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GENERAL BIOLOGY

Plasma Corticosterone Concentrations in European Robins during Spring and Autumn Migration[1]

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Abstract—To estimate differences in hormonal mechanisms of regulation of spring and autumn migration in European robins *Erithacus rubecula,* the plasma corticosterone (CORT) concentrations were compared in birds caught during both migratory seasons. A total of 414 blood samples were analyzed. It was found that the baseline and stress-induced CORT concentrations in free-living robins during spring migration were practically twice as high as during autumn passage. Our results demonstrate that autumn and spring migrations are independent stages of the avian annual cycle. Probably, the increase in the CORT concentrations in spring can be considered to be physiological preparation for the breeding season.

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Spring and autumn migrations are the stages of the annual cycle in most passerine species breeding at temperate and Arctic latitudes [1]. During both migratory seasons, the birds cover large distances and often follow the same routes and expend similar amounts of energy [2]. As a result, similar suites of morphological, physiological and behavioral adaptations develop in spring and in autumn, known as migratory disposition [3]. However, speed and duration of migration [4], environmental factors (temperature, wind conditions, food abundance etc.), photoperiod, gonadal development and circulating level of sex hormones significantly differ between the seasons [5].

The hypothalamic–pituitary–adrenal axis (HPA) and corticosterone (CORT), the final hormone of this neuroendocrine pathway, play a great role in regulation of behaviour and physiology of birds [6]. Distinction is made between the baseline concentration of the hormone, which reflects the intensity of its secretion during the everyday forms of activity, and stress-induced concentration, when CORT is produced as a response to stressful situations, which reflects the reactivity of HPA [7]. To measure the baseline and stress-induced CORT concentrations in birds, a standardized protocol is used [8]. The first blood sample is usually taken within 3 min after capture to characterize the baseline CORT level, after which the bird is put into a cotton bag. The second sample which reflects the response of HPA to the external stimulus is taken 20–30 min after capture.

The baseline CORT level regulates the activity of the cardiovascular system, immune system, and metabolism, and also enhances locomotor activity [7, 9]. The CORT concentrations that characterize the adrenocortical response to external stimuli inhibit growth, immune response, and sexual behavior. At the same time, vascular tone and catabolism of lipids and proteins increase, and locomotor activity is enhanced, which results in redistribution of resources necessary to avoid the negative impact of the stressful event [7, 8].

CORT plays an important role during bird migrations as stages of the annual cycle with an increased energy metabolism and locomotor activity, when the birds regularly face unknown distributions of resources and threats [10]. However, seasonal variations of environmental conditions and the place of migrations in the annual cycle may result in different mechanisms of regulation of spring and autumn migrations. It still remains little known whether CORT plasma concentrations differ between spring and autumn migratory seasons, with the data available being equivocal [11, 12].

To test the hypothesis of different mechanisms of regulation of spring and autumn migrations, we compared the baseline and stress-induced CORT concentrations in European robins (*Erithacus rubecula*)

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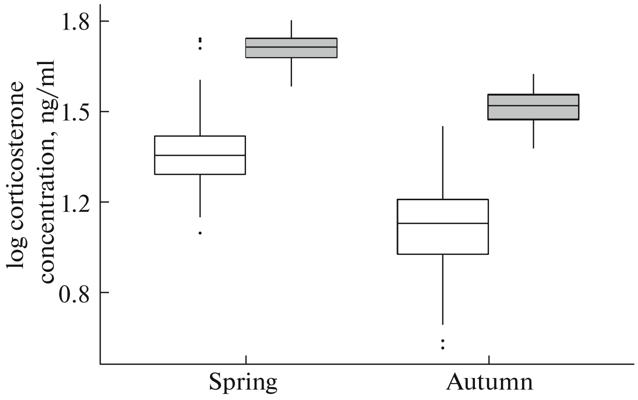

Seasonal variation in baseline (white bars; spring, $n = 85$; autumn, $n = 180$) and stress-induced (grey bars; spring, $n = 63$; autumn, $n = 86$) corticosterone concentrations in European robins captured on the Curonian Spit during seasonal migrations. Pooled data for 2013–2015. Median value and quartile range.

during both migratory seasons in three consecutive years on the Curonian Spit on the Baltic Sea. The results of this study are reported here.

Trapping of birds was conducted at the two field sites of the Rybachy Biological Station of the Zoological Institute of the Russian Academy of Sciences and followed the standard protocol [8]. The time elapsed between capture of a bird and the end of blood sampling was, on average, 2 min 53 s. Blood sampling from the stressed birds occurred, on average, 23 min after capture. It was not always possible to collect the second sample; therefore, the sample sizes for baseline and stress-induced CORT concentrations are not equal. Blood was taken from the brachial vein in heparinized microtubes. The volume of each sample did not exceed 75 μl. After blood sampling, each bird was weighed, wing length was measured as a proxy for size, and fat was scored using the standard scale, after which the birds were ringed and released into the wild. Blood samples were centrifuged in an Elmi CM-70 haematocrit centrifuge (Elmi, Latvia). Plasma samples were put into 0.5 ml Eppendorf tubes and frozen at –20°C until further analysis. CORT concentrations were measured at the Laboratory of Behavior and Behavioral Ecology of the A.N. Severtsov Institute of Ecology and Evolution by means of ELISA kits (EIA-4164, DRG International, United States) following the manufactory guidelines. CORT antibody cross-reactivity to other steroids was from 0.1 up to 7.4%. Intra-assay coefficient of variation was 2.0% ($n = 379$), inter-assay coefficient of variation was 19.76% ($n = 18$). We analyzed a total of 265 samples for baseline CORT concentrations and 149 samples for the stress-induced concentrations.

Statistical analysis was performed using the R version 3.3.1 software (R Core Team, 2016). To normalize the distributions, CORT concentrations were log-transformed. Plasma CORT concentrations in spring and in autumn were compared by means of the linear mixed effects model [13] using the *lme4* package. Apart from the season factor (spring vs. autumn), we included in the model following independent fixed factors: time elapsed between capture and blood sampling, size-corrected body mass, time of capture within the day and capture date. Year and field site were included as random factors. Difference of the CORT concentrations in spring and in autumn was tested for significance by the *summary* function of the *lmerTest* package.

The baseline plasma CORT concentration in European robins captured in both migratory seasons varied between 1.13 and 256.80 ng/ml ($n = 265$). Concentration values exceeding 100 ng/ml ($n = 4$) were considered to be outliers and were excluded from further analyses. In spring, the baseline plasma CORT concentrations were significantly higher than in autumn ($p < 0.0001$, figure). The mean values predicted by the model were 13.2 ± 0.3 ng/ml for autumn and 23.2 ± 0.7 ng/ml for spring.

The CORT concentrations in stressed birds varied between 6.55 and 326.55 ng/ml ($n = 149$). The values exceeding 200 ng/ml ($n = 3$) were outliers to the overall distribution and were excluded from further analyses. In spring, the stress-induced CORT concentrations were significantly higher than in autumn ($p < 0.0001$, figure). The means predicted by the model were 32.9 ± 0.5 ng/ml in autumn and 51.1 ± 0.8 ng/ml in spring. In autumn, a marginally significant correlation between the baseline and the stress-induced CORT concentrations was found ($r_p = 0.22, p = 0.05$). In spring, the individuals with higher baseline CORT concentrations also showed higher stress response ($r_p = 0.31, p = 0.02$).

Our results agree with the data on seasonal variation of CORT values in white-crowned sparrows (*Zonotrichia leucophrys*) in North America [11, 14]. However, northern wheatears (*Oenanthe oenanthe*) and blackbirds (*Turdus merula*) in Europe displyed no seasonal variation in baseline CORT levels [12]. The reasons behind this disagreement are not obvious. However, the data collected on the Curonian Spit during three years clearly demonstrate that in free-living European robins baseline CORT concentration, as well as the reactivity of HPA, during spring migration are practically twice as high as during autumn passage (figure).

Several hypotheses have been suggested to explain the seasonal variation of plasma CORT concentrations in birds [14]. The most well-founded is the "preparatory hypothesis," which postulates that HPA activity is increased before the periods when the probability to encounter a stressful event is higher. If the breeding season is considered to be such a period (taking into account the necessity to defend a breeding territory, find a mate, increased activity when feeding the offspring, etc.), the increased baseline CORT concentration and HPA reactivity in spring may be treated as the physiological preparation for the breeding season [14].

The mechanism of increasing CORT concentrations in spring might be a combination of photoperiodic stimulation and endogenous rhythm of HPA activity, which may result in an increase of adrenal gland mass and CORT secretion intensity [10]. In addition, it might be related to the increased androgen secretion, which stimulates the HPA irrespectively of the development of vernal migratory disposition [15]. Seasonal variation in the impact of various environmental factors may also influence CORT secretion intensity [14].

The question arises, how similar adjustments of behavior and physiology during seasonal migrations are governed by different CORT concentrations? A possible mechanism includes seasonal variation of cell sensitivity to CORT [14]. Another possibility is the regulation at the level of corticosterone binding globulin, CBG, which enables CORT transport in the blood. If CBG not only plays the transport role, but also can be a buffer for excessive CORT, increasing CBG concentration in spring may decrease CORT availability for target tissues [14].

Apart from the seasonal variation of the baseline CORT concentration and HPA reactivity, we found that strength of relationship between baseline and stress-induced values differs between the seasons.

Thus, our results demonstrate that, in spite of the apparent similarity between components of migratory disposition, spring and autumn migrations are distinct stages of the annual cycle that are characterized by dissimilar CORT concentrations and possibly by different mechanisms of endocrine regulation.

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