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Endocrine, metabolic, and behavioral effects of and recovery from acute stress in a free-ranging bird

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ABSTRACT

Acute stress in vertebrates generally stimulates the hypothalamo-pituitary-adrenal axis and is often associated with multiple metabolic changes, such as increased gluconeogenesis, and with behavioral alterations. Little information is available, especially in free-ranging organisms, on the duration of these reversible effects once animals are no longer exposed to the stressor. To investigate this question, we exposed free-ranging adult male Rufous-winged Sparrows, *Peucaea carpalis*, in breeding condition to a standard protocol consisting of a social challenge (conspecific song playback) followed with capture and restraint for 30 min, after which birds were released on site. Capture and restraint increased plasma corticosterone (CORT) and decreased plasma testosterone (T), glucose (GLU), and uric acid (UA). In birds that we recaptured the next day after exposure to conspecific song playback, plasma CORT and UA levels no longer differed from levels immediately after capture the preceding day. However, plasma T was similar to that measured after stress exposure the preceding day, and plasma GLU was markedly elevated. Thus, exposure to social challenge and acute stress resulted in persistent ($\geqslant$24 h) parameter-specific effects. In recaptured sparrows, the territorial aggressive response to conspecific song playback, as measured by song rate and the number of flights over the song-broadcasting speakers, did not, however, differ between the first capture and the recapture, suggesting no proximate functional association between plasma T and conspecific territorial aggression. The study is the first in free-ranging birds to report the endocrine, metabolic, and behavioral recovery from the effects of combined social challenge and acute stress.

1. Introduction

In vertebrates, acute stressors activate the hypothalamo-pituitary-adrenal (HPA) axis, resulting in elevated plasma glucocorticoids (GCs; primarily corticosterone [CORT] in birds; Astheimer et al., 1995; Cockrem et al., 2016; Deviche et al., 2014; O'Reilly and Wingfield 2001). Stressors can also induce metabolic changes including an increase in glycemia (cat, *Felis catus*: Rand et al., 2002; Japanese Quail, *Coturnix coturnix japonica*: Gildersleeve et al., 1985; turkey, *Meleagris gallopavo*: Donaldson et al., 1991; chicken, *Gallus gallus*: Debut et al., 2005) and so enhance the amount of glucose (GLU) available to metabolically active tissues. Stimulatory effects of stress on glycemia are, however, not observed universally. In particular, several avian studies found that plasma GLU did not increase (Curve-billed Thrasher, *Toxostoma curvirostre*: Fokidis et al., 2011; European Starling, *Sturnus vulgaris*: Remage-Healey and Romero, 2002 Abert's Towhee, *Melozone aberti*: Davies et al., 2013) or even decreased (Rufous-winged Sparrow, *Peucaea carpalis* [RWSP]: Deviche et al., 2014, 2016; see below) during acute stress. Furthermore, GCs are generally considered to play an important role in mediating the stimulatory effects of stress on glycemia, but the generality of this conclusion in birds is not established. Indeed, the increase in plasma CORT that is observed during stress was associated with elevated plasma GLU in some cases (Debut et al., 2005; Herring Gull, *Larus argentatus*: Jeffrey et al., 1985) but not others (pigeon, *Columba livia*: Viswanathan et al., 1987; chicken: Xie et al., 2015). These differences may stem from CORT stimulating glycemia in some species and not others. Alternately, methodological differences between studies may account for the observed variation in relationship between plasma CORT and GLU. For example, in the European Starling, acute stress elevated plasma CORT, but plasma GLU increased during stress at night and not during

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daytime (Remage-Healey and Romero, 2001). In the same study CORT administration at supraphysiological dose did by itself not influence plasma GLU, but this treatment hastened recovery from the hypoglycemic effect of concurrent insulin injection.

Acute stress can also promote oxidative damage (Lin et al., 2004; Huang et al., 2015) and in some avian species, it decreases the plasma level of the main circulating antioxidant, uric acid (UA; Cohen et al., 2007). At the individual level, the decrease in plasma UA during acute stress correlates with the corresponding increase in plasma CORT (Deviche et al., 2016). This correlation suggests a causal relationship whereby elevated plasma CORT lowers plasma UA, but whether this is the case remains unclear. Indeed, UA is a main product of protein degradation and in the chicken, CORT treatment during growth decreased muscle mass and increased UA excretion, suggesting increased UA production (Siegel and Van Kampen, 1984). Furthermore, CORT treatment to chickens increased plasma UA within hours (Simon 1984). Acute stress also often reduces the plasma levels of reproductive hormones such as testosterone (Lynn et al., 2010; Deviche et al., 2014; Davies et al., 2016). In mammals, GCs are thought to contribute to this reduction by acting on gonads (Orr and Mann, 1992; Hardy et al., 2005; Dong et al., 2004; Monder et al., 1994), but few studies have researched this subject in non-mammalian vertebrates.

Most of the avian studies described above investigated captive subjects or domesticated species, and so we know little about relationships between plasma CORT, T, UA, and GLU in free-ranging birds. To address this question, our recent work used free-ranging adult male RWSP, the same species as used in the present work. Exposing RWSP to acute stress (capture and restraint for 30 min) consistently increased plasma CORT and decreased plasma UA (Deviche et al., 2012, 2014, 2016). However, acute stress influenced plasma T and GLU in a life history stage-dependent manner. During stress plasma T decreased in pre-breeding and breeding condition males, when plasma T was initially (i.e., before experimental stress) high, but not in molting and non-breeding males, when levels of this hormone were lower (Deviche et al., 2016). Furthermore, in the same study GLU increased during acute stress in pre-breeding birds, did not change in late-breeding birds, and decreased in birds sampled during post-breeding molt and in winter. We also found that blood hormone and metabolites were selectively influenced by social factors: a social challenge consisting in conspecific song playback exposure for 30 min increased plasma CORT and decreased plasma UA (Deviche et al., 2014) as effectively as restraint for the same duration, but did not affect plasma T or GLU. The *first objective* of the present work was to confirm and extend these findings. For this, we exposed males to song playback, caught them and took a blood sample, restrained them for 30 min, and took a second blood sample. If song playback exposure increases plasma CORT and decreases plasma UA maximally, we predicted that subsequent acute stress exposure would have no additional effect on these parameters. Alternatively, if song playback exposure does not maximally stimulate and depress plasma CORT and UA, respectively, we predicted acute stress to further elevate plasma CORT and decrease plasma UA. The present investigation was conducted using sparrows in breeding condition and so based on the above-described studies, which also used birds in breeding condition, we predicted acute stress to decrease plasma T and either to decrease or to not influence plasma GLU.

Once individuals are no longer exposed to an acute stressor, endocrine effects of stress generally subside. The latency to restore homeostasis has been investigated in humans (Annerstedt et al., 2013) and domesticated animals (Ericsson et al., 2014). For example, in the chicken, plasma CORT increased within 10 min of restraint and had returned to pre-restraint level 50 min after birds were released into their cages (Ericsson et al., 2014). In captive European Starlings, plasma CORT returned to levels approximating initial (pre-stress) levels three hours after stress exposure (Remage-Healey and Romero, 2001). It should be pointed out that these studies provide no information about the time course of recovery from a singular stressful event in free-ranging vertebrates and only one study has, to our knowledge, investigated this question in birds. In this study on male RWSP, plasma T decreased within 10 min of capture and restraint, and had not recovered after birds were released and recaptured, on average, three hours later (Deviche et al., 2012). The *second objective* of the present work was to further characterize how birds hormonally and metabolically recover from acute stress. To this aim, we measured plasma CORT, T, GLU, and UA in free-ranging adult male RWSP one day after birds were socially challenged, caught, exposed to acute stress as described above, and released on site. Based on the above studies on chickens and captive European Starlings, we predicted plasma CORT levels to be similar at recapture to those measured at the time of first capture the preceding day. If so and if plasma CORT regulates plasma T, GLU, and UA on a short-term basis, we predicted the values of these parameters at the time of recapture to also be similar to those at the time of initial capture. Alternatively, if CORT, but not T, GLU, and/or UA, had returned to initial levels at the time of recapture, this would suggest that changes in plasma T, GLU, and/or UA are proximately controlled by factors other than or in addition to plasma CORT.

Across vertebrates, testosterone (T) generally increases the expression of aggressive behavior (Wingfield et al. 2006). An association between T and the expression of aggressive behavior is supported by the increase in aggression coincident with elevated T during transition to the breeding period, but the correlation between plasma T and within- as well as between-individual variation in aggression is less clear (Wingfield et al., 1987). The *third objective* of the present investigation was to address this question. To this aim we determined the relationships between plasma T and territorial aggression toward conspecific song playback before initial capture and again immediately before recapture the next day. If, at the time of recapture, plasma T was still low compared to initial levels the previous day, and if plasma T proximately regulates territorial aggression, we predicted that birds would be less responsive to song playback one day after the initial capture than on the previous day. To our knowledge, this is the first study to examine the time course of endocrine and metabolic recovery from acute stress in free-ranging birds, as well as the territorial aggressive behavioral correlates of this recovery.

2. Materials and methods

2.1. Species and study site

We sampled 17 experimentally naïve adult male RWSP (17th – 20th July 2014; 6 am – 3 pm MST) during the breeding period of the species at the Santa Rita Experimental Range (Pima Co., AZ, USA; altitude: 979 m.a.s.l.; latitude: 31° 49′ N; longitude: 110° 55′ W). Breeding in this species closely follows the onset of the summer monsoon which, in the region where the study was conducted, usually begins in early July (Lowther et al.,1999). Rufous-winged Sparrows are sedentary, males are territorial year-round, and they have been investigated extensively (Lowther et al., 1999; Deviche et al., 2006, 2014, 2016).

2.2. Behavioral quantification, capture, and blood sampling

On Sample Day 1, we located males based on spontaneous singing or brief (⩽2 min) response to conspecific song playback and exposed them to a standard protocol consisting of song playback