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The acoustic expression of stress in a songbird: Does corticosterone drive isolation-induced modifications of zebra finch calls?

Emilie C. Perez [a,*], Julie E. Elie [a,1], Christophe O. Soulage [b], Hédi A. Soula [b],
Nicolas Mathevon [a], Clémentine Vignal [a]

[a] *Université de Saint-Etienne, Université de Lyon, ENES/CNPS CNRS UMR8195, 42023 Saint-Etienne, France*
[b] *Université de Lyon, INSA de Lyon, CarMeN, INSERM U1060, Univ Lyon-1, F-69621, Villeurbanne, France*

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ABSTRACT

Animal vocalizations convey multiple pieces of information about the sender. Some of them are stable, such as identity or sex, but others are labile like the emotional or motivational state. Only a few studies have examined the acoustic expression of emotional state in non-human animals and related vocal cues to physiological parameters. In this paper, we examined the vocal expression of isolation-induced stress in a songbird, the zebra finch (*Taeniopygia guttata*). Although songbirds use acoustic communication extensively, nothing is known to date on how they might encode physiological states in their vocalizations. We tested the hypothesis that social isolation in zebra finches induces a rise of plasma corticosterone that modifies the vocal behavior. We monitored plasma corticosterone, as well as call rate and acoustic structure of calls of males in response to the playback of female calls of varied saliences (familiar versus stranger) in two situations: social isolation and social housing. Social isolation induced both a rise in plasma corticosterone, and a range of modifications in males' vocal behavior. Isolated birds showed a lower vocal activity, an abolition of the difference of response between the two stimuli, and evoked calls with longer duration and higher pitch. Because some of these effects were mimicked after oral administration of corticosterone in socially housed subjects, we conclude that corticosterone could be partly responsible for the isolation-related modifications of calls in male zebra finches. To our knowledge, this is the first demonstration of the direct implication of glucocorticoids in the modulation of the structure of vocal sounds.

Introduction

Animal vocalizations can convey information about context and events in the environment, as well as multiple pieces of information about the sender such as identity, physical characteristics, emotional or motivational state (Seyfarth and Cheney, 2003). Within a species, individuals often produce different context-specific types of calls that are characterized by different structures (amplitude, frequency, duration, etc). These calls generally carry stable characteristics related to the emitter's sex or individual identity, but also more instantaneous information such as the sender's motivation or physiological state. For instance, in the presence of a male, females of the South African clawed frog *Xenopus laevis* can produce two call types both consisting of repetitive click trains: a fast fertility call and a slower unreceptive call (Tobias et al., 1998). While these two call types differ in click rate, both are slower than male calls (Tobias et al., 1998). Thus, these calls encode stable information about the sender's sex and social context, but also labile information about reproductive state (Tobias et al., 1998; Vignal and Kelley, 2007).

The emotional state of the emitter is an example of transient information conveyed in the vocalizations. Because of its importance in speech perception, several studies have focused on vocal expression of emotions in humans (Bachorowski, 1999; Bachorowski and Owren, 2003), and speech rhythm and intonation seem to be the main parameters affected by emotional state (Nygaard and Queen, 2008). In contrast, few studies have examined the acoustic expression of emotional state in non-human animals. One notable exception is the acoustic expression of distress during mother–young separation or during isolation from affiliated individuals in mammalian species. In infant rhesus monkeys, the isolation from the mother induces behavioral agitation and modification of the acoustic structure of vocalizations (Bayart et al., 1990; Levine et al., 1985). In several species of primates, isolation from the mother in young or separation from affiliated individuals in adults has been reported to elicit the emission of specific vocalizations named separation calls (Masataka and Symmes, 1986; Mendoza and Mason, 1986; Norcross and Newman, 1999; Scheumann et al., 2007). In rodents, isolated pups produce specific ultrasonic vocalizations that provoke a fast return of the mother to the nest (Ehret, 2005; Shair, 2007), suggesting that these separation calls convey information

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* Corresponding author.
E-mail address: emilie.perez@univ-st-etienne.fr (E.C. Perez).

[1] Present address: 3210 Tolman Hall, University of California, Berkeley, CA 94720, USA.

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about the emotional distress of the young and promote physical reunion with the mother (D'Amato et al., 2005).

Physiological stress is a good candidate as proximate mechanism of the acoustic expression of separation distress. Indeed, the perturbation of social interactions is one of the most efficient stressors in animals (DeVries et al., 2003). Social isolation in particular provokes a rise of plasma glucocorticoids in both young and adults of many mammalian and avian species. In infant squirrel monkeys and rhesus monkeys, isolation from the mother elicits a rise of plasma cortisol (Levine and Wiener, 1988; Levine et al., 1985). In marmosets (Norcross and Newman, 1999) and titi monkeys (Mendoza and Mason, 1986), separation of adult pair-mates elevates cortisol levels. In pigs, young females isolated from the social group show an increase of plasma cortisol (Ruis et al., 2001). In European starlings, isolation from group members increases plasma corticosterone, the main stress hormone in birds (Apfelbeck and Raess, 2008). Thus, social isolation may be perceived as a strong stressor, triggering a hypothalamic–pituitary–adrenal (HPA) axis response through a glucocorticoid release. This physiological stress response could provoke the emission of modulated vocalizations that could be considered as the vocal expression of stress. To the best of our knowledge, this hypothesis has not yet been directly tested.

The present study aimed at investigating the vocal expression of stress and its control by glucocorticoids using a songbird as a model system. Although songbirds use acoustic communication extensively during social interactions, nothing is known to date on how they encode emotional and physiological states like stress in their vocalizations. Our study focused on a songbird model species, the zebra finch (*Taeniopygia guttata*). This gregarious bird forms monogamous life-long pair bonds, and is thus highly social (Zann, 1996). Perturbations of social interactions in this bird such as separation from the mate (Remage-Healey et al., 2003) or isolation (Banerjee and Adkins-Regan, 2011) are already suspected to provoke physiological stress but nothing is known on the related modifications of acoustic communication. We hypothesized that social isolation in zebra finches provokes a rise of plasma corticosterone that modifies the acoustic structure of calls and the call rate of emission. We monitored plasma corticosterone concentration, as well as call rate and acoustic structure of calls of males in response to the playback of female calls of varied saliences (familiar versus stranger) in two situations: social isolation and social housing. To test whether the vocal modifications induced by social isolation are triggered by a physiological stress, we investigated whether oral administration of corticosterone can mimic the effects of isolation in socially housed subjects.

Materials and methods

Subjects and housing conditions

Birds used for this study were zebra finches (*T. guttata*) bred in our colony (ENES lab, University of Saint-Etienne). Thirty male subjects were used to study the kinetics of circulating level of corticosterone after oral administration of exogenous hormone (Experiment 1). Eighteen male subjects were used in the playback experiment (Experiment 2). All male subjects ($n=48$) were housed in individual cages (dimensions $24\times29\times39$ cm) for the duration of the experiments (light conditions: 14:10 h light:dark; temperature from 22 to 24 °C) to acclimate them to the experimental condition. Food (mixed seeds) and water were provided ad libitum. Birds had free access to a water pool for bathing and were also supplemented with fresh salad once a week.

For at least two weeks before the playback experiment, subjects shared the room with 10 breeding pairs (referred to as “audience birds” in the protocol; each breeding pair was housed in an individual cage) and six females (whose calls were used as familiar acoustic stimuli during playback experiments; each female was housed in an individual cage). Because all cages were in the same room allowing visual and acoustic contacts between birds, all birds in the room can be considered as familiar to each other.

Six females unknown by the other birds were housed in individual cages and kept in a second room without any acoustic or visual contact with the subjects. Distance calls from theses females were used as stranger acoustic stimuli during the playback experiment. The corticosterone baseline of our birds (see results, mean = 3.50 ng/mL) was not different from corticosterone baseline of birds living in aviaries of other studies (Remage-Healey et al., 2003, 3.80 ng/mL for pair housed birds, 3.50 ng/mL for group housed birds). Thus, we assumed that our housing conditions did not modify the baseline stress level and thus the basal behavior of our birds.

Experiments were performed under the authorization no. 42-218-0901-38 SV 09 (ENES Lab, Direction Départementale des Services Vétérinaires de la Loire) according to the guidelines laid down by the French Ministère de l'Agriculture (no. 87-848) and the E.U. Council Directive for the Care and Use of Laboratory Animals of November 24th, 1986 (86/609/EEC).

Corticosterone manipulation

To non-invasively induce an acute increase of plasma corticosterone, a procedure of oral administration was used. The subjects were fed with 300 mg of seeds sprinkled either with corticosterone dissolved in peanut oil (CORT condition in Experiment 1 and 2) or peanut oil alone (Control condition in Experiment 1, NOCORT and ISOLATION conditions in Experiment 2). To elicit a huge increase of plasma corticosterone in few minutes (Spencer and Verhulst, 2007), a dose of 0.0125 mg of exogenous corticosterone was used (50 μL, concentration: 0.25 mg/mL; Sigma Aldrich ref: 27840). Seeds were presented via a trap-door at the bottom of the experimental cage. The cage was inside an acoustically-isolated chamber (internal dimensions: 2.00 m H × 1.35 m W × 1.00 m D; Silence Box model, Tiptop Wood, Saint-Etienne, France). To make sure that subjects ate the entire dose of seeds in less than 5 min, any food was removed from the cage the day before the experiment (15 h30 ± 30 min before the experiment). To monitor when the subject started eating the seeds (denoted as $t=0$) and the time spent eating, the experimenter observed the bird through a one-way mirror and was thus outside the chamber.

Blood sample collection and hormone assay

All blood samples (<150 μL) were obtained by puncturing the alar vein with a 25-gauge needle. Blood was collected in heparinized tips, and plasma was separated under centrifugation (15 min, 3800 × *g*) and stored at −80 °C until hormone assay. Blood samples were taken within 3 min so as to measure corticosterone baseline and not corticosterone due to handling (Wingfield et al., 1982). No bird was bled more than once per week to ensure that they had sufficiently replenished their blood volume between bleeds and HPA axis function had recovered from previous sampling (Romero and Reed, 2008).

Because circulating level of endogenous corticosterone shows circadian variations in many bird species with a peak just before the active period and a rapid decrease during the first hours of activity, all experiments were performed between 8 and 11 am (Remage-Healey and Romero, 2000; Tarlow et al., 2003).

Hormone assays were performed using the ELISA method (Kit Corticosterone EIA, Cayman, #5000561) according to the manufacturer's recommendations. All samples were run in duplicate in three different assays: all samples from Experiment 1 were run in one assay and samples from Experiment 2 in two other assays. The detection limit was 0.2 ng/mL, intra-assay coefficient of variation was 0.14, and inter-assay coefficient of variation was 0.09.