



Early life stress increases testosterone and corticosterone and alters stress physiology in zebra finches



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ABSTRACT

Early life stress has enduring effects on behavior and physiology. However, the effects on hormones and stress physiology remain poorly understood. In the present study, parents of zebra finches of both sexes were exposed to an increased foraging paradigm from 3 to 33 days post hatching. Plasma and brains were collected from chicks at 3 developmental time points: post hatching days 25, 60 and adulthood. Plasma was assayed for testosterone (T), estradiol (E2), and corticosterone (CORT). The paraventricular nucleus of the hypothalamus was assessed for corticotrophin releasing factor (CRH) and glucocorticoid receptor (GR) expression. As expected, body mass was lower in nutritionally stressed animals compared to controls at multiple ages. Nutritionally stressed animals overall had higher levels of CORT than did control and this was particularly apparent in females at post hatching day 25. Nutritionally stressed animals also had a higher number of cells expressing CRH and GR in the paraventricular nucleus of the hypothalamus than did controls. There was an interaction, such that both measures were higher in control animals at PHD 25, but higher in NS animals by adulthood. Females, regardless of treatment, had higher circulating CORT and a higher number of cells expressing CRH than did males. Nutritionally stressed animals also had higher levels of T than did control animals, and this difference was greatest for males at post hatching day 60. There were no effects of nutritional stress on E2. These findings suggest that nutritional stress during development has long-lasting effects on testosterone and stress physiology.

Early life conditions (adverse or positive) have lasting effects on behavior and physiology (Buchanan et al., 2003). Early life adversity, or developmental stress, can be induced in birds through nutritional stress (NS), in which parents must spend more time foraging and, presumably, less time taking care of hatchlings. NS negatively impacts learning and memory, as evidenced by song quality deficits in males (Brumm et al., 2009; Spencer et al., 2003; Spencer et al., 2005; Zann and Cash, 2008).

One way in which NS may alter song learning in male birds is by chronically increasing corticosterone (CORT) and permanently altering the hypothalamic-pituitary-adrenal (HPA) axis. Chronic increases in CORT lead to immunosuppression (Buchanan and Catchpole, 2000) and deficits in neural development (McEwen et al., 1995). However, multiple studies have found no effects of NS on circulating CORT after the cessation of NS (Brumm et al., 2009; Buchanan et al., 2003; Spencer et al., 2003). One study found increased CORT during NS (Kriengwatana et al., 2014). These findings suggest CORT is only elevated for a brief period following the onset of the stressor and returns to baseline, even perhaps when the stress is ongoing (Pravosudov and

Kitaysky, 2006). To assess when CORT is elevated relative to NS, we measured CORT both during and after NS.

NS may also affect learning through other mechanisms than elevated CORT. Estradiol (E2) and testosterone (T) are crucial for song production in male zebra finches (Konishi and Akutagawa, 1985). E2 treatments masculinize the female song system (Grisham and Arnold, 1995; Gurney and Konishi, 1980; Holloway and Clayton, 2001) and cause females to produce male-like song (Gurney and Konishi, 1980; Simpson and Vicario, 1991). In males, intracranial injections of an estrogen receptor antagonist demasculinize the song system (Bender and Veney, 2008). Such findings would lead us to conclude that males have higher circulating levels of E2 than do females during development. Hutchison et al. (1984) found that males did have higher levels of E2 than did females at post hatching day (PHD) 4. Song learning, however, occurs during the first 60 days post hatching, and other work suggests no significant sex differences through PHD 54, although further tests revealed a male-biased sex difference at PHD 12 and 14 (Adkins-Regan et al., 1990). In the present study, we aimed to replicate findings from the Adkins-Regan et al. (1990) study. Furthermore, if song quality is E2

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dependent, then we predicted that NS males would have lower E2 than would control males.

E2 works in concert with androgens to promote song learning. E2 treatments increase androgen receptor (AR) mRNA. AR also increases and reaches its peak at the time ER α expression begins to decrease (Kim et al., 2004). This pattern of expression may define the sensitive period for song learning, in that E2 may be responsible for maintaining plasticity during the sensitive period for song learning, while T closes this sensitive period (Schlinger, 1997). Therefore, excessive T exposure prior to PHD 45 (when song learning normally ends) may close the sensitive period for song learning early and affect later production of song. Indeed, administration of adult levels of T in male zebra finches from PHD 20–40 significantly reduces song repertoire size compared to controls (Korsia and Bottjer, 1991).

At least one study has examined the effects of NS on song nuclei in the brain. Buchanan et al. (2003) found decreases in the volume of HVC in zebra finches that had experienced NS until post-hatching day 30. Interestingly, the volume of HVC was quantified in the study *via* expression of androgen receptor mRNA. This is further support that NS could impact neural E2, as E2 is necessary for the normal development of ARs in HVC (Nordeen et al., 1986).

Thus, we hypothesized NS males would have increased exposure to T at PHD 25 compared to control males, which would account for reduced song quality. Some research supports this hypothesis. Experimental brood size enlargement, a stressor, significantly increases levels of circulating T (Naguib et al., 2004; Spencer et al., 2004). Similarly, unpredictable food supply reduces repertoire sizes and elevated T in male European starlings (Spencer et al., 2004).

Finally, we were the first, to our knowledge, to test the hypothesis that NS has permanent effects on the neural stress response. To do this, we measured glucocorticoid (GR) and corticotropin releasing hormone (CRH) expression in the paraventricular nucleus of the hypothalamus (PVN). Long-term stress causes detriments to psychological and physiological function [see McEwen, 2006 for review]. For example, high levels of CORT activate glucocorticoid (GR) receptors. Increased activation decreases synaptic plasticity, whereas experimentally blocking GR restores synaptic plasticity (Krugers et al., 2006). However, other research suggests that GR expression *decreases* after chronic stress (Banerjee et al., 2012; Dickens et al., 2009). Expression of CRH is released by the hypothalamus to activate the HPA axis during stress, such that increases in CORT *increase* CRH expression in the PVN (Liu et al., 2001; Makino et al., 2002). We therefore predicted that NS would increase CRH and decrease GR expression.

In sum, the goal of the present study was to test the hypothesis that NS alters sex steroids and stress physiology. To accomplish this, birds were exposed to NS from PHD 3 to 33. Birds were sacrificed at 3 time points (PHD 25, 60 and adult). Trunk blood was collected at the time of sacrifice to assay CORT, E2, and T, and brains were also collected to measure CRH and GR mRNA in the PVN. We hypothesized that NS would increase CORT. We also predicted that NS would increase T and decrease E2 in zebra finches. Furthermore, we predicted that NS would increase expression of CRH and decrease expression of GR.

1. Method

1.1. Breeding

To obtain subjects for the experiment, adult male and female zebra finches were housed in aviaries (10 of each sex) and allowed to pair for 1 week. They were then given nest boxes and sanitary nesting materials to encourage synchronized egg laying. Each aviary was randomly assigned to NS or control. All birds were given *ad libitum* access food, water and grit and twice per week supplements of hard-boiled chicken egg mixed with bread. To control for the effects of parenting, each pair only raised one nest. Each nest was limited to 4 chicks to control for the effects of brood size. Breeding conditions were repeated with new pairs

of adults until a minimum of 8 animals were obtained in each condition.

1.2. NS

In the control condition, birds received 2 dishes each containing 200 mg of seed, replaced every 48 h. When the first nestling in the aviary reached PHD 3, animals in the NS condition received the same amount of seed as controls, but the seed was mixed with wood chips at a ratio of 1:3, as in earlier work (Kriengwatana et al., 2014). After the first nestling reached PHD 6, eggs were removed from nests that had not yet produced nestlings, to ensure that all nestlings experienced NS during a similar time frame. Seed ratios were reset every 48 h. When the first offspring in an aviary reached post hatch day 33, birds once again received *ad libitum* access to seed. This protocol has been used successfully in previous studies (Kriengwatana et al., 2014; Spencer et al., 2003; Zann and Cash, 2008).

1.3. Subjects

A total of 84 subjects (40 NS and 44 control; 41 males and 43 females) were included in the study. Subjects were randomly assigned to 1 of 3 age groups: PHD 25, PHD 60, and adults. To control for the effects of family, subjects within the same nest were assigned to different age groups. Subjects were marked with food coloring at birth. Once the leg was large enough to support a band, subjects were banded with unique color/number combinations. Subjects remained in the home cage with their parents until the end of the experiment, with the exception of birds in adult condition; these animals were removed from their home cage at PHD 60 and placed in same-sex aviaries and allowed to grow up. See Table 1 for final animal numbers in each treatment group by sex. This research was conducted in strict accordance with the IACUC standards for animal use in research at Wayne State University.

1.4. Body weight measurements

Subjects were weighed 4 times per week for 3 weeks, and then weighed again before brain collection. To do this, birds were placed on a scale in the aviary, to minimize time away from the nest. Once birds fledged (around PHD 14), they were placed into a small black sock to prevent them from flying while being weighed.

1.5. Sex determination

To maximize the efficiency of obtaining target numbers of each sex

Table 1
The Number of subjects in each experiment.

Condition	CORT	T	E2	CRH/GR
NS male				
PHD25	7	6	6	5
PHD60	3	4	4	5
PHD90	6	6	6	7
Control male				
PHD25	6	6	6	6
PHD60	4	5	5	5
PHD90	6	6	6	9
NS female				
PHD25	6	6	6	5
PHD60	4	4	5	5
PHD90	8	7	5	7
Control female				
PHD25	6	5	6	5
PHD60	5	7	8	7
PHD90	5	5	8	5

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