General and Comparative Endocrinology 196 (2014) 123–129

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Corticosterone exposure during development has sustained but not lifelong effects on body size and total and free corticosterone responses in the zebra finch

O.L. Crino [a,b,*], Stephanie C. Driscoll [b], C.W. Breuner [b]

[a] Department of Biological Sciences, Macquarie University, 2122 New South Wales, Australia
[b] Division of Biological Sciences, University of Montana, Missoula, Montana 59812, United States

ARTICLE INFO

Article history:
Received 15 March 2013
Revised 30 August 2013
Accepted 10 October 2013
Available online 1 November 2013

Keywords:
Developmental stress
Corticosterone
Nestling
Passerine
Body size
Brood size

ABSTRACT

Animals exposed to stress during development experience sustained morphological, physiological, neurological, and behavioral consequences. For example, elevated glucocorticoids (GCs) during development can increase GC secretion in adults. Studies have examined the sustained effects of elevated developmental GCs on total GC responses, but no study to date has examined the effect of developmental stress on corticosteroid binding globulin (CBG). CBG is a protein which binds to GCs and facilitates their transportation in blood. When bound to CBG, GCs are unavailable to interact with target tissues. Exposure to stress can decrease CBG capacity and, thus, increase free GCs (the portion of unbound GCs). We examined the long-term effects of elevated corticosterone (CORT) during development (12–28 days post-hatch) on acute stress responses, negative feedback, and CBG capacity at 30, 60, and 90 days post-hatch in zebra finches. Additionally, we evaluated the effect of CORT treatment on body size and condition at 28, 60, and 90 days post-hatch. CORT exposed birds had higher acute stress responses at 30 days post-hatch compared to control birds. However, there was no treatment effect at 60 or 90 days post-hatch. CBG levels were not affected by treatment, and so free CORT estimations reflected patterns in total CORT. CORT treatment decreased growth and condition in zebra finches at 28 days post-hatch, but these differences were not present at later life history stages. However, brood size had a sustained effect on body size such that birds reared in medium sized broods were larger at 28, 60, and 90 days post-hatch. These results demonstrate the complexity of early environmental effects on adult phenotype and suggest that some conditions may have stronger programmatic effects than others.

Published by Elsevier Inc.

1. Introduction

Glucocorticoids increase in response to external perturbations and promote behavioral and physiological changes to restore homeostasis. In this way, glucocorticoids (GCs) have *activational* effects on adult phenotype and behavior. In developing animals, GCs can have similar effects on short-term behavior and physiology. In addition, there is a growing body of literature from across taxonomic groups which suggest that GCs have *organizational* effects on developing animals (a process known as developmental programming; (Mcmillen and Robinson, 2005). Specifically, animals exposed to elevated levels of GCs during development can experience sustained morphological, physiological, neurological, and behavioral consequences (reviewed in Matthews (2005), Nesan and Vijayan (2005), Schoech et al. (2012)). In some cases, these phenotypic effects appear to be life-long and can even be transmitted across generations (Catalani et al., 2000; Liu et al., 1997; Schöpper et al., 2012; Weaver et al., 2000).

Recent research has focused on the organizational effects of developmental stress on hypothalamic–pituitary–adrenal (HPA) axis activity in birds. Exposure to pre- and postnatal stress (via elevated GCs, reduced maternal condition, food restriction), can significantly affect HPA axis function at later life history stages. In general, developmental stress causes sustained elevation of HPA function such that animals exposed to stress during development respond more strongly to stressors as adults (e.g. Marasco et al., 2012; but see Lendvai et al., 2009; Love and Williams, 2008). For example, chicks from CORT-implanted Japanese quail (*Coturnix coturnix japonica*) grew more slowly and had significantly higher HPA responses to stressors at eight weeks of age compared to controls (Hayward and Wingfield, 2003). Postnatal GC exposure has

Abbreviations: CBG, corticosteroid binding globulin; CORT, corticosterone; EPC, extra pair copulation; GC, glucocorticoid; HPA, hypothalamic pituitary adrenal.

* Corresponding author at: Department of Biological Sciences, Macquarie University, 2122 New South Wales, Australia.
E-mail addresses: andrea.crino@mq.edu.au, andrea.crino@umontana.edu (O.L. Crino).

0016-6480/$ - see front matter Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.ygcen.2013.10.006

been shown to have similar effects on HPA axis function. Zebra finches (*Taeniopygia guttata*) fed CORT dissolved in peanut oil during the nestling period (12–28 days post-hatch) had elevated levels of CORT following an acute stressor compared to control finches at 60 days post-hatch (Spencer et al., 2009). Finally, western scrub jays (*Aphelocoma californica*) raised on a food restricted diet (65% of *ad libitum*) had higher levels of baseline CORT as nestlings and elevated levels of stress-induced CORT at one year of age (Pravosudov and Kitaysky, 2006). These studies, along with many studies from mammals (e.g. Liu et al., 1997, reviewed in Matthews, 2005), demonstrate that exposure to developmental stress (i.e. elevated GCs and food restriction) can have sustained effects on HPA activity at later life history stages.

Although there is substantial support demonstrating the sustained effects of developmental stress (i.e. elevated GCs) on HPA function, no study to date has examined the long-term effects of developmental stress on corticosteroid binding globulin (CBG) capacity. CBG is a protein that binds to GCs with high affinity and facilitates transportation of lipophilic GC molecules through the blood. CBG regulates stress responsiveness by binding to GCs and preventing them from interacting with target tissues (the "free hormone hypothesis;" Mendel, 1989; Malisch and Breuner, 2010; Breuner et al., 2013). Elevated GCs can transiently decrease CBG levels which may increase the amount of CORT available to interact with target tissues. For example, Breuner et al. (2006) showed a reduction in CBG capacity following 60 min of restraint stress in zebra finches suggesting a regulatory role for CBG in response to acute stress. Other studies have shown a decline in CBG capacity 24 h following acute stressors (Malisch et al., 2010, but see Mueller et al., 2009). Therefore, measuring free CORT (the portion of CORT not bound to CBG) can provide additional information about how animals respond to stressors.

We investigated the long-term effects of elevated CORT during development on HPA function, CBG capacity, negative feedback, body size, and condition in zebra finches. We elevated endogenous CORT by orally administering CORT dissolved in peanut oil to nestling zebra finches for 16 days during the nestling period (from 12 to 28 days post-hatch). We predicted that CORT-exposed nestlings would have elevated total CORT, lower CBG capacity, and reduced negative feedback as adults, compared to controls. We also expected that CORT treatment would result in reduced body size and body condition metrics. These results would support and expand recent studies demonstrating sustained effects of elevated developmental GCs on phenotype and physiology.

2. Methods

2.1. Parental birds – housing and breeding

Ten female and ten male zebra finches were purchased from six pet stores across Montana and Washington. We banded the birds with a unique combination of color bands in order to identify individual birds. Throughout the course of the experiment, 3 males and 2 females were replaced due to mortality. Breeding finches were housed in a 20×25 ft. room where they were allowed to interact freely with all other birds. We housed the birds on a 14:10 light/dark cycle at 26–27 °C with 20–30% humidity. Birds had access to 12 nest boxes and shredded burlap nesting material. We fed birds commercial finch seed (Silver Song West) and spray millet *ad libitum* and supplemented their diet daily with hard boiled eggs, spinach, and crushed egg shells. Nest boxes were monitored daily for signs of nest building and egg laying. Over the course of the experiment, 48 clutches of nestlings were produced.

2.2. Nestlings– treatment and measurements

Starting on hatch day, we marked nestlings with an individual combination of leg markings using a black Sharpie marker. Between three and four days after hatching, we banded nestlings with a numbered plastic leg band. Twelve days after hatching, we weighed nestlings to the nearest 0.1 g and measured tarsus length (posterior to anterior tarsus) and wing chord (carpus to longest primary feather) to the 0.1 mm. Within clutches, nestlings were randomly assigned to treatment groups such that roughly half of each clutch was CORT-treated and half control. Nestlings exposed to the CORT treatment were fed oral boluses (25 μl) of CORT (Sigma Aldrich) dissolved in peanut oil twice daily approximately 5 h ± 1 h apart. From 12 to 15 days post-hatch, nestlings received 0.124 mg/ml of CORT in peanut oil for a total daily dose of 6.2 μg of CORT. Starting 16 days post-hatch, the dose was increased to 0.163 mg/ml for a total daily exposure of 8.15 μg of CORT. Control nestlings were fed 25 μl of peanut oil on an identical feeding schedule. Nestlings were exposed to treatments from 12 to 28 days post-hatch (methods as per Spencer et al., 2009).

Nestling zebra finches fledge as early as 17 days post-hatching. Before fledging, we identified the social parents of a nest by observing incubating and provisioning behaviors. If we were unable to identify parents based on these behaviors, we recorded parental behavior using VehoMuvimicroDV camcorders and identified parents from the resulting videos. After determining social parents, we moved the nest box and parents to wire cages ($70 \times 40 \times 44$ cm^3) where they were housed until the nestlings reached nutritional independence at 28 days post-hatch (Spencer et al., 2009). Following nutritional independence, we returned the parents to the breeding aviary. Nestlings remained in the cages and were fed a diet of commercial finch food, spray millet, boiled eggs, and spinach. We measured tarsus, wing chord, and mass at 28, 60, and 90 days post-hatch. We calculated condition for birds at 28, 60, and 90 days post-hatch using the scaled mass index (Peig and Green, 2009; Peig and Green, 2010). The scaled mass index accounts for errors associated with residual body mass measurements by using a scaling relationship derived from the population of interest to calculate the expected mass of each individual at a fixed body size. In this way, the scaled mass index standardizes all animals to the same growth phase or body size and is considered to be a more accurate measure of condition (Peig and Green, 2010).

2.3. HPA function at 30, 60, and 90 days post-hatch

We measured the effects of CORT treatment during development on HPA function 30, 60, and 90 days post-hatch ($n = 29$, 25, and 38, respectively). We measured stress responses by exposing finches to a standardized restraint stress protocol (Wingfield, 1994). We obtained one blood sample within three minutes of disturbing birds (baseline CORT). After initial blood samples were obtained, we placed finches in cloth bags and collected two more samples 10 and 30 min after initial disturbance. To collect blood, we punctured the alar vein with a 26-gauge needle and collected 25–50 μl of blood with heparinized microcapillary tubes. Immediately after collection, blood was kept in a 4 °C refrigerator on ice (<1 h) until it could be centrifuged to separate plasma from red blood cells (3000 rpm for seven minutes). After separation, the plasma was isolated and stored at −20 °C.

Plasma CORT typically does not increase within three minutes of stress exposure (Romero and Reed, 2005). However, samples obtained within three minutes of disturbance in our experiment ($\bar{x} = 1.80$ min, $SD = 0.77$) did show a significant increase in CORT ($N = 118$, $F_{1,115} = 24.99$, $r_s = 0.18$, $P < 0.01$). To account for this, we used the time to collect the initial blood sample as a scaled weight variable in our analyses.