tests. *P*-values were Bonferroni-adjusted.

Effects of RU486 on activity and energy metabolism during a fast

Spring migrants and wintering birds were given a second injection of RU486 or vehicle 2 days after the first injection (see Fig. 1B). On the morning following the second injection, food was removed from each cage for 4 h to determine how RU486 affects energy metabolism (as determined from plasma metabolites) and locomotor activity in association with an energetic challenge. Birds in control chambers were held on *ad libitum* food. We continued to monitor locomotor activity after the conclusion of the food trial and also measured food intake to determine how RU486 affected recovery. A two-way ANOVA was used to statistically examine differences in locomotor activity and food intake between treatment groups. Feeding condition (fasted or *ad libitum*) was included in the test as a factor.

Collection of behavioral data

Locomotor activity was recorded throughout the day and

night in registration cages equipped with motion detectors (Radio Shack, Fort Worth, TX, USA; Ramenofsky et al., 2003; Sperry et al., 2003). When birds crossed an infrared beam running parallel to and above their perch, the electric signal was transmitted to a computer and catalogued as an instance of activity by Labview Program (National Instruments, Austin, TX, USA). Activity (hops min^{-1}), as determined by infrared beam-breaks, is reported for the following three periods: early-day (90 min after lights-on until afternoon), late-day (afternoon until 30 min before lights-off) and night (30 min after lights-off until 30 min before lights-on).

Food intake was monitored by weighing food trays with bird food mix before and after a feeding period and is reported here as grams of food consumed per day. Food spillage from trays was minimized by allowing only a small opening in trays for food access.

Blood sampling

To determine effects of RU486 treatment on baseline levels of plasma corticosterone, blood samples were collected in the early afternoon (5 h after lights-on) one day after the first RU486 injection. We collected samples within 3 min of disturbance, as described in the validation study above. Differences in baseline corticosterone between RU486-treated birds and controls were examined with a two-way ANOVA. Life-history stage (spring migrants or wintering birds) was included as a factor.

To determine effects of food removal on baseline plasma corticosterone, we also collected blood samples at the conclusion of the 4-h food manipulation trial (again, 5 h after lights-on). Differences in corticosterone between birds held on the two feeding regimes (fasted or *ad libitum*) were tested with a two-way ANOVA, with treatment group (RU486-treated birds or controls) included as a factor.

Blood collected at the conclusion of the food manipulation trial was also evaluated for concentrations of six plasma metabolites: triglycerides, free fatty acids, glycerol, uric acid, β-hydroxybutyrate and glucose. Triglycerides are the storage form of lipids. They are synthesized in the liver and are transported to peripheral tissues for deposition into fat bodies, but can also enter the blood *via* dietary absorption (Robinson, 1970). Free fatty acids and glycerol are released when triglycerides are hydrolyzed and indicate lipid mobilization from adipose tissue (Elia et al., 1987; Scow and Chernick, 1970). Uric acid results from the breakdown of protein, which can originate from either the muscle or the diet (Lindgård et al., 1992; Robin et al., 1987). β-hydroxybutyrate is synthesized predominantly during fasting to replace part of the glucose supply and suggests lipid catabolism and glucose shortage (Robinson and Williamson, 1980). Concentrations of these six plasma metabolites were compared between RU486-treated birds and controls with a two-way ANOVA. Feeding regime (fasted or *ad libitum*) was included as a factor. Plasma metabolite concentrations were $\log_{10}$-transformed for use in statistical analyses.

Plasma assays

Plasma for later determination of corticosterone and metabolites was stored in micro-centrifuge tubes at –20°C and at –80°C, respectively. Corticosterone concentrations were determined at the University of Washington by direct RIA as described by Wingfield et al. (1992). Plasma samples of 20 μl were combined with 180 μl distilled H_2O. 2000 c.p.m. [^{3}H]corticosterone (NET-399, PerkinElmer, Boston, MA, USA) was added and allowed to equilibrate for at least 30 min at 4°C for the determination of percent recovery of steroid. Steroids were extracted for 2 h with 4 ml redistilled dichloromethane and dried under nitrogen gas at 37°C. Dried extracts were resuspended in 550 μl phosphate-buffered saline (0.1% gelatin). Duplicate samples of 200 μl were used for the RIA. Corticosterone concentration was calculated from a standard curve that ranged from 7.8 pg to 2000 pg. Of the remaining re-suspended extract, 100 μl was used to determine the percent steroid recovered after extraction. Corticosterone concentrations determined from the RIA were adjusted to account for percent steroid lost during extraction. We set the limits of detectability for the assay at 15 pg. As calculated from known standards run within each assay, inter-assay variation was 6.0%. As previously determined from 10 known standards analyzed in a separate assay, intra-assay variation was 8.7%.

Metabolite concentrations in blood plasma were determined at the University of Montana, Missoula on a powerwave 340× microplate spectrophotometer (BioTec Instruments, Winooski, VT, USA). Assays were run in 400 μl flat-bottom, 96-well polystyrene microtitre plates (NUNC, Roskilde, Denmark). Glycerol and triglycerides were measured sequentially by endpoint assay (Sigma; GPO-Trinder Reagents A and B; 5 μl plasma, 240 μl reagent A, 60 μl reagent B). Glucose was measured by endpoint assay (Sigma; Infinity Glucose Reagent; 3 μl sample, 300 μl reagent). Free fatty acids were measured by endpoint assay (WAKO Diagnostics, Richmond, Virginia; 3 μl sample, 120 μl reagent A, 240 μl reagent B). Uric acid was measured by endpoint assay (WAKO Diagnostics; 5 μl sample, 300 μl reagent). β-hydroxybutyrate was measured by kinetic assay (Sigma; β-HBA reagent; 10 μl sample, 4 μl β-HBDH enzyme, 250 μl reagent) according to the method described in Guglielmo et al. (2002).

Results

RU486 validation study

Results from the validation study indicate that RU486 is an effective corticosterone antagonist in the Gambel's white-crowned sparrow. RU486 treatment inhibited corticosterone-induced fat deposition (H=7.040, d.f.=2, P=0.030; Fig. 2). In comparison to controls, birds given an implant of corticosterone (the B group) increased in fat score over the course of the experiment (Dunn's *post-hoc* test, P<0.05). By contrast, fat score changed similarly between controls and birds given a corticosterone implant plus an RU486 injection (the B+RU486 group) (P>0.05).

Food intake changed differently among the three groups as

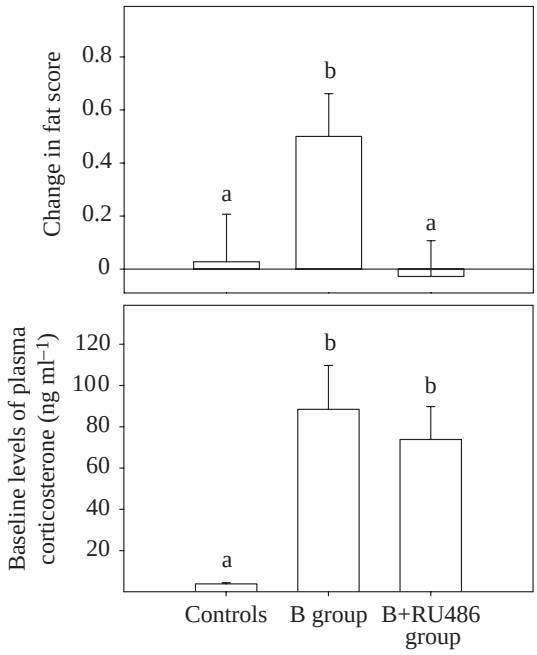

Fig. 2. Validation of RU486 as a corticosterone antagonist in the Gambel's white-crowned sparrow. Changes in fat score and plasma corticosterone (ng ml^{-1}) were compared among three treatment groups: (1) birds with empty implants and vehicle injections (controls), (2) birds with implants of corticosterone and vehicle injections (B group) and (3) birds with implants of corticosterone and injections of 50 mg kg^{-1} RU486 (B+RU486 group). Corticosterone concentrations were determined from blood samples collected in under 3 min of disturbance. Corticosterone implants were effective in elevating plasma corticosterone levels, and caused an increase in fat score. RU486 treatment inhibited corticosterone-induced fat deposition. Nine animals were included in each treatment group. Error bars represent S.E.M. Different letters above bars indicate significant differences among groups.

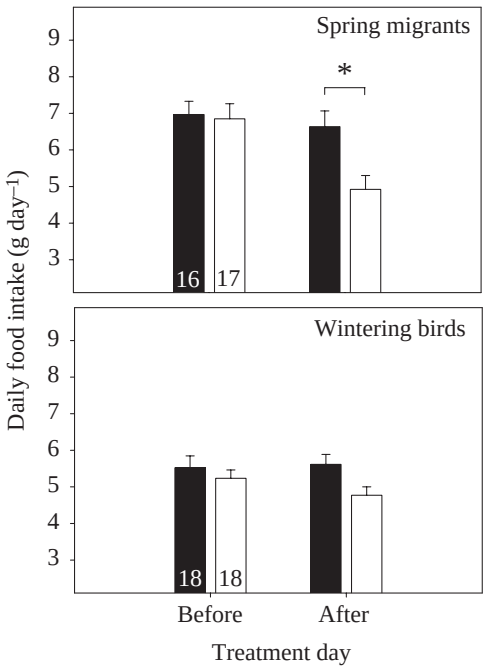

Fig. 3. Effects of RU486 treatment on daily food intake (g day^{-1}) of spring migrants and wintering birds. Administration of RU486 significantly decreased food intake only in spring migrants. Daily food intake is shown before and after RU486 injections. Black bars, controls; white bars, RU486-treated birds. Sample size is indicated. Asterisks indicate significant differences between treatment groups. Error bars represent S.E.M.

a consequence of treatment ($F_{2,24}$=3.767, P=0.038). Food intake of the B+RU486 group decreased in comparison to that of controls (Tukey *post-hoc* test, P<0.05), while food intake of the B group and the controls changed similarly (P>0.05). In response to treatment, daily food intake (± S.E.M.) of the B+RU486 group, the B group and the controls decreased by 2.3±0.3 g, 1.5±0.1 g and 1.0±0.5 g, respectively.

One day after implantation procedures, baseline levels of plasma corticosterone were different among treatment groups ($F_{2,24}$=44.589, P<0.001; Fig. 2). Plasma corticosterone was higher in the B group and the B+RU486 group than in controls (Tukey *post-hoc* tests: P<0.05), suggesting that corticosterone implants were effective in elevating plasma levels of this hormone. Plasma corticosterone was similar between the B group and the B+RU486 group (P>0.05).

Effects of RU486 treatment on activity and food intake in ad libitum *food conditions*

We found that RU486 administration affected the daily food intake of white-crowned sparrows during spring migration (interaction term, $F_{1,31}$=14.992, P<0.001; Fig. 3). Pairwise *post-hoc* tests indicate that food intake in RU486-treated birds and controls was similar before treatment (P>0.05) but was lower in RU486-treated birds after treatment (P<0.05). RU486 did not affect food intake in wintering birds (interaction term, $F_{1,34}$=2.082, P=0.158; Fig. 3).

Locomotor activity in spring migrants and wintering birds was not altered by RU486 treatment during any of the three examined periods: early-day (interaction term, $F_{1,31}$=0.131, P=0.720 and $F_{1,30}$=0.352, P=0.557 for spring migrants and wintering birds, respectively), late-day ($F_{1,31}$=1.713, P=0.200 and $F_{1,30}$=0.043, P=0.838, respectively) and night ($F_{1,31}$=1.330, P=0.258 and $F_{1,30}$=0.393, P=0.535, respectively) (Fig. 4).

As expected, daily food intake (t=1525.00, P<0.002) and night-time locomotor activity (t=688.00, P<0.002) were significantly higher in spring migrants than in wintering birds (see Figs 3, 4), suggesting that the long-day photoperiod conditions employed in this study were effective in inducing birds to enter into a migratory state (Ramenofsky et al., 2003).

Baseline levels of plasma corticosterone were lower in birds treated with RU486 than in controls ($F_{1,65}$=17.740, P<0.001; Fig. 5). Consistent with Romero et al. (1997), baseline corticosterone showed a trend to be higher in spring migrants than in wintering birds ($F_{1,65}$=3.364, P=0.071). The interaction

between season and treatment was not significant ($F_{1,65}$=0.836, P=0.364).

Effects of RU486 on activity and energy metabolism during a fast

Fasting caused an increase in daytime locomotor activity ($F_{1,29}$=45.229, P<0.001 and $F_{1,28}$=18.268, P<0.001 for spring migrants and wintering birds, respectively; Fig. 6). Locomotor activity was not affected by RU486 treatment ($F_{1,29}$=0.181, P=0.674 and $F_{1,28}$=1.115, P=0.300, respectively; Fig. 6). The interaction between feeding regime (fasting or *ad libitum*) and treatment was not significant ($F_{1,29}$=1.273, P=0.268 and $F_{1,28}$=0.792, P=0.381, respectively).

Food manipulation significantly affected plasma metabolite levels. Fasting birds displayed elevated free fatty acids and β-hydroxybutyrate in comparison with birds provided an *ad libitum* diet, both during spring migration and winter (Tables 1, 2). Glycerol also increased with fasting, but only in spring migrants. By contrast, fasting decreased plasma levels of trigyclerides, glucose and uric acid in spring and winter.

RU486 treatment significantly affected plasma levels of metabolites. RU486 decreased free fatty acids in spring migrants but only if birds had been subjected to a fast (Fig. 6; Table 2). By contrast, during winter, RU486 decreased plasma free fatty acids both in fasting and in *ad libitum* birds. RU486 administration did not consistently affect plasma concentrations of any of the other measured metabolites: glucose, triglycerides, glycerol, uric acid or β-hydroxybutyrate (Tables 1, 2).

As expected, baseline levels of plasma corticosterone were higher in fasting birds than in birds held on *ad libitum* food ($F_{1,28}$=6.261, P=0.018 and $F_{1,31}$=18.635, P<0.001 for spring migrants and wintering birds, respectively; Fig. 6), suggesting that birds experienced fasting as a challenge (Lynn et al., 2003; Richardson, 1997). RU486 treatment significantly decreased baseline concentrations of corticosterone ($F_{1,28}$=5.373, P=0.028 and $F_{1,31}$=6.673, P=0.015, respectively; Fig. 6). The interaction between treatment and feeding regime was not significant ($F_{1,28}$=3.999, P=0.055 and $F_{1,31}$=2.433, P=0.129, respectively).

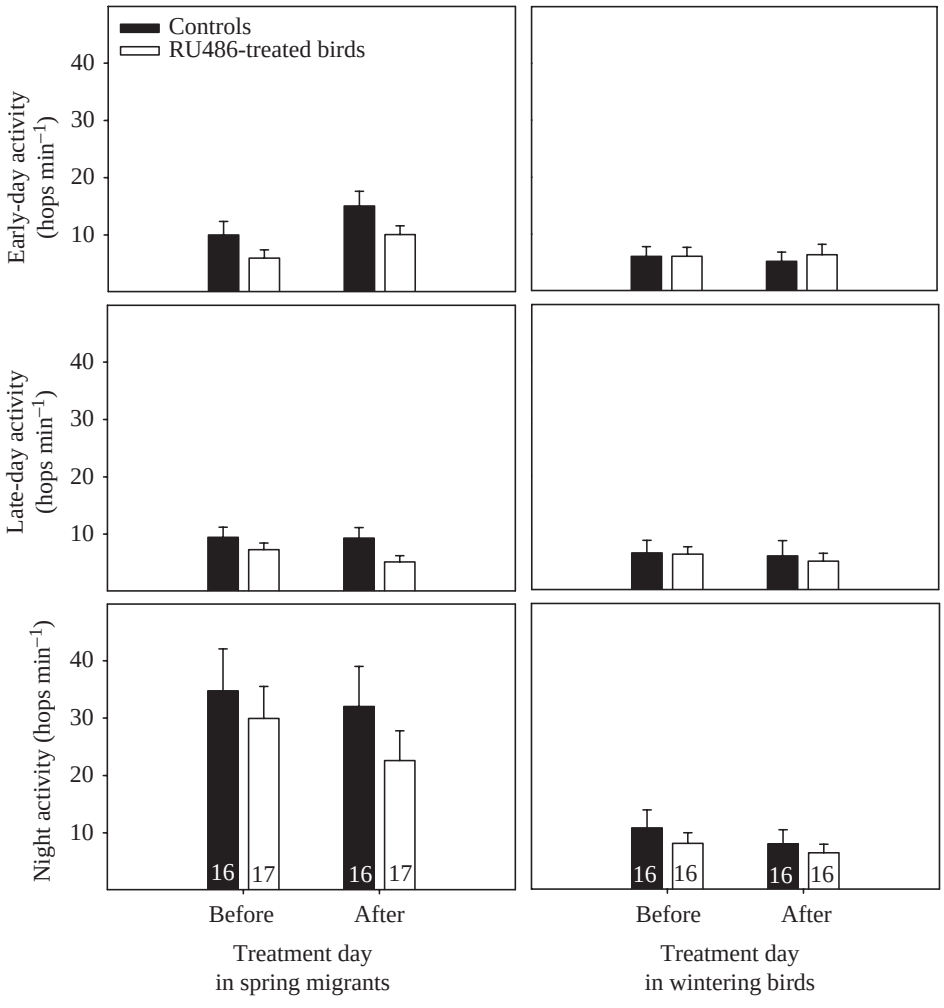

Fig. 4. Effects of RU486 treatment on locomotor activity (hops min^{-1}) in spring migrants and in wintering birds. Locomotor activity was determined with motion detectors in registration cages and was investigated during three periods of the day: early-day, late-day and night. In response to treatment, locomotor activity changed similarly in controls and in RU486-treated birds during all examined periods. Locomotor activity is shown before and after RU486 injections. Sample size is indicated. Error bars represent S.E.M.

Effects of RU486 on activity and food intake during recovery

History of food availability did not affect feeding behavior in spring migrants: food intake was similar between migrants that had been fasted and migrants that had been held on *ad libitum* food ($F_{1,28}$=0.012, P=0.915). By contrast, wintering birds responded to food deprivation with increased feeding ($F_{1,32}$=5.496, P=0.025; Fig. 7).

Administration of RU486 to spring migrants decreased food intake regardless of whether birds had been fasted or kept on *ad libitum* food ($F_{1,28}$=9.961, P=0.004; Fig. 7). The interaction between treatment and feeding history was not significant ($F_{1,28}$=0.229, P=0.636). By contrast, in wintering birds, RU486 decreased feeding only following a fast (interaction term, $F_{1,32}$=5.723, P=0.023; see Fig. 7). RU486 had no effect on overall food intake in wintering birds ($F_{1,32}$=1.391, P=0.247).

During the night following the recovery period, RU486-treated birds

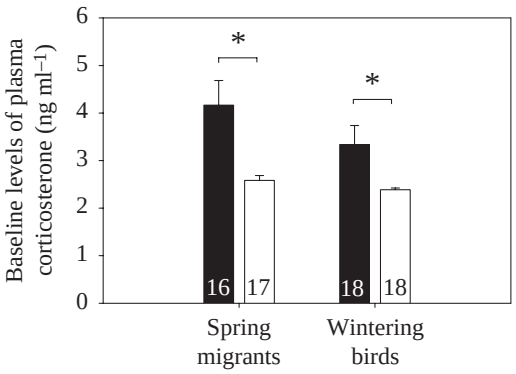

Fig. 5. Effects of RU486 treatment on plasma corticosterone (ng ml^{-1}) in spring migrants and wintering birds. Corticosterone concentrations were determined from blood samples collected in under 3 min of disturbance. Administration of RU486 caused a decrease in plasma corticosterone during both life-history stages. Black bars, controls; white bars, RU486-treated birds. Sample sizes are shown. Asterisks indicate significant differences between treatment groups. Error bars indicate S.E.M.

and controls displayed similar locomotor activity ($F_{1,29}$=0.330, P=0.570 and $F_{1,28}$=0.862, P=0.361 for spring migrants and wintering birds, respectively; Fig. 7). Night-time locomotor activity was also similar between birds that had been fasted and birds that had been held on *ad libitum* food ($F_{1,29}$=0.661, P=0.423 and $F_{1,28}$=3.741, P=0.063, respectively; Fig. 7). The interaction between treatment and feeding history was not significant ($F_{1,29}$=0.265, P=0.611 and $F_{1,28}$=3.221, P=0.083, respectively).

Discussion

RU486 validation study

The validation study demonstrated that RU486 inhibits effects of corticosterone in the Gambel's white-crowned sparrow: administration of RU486 prevented corticosterone-induced fat deposition in this species. RU486 also suppressed food intake. We suggest that effects were generated through antagonism of the GR, because previous research has demonstrated that RU486 binds to a GR-like cytosolic receptor in the white-crowned sparrow (Breuner et al., 2001). Data from other avian systems would strengthen the case for the antagonistic role of RU486 – a potentially valuable tool in determining how endogenous corticosterone levels affect avian behavior and physiology. However, because RU486 also blocks the progesterone receptor, it is important to note that there are limitations to the use of this compound in the investigation of glucocorticoid action.

Although we anticipated that RU486 would antagonize corticosterone-induced responses, effects on circulating corticosterone concentrations were unexpected. In mammals, administration of RU486 typically elevates plasma corticosterone due to elimination of negative feedback (e.g. Healy et al., 1985; Langley and York, 1990), although some studies report the opposite (e.g. Laue et al., 1988). In the present study, RU486 decreased plasma corticosterone (see Fig. 5), an effect that was already evident after 2 h of RU486 treatment (M. Landys, unpublished observation).

Decreased plasma corticosterone commonly results after stimulation of glucocorticoid receptors (e.g. Bugajski et al., 2001; Rich and Romero, 2001), suggesting that RU486 may act as an agonist in relation to corticosterone feedback mechanisms. However, we are confident that, in the white-crowned sparrow, RU486 functions as an antagonist with respect to GR-mediated behavior and energy metabolism. First, we found that RU486 administration inhibits corticosterone-induced fattening in this species. Also, observed effects on food intake and plasma free fatty acids are consistent with those of a GR antagonist (e.g. Castonguay et al., 1984; Dallman et al., 1989; Mukherjee and Mukherjee, 1973). In an effort to explain the variability of RU486 action, we suggest that effects may depend on the presence of specific co-activators or co-repressors at the site of transcription (McKenna and O'Malley, 2000).

The role of corticosterone during migration

Given previous findings (Landys, 2003; Landys-Ciannelli et al., 2002; Piersma et al., 2000), we hypothesized that elevations in corticosterone during migration in the Gambel's white-crowned sparrow would contribute to the regulation of flight-associated processes, such as migratory restlessness and energy mobilization. Because glucocorticoids exert permissive effects on food intake, we also hypothesized that corticosterone would participate in the regulation of migratory hyperphagia. We used the GR antagonist RU486 to examine our predictions.

We found that RU486 treatment decreased feeding in migratory individuals, suggesting that GR binding is required for the expression of hyperphagia. These results demonstrate that, as in mammals (e.g. Langley and York, 1990; Santana et al., 1995), the GR participates in the expression of feeding behavior in a passerine bird. Many studies have characterized corticosterone as an important regulator of food intake. In mammals, food intake decreases with adrenalectomy and can be restored with physiologically low doses of corticosterone (e.g. Dallman et al., 1989; King, 1987). However, corticosterone treatment in intact animals typically does not increase feeding further (e.g. Astheimer et al., 1992; Davison et al., 1983; Gray et al., 1990), suggesting that corticosterone affects food intake only in a permissive capacity (Dallman et al., 1993; King, 1987). For example, corticosterone may influence food intake by altering responsivity to other hormones or neurotransmitters, such as neuropeptide Y (e.g. Stanley et al., 1989; Tempel and Leibowitz, 1993; Zakrzewska et al., 1999) or norepinephrine (e.g. Leibowitz et al., 1984; Tempel and Leibowitz, 1993). The RU486 validation study conducted here underlines the permissive nature of corticosterone's effects: food intake was not affected by corticosterone implants but was suppressed after co-administration of RU486.

Whereas spring migrants responded to RU486 by decreasing food intake, RU486 did not affect feeding in wintering birds.

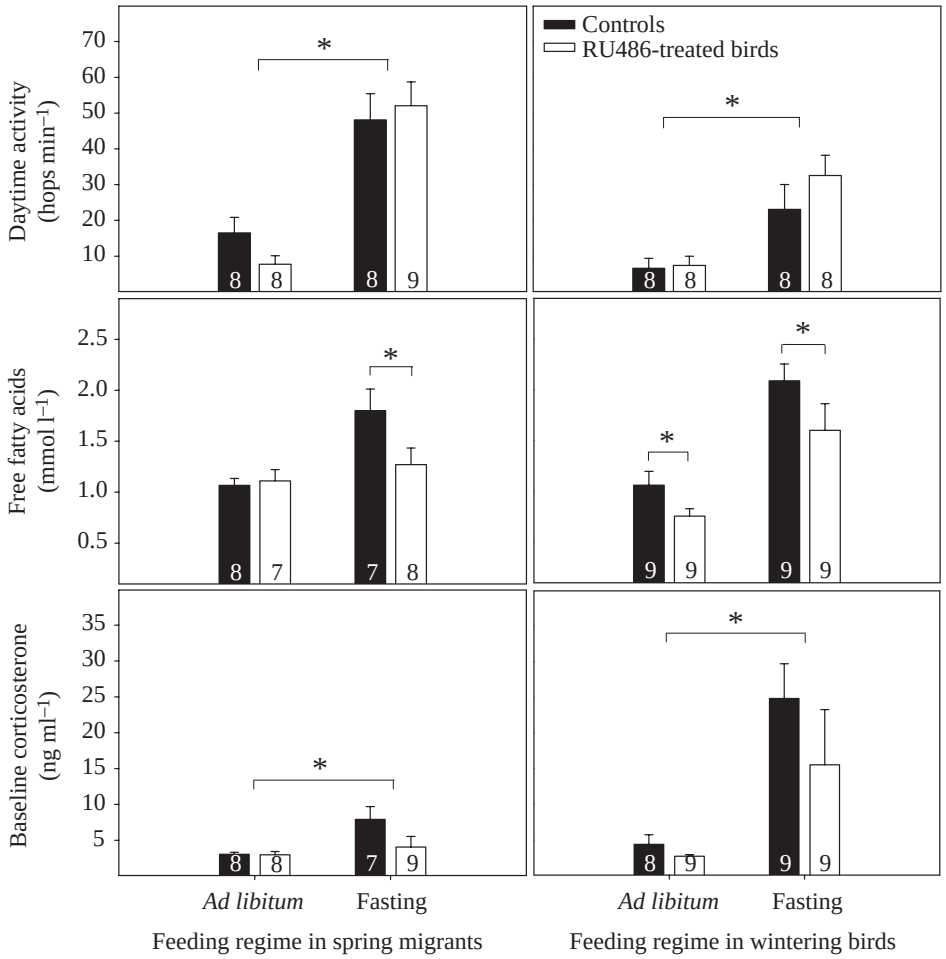

Fig. 6. Daytime locomotor activity (hops min^{-1}), plasma free fatty acids (mmol l^{-1}) and plasma corticosterone (ng ml^{-1}) during the food manipulation trial, both in spring migrants and in wintering birds. White-crowned sparrows were treated with RU486 or with vehicle and were either in a fasting state or were provided with *ad libitum* food. Locomotor activity was higher in fasting birds than in birds held on *ad libitum* food. Plasma free fatty acids and plasma corticosterone also increased with fasting, and RU486 suppressed the fasting-associated increase in fatty acids. Corticosterone concentrations were determined from blood samples collected in under 3 min of disturbance. Sample size is indicated. Asterisks indicate significant differences between groups. Error bars represent S.E.M.

Table 1. *Effects of RU486 treatment and feeding regime on plasma metabolite concentrations (mmol l^{-1})*

	Ad libitum birds		Fasting birds	
	Controls	RU486 birds	Controls	RU486 birds
Spring migrants				
Free fatty acids	1.03±0.07	1.16±0.12	1.83±0.24	1.27±0.16
Glycerol	0.44±0.07	0.37±0.05	0.82±0.08	0.90±0.12
Triglycerides	6.75±0.70	5.81±0.80	2.39±0.23	2.48±0.17
Glucose	27.04±0.78	25.88±0.60	20.55±0.72	20.39±0.74
Uric acid	1.39±0.07	1.44±0.14	0.79±0.10	0.79±0.10
β-Hydroxybutyrate	0.43±0.07	0.32±0.01	2.96±0.19	2.72±0.18
Wintering birds				
Free fatty acids	1.06±0.14	0.76±0.07	2.09±0.17	1.60±0.26
Glycerol	0.79±0.09	0.65±0.09	0.65±0.08	0.53±0.07
Triglycerides	8.33±0.69	7.05±0.90	2.07±0.19	2.46±0.24
Glucose	33.08±1.63	27.54±1.28	18.93±0.79	19.38±0.52
Uric acid	1.71±0.11	1.70±0.14	1.02±0.11	0.78±0.05
β-Hydroxybutyrate	0.30±0.00	0.37±0.04	4.32±0.64	5.21±0.38

Data are shown for the life-history stages of spring migration and winter. Birds were treated with 50 mg kg^{-1} RU486 or vehicle and were either subjected to a fast or maintained on *ad libitum* food. Sample size was 31 for spring migrants and 36 for wintering birds (for specific group size, see Fig. 6). Data are means ± S.E.M.

This suggests that the observed effects of RU486 in spring migrants were not pharmacological and also that the GR does not regulate feeding behavior during the life-history stage of winter.

Although the GR appears to be necessary for the expression of daytime feeding behavior during spring (white-crowned sparrows do not feed at night; Morton, 1967), the fact that spring migrants show an elevation in corticosterone mainly in association with night-time migratory flight (Landys, 2003) suggests that hyperphagia is not regulated at the level of plasma glucocorticoids. We suggest that variation in receptor number may play a more important role in the seasonal regulation of feeding. For example, hyperphagia in spring migrants may be stimulated through an increased population of GRs. In support of such a prediction, we found that RU486 was effective in suppressing daily food intake in migratory individuals but not

Table 2. *Results of the two-way ANOVA tests used to examine effects of RU486 treatment and feeding regime on plasma metabolite concentrations (mmol l^{-1})*

	Treatment		Feeding regime		Interaction	
	F	*P*	*F*	*P*	*F*	*P*
Spring migrants						
Free fatty acids	1.876	0.182	9.003	0.006	5.199	0.031
Glycerol	0.005	0.946	29.548	<0.001	0.503	0.484
Triglycerides	0.340	0.565	67.480	<0.001	0.874	0.358
Glucose	0.638	0.431	63.722	<0.001	0.270	0.608
Uric acid	0.010	0.920	33.763	<0.001	0.031	0.861
β-Hydroxybutyrate	2.557	0.122	583.078	<0.001	0.006	0.938
Wintering birds						
Free fatty acids	6.931	0.013	35.163	<0.001	0.135	0.716
Glycerol	2.364	0.134	2.128	0.154	0.046	0.831
Triglycerides	0.173	0.680	114.139	<0.001	2.920	0.097
Glucose	3.352	0.076	115.171	<0.001	6.234	0.018
Uric acid	1.935	0.174	60.167	<0.001	1.448	0.238
β-Hydroxybutyrate	3.181	0.084	480.451	<0.001	1.189	0.284

Data were evaluated separately for the life-history stages of spring migration and winter. Birds were treated with 50 mg kg^{-1} RU486 or vehicle and were either subjected to a fast or maintained on *ad libitum* food. Based on 31 spring migrants, the degrees of freedom for treatment, feeding regime, the interaction and the error term were 1, 1, 1 and 27, respectively. Based on 36 wintering birds, the degrees of freedom for treatment, feeding regime, the interaction and the error term were 1, 1, 1 and 32, respectively.

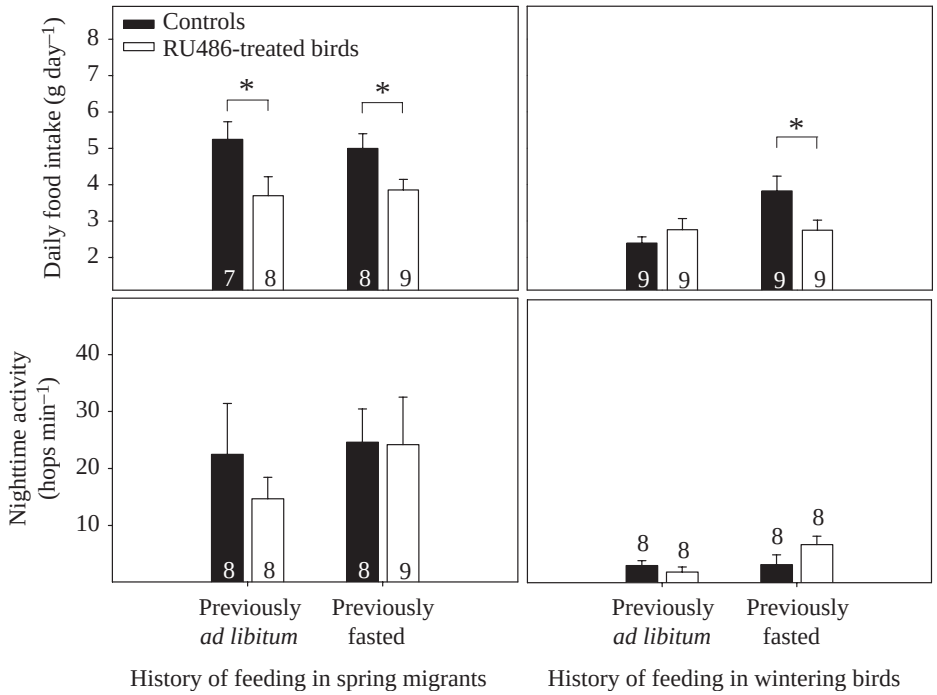

Fig. 7. Daytime food intake (g day^{-1}) and night-time locomotor activity (hops min^{-1}) during the recovery period following the food manipulation trial, both in spring migrants and in wintering birds. Birds with a history of fasting were compared with birds that had been maintained on *ad libitum* food. Fasted birds showed an increase in food intake during the recovery period in winter but not in spring. In spring migrants, RU486 decreased food intake both in fasted birds and in birds that had been maintained on *ad libitum* food. By contrast, during winter, RU486 decreased food intake only in fasted birds. Night-time locomotor activity was unaffected by RU486 treatment or by history of feeding conditions. Sample size is indicated. Asterisks indicate significant differences between groups. Error bars represent S.E.M.

in wintering birds. Research has already demonstrated that the GR population of house sparrows (*Passer domesticus*) varies with life-history stage (Breuner and Orchinik, 2001).

RU486 treatment did not affect intensity of migratory activity during the night, suggesting that corticosterone does not regulate migratory restlessness through the GR. Elevations in corticosterone may only be a byproduct of increased activity, as shown in homing pigeons (*Columba livia*) that have completed a bout of flight (Haase et al., 1986). On the other hand, it is possible that elevations in corticosterone regulate migratory restlessness through a non-genomic receptor. Whatever the case may be, our results suggest that migratory restlessness and hyperphagia, the two behaviors that characterize migration, are regulated by different mechanisms, as has been previously suggested by King and Farner (1963).

The role of corticosterone during an energetic challenge

Because white-crowned sparrows held in captivity are not exposed to the energetic demands typical of migration (Landys, 2003), we simulated energy shortage in birds by subjecting them to a fast. Consistent with previous research (Lynn et al., 2003; Richardson, 1997), fasting in this species caused an increase in plasma corticosterone and in attempts to escape. We predicted that during an energetic challenge, increased corticosterone may participate in the regulation of this escape activity and in the correlated mobilization of energy stores (Richardson, 1997).

Results showed that in response to food removal, locomotor activity increased similarly in RU486-treated birds and controls. Therefore, as mentioned above, the GR does not seem to participate in the regulation of locomotor activity. Results from past studies support this prediction. For example, although adrenalectomy decreases locomotion in fasted rats, RU486 treatment does not (Challet et al., 1995). Also, rapid locomotor responses that follow corticosterone administration are not inhibited by administration of genomic receptor antagonists (Sandi et al., 1996). Rapid responses to corticosterone have been noted in a number of species (e.g. Orchinik et al., 1991; Sandi et al., 1996), including the white-crowned sparrow (Bruener et al., 1998), suggesting that elevations in corticosterone may affect activity through a non-genomic receptor. This would allow for swift responses to unpredictable and challenging perturbations. Corticotropin-releasing factor (CRF), which has also been shown to promote locomotion, may play a mediating role (e.g. Lowry and Moore, 1991; Maney, 1997).

In conjunction with the increase in locomotor activity, fasting caused the mobilization of energy depots. Metabolite data demonstrate that energy was primarily mobilized from lipid stores: fasting resulted in elevated levels of plasma glycerol, free fatty acids and β-hydroxybutyrate. Data are consistent with a past study on this species (Richardson, 1997). Thus, unlike the sparing of adipose tissue that occurs during fasting in small migrant songbirds (Jenni-Eiermann and Jenni, 1996), migratory white-crowned sparrows seem to rely on the mobilization of lipid stores during periods of energetic demand.

We found that RU486 inhibited an increase in free fatty acids during fasting, both in spring migrants and in wintering birds. Thus, elevations in corticosterone appear to act through the GR to regulate the availability of lipid energy substrates, thereby ensuring that energy metabolism is balanced in response to changing energy requirements (McEwen, 2000). Because RU486 did not affect plasma glycerol, which is a general indicator of lipolysis (Hetenyi et al., 1983), corticosterone does not appear to increase free fatty acid availability through the breakdown of lipid stores. Catecholamines and/or growth hormone may play a more dominant role in this process (Hadley, 1999). Corticosterone may instead produce effects by altering the re-esterification rate of free fatty acids at adipose tissue sites (Mukherjee and Mukherjee, 1973). Further study is required to test this hypothesis.

Spring migrants provided with *ad libitum* food did not decrease free fatty acid levels after RU486 treatment. Blood samples for the determination of plasma metabolites were collected during the day, when migratory white-crowned sparrows show a nadir in plasma corticosterone (Landys, 2003). These comparatively low levels may be less effective in binding the low-affinity GR to elicit effects related to energy metabolism (Kalman and Spencer, 2002). Past studies verify that only elevated corticosterone levels affect lipid mobilization. In rats, glucocorticosteroid treatment stimulates the release of free fatty acids in a dose-dependent fashion (Mukherjee and Mukherjee, 1973), and differences in adipose tissue mass between adrenalectomized animals and controls are only evident after a challenge (Santana et al., 1995).

Following the fast, re-feeding was evaluated to determine how history of energetic stress affects recovery. Only wintering birds displayed an increase in feeding after the fast, suggesting that fasting-induced elevations in corticosterone may have increased food intake through additional GR binding. Thus, elevations in corticosterone may be capable of increasing food intake above levels appropriate to a particular life-history stage if original plasma concentrations do not fully activate the GR. Consistent with this prediction, an increase in food intake after glucocorticoid treatment has been observed in non-breeding ring doves (Koch et al., 2002), in which endogenous corticosterone levels are low (Lea et al., 1992).

Finally, history of fasting did not affect migratory restlessness: migratory activity was similar between fasted birds and birds that had been maintained on *ad libitum* food. Thus, unlike garden warblers (*Sylvia borin*; Gwinner et al., 1988), white-crowned sparrows do not seem to alter intensity of migratory activity in response to short-term food deprivation. Different responses between species may be explained by employed migration strategies. Garden warblers undertake relatively longer bouts of migratory flight that are energetically more demanding. Thus, these birds may be more sensitive to fasting-induced changes in energy stores.

Summary

First, results from this study demonstrate that, as in mammals, corticosterone acts through the GR to exert permissive effects on food intake in a passerine migrant. Corticosterone-induced effects on food intake may be mediated through seasonal differences in GR number rather than though changes in plasma glucocorticoids – during migration, corticosterone levels are low in association with periods of feeding (Landys, 2003). Second, data indicate that elevated levels of corticosterone do not stimulate migratory restlessness or escape activity through the GR. Previous studies have suggested that corticosterone regulates locomotor activity *via* a non-genomic receptor. Thus, it is interesting to note that hyperphagia and migratory restlessness, the two behaviors that characterize migration, may be regulated by different mechanisms. Third, data suggest that elevated levels of corticosterone act through the GR to regulate energy substrate availability during energetic challenge. Thus, variations in plasma corticosterone (and/or GR number) may be key in ensuring that behavior and physiology are adjusted appropriately to meet changing requirements during the life-history stage of migration. Future studies will hopefully elucidate the importance of seasonal variations in GR number in the regulation of migratory processes and will begin to address how corticosterone may interact with other hormones and neurotransmitters to regulate behavior and physiology during the period of migration.

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