Hormones and Behavior 54 (2008) 435–441

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Behavioural and hormonal effects of social isolation and neophobia in a gregarious bird species, the European starling (*Sturnus vulgaris*)

Beate Apfelbeck *, Michael Raess

Department of Behavioural Neurobiology, Max Planck Institute for Ornithology, Eberhard-Gwinner-Str.7, D-82319 Seewiesen, Germany

ARTICLE INFO

Article history:
Received 28 November 2007
Revised 7 April 2008
Accepted 10 April 2008
Available online 23 April 2008

Keywords:
Social isolation
Neophobia
Novelty
Corticosterone
Testosterone
Flocking behaviour
Starling

ABSTRACT

Separating gregarious individuals from their group members often results in behavioural and physiological changes, like increased levels of corticosterone. Testosterone and corticosterone, in particular, have been implicated in the response of mammals to novelty. Data in birds are, however, rare. The presence or absence of group members may also influence an individual's response to novel stimuli. We assessed the behaviour and hormonal response of European starlings (*Sturnus vulgaris*) to a novel object in two different situations and seasons: each starling was tested when separated and when in contact with its group members in May/June (breeding season) and again in September/October (non-breeding season). Starlings are gregarious throughout the year, but as foraging flocks are small during the breeding season and large during the non-breeding season, we assumed that non-breeding starlings would be more affected by social isolation. Overall, starlings had higher levels of corticosterone, lost more body mass, and were more active when they were separated from their group. Isolated individuals, however, did not show a greater neophobic response than individuals in the presence of their group members in either season. Circulating levels of testosterone and corticosterone were higher after a test with novel object than after a test with only the familiar feeding dish in both sexes and seasons. However, control tests for handling effects confirmed only the increase in testosterone. Our study shows that social isolation is stressful for unrelated and unpaired members of a wild flocking bird species and demonstrates that novelty can lead to a rise in testosterone in birds.

Introduction

Many explanations exist for the advantages of group living (Krause and Ruxton, 2002). In contrast, surprisingly little is known about the underlying physiology. In several gregarious vertebrate species social isolation causes behavioural and hormonal changes indicating that it is perceived as stressful (Hennessy, 1997). Furthermore, the presence or absence of conspecifics can modulate the physiological and behavioural responses to novelty e.g., the confrontation with a novel environment or a previously unknown object in a familiar environment. For example, although male mice housed either in groups or individually do not differ in baseline levels of corticosterone (Bartolomucci et al., 2003), individually housed males express higher corticosterone concentrations after the confrontation with a novel environment than group-housed males. When birds are confronted with a novel object beside their familiar feeding dish they often show a neophobic reaction that can be measured as the latency to approach and feed (Greenberg and Mettke-Hofmann, 2001). In such a situation budgerigars (*Melopsittacus undulates*) or zebra finches (*Taeniopygia guttata*) feed more readily in the presence of their group mates than when alone (Coleman and Mellgren, 1994; Soma and Hasegawa, 2004).

Many studies have shown that novelty is a stressor triggering changes in behaviour and the release of corticosterone (e.g., in mice and rats: Hennessy, 1991; Marquez et al., 2006). In birds, only a few studies have investigated corticosterone in this context and the hormonal response to novelty across these studies was not consistent (Mettke-Hofmann et al., 2005; Nephew et al., 2003; Richard et al., 2008). This is, however, probably due to differences in methodology and the point in time when the blood sample was obtained after presentation of the novel stimulus.

In addition to corticosterone, there are indications that testosterone could play a role in the reaction of animals to novelty. Chicks (*Gallus gallus domesticus*) treated with testosterone are more persistent to search for a particular preferred type of food than control chicks (Andrew, 1972; Andrew and Rogers, 1972) and cocks show stronger avoidance towards a novel object in their food trough than capons (Jones and Andrew, 1992). The effects demonstrated in these studies are based on sustained differences in testosterone levels. To our knowledge, however, it has not yet been investigated whether confrontation with a novel object in the familiar feeding environment directly leads to transient changes in circulating testosterone levels in birds.

European starlings (*Sturnus vulgaris*) are gregarious throughout the year and the individuals of a starling flock frequently interact

* Corresponding author.
E-mail address: apfelbeck@orn.mpg.de (B. Apfelbeck).

0018-506X/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.yhbeh.2008.04.003

(Fernandez-Juricic et al., 2004; Fernandez-Juricic et al., 2005). However, flocking behaviour in starlings varies seasonally, with flocks being small during spring and early summer (especially during the breeding season) and large during autumn migration and winter (Fischl and Caccamise, 1985; Williamson and Gray, 1975). It is therefore likely, that the importance of the group changes for an individual in the course of the year. Similarly, Dickens et al. (2006) showed that the response of starlings to intruders in their home cage is more pronounced when they are held on a long day light–dark cycle than when held on a short day light–dark cycle.

In this study we separated individual European starlings from their group members while either maintaining or blocking visual contact amongst members of the group. In both situations, we first measured the behavioural reaction of the isolated bird to a familiar feeding dish and then measured circulating corticosterone and testosterone concentrations. Two hours after the first test we attached a novel object to the feeding dish and again measured behaviour and hormone levels. To control for possible effects of time of day and testing sequence, we repeated the same measurements in control birds that did not receive a novel object in the second test. To check for seasonal influences we conducted the experiments with the same starlings twice: once during May/June and again in September/October. We tested the hypothesis that social isolation elicits a stress response in starlings resulting in higher levels of corticosterone and changes in behaviour. We did not expect changes of testosterone levels in response to social isolation because there are no indications from other studies that testosterone plays a role in this context. We predicted that a novel object beside a familiar feeding dish would provoke a neophobic reaction that occurs concurrently with a rise of corticosterone and testosterone. Furthermore, we expected that the starlings' neophobic reaction would be stronger and the hormonal response more pronounced when the birds are in visual isolation to conspecifics than when visual contact to group members is possible. With regard to seasonal influences, we proposed that both the reaction to social isolation and the social modulation of the neophobic response is more pronounced during autumn and winter than during spring and summer.

Materials and methods

Animals and housing

Thirty-two European starlings (*S. vulgaris*) taken as nestlings from three different breeding colonies (Upper Bavaria, 48°N 11°E) in 2005 were raised to one year of age. The birds were kept in eight adjacent roofed outdoor aviaries (each 322*193*265 cm) in groups of four unrelated birds consisting of two males and two females. The starlings experienced the natural light–dark cycle and natural temperature conditions. They were provided with food and water *ad libitum*, and had free access to a water tub for bathing. Experiments were approved by the government of Upper Bavaria (permit no. 55.2-1-54-2531-121-05) and in accordance with the German animal protection law.

Experimental setup

Each group member was tested in two social settings: in visual isolation from its group members and with visual contact to them maintained. In addition, the experiments were carried out once in spring/summer (5 May–5 July 2006), at a time when naturally occurring group sizes are small, and a second time in autumn (11 September–3 November 2006) when natural group sizes are large (Fischl and Caccamise, 1985; Williamson and Gray, 1975). Thus, each bird was tested four times in a semi-random design (Table 1). The order of the tested aviaries was fixed (to avoid two adjacent aviaries being tested on the same day); however, the initial order of the birds tested in each aviary and the social setting (visual isolation/contact) was chosen randomly. Once all birds had been tested in their first social setting, this test order was repeated with the second social setting. The tests of the two different social settings were four weeks apart for each bird. In September/October we maintained the testing order established in May/June to assure that the time period between tests was the same for all birds. The novel object used, a soft, orange-coloured woollen ball slightly smaller than the birds, was the same in all tests (Fig. 1D).

The experiments took place in the aviaries where the starlings were housed. Each aviary was divided into two compartments by a partition. The partition was composed of a permanent lower wooden part (height: 1 m) and two exchangeable frames constituting the upper part of the partition (Figs. 1A, B.). When the starlings were tested in visual isolation, the two frames were made up of opaque wood, separating the focal bird visually, but not acoustically, from its three group members (Fig. 1A). In the visual contact setting the frames were made up of a wire lattice allowing contact between the focal bird and its group members (Fig. 1B). The permanent lower part of the partition ensured that the group members could not see the novel object (to exclude learning effects). Four weeks prior to and also during each experimental phase the birds were trained twice a day (at around 0800 and 1100 h) to feed from a dish containing mealworms (a preferred food item of starlings) that we put next to the lower part of the partition (see Fig. 1C).

Test procedure

To allow the subjects to get used to the experimental setting, the frames were installed on the day before the experiment at 1300 h. At this time all birds were caught with a dip net and biometric measures were taken (Table 2). The compartment with the focal bird (Fig. 1C) was provided with normal food and water. Although a period of food restriction before the novel object test may have enhanced the motivation of the focal bird to feed with novel object present, we did not restrict access to food because fasting has been shown to interact with circulating levels of corticosterone (Astheimer et al., 1992). After approximately 15 min the focal bird was released into the experimental compartment and the other three birds into the group compartment. On the next day (the experimental day) the focal bird was tested two times for 30 min, starting at 0830 h and again at 1100 h (Table 2). A recovery period of 2 h has been shown to be sufficient to let corticosterone levels return to basal in captive starlings (Rich and Romero, 2005). At the beginning of each test the food dish was replaced by a dish containing four mealworms, with the difference that at 1100 h the novel object was attached to the mealworm dish (Fig. 1D). The behaviour of the focal bird was recorded by a digital camera (Table 2). Immediately after each behavioural test the focal bird was caught, bled within 3 min after entering the aviary (Romero and Reed, 2005; Romero and Romero, 2002) and weighed. Between the two tests the focal bird was provided with its normal food. During the autumn test series (September/October) not only the focal bird, but also its other group members were weighed after removal of the frames to check for an influence of the partition on the birds kept in the group.

To minimize potential effects of diel variations in hormone levels, the experiments were carried out only in the mornings (0830–1145 h). In the time between the two experimental phases in spring and in autumn one female died and was replaced by a

Table 1
Experimental design: the sequence of testing with respect to visual isolation or visual contact was chosen randomly

Experiment								Control				
May/June 06				September/October 06				May 07				Season
Visual isolation		Visual contact		Visual isolation		Visual contact		Visual isolation		Visual contact		Social setting
0830–0900	1100–1130	0830–0900	1100–1130	0830–0900	1100–1130	0830–0900	1100–1130	0830–0900	1100–1130	0830–0900	1100–1130	Time of day
No object	Object	No object	Object	No object	Object	No object	Object	No object	No object	No object	No object	Treatment
30	30	30	30	30	30	30	30	10	10	10	10	Sample size
Measurements:								Measurements:				
Corticosterone								Corticosterone				
Testosterone								Testosterone				
Body mass												
Food intake												
Behaviour												

The order of the treatment was fixed. Each individual starling went through each treatment and each social setting in both seasons. During the control tests 10 of the birds tested in the experiment experienced the visual isolation setting and 10 different (!) birds experienced the visual contact setting.