

*Non-invasive corticosterone treatment changes foraging intensity in red-eyed vireos *Vireo olivaceus**

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Corticosterone is thought to play an important role in food caching and foraging behaviour. However, the direct influence of increased plasma corticosterone on feeding behaviour is still unclear. In this study the effect of increased corticosterone on feeding behaviour in migratory active red-eyed vireos *Vireo olivaceus* was investigated. We hypothesized that if increased corticosterone levels facilitate foraging behaviour, an increased number of visits to the food bowl by corticosterone treated birds would be seen. In addition to ad lib food during the experiment, the vireos were fed every full hour between 09.00–13.00 h with one meal worm injected either with 4 μg corticosterone dissolved in 20 μl DMSO, or with DMSO only as a control treatment. The presence or absence of a bird in the food bowl was recorded by a motion detector between 09.00–15.00 h. The non-invasive corticosterone treatment increased plasma corticosterone levels and caused vireos to visit the food bowl more frequently compared to control treated individuals between 11.00–12.00 h and 13.00–15.00 h. Our data indicate that corticosterone has an effect on feeding behaviour in birds.

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Corticosterone is the primary glucocorticoid hormone in birds. The involvement of this hormone in the regulation of foraging behaviour has been discussed in a number of studies (Gray et al. 1990, Wingfield et al. 1990, Kitaysky et al. 2001, Koch et al. 2002, Pravosudov 2003, Dallman et al. 2004, Long and Holberton 2004) generally suggesting a facilitating effect on food intake (Dallman et al. 2004).

However, the direct role of corticosterone in feeding behaviour of birds is not clear (Piersma et al. 2000). For example, no effect of corticosterone treatment was observed on either foraging behaviour or food consump-

tion in captive dark-eyed juncos *Junco hyemalis* under simulated winter conditions (Gray et al. 1990). On the other hand, chronic but moderate increase in corticosterone levels increased feeding, food caching and spatial memory in mountain chickadees *Poecile gambeli* (Pravosudov 2003). Migratory active (individuals displaying migration related signs such as hyperphagia and *zugunruhe*) shore birds do not seem to require elevated levels of corticosterone to feed during the period of hyperphagia, as the corticosterone levels are not increasing during the period of fattening (Piersma et al. 2000, Landys-Ciannelli et al. 2002). Still, corticosterone appears to have a permissive role in hyperphagia and feeding (Landys et al. 2004) and has been suggested to help the replenishment behaviour at stop-over sites in migratory active passerines (Long and Holberton 2004).

To avoid stressing birds due to handling, Breuner et al. (1998) tested a new method of non-invasive corticosterone administration in white-crowned sparrows *Zonotrichia leucophrys gambelii*. Birds were fed mealworms *Tenebrio molitor* injected with corticosterone which resulted in an increase in plasma corticosterone (highest recording approximately seven minutes after the consumption of the worm) paralleled with a rapid increase in perch hopping. Following the method of Breuner et al. (1998), we investigated the effect of corticosterone on feeding behaviour in migratory active red-eyed vireos. The experimental birds consumed one mealworm containing 4 μg of corticosterone at each full hour between 09.00 and 13.00. As an indication of the involvement of corticosterone levels in foraging, we expected to observe an increased number of visits to the food bowl by corticosterone treated birds as compared to control treated birds.

Material and methods

The study was performed on the Ft. Morgan peninsula within the Bon Secour National Wildlife Refuge in Alabama; U.S.A. Migratory active red-eyed vireos were captured in mist-nets (12 × 2.6m, 30 mm mesh) between 5 and 20 Sept. and transferred to the experimental housing. Vireos were habituated approximately two weeks prior to the experiment. Birds were given fresh water supplemented with vitamins and fed moist monkey biscuits (Hill's Products, Premium Nutritional Products, Mission, Kansas), meal worms, and berries ad libitum.

Vireos were randomly divided into control and experimental birds eventually producing valid data from 14 control and 12 corticosterone treated individuals. In the evening before the experimental day (8 occasions between 19 Sep and 5 Oct 2002) each bird was weighed to the nearest 0.1 g using an electronic balance. During the experiment birds consumed, in addition to the ad libitum monkey biscuits, one meal worm each, every full hour between 09.00–13.00 h. Meal worms given to birds in the corticosterone group were injected with 4 µg corticosterone dissolved in 20 µl dimethyl sulfoxide (DMSO) whereas worms fed to the control group were injected with the same amount of DMSO only.

Video observations of seven birds showed that they consumed their mealworm within less than three minutes after we left the experimental room. Motion detectors (Mouse-e-motion, Infra-e-motion activity monitoring systems, Hamburg, Germany) mounted at the top of the food bowl recorded the presence or absence of a bird in the food bowl over three-second intervals, and from these recordings we calculated the number of visits to the food bowl (assuming that birds were not doing several visits within three seconds). Earlier visual observations had confirmed that birds at 98% of recorded visits consumed food. Data were pooled into 1 h intervals and effects of corticosterone over time were tested with repeated-measures analysis of variance (rm-ANOVA). A multivariate test (MANOVA) was used to test for differences between treatments during individual hours. Response variable was the number of visits per hour.

A second group of vireos, divided into two subgroups, was used for collecting blood samples. The first subgroup did not receive a meal worm providing us with blood samples from non-manipulated birds. All birds belonging to the second subgroup consumed one mealworm each injected with either DMSO only (the control birds), or with corticosterone dissolved in DMSO as described above (the experimental birds). One blood sample per individual from the second subgroup was drawn seven minutes after the birds had consumed the worm. The blood samples were taken within one minute from capturing by punctuating the wing vein with a

27-gauge needle and collecting 20–50 µl of blood in a heparinized capillary tube. Birds in cages had no visual contact with each other or with the person catching another bird. Samples were centrifuged and the plasma was removed with a 50 µL Hamilton syringe. Plasma was kept frozen in microcentrifuge tubes until assayed for corticosterone by radioimmunoassay (Wingfield et al. 1992, Cash and Holberton 1999).

Blood data were log₁₀ transformed to make variances more homogenous, and differences between samples from non-manipulated birds (first subgroup), and from control and corticosterone treated birds (belonging to second subgroup) were tested with a one-way analysis of variance (ANOVA) followed by a Scheffé post-hoc test.

Results

Birds in the control and corticosterone groups were of equal weights. Control birds weighted 16.6 g ± 0.42 (SE) and corticosterone treated birds 16.5 g ± 0.43. Overall, corticosterone treated birds visited the food bowl more frequent (between-subjects effect: $F_{1,24} = 6.1$, $P = 0.021$; Fig. 1) and this difference was consistent throughout the observation period (time × treatment; Huyhn-Feldt $\epsilon = 0.75$, $F_{3,8,90.5} = 1.5$, $P = 0.20$). Specific analyses between corticosterone treated and control birds for each time period showed that the differences were significant between 11:00–12:00 h ($F_{1,24} = 7.35$, $P = 0.012$) and 13:00–15:00 h (13:00–14:00 h: $F_{1,24} = 5.21$, $P = 0.032$; 14:00–15:00 h: $F_{1,24} = 8.26$, $P = 0.008$). Averaged over the entire observation period, corticosterone treated birds visited the food bowl 24.7 times (± 2.0 SE) per

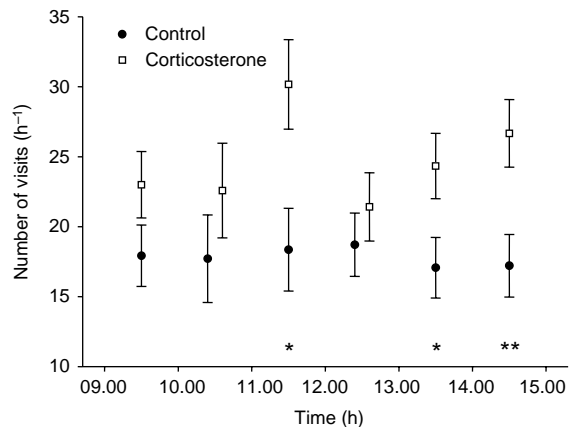


Fig. 1. Foraging behaviour of red-eyed vireo on ad lib monkey biscuit fed once every full hour with a meal worm prepared with corticosterone or vector only (control). The figure is representing the average number of visits per hour with standard error of mean. Asterisks below points denote significant differences between corticosterone and control treated birds within the time-interval where * = $P < 0.05$ and ** = $P < 0.01$.

hour whereas control birds visited the food bowl 17.8 times (± 1.9) per hour.

Plasma corticosterone levels differed between birds from first subgroup (non-manipulated) and experimental and control birds from second subgroup ($F_{2,18} = 33.4$, $P < 0.001$). The basal level of corticosterone in non-manipulated birds was 9.3 ng ml^{-1} (± 1.1 SE). Control birds had the same level 7 min after the treatment ($8.9 \text{ ng ml}^{-1} \pm 1.1$; Scheffé post-hoc $P = 0.94$) whereas experimental vireos had significantly higher levels of corticosterone than non-manipulated and control birds ($22.3 \text{ ng ml}^{-1} \pm 1.1$; $P < 0.001$ for both comparisons).

Discussion

We observed an alteration in feeding behaviour in migratory active red-eyed vireos as a result of non-invasive treatment with corticosterone. Birds visited the food bowl more often than did the control individuals, particularly after two and four hours of treatment. Corticosterone treatment also caused a significant elevation of plasma corticosterone.

Similar non-invasive corticosterone treatment in white-crowned sparrows resulted in a rapid increase in corticosterone levels, contemporarily with increased perch hopping activity (Breuner et al. 1998). The rapid effect on behaviour was suggested to be a consequence of corticosterone acting through neuronal membrane glucocorticoid receptors since a behavioural effect through intracellular receptors would not be detectable before 30–60 min after hormone treatment and would last after circulating levels of corticosterone had returned to normal. The effect on feeding, on the other hand, has been shown to be mediated by the intracellular receptors (Landys et al. 2004). Hence, in our study the significant effect on the frequency of visits to the food bowl first appeared considerably later than the initial increase in plasma corticosterone, supporting the idea that the behaviour was mediated by intracellular receptors. Since the treatment in this paper was repeated at every full hour it is possible that a cumulative effect of the hormone was needed to produce a significant behavioural effect.

In some earlier studies the stimulatory role of corticosterone on feeding has been suggested to be permissive rather than casual since corticosterone treatment in many cases increased feeding in adrenalectomized animals (King 1987, Dallman et al. 1989, Astheimer et al. 1992, Dallman et al. 1993). However, other studies have shown a change in feeding behaviour as a response to corticosterone administration in organisms with intact adrenals (e.g., Pravosudov 2003). It is possible that the differences in results between studies depend on the concentrations of corticosterone used.

For example only moderate elevations of plasma corticosterone levels has been seen to increase the perch hopping activity in passerines whereas high levels do not have this effect (Breuner et al. 1998, Breuner and Wingfield 2000). The behavioural response is not expected to increase linearly with the circulating hormone concentrations; hence, it is possible that only a certain level of increased plasma corticosterone can induce an enhanced feeding behaviour.

Moderate increase in corticosterone levels, leading to enhanced foraging behaviour, may be adaptive during demanding environmental conditions (Pravosudov 2003). According to Breuner and Orchinik (2001) it is likely that multiple components of the corticosterone response are seasonally variable and the interactions between these components regulate the seasonal differences in behaviour and physiology. Since migration is an energy demanding event that requires special adaptations regulated by altered signalling mechanisms, it is possible that migratory active birds are more sensitive to intermediate changes in corticosterone levels than are for example wintering birds.

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