Adrenocortical responses in zebra finches (Taeniopygia guttata): Individual variation, repeatability, and relationship to phenotypic quality

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Abstract

Although individual variation is a key requirement for natural selection, little is known about the magnitude and patterns of individual variation in endocrine systems or the functional significance of that variation. Here we describe (1) the extent and repeatability of inter-individual variation in adrenocortical responses and (2) its relationship to sex-specific phenotypic quality, such as song duration and frequency and timing of egg laying. We measured adrenocortical responses to a standardized stressor in zebra finches (Taeniopygia guttata) at two life history stages: ~day 16 (nestlings) and 3 months of age (sexually mature adults). Subsequently, we assessed phenotypic (reproductive) quality of all individuals as adults. Marked inter-individual variation in the adrenocortical response was seen in both sexes and ages, e.g., stress-induced corticosterone ranged from 2.2 to 62.5 ng/mL in nestlings and 5.0–64.0 ng/mL in adults. We found sex differences in (a) inter-individual variation in the adrenocortical response, (b) repeatability, and (c) relationships between corticosterone levels and phenotypic quality. In males, variation in nestling corticosterone was weakly but positively correlated with brood size and negatively correlated with nestling mass (though this relationship was dependent on one individual). There was no significant correlation of adrenocortical responses between two stages in males and adult phenotypic quality was significantly correlated only with adult corticosterone levels. In contrast, in females there was no relationship between nestling corticosterone and brood size or mass but adrenocortical response was repeatable between two stages ($r^2=0.413$). Phenotypic quality of adult females was correlated with nestling baseline and adrenocortical response.

Keywords: Adrenocortical response; Individual variation; Phenotypic quality; Taeniopygia guttata

Introduction

Although it is a widely held view that there is an extensive physiological diversity among individuals of the same species (e.g., Adkins-Regan, 2005), it is still very rare for studies to present, let alone formally analyze, inter-individual variation in endocrine or physiological traits (

have been documented, though these are typically non-linear and take the form of threshold or step functions (Adkins-Regan, 2005). It is far less clear how individual variation in hormone titers relates to variation in continuous traits (or variation within specific behaviors or morphs), although it has been suggested that for behavioral traits these correlations can often be near zero or even negative (Adkins-Regan, 2005).

Here we describe inter-individual variation and repeatability in hypothalamic–pituitary–adrenal (HPA) axis reactivity to a standardized stressor in nestling and adult zebra finches (Taeniopygia guttata) and relate this variation to sex-specific measures of phenotypic quality. Mammalian and avian studies have shown that early hormonal and behavioral experiences have permanent effects on glucocorticoid-driven stress physiology: pre- and post-natal stress results in enhanced stress reactivity later in life (reviewed in Caldji et al., 2001). In addition, variation in adrenocortical response in adults is widely assumed to have fitness consequences. For instance, glucocorticoids have been negatively correlated with foraging success (Angelier et al., 2007), numbers of offspring successfully raised (Bonier et al., 2007; Good et al., 2005), and survival (Blas et al., 2007; Romero and Wikelski, 2001) (but also see Comendant et al., 2003). Several studies have described inter-individual variation in the adrenocortical response of adult animals (birds, Littin and Cockrem, 2001; Cockrem and Silverin, 2002; mammals, Guimont and Wynne-Edwards, 2006) and individual variation in adrenocortical response can be repeatable (Cockrem and Silverin, 2002; Schjolden et al., 2005). Furthermore, in hamsters post-stress corticosterone (CORT) levels are positively correlated with pre-stress baseline CORT suggesting that individual variation in “baseline”, non-manipulated hormone levels can be informativecan was then separated and stored at −80 °C until they were shipped on dry ice to the University of Texas at Austin, where they were analyzed for CORT and corticosteroid binding globulin (CBG).

Assessment of phenotypic quality of adults

Upon completion of the adult stress series at 3 months of age, one female and one male per brood were selected (matched for body mass and age) for a breeding attempt with an experienced male, or a courtship trial with an experienced female, respectively. For each individual, we conducted two breeding or courtship trials so we could determine repeatability of phenotypic traits that would be reliable indicators of individual quality.

Each selected female was paired and allowed to breed with a randomly chosen experienced male under standard conditions as described above. We recorded laying interval (i.e., days between pairing and initiation of egg laying), egg mass, and clutch size. In addition, blood samples were collected from each female on the day the first egg was laid to measure plasma yolk precursor levels (vitellogenin (VTG) levels measured as plasma zinc levels, and very low density lipoprotein (VLDL) levels measured as plasma triglyceride levels; following Mitchell and Carlisle, 1991; Williams and Christians, 1997). Clutches were considered complete following 2 days of no additional eggs, and we then rested females for approximately five weeks before repeating the breeding trial.

Male courtship trials were conducted as follows: an experienced wild-type female was randomly chosen and placed in a cage (61 × 46 × 41 cm) for 5 min to acclimate alone. Different females were randomly chosen for each male and trial. The cage contained a perch, a grit, a cuttlefish bone, but no water or food inside, and a microphone and timer on the outside. The cage was visually but not acoustically isolated from other cages. Each male in this study was placed in the cage with the experienced female, and the behaviors of both the male and the female were recorded for 15 min. All of the courtship trials were performed between 0900 and 1200 h. Six typical male courtship displays (described in Zann, 1996) were recorded during the experiment: invitation (Y or N), bill wiping (number of wipes), head or tail twisting (scored per left to right cycle), following (number of times the male followed the female), singing (duration and frequency), and mounting/copulation (number of mount, number of successful copulation, and time in seconds to initial attempt).

50 μL buffer, and 50 μL plasma, while non-specific binding was measured using 50 μL 3 H CORT, 50 μL 1 μM cold CORT, and 50 μL plasma. After the 2-h incubation, samples were filtered with chilled rinse buffer to trap 3 H CORT-bound CBGs, which were later counted for their radioactivity. All the samples were randomly distributed among three point sample assays that were run in two consecutive days. An average of 17.81 nM 3 H CORT was used in these assays, which should occupy 77.6% (nestlings) and 90.3% (adults) of total binding sites. Thus, we adjusted the CBG capacity to 100% for the free hormone analysis. Intra- and inter-assay variations were 9.3% and 18.5%, respectively.

To estimate the free hormone levels, we used the equation by Barsano and Baumann (1989):

$$H_{free} = 0.5 \times \left[H_{total} - B_{max} - 1/K_a \pm \sqrt{(B_{max} - H_{total} + 1/K_a)^2 + 4(H_{total}/K_a)} \right]$$

where K_a is $1/K_d$ (nM), K_d is affinity of CORT for CBG, B_{max} is total CBG capacity, and H_{total} is total plasma hormone concentration. K_d was determined in equilibrium binding analysis using pooled plasma (Wada et al., in review).

Table 2
Summary of multiple regression analysis for males (a) and females (b) with environmental and body condition factors against corticosterone levels

		Model summary		Factors	Standardized beta	Significance
		Adjusted r^2	Significance			
(a) Males						
Nestling	Total baseline	0.483	0.006	Brood size	0.368	0.058
				Nestling mass	−0.506	0.02
				Nestling tarsus		ns
	Total integrated	0.046	0.323	Brood size		
				Nestling mass		
				Nestling tarsus		
	Free baseline	0.474	0.01	Brood size	0.411	0.047
				Nestling mass	−0.527	0.019
				Nestling tarsus		ns
	Free integrated	−0.019	0.466	Brood size		
				Nestling mass		
				Nestling tarsus		
Adult	Total baseline	0.153	0.103	Adult mass		
				Adult tarsus		
	Total integrated	0.199	0.066	Adult mass		
				Adult tarsus		
	Free baseline	0.1	0.167	Adult mass		
				Adult tarsus		
	Free integrated	0.277	0.029	Adult mass		ns
				Adult tarsus	−0.451	0.08
(b) Females						
Nestling	Total baseline	−0.182	0.943	Brood size		
				Nestling mass		
				Nestling tarsus		
	Total integrated	0.007	0.406	Brood size		
				Nestling mass		
				Nestling tarsus		
	Free baseline	−0.183	0.945	Brood size		
				Nestling mass		
				Nestling tarsus		
	Free integrated	−0.188	0.956	Brood size		
				Nestling mass		
				Nestling tarsus		
Adult	Total baseline	−0.082	0.659	Adult mass loss		
				Adult mass		
				Adult tarsus		
	Total integrated	−0.036	0.518	Adult mass loss		
				Adult mass		
				Adult tarsus		
	Free baseline	−0.159	0.91	Adult mass loss		
				Adult mass		
				Adult tarsus		
	Free integrated	− 0.ult -1.ted	−			

Data analysis

Statistical analyses were performed using SPSS 15.0 and JMP 5.0.1. Multiple regression analyses examined relationships between CORT and possible factors influencing the individual variation in HPA axis reactivity. Repeatability of the adrenocortical response (log-transformed) and reproductive traits were determined using the equation by Lessells and Boag (1987)

Multiple regression analyses showed that in males both total ($p = 0.02$) and free ($p = 0.019$) baseline CORT were negatively correlated with nestling mass (Table 2 and Fig. 2). In contrast, both total ($p = 0.058$) and free ($p = 0.047$) baseline CORT were positively correlated with brood size. However, these relationships are largely driven by one extreme data point. Excluding this value these correlations were all non-significant ($p > 0.20$). Once males reach adulthood, adult tarsus length was marginally correlated with free integrated CORT ($p = 0.08$), but other measures of body condition and body mass were independent of hormone levels. In females, none of the hormone levels correlated with nestling “environmental” or “body condition” total CORT $F = 5.60$, $p = 0.037$; free CORT $F = 15.03$, $p = 0.003$). However, number of bill wipes was not significantly correlated with any of the measured components of the adrenocortical response at either stage in males ($p > 0.05$).

Discussion

In this study, we found marked inter-individual variation in the adrenocortical response in both sexes and ages, e.g., total and free CORT varied as much as 29-fold and 70-fold between individuals, respectively. On average, ~day 16 nestlings had a significantly greater adrenocortical response than adults, both for total and free CORT levels, and this difference was significant by 15 min post-handling. We found sex differences in inter-individual variation in the adrenocortical response, repeatability, play a larger role in determining individual variation in zebra finches. This points to the importance of confirming repeatability/predictability of endocrine responses if one uses developmental responses to predict future quality.

Inter-individual variation in corticosterone in relation to phenotypic quality

Chronically elevated CORT in adults inhibits reproduction in many taxa (see Sapolsky et al., 2000). More interestingly, an early exposure to CORT during embryonic or neonatal development modifies adult reproduction as well (Spencer et al., 2003). For example, CORT administration and food reduction during pre- and post-fledging have been shown to have pleiotropic effects, reducing song duration and complexity as well as growth in zebra finches. Here we have shown that natural variation in CORT is correlated with reproductive quality of sexually mature adults. In both sexes, low total baseline CORT was correlated with high

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