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Fecal corticosteroids in a territorial bird selected for different personalities: daily rhythm and the response to social stress

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Abstract

In this study we tested the hypothesis that in a passerine bird (great tit, *Parus major*) individuals differing for coping strategies differ in the magnitude of the adrenocortical response to social stress as well. Furthermore, we aimed at characterizing daily rhythms in corticosteroid release before and after social stress. We used 16 males from either of two lines bidirectionally selected for different coping strategies (fast and slow explorers). Social stress was induced by confrontation with an aggressive resident male. Corticosteroid metabolites were analyzed in feces collected at 90-min intervals from 900 to 1630 h on a baseline day, on the day of the social conflict, and on the following day. In both days and in both lines levels varied with time of day in a robust rhythm with a peak in the first sample of the morning and a trough at the end of the light phase. This rhythm correlates with activity (perch hopping). An overall increase in levels relative to baseline day was observed between 30 and 140 min after the challenge. Birds of the less aggressive and more cautious line (slow explorers) showed a trend for a higher response compared to birds of the more aggressive and bolder line (fast explorers), which showed almost no response. On the day after the challenge the birds of the slow line exhibited significantly reduced corticosteroid secretion, probably due to an increased negative feedback. The results provide evidence for a physiological basis of different coping strategies in birds, emerging in response to social stress and with a pattern similar to that in other vertebrates.

Keywords: Birds; Social stress; Personality; Coping; Great tit; Corticosterone; Metabolites; Androstane; Rhythm; Feces; Activity

Introduction

Identification of the dynamics of hormone secretion in vertebrates is essential for the integration of proximate and ultimate explanations of behavior. Elevated levels of glucocorticoids in response to stressful events induce short- and long-term physiological and behavioral changes (reviews in Orchinik, 1998; Wingfield and Romero, 2000; Levine and Cirulli, 2001), whereas daily fluctuations are thought to regulate homeostatic mechanisms (Widmaier, 1992; Atkinson and Waddell, 1995). While studies using laboratory rodents are carried out with the main goal to unravel mechanisms of stress-related disorders (e.g., Korte, 2001; Sgoifo et al., 2001), studies using wild species may allow to take into account ecological aspects of hormone–behavior interactions. Since the ecology is well studied in many wild avian species, these offer a particularly suitable study object. For example, alterations in plasma corticosterone (CORT) following environmental challenge may alter memory-reliant behaviors which promote survival in the food-caching mountain chickadee (Saldahna et al., 2000); an ecological role of CORT in redirecting behavior toward dispersal by influencing body condition, locomotion, and foraging activity has been hypothesized in tits and owls (Silverin, 1997; Belthoff and Dufty, 1998).

Most of the procedures used to induce an adrenocortical response include stressors which bear little relationship with naturally occurring stressors such as, in birds, the classical

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handling and restraint paradigm (e.g., Wingfield et al., 1992; Dufty and Belthoff, 1997; Rich and Romero, 2001; van Hierden Korte et al., 2002). A successful attempt to modify this approach has been made by testing the effect of social defeat in territorial species. However, this approach has been used mainly in laboratory rodents (e.g., Koolhaas et al., 1997b; Meerlo et al., 1999). In rats and mice a social defeat elicits a general increase in sympathetic activity (heart rate and body temperature) lasting from 1 to 2 h, an increase in CORT lasting at least 4 h, and an impairment of social and explorative activity lasting up to several weeks (Koolhaas et al., 1997a; Koolhaas et al., 1997b). Effects of social defeat have been tested in a territorial wild bird species, showing similarities with rodent studies, but the data did not include information on the adrenocortical response (Carere et al., 2001). A recent study in geese showed that a social confrontation between two opponents increases fecal CORT metabolites (Kotrschal et al., 2000).

Individuals greatly differ in stress-induced glucocorticoid levels reflecting interindividual differences in response to stress (Piazza et al., 1993; Schwabl, 1995). Such variation reflects the individual capacity to cope with environmental demands. A coping strategy can be defined as a coherent set of behavioral and physiological responses which is consistent over time and context and which is characteristic of a certain group of individuals (Koolhaas et al., 2001). In this respect, the within- and between-individuals covariation of behavioral traits makes a coping strategy comparable with human personalities. In rats proactive coping is associated with high neurosympathetic activity and low CORT levels, whereas reactive coping is associated with high cardiac parasympathetic activity and high CORT levels (Koolhaas et al., 2001). Recent data on CORT response following a social conflict in mice of the SAL (short attack latency, proactive style) and LAL (long attack latency, reactive style) lines, an important model for coping strategies (Benus et al., 1991), indicate a higher HPA reactivity in individuals of the LAL line (Veenema et al., 2003). Similarly, it was found that hens of a low feather pecking line, thought to reflect a reactive style, had higher basal and stress-induced (manual restraint) plasma CORT levels than hens of a high feather pecking line, thought to reflect a proactive style (Korte et al., 1997; van Hierden et al., 2002).

Recently, evidence has accumulated that two such coping strategies exist in the great tit (*Parus major*), a passerine bird. Hand-reared birds from wild populations can be categorized along an axis ranging from slow to fast explorers with a combined score of the exploration of an unknown environment and the response to novel objects in a familiar environment performed around 40 days after hatching (Verbeek et al., 1994; Drent et al., 2003). Artificial selection combined with cross fostering resulted in clear evidence for a genetic basis of these traits (heritability of $54 \pm 5\%$ based on four generations, Drent et al., 2003). The behavioral characteristics of the fast and slow great tits show analogy to the characteristics of, respectively, the proactive (active) and reactive (passive) coping strategies described in other vertebrates (see Verbeek et al., 1994; Verbeek et al, 1996; Verbeek et al., 1999, for great tits; Benus et al., 1990; Benus et al., 1991, for rodents; Ruis et al., 2000, for pigs; Malkvist and Hansen, 2002, for farm minks). Proactive copers are more guided by internal mechanisms than by environmental stimuli, and they easily develop routines; in contrast, reactive copers are more flexible and react more to environmental stimuli. One of the main differences between proactive and reactive coping strategies is in the degree of behavioral plasticity (Koolhaas et al., 2001). Since a differential HPA axis (re)activity between the two strategies is suggested to underlie these difference (Koolhaas et al., 1999), we hypothesize that the slow line of the great tits would show higher CORT levels than the fast line in response to social stress.

In studying glucocorticoid release daily fluctuations must be taken into account. Daily rhythms in glucocorticoids release have been amply described in humans, laboratory mammals (e.g. Ader et al., 1967; Krieger, 1979), amphibians, and reptiles (Pancak and Taylor, 1983; Tyrrell and Cree, 1998), as well as in some domesticated avian species (e.g., Joseph and Meier, 1973; Boissin, Nouguier-Soule, and Assenmacher, 1975). Among passerine birds daily rhythms have been established for four species, the white-throated sparrow, *Zonotrichia albicollis* (Dusseau and Meier, 1971), the white-crowned sparrow, *Zonotrichia leucophrys* (Breuner et al., 1999), the starling, *Sturnus vulgaris* (Romero and Remage-Healey, 2000), and the house sparrow, *Passer domesticus* (Rich and Romero, 2001). In these species a distinct unimodal rhythm with more corticosterone released at the end of the dark phase was found. However, Marra and co-workers (Marra et al., 1995), did not detect any clear rhythm in the two Zonotrichia species. As regards the relationship between stress-induced levels and rhythm, it seems that the intensity of the CORT response to stressors is dependent upon the time of day at which the stimulus is presented: the stress response has a daily rhythm which approximately mimics the pattern of the basal fluctuation, at least on a winter photoperiod (Breuner et al., 1999; Romero and Remage-Haley, 2000; Rich and Romero, 2001).

The effect of stress on the CORT rhythm itself has never been assessed in birds. The main reason is that studies assessing rhythms obtained hormonal profiles with bleeding and handling procedures at 3- to 6-h intervals, sampling each bird at one time point per day. Usually the patterns are extrapolated from samples deriving from different birds at different time points during the day or from the same bird on different days, to ensure replenishment of blood volumes and to minimize the number of handling events so that CORT levels represent indeed basal levels. Moreover, such long intervals might mask more subtle ultradian variation. Also, in case of stress-induced responses an invasive evaluation of CORT is used which may provoke confounds, the only valid alternative being the cannulation method (Le Maho et al., 1992), which is difficult to apply in small birds.

The analysis of CORT metabolites in feces, enabling stress-free investigation, may remove sources of unpredictable variation and allow testing the effect of stress on rhythms. Recently it has been used in birds (geese) to describe the effect of social challenges in relation to dominance status and season as well as ontogenetic/seasonal patterns (Kotrschal et al., 1998; Kotrschal et al., 2000; Frigerio et al., 2001). In these studies inference on hormonal status has been validated via the results obtained or via ACTH injection. A recent study in hummingbirds demonstrated that changes in cloacal fluid concentration due to administration (via food) of exogenous CORT do reflect plasma levels with a delay, with peaks appearing in samples pooled over 30 min and disappearing within 90 min over termination of the handling stress (Hiebert et al., 2000).

Great tits are passerine, territorial, nonmigratory birds inhabiting woods and parks. Males start establishing their territory already in winter, thereafter forming monogamous pair bonds. Intermale competition and social conflicts are very common, including a well-defined repertoire of agonistic behavior displayed both in territorial contests and in foraging flocks (Blurton-Jones, 1968; Wilson, 1992). In the present study we used fecal CORT metabolites as a noninvasive marker of adrenocortical activity to test whether individual great tits genetically different for coping strategy differ in their basal levels and in the magnitude of the stress response. At the same time we wanted to characterize the daily rhythm in corticosteroids, if any, before and after social stress.

Methods

Animals

The subjects were 16 adult male great tits originating from a program of bidirectional artificial selection started in 1993 on the basis of the outcome of exploration tests carried out at the age of 30–40 days (see Introduction). Lines did not differ in body mass or tarsus (e.g., body mass means ± SEM: 17.7 ± 0.36 and 17.8 ± 0.60 g for the fast and the slow line, respectively, $t = -0.15$, $P = 0.88$). The birds belonged to the 3rd and 4th generations, 6 of the slow and 10 of the fast line. Birds were individually housed in standard cages (80 × 40 × 40 cm) with wooden bottom, top, side, and rear walls, a wire-mesh front, and three perches. The bottom was covered with shellsand. The birds were kept in a room with natural winter daylight augmented with fluorescent light tubes from 800 to 1700 h. They were provided with ad libitum water, sunflower seeds, and a commercial dry mixture (proteins, trace elements, minerals, and vitamins). Every 2 days the animals were also provided with a fresh mixture of raw heart. Live mealworms were given three times a week.

Social conflict

For the social confrontation an experimental bird was transferred to a cage, similar to the home cage, 3 days before the social conflict, and remained there until day 6 after the social interaction. Adjacent to the cage of the experimental bird an identical cage was positioned, containing one of two so-called resident birds. Experimental birds were randomly allocated to these two resident males. The latter were kept in these cages under the same housing conditions of the experimental birds for several weeks to become territorial. The two resident birds were unselected adult males chosen based on the fact that they always attacked an intruder male in a series of 5-min tests. The cages of the experimental and the resident birds were separated from each other by a removable opaque partition. The cage of the resident contained an identical removable partition that divided the cage into two equal parts. During the day before the social interaction the experimental birds underwent a series of five training sessions to allow the social interaction to occur without handling or forced chasing. Each session consisted in confining the resident bird to one-half of his cage that was opposite to the cage of the experimental bird. Next, a dish with mealworms was placed in the remaining part of the cage. The partition dividing the resident's cage from the cage of the experimental bird was opened allowing it to enter and eat one mealworm. During the next day the social interaction took place in the cage of the resident between 930 and 1230 h, according to the following procedure: (1) the resident bird was confined with the same procedure used for the training sessions; (2) after a few seconds the partition dividing the cage of the resident from the cage of the experimental bird was removed; (3) as soon as the experimental bird entered the area (due to the training this occurred within 10–30 s), the partition separating the cages was closed, the partition confining the resident male was removed, and the social interaction started. After the social interaction the original situation was reinstated. The removal of the partitions was remotely controlled by means of ropes. The resident bird was always exposed to a maximum of two interactions per day and was not fed before the social interaction. Next, some behavioral observations were done (Carere et al., 2001). Thereafter, a second interaction with a standard cutoff time of 5 min was given to the experimental bird immediately after the hopping measurement with the same resident male and the same procedure described above. The first interaction was stopped after 20 min or as it escalated, i.e., when one of the following three criteria was met: one of the two birds was (1) sitting quietly (freezing) for 5 min, (2) being chased for 3 min, or (3) being attacked 10 times.

The birds were also caught once on the day before (day −1) and once on the day after (day 1) the social interaction in order to take measurements of body temperature and breath rate. This was done at the end of the dark period (630 h) because in the darkness catching time is short (1–10 s),

minimizing possible confounds. Details of the procedures are reported elsewhere (Carere et al., 2001). The experiments were carried out in January and February of 2000. The birds were tested in series of four individuals to which they were assigned randomly, but fast and slow individuals alternated within the series.

Fecal sampling and CORT metabolites assay

Fresh feces were collected at 90-min intervals from 900 to 1630 h on a baseline day (day −5), at 60-min intervals from 900 to 1700 h on the day of the social conflict (day 0), and again at 90-min intervals on the day after the social interaction (day 1). Since the amount of fresh samples was low and in some cases no feces were found with the shortest interval, eventually we used a 90-min interval for the conflict day in most birds. When necessary the matching was achieved by averaging the CORT values of the samples at 1000–1100, 1300–1400, and 1600–1700 h on the conflict day. Samples at 900 h were also collected on each subsequent day. We also tried to sample 2 h after the onset of the dark phase. Feces were collected from a white paper sheet previously positioned on the bottom of each cage with tweezers and stored at −20°C within 10 min of collection (i.e., up to 100 min after being excreted by the bird). At each time we always pooled two to five fresh samples of similar appearance.

Glucocorticoids are extensively metabolized and the steroid excretion in birds may take place via the gut or via urine in conjugated or unconjugated form. For measuring CORT metabolites in the fecal samples of great tits three enzyme immunoassays were tested with and without enzymatic hydrolysis of the samples: corticosterone (Palme and Möstl, 1997), 11β-hydroxyoxoetiocholanolone (5β-androstane-3α, 11β-diol,-17-one), and 11-oxoetiocholanolone (5β-androstane-3α,11,17-dione) as described elsewhere (Möstl et al., 2002).

In the first experiment immunograms were performed. Pooled fecal samples were extracted with 60% methanol. After centrifugation, the supernatant was diluted 1 + 10 with water and extracted using a Sep-Pak C_{18} cartridge (Fa. Waters, Milford, MA, USA). After the cartridge was primed (according to the instruction of the manufacturer), the diluted sample was passed slowly through the cartridge, which was washed with 5 ml water. Afterward, the steroids were eluted from the column using 4 ml methanol. The methanol phase was evaporated and redissolved in 300 μl water/methanol (80/20 v/v) and the extract was purified by HPLC (Novapac C_{18} column 0.39 × 15 cm, Fa. Waters; solvent: water/methanol, linear gradient from 20% methanol to 100% within 30 min; flow 1 ml/min; three fractions/min were collected). All individual fractions were divided in half, and the two sets of tubes were evaporated. The one series of fractions were measured without hydrolysis and the other series after hydrolysis (Kotrschal et al., 1998). Immunoreactive HPLC fractions could be measured only after hydrolysis and using the assay against 11-oxoetiocholanolone. Therefore, this assay system was used for the analysis of the CORT metabolites in tit feces. The details of the assay are described elsewhere (Möstl et al., 2002). In brief, the carboxymethyloxime of 11-oxoetiocholanolone was prepared and linked to bovine serum albumin. The antibody was raised in a rabbit and was used in a working dilution of 1:60,000. As label, the 11-oxoetiocholanolone was biotinylated and was used in a working dilution of 1:2,000,000. As standard, 11-oxoetiocholanolone was used and the standard curve ranged from 2 to 500 pg/well; the 50% intercept was about 20 pg. Because of the linking of the steroid via position 17 of the molecule the assay showed cross-reactions with some C-21 and C-19 steroids: 5β-androstane-3α-ol-11,17-dione, 100%; 5β-pregnane-3α-ol-11,20 dione, 37%; 5β-androstane-3α,11β-diol-17-one, 3.3%; and 5β-androstane-3,11,17-trione, 1.2%. Other tested steroids (11-ketoandrosterone, etiocholanolone, pregnanediol, tetrahydrocortisol, 5β-dihydrocortisol, cortol, 5β-pregnane-3α,11β,21-triol, 20-one, 5β-pregnane-3α,11β,17α,20α,21-pentol, 5β-pregnane-3β-ol, 11,20-dione, and 5β-pregnane-3α,11β-diol, 20-one) had cross-reactions below 1%. Fecal sample extraction and hydrolysis was performed as described by Kotrschal and co-workers (1998) for the determination of CORT immunoreactive substances with the exception that smaller amounts of feces (about 0.1 g) were used. The intra- and interassay variations were 13.8 and 14.2%, respectively.

Statistical analysis

Data were normalized through log transformation (log (x + 1)) and then analyzed with SPSS 10.0. A nested analysis of variance for repeated measures (day, two levels, time of day, six levels) was used to test the daily patterns of fecal CORT before (baseline day) and after (day 1) the social conflict. Data collected in the dark phase were too few to allow inferential statistics. Since the social conflict occurred between 930 and 1230 h at different times for different birds and the rhythm itself might have affected the response, we used differences with the levels at the same time of the baseline day to analyze whether the different interval times after the social conflict differed from the levels before the social conflict. In this model the within-subjects factors had four levels, namely the prestress sample (last sample before the conflict), and three poststress samples, collected between 30 and 230 min after the conflict (time course of the response, based on Hiebert et al., 2000; see Introduction). In the model we used a simple contrast by which the data of each time after the social conflict are compared with the prestress levels. Finally, for each individual the total amount of CORT (prestress sample plus three post-stress samples) was computed over the baseline day, the day of the social conflict, and the day after the social conflict. In this case an analysis of variance for repeated measures with three with-

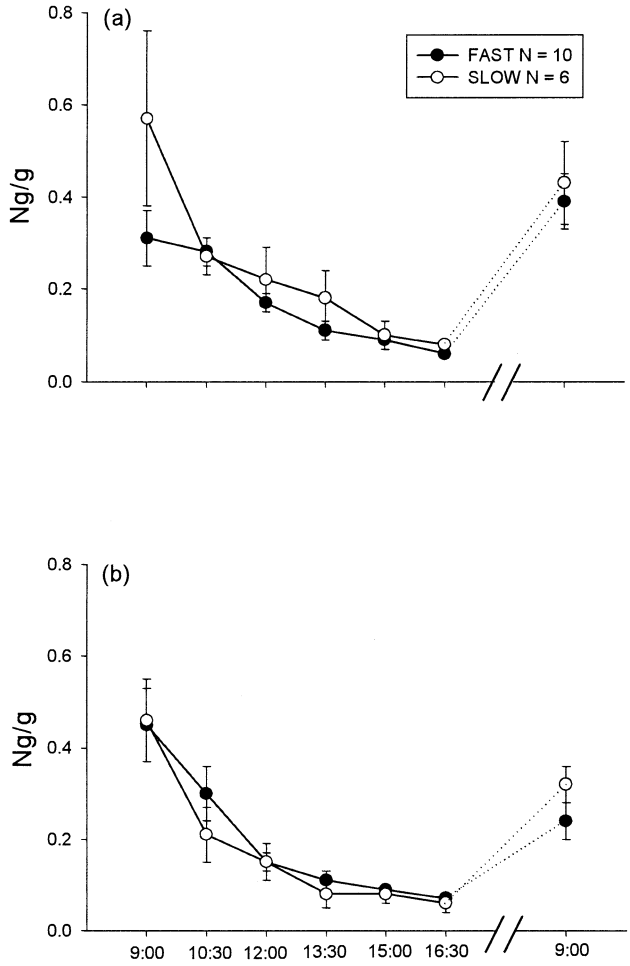

Fig. 1. (a, b). Daily pattern of mean (±SEM) CORT metabolites detected in fecal samples of birds of the two selection lines on the baseline day (a) and on the day after the social challenge (b). Lights on: 800–1700.

in-subjects factors was used. In all models line formed the between-subjects factor.

Results

Social conflict

With the criteria reported in a separate paper (Carere et al., 2001) we considered clearly defeated 6 of 6 slow birds and 6 of 10 fast birds In the analysis we included also the 4 fast birds that were not clearly defeated, since no difference was found between defeated and not clearly defeated birds within the fast line.

Daily rhythm

On both the baseline day and the day after the social conflict, hormonal levels showed a robust daily rhythm with relatively high levels at the start of the active period and a trough at its end (time of day, F5, 70 = 46.5; $P < 0.01$, Fig. 1). Neither line nor the interaction between line and time of day contributed significantly to the explained variance in CORT metabolites. On the day after the social interaction individuals of the slow line showed lower concentrations than on the baseline day (day × type, F1, 14 = 6.0; $P < 0.03$, Fig. 1). Both the interactions day × time of day and time of day × hour × line were not significant, indicating that the rhythmicity was not affected. Samples from four slow and three fast birds collected at 1900 h on the baseline day indicate that in the slow line concentrations are increasing after the onset of the dark period.

Response to social stress

No correlation was found between CORT response and body condition on any of the 3 days on which samples were collected The analysis of the differences in fecal CORT with the baseline day showed a rise from basal to stress-induced concentrations, which returned soon to basal levels. This pattern showed a significant quadratic response (F3, 42 = 7.8; $P = 0.01$, Fig. 2). The highest concentration was found in the first sample after the social challenge (T1 vs T, F1, 14 = 5.3; $P = 0.04$, Fig. 2), collected 30–45 min after the end of the confrontation. In the second sample, collected after 120–140 min, values were just above the significance level (T2 vs T F1, 14 = 4.1; $P = 0.06$, Fig 2). In the third sample, after 210–230 min, values were similar to those before the confrontation. No significant effect of line nor a significant interaction effect between line and time in relation to social stress was found. However, the increase after the conflict was mainly due to individuals of the slow line (T1 vs T for the slow line F1, 5 = 5.2; $P = 0.05$, Fig. 2); The analysis of the total CORT over the 3 days revealed a significant effect of the social challenge (day, F1, 14 = 8.9; $P = 0.01$) as well as that lines reacted differently to it over

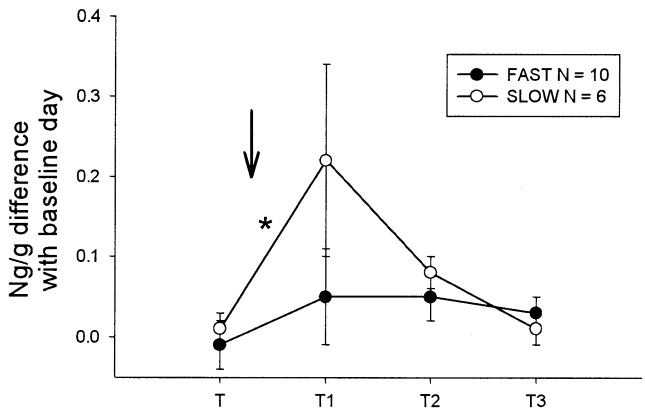

Fig. 2. Time course of the increase of CORT metabolites detected in fecal samples of birds of the two selection lines collected before (T = 0–30 min before) and after (T1 = 30–45 min after; T2 = 120–140 min after; T3 = 210–230 min after) T the social challenge, indicated by an arrow. Values are mean differences (±SEM) with baseline day. *$P = 0.05$.

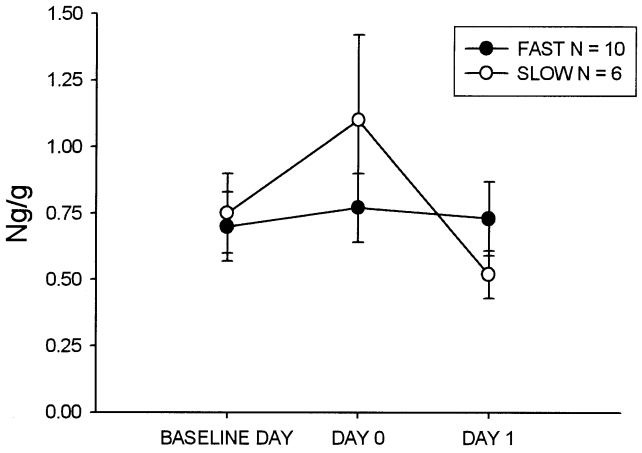

Fig. 3. Total CORT metabolites secretion (four samples 0–230 min after time of the conflict pooled) in both lines under nonstressed (baseline, day −5) *vs* stressed (social conflict, day 0) *vs* post conflict (day 1). Values are means ± SEM.

the time course of the experiment (day × line F1, 14 = 4.7; $P = 0.045$, Fig. 3).

Discussion

Daily rhythm

We observed a robust daily rhythm of CORT metabolite concentration. No ultradian variation was detected and the rhythm was not disrupted by social stress. A peak was detected in the first sample, at 900 h, after the onset of the daily photoperiod. There was also a rapid decline after 900 h, so that low concentrations were observed during the major fraction of the day. Although the fecal steroid analysis has not been validated in this species, the rhythmicity data constitute themselves a strong validation. The rhythm has been repeatedly detected, and its pattern is very similar to the one described in closely related species, such as sparrows and starlings, through blood sampling. The great tit pattern shows striking similarities with the pattern found in the migratory passerine white-throated sparrow (*Z. albicollis*) when kept under a similar short photoperiod. In this species the daily rise occurred 12 h after the onset of darkness, suggested as the entraining element for the rhythm (Meier and Fivizzani, 1975). The great tit rhythm is also similar to the one found in starlings (*S. vulgaris*) and house sparrows (*P. domesticus*), where levels are high during the dark hours and low throughout the active period (Romero and Remage-Healey, 2000; Rich and Romero, 2001). Considering the delay in the feces, we presume that in our birds the actual CORT peak in the plasma occurred during or at the end of the dark hours.

A study in the white-throated sparrow led to the hypothesis that the daily rhythm of locomotor activity causes the rhythm of CORT binding activity, which in turn may be responsible for the daily rhythm of plasma concentration (Meier et al., 1978). As regards the relationship with activity, for other purposes we have taken measurements of the frequency of perch-hopping during the active period at 900, 1200, 1500, and 1800 h in a number of juvenile great tits kept under the same conditions, but on a summer photoperiod. Data showed a pattern of activity which parallels that of CORT metabolites of our adult males, with a peak at 900 h and levels decreasing with time of day (F1, 9 = 4.1; $P = 0.02$, Fig. 4), suggesting an association between daily CORT variation and activity levels. Indeed, a link between CORT and locomotor activity has been hypothesized in other bird species (e.g., in owls and in white-crowned sparrows, Belthoff and Dufty, 1998; Breuner et al., 1998; see also next section).

The general daily pattern of CORT levels in songbirds corresponds to some extent to the results of much more extensive studies in mammals (for a review see Krieger, 1979). In rats and mice CORT rises in the late hours of the (inactive) day and peaks just prior to the onset of activity. During the night levels gradually drop to reach the lowest levels around the beginning of rest (Ader et al., 1967; Krieger, 1979). Also in humans peak levels are typically observed around awakening (Aschoff, 1978) and, in addition to the circadian periodicity, there are six to nine minor synchronous peaks of plasma ACTH and cortisol throughout the day (Krieger, 1979). Another difference in our data with the mammalian situation, and also with part of the avian data, seems to be the sharp drop in CORT metabolites occurring in the first hour of the day. It is possible that the first fecal sample is particularly rich in CORT metabolites due to the accumulation of renal excretion over the hours prior to the daily onset of activity and of defecation. Be this as it may, the data presented provide a baseline to be taken into account in behavioral studies of songbirds. Whether

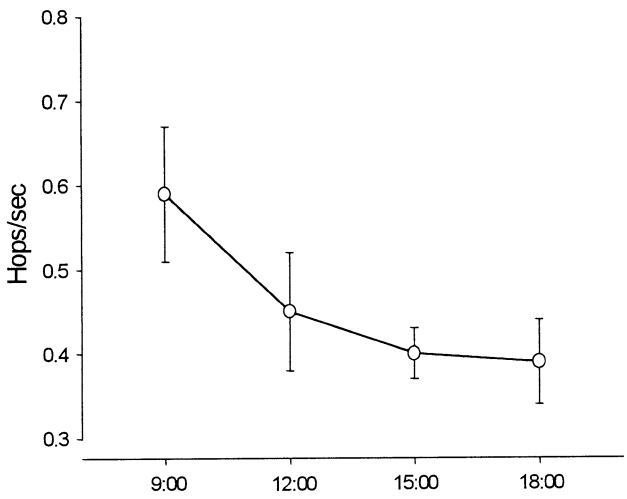

Fig. 4. Daily activity pattern (mean ± SEM) in juvenile great tits ($n = 10$; age, 60 days) of the slow selection line. Lights on: 700–1900.

this periodicity is under endogenous control of a circadian pacemaker remains an open question, requiring experiments under constant conditions.

Tits species are extensively used in behavioral and ecological research both in the field and in captivity. It is important that the spontaneous daily variation of CORT levels is taken into account in the planning of experiments at appropriate times of day. For example, circulating CORT levels can be experimentally elevated during most of the daylight hours without confounding effects of endogenous origin.

Response to social stress

In this study we demonstrated a difference in CORT secretion in response to social stress in two types of individuals adopting different and heritable coping strategies. Together with other findings (Kotrschal et al., 2000; Carere et al., 2001), it is clear that a social conflict induces a stress response in birds, which has a similar impact on behavior and physiology as in mammals. Stress of social origin induced a moderate, but clear glucocorticoid response in great tits. The peak of the stress response occurred in fecal samples collected within 30–45 min after the social conflict, a figure similar to the one found in cloacal fluids of hummingbirds (Hiebert et al., 2000). The magnitude of the response was lower than those following bleeding and handling stress found, for example, in starlings and house sparrows kept under similar photoperiods (Romero and Remage-Healey, 2000; Rich and Romero, 2001). It was hypothesized that birds of the slow line would show a higher HPA reactivity than birds of the fast line (see Introduction). In accordance with this we found a clear response only in birds of the slow line, while birds of the fast line did not show evidence of a response. Interestingly, reduced baseline levels were observed in the slow line on the post-conflict day. A similar phenomenon, maybe due to an enhanced negative feedback by corticosteroids in the brain, has been described in humans (Yehuda, 2001). It may indicate an alteration of the HPA axis as a consequence of the social stress, which could exert effects on the reaction to subsequent challenges.

These data provide the first evidence of an adrenocortical response to a social conflict in a songbird; at the same time they provide evidence for a physiological basis of different coping strategies in birds. We conclude that the two types of birds display a different reactivity in the adrenocortical response and this strengthens the idea that the fast and slow lines of great tits are representatives of, respectively, the proactive and reactive coping strategy (see Introduction).

In the same individuals social stress has been shown to: (1) substantially increase body temperature for at least one day; (2) impair activity (perch-hopping) on a short-term basis more markedly in the fast line; and (3) substantially decrease the tendency to approach to an unknown conspecific (Carere et al., 2001). In rodents stress-induced hyperthermia is simultaneously accompanied by increases of plasma CORT, while lipopolysaccaride-induced fever also increases the amount of biologically available CORT (Groenink et al., 1994; Cabrera et al., 2000). Although we could not get simultaneous measurements of body temperature and CORT levels, our results suggest that the situation in birds might be different. We did not find any indication for a line difference in body temperature in both basal and stress-induced levels (after about 18 h), while we did find a line effect for CORT.

Instead, lines differed in the magnitude of the impairment of activity levels. The slow birds, which had a higher CORT response, were not impaired in activity compared to fast birds, which showed a weak CORT response (Carere et al., 2001). Given the increasing evidence that CORT treatment at intermediate levels has a rapid and transient effect (within 15 min) in stimulating perch-hopping in passerines (Breuner et al., 1998; Breuner and Wingfield, 2000; but see Astheimer et al., 1992), our data showing impairment in the first minutes after the challenge yield an apparent contradiction. It is possible that in birds of the slow line the occurring rise in CORT levels has buffered the tendency to decrease activity more than in birds of the fast line. However, it must be noted that a clear effect of CORT on activity in other species is evident only on long days (Breuner and Wingfield, 2000), while our birds were on short days.

Our results suggest that interindividual differences in sensitivity to stressors occur and relate to behavioral differences with a genetic component maintained in wild populations (see also discussion in Schwabl, 1995). Hence, in the natural situation social stress may have important consequences for population dynamics. Field and theoretical studies indicate an ecological role of CORT in promoting dispersal via stimulation of locomotion and foraging activity (Silverin, 1997; Belthoff and Dufty, 1998). The two types of birds of our selection lines originate from a wild population where a coexistence of strategies does occur (Verbeek et al., 1994; Dingemanse et al., 2002). Given the line differences we found, we hypothesize that social stress may affect differently individuals adopting alternative coping strategies.

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