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Effects of predictable and unpredictable food restriction on the stress response in molting and non-molting European starlings (*Sturnus vulgaris*)

Carolyn M. Bauer *, Laura W. Glassman, Nicole E. Cyr [1], L. Michael Romero

Department of Biology, Tufts University, 163 Packard Avenue, Medford, MA 02155, USA

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ABSTRACT

This study tested whether an ethologically relevant stressor, a three-week period of food restriction where food was unavailable for four hours a day, caused chronic stress in molting and non-molting captive European starlings. Although all birds increased weight during the Food Restriction period, only non-molting birds increased food intake. Morning baseline heart rates increased during the Food Restriction period and all birds showed a decrease in heart rate when food was absent from the cage. In non-molting birds, there were no differences in either baseline or stress-induced corticosterone (CORT) concentrations, whereas molting birds showed attenuated baseline CORT, stress-induced CORT, and fecal glucocorticoid metabolite levels over the Food Restriction period. Although several parameters, such as increased morning heart rate, are consistent with chronic stress, the majority of these data suggest that restricting food availability is not chronically stressful. Furthermore, making the timing of food removal less predictable by randomizing when food was removed during the day did not enhance any of the above responses, but did alter the frequency of maintenance and feeding behaviors. In conclusion, starlings appear resistant to developing symptoms of chronic stress from repeated food restriction.

1. Introduction

The majority of stress research has been carried out on domesticated animals in the laboratory. Since wild animals have been shown to have different hypothalamic–pituitary–adrenal (HPA) axis function than their domesticated counterparts (e.g. Kunzl and Sachser, 1999; Oskina et al., 2000), there has been an increase in the use of wild animals in laboratory stress research. Common techniques used to elicit acute or chronic stress responses in laboratory animals include restraint, loud noises, or other anthropogenic stressors. However, using more ethologically-relevant stressors, such as food restriction, would provide a better bridge for laboratory research that is applicable to free-living animals. One goal of this study was to test the hypothesis that sustained daily food restriction causes chronic stress.

Food resources in the wild are often patchily distributed over space and time, and many animals dedicate the majority of their time budget to foraging (Schoener, 1971; Smith and Dawkins, 1971; Smith and Sweatman, 1974). Limited food has often been linked with an increase in stress, and several studies on many different bird species have shown that decreased food access causes elevated plasma corticosterone (CORT) concentrations (Kitaysky et al., 2001; Lynn et al., 2001; Pravosudov et al., 2001; Buck et al., 2007). Conversely, food supplementation experiments have demonstrated that increased availability of food advances time of reproduction (Boutin, 1989; Schoech and Hahn, 2008) and can decrease CORT concentrations (Clinchy et al., 2004). Furthermore, unpredictability is a major component of what makes a stimulus stressful (Levine and Ursin, 1991) and is thought to enhance the stressfulness of limited food availability (e.g. Acquarone et al., 2002). For example, unpredictable food availability causes an increase in body mass in European starlings (*Sturnus vulgaris*) (Cuthill et al., 2000) but not in European magpies (*Pica pica*) (Cucco et al., 2002). In the present study, we have modeled long-term food restriction by using a food restriction protocol previously used in our own and other laboratories (Witter et al., 1995; Buchanan et al., 2003; Strochlic and Romero, 2008). In addition to making food unavailable for a few hours everyday, we also included variable food availability schedules to test a second hypothesis: unpredictable food availability is more stressful than predictable food availability.

In order to appropriately respond to stressful stimuli, vertebrates must alter and coordinate their cardiovascular, behavioral, and endocrine systems. The cardiovascular stress response results from an activation of the sympathetic-adrenal-medulla axis which, through the release of catecholamines, causes an increase in heart rate, blood pressure, and diversion of blood to muscle tissue (Sapolsky et al.,

* Corresponding author. Tel.: +1 617 627 5346; fax: +1 617 627 3805.
E-mail addresses: Carolyn.Bauer@tufts.edu (C.M. Bauer), LauraG02@gmail.com (L.W. Glassman), Nicole_Cyr@brown.edu (N.E. Cyr), Michael.Romero@tufts.edu (L.M. Romero).
[1] Present address: Division of Endocrinology, Department of Medicine, The Warren Alpert Medical School of Brown University/Rhode Island Hospital, 55 Claverick St., Providence, Rhode Island, 02903, USA.

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2000). The endocrine stress response is controlled by the HPA axis. Activation of the HPA axis leads to increased secretion of glucocorticoids, which cause a suite of physiological and behavioral changes affecting metabolism, immune function, reproduction, and escape behavior (Sapolsky et al., 2000). Increased secretion of catecholamines, termed the "fight-or-flight response" happens within seconds of an animal perceiving a stressor and is the first wave of the stress response. CORT release from the adrenal glands is a little slower, and is considered the second wave of the stress response because CORT-mediated physiological changes usually take minutes or hours to occur (Sapolsky et al., 2000). While increased secretion of catecholamines and glucocorticoids in response to a stressor has been shown to be adaptive in the short term, long-term activation of these systems is thought to be maladaptive (McEwen, 1998; Sapolsky et al., 2000). Chronically elevated levels of catecholamines have been associated with a variety of cardiovascular pathologies including hypertension, myocardial infarction, increased cardiac output, and arrhythmias (Sapolsky and Share, 1994; Rupp, 1999). Long-term, amplified levels of glucocorticoids have been linked to several physiological consequences including diabetes, suppression of the reproductive system, muscle breakdown, and immunosuppression (Wingfield et al., 1997; Sapolsky et al., 2000). The relationship between an animal's health and activation of the stress response is tightly linked, and the ability to quickly identify and detect chronic stress can help conservation biologists assess the general health of at-risk populations (Creel et al., 1997; Wasser et al., 1997; Mason, 1998; Cockrem, 2005).

Previous studies in our laboratory have applied a series of randomly, rotating psychological stressors to induce chronic stress in wild animals (Rich and Romero, 2005; Cyr et al., 2007; Cyr and Romero, 2007; Kostelanetz et al., 2009; Awerman and Romero, 2010). However, applied stressors were anthropogenic (such as restraint, cage disturbance, radios, etc.) and may not be relevant to a free-living animal. Because animals typically experience food shortages or unpredictable food availability in the wild (Newton, 1998), food deprivation or restriction are considered more-ethologically relevant stressors (Acquarone et al., 2002; Cucco et al., 2002). Earlier work indicated that a four-hour period of food removal when food was otherwise available ad libitum resulted in significant CORT elevation — consistent with an acute stress response (Strochlic and Romero, 2008). The principal aim of this study was to test the hypothesis that a sustained daily food restriction causes chronic stress. Furthermore, because responses to chronic stress differ in molting and non-molting birds (Kostelanetz et al., 2009), we included tests in birds under both physiological conditions. A second aim was to determine if unpredictable food availability was more stressful than predictable food availability. The ultimate goal of the study was to determine whether food restriction, a stressor highly relevant to birds, would result in similar markers of chronic stress demonstrated in response to anthropogenic stressors.

2. Methods

2.1. Animals and housing

We used European starlings (*S. vulgaris*) in this study because they are invasive to North America, have been used in a wide range of stress physiology studies, and are large enough to accept heart rate transmitters while still possessing a wide range of motion. Wild starlings were captured from eastern Massachusetts between January and March 2009. Birds were housed outside in sheltered aviaries at Tufts University (Medford, MA, USA). Food and water were provided ad libitum. Birds were fed on an 18% protein diet (Start and Grow SunFresh, Purina Mills). All experiments were approved by the Institutional Animal Care and Use Committee at Tufts University and were carried out according to the guidelines of the Association for Assessment of Laboratory Animal Care.

2.2. Experiment 1: Predictability of food availability in non-molting starlings

Twenty starlings were moved to indoor facilities in January 2010 and were placed in individual cages (45 cm × 37 cm × 33 cm). The cages were housed in the same room and birds were able to see and hear one another. Birds were held on a short-day light cycle (10 L:14D) at 22 °C. Ten birds were then implanted with heart rate transmitters (Data Sciences International model TA 10EA-F20 transmitters, St Paul, MN, USA). For implantation procedures see Nephew and Romero (2003). Surgeries were completed six days before switching to a long-day light cycle (14L:10D). Birds were then given a ten-day adjustment period before starting the experiment.

We split our birds into either the Predictable Food Availability Treatment (henceforth abbreviated as Predictable) or the Unpredictable Food Availability Treatment (henceforth abbreviated as Unpredictable). Each treatment group had five starlings with heart rate transmitters and five starlings without heart rate transmitters. We divided males and females evenly between our treatment groups (6 females and 4 males in each group), although past studies have shown no sex differences in the starling stress response while in captivity (Romero and Remage-Healey, 2000).

Our experiment consisted of three stages: Control (7 days), Food Restriction (20 days), and Recovery (15 days). During the Control and Recovery stages, all birds had ad libitum food and water. During the Food Restriction stage, all birds had their food removed from their cage every day for four continuous hours. Otherwise, food was available ad libitum. Food removal only occurred during the lights-on period because starlings usually eat only during the day (Feare, 1981). Food was never removed within the first hour of lights turning on or within one hour of lights turning off. Water was always available ad libitum. Predictable birds always had their food removed 5 h after lights-on, whereas the Unpredictable individuals had their food removed at an unpredictable time alternating randomly between 1, 3, 5, or 7 h after lights-on. To summarize, both treatment groups experienced the same four-hour duration where food was unavailable, but differed in the predictability of when the food was removed.

To determine if there were caloric intake differences between the Predictable and Unpredictable treatments, we measured the daily food consumption rate of each bird. Since starlings are messy eaters and tend to throw their food around, we built open-topped, clear Plexiglas boxes (43.5 cm × 38 cm × 25 cm) around each cage so we could collect any spilled food. Food consumption rate was determined by subtracting the mass of the remaining food in the food dish, plus any food that spilled on the cage bottom or into the Plexiglas cage barrier, from the mass of the food prior to putting it in the cage the day before.

We took baseline and stress-induced blood samples every three days within one hour of lights on so we could measure corticosterone (CORT) concentrations. We took blood samples during the morning so as to measure the chronic effect of food restriction on CORT levels. An earlier study already established that food removal acutely increases CORT (Strochlic and Romero, 2008). While taking blood samples, we also weighed the birds to the nearest half-gram. Each day we took heart rate measurements on a total of four birds (four being the limit of our heart rate measurement software). The implanted heart rate transmitters sent out signals to receiver plates attached to the side of the cage. These data were then transferred to a computer and analyzed with Dataquest Advanced Research Technology Gold 4.0. Heart rate was collected continuously and averaged every 30 s. We took 30-min heart rate measurements three times a day: half an hour after lights turned on (Morning), half an hour after the food was removed from the cage (Food Out), and half an hour after food was returned to the cage (Food Back In) (see Fig. 1). We always measured heart rate half an hour after entering the room because this level of human disturbance transiently elevates heart rate with a return to