

Post-natal glucocorticoid elevation affects GnRH-induced luteinizing hormone concentration in female house sparrows

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ABSTRACT

Most non-mammalian studies investigating the long-term effects of early-life stressor exposure on endocrine regulation have focused on the hypothalamic-pituitary-adrenal (HPA) axis. However, the hypothalamic-pituitary-gonadal (HPG) axis may more directly affect fitness by regulating reproduction. Changes in HPG axis regulation could allow vertebrates to adaptively mitigate negative effects of early-life stressor exposure. However, only a few studies have examined long-term effects of early-life stressor experience on the HPG axis, and these have found mixed results. Here, we evaluate long-term effects of post-natal corticosterone exposure on the HPG axis in adult female house sparrows (*Passer domesticus*). We elevated circulating corticosterone non-invasively in wild nestling house sparrows between 8 and 11 days post-hatching, and then brought birds into captivity at fledging. Early in their first breeding season (ages 285–353d post-hatching), females were given a gonadotropin releasing hormone (GnRH) challenge. We found that early-life corticosterone exposure interacted with current condition such that females exposed to elevated post-natal corticosterone had higher baseline and GnRH-induced luteinizing hormone (LH) concentration than control females, but only if they had a high mass. Our results suggest that female house sparrows may mitigate negative impacts of early-life corticosterone exposure by investing in early reproduction, but only when current energetic condition allows.

1. Introduction

Early life exposure to stressors and the associated increase in circulating glucocorticoids can have long-term effects on physiology and neurology (Welberg and Seckl, 2001). These “programming” or “organizational” effects (Seckl, 2001; Welberg and Seckl, 2001) often involve changes to neuroendocrine axes. For example, captive rodent and human studies suggest that early-life stressor exposure is associated with a hyperactive hypothalamic–pituitary-adrenal (HPA) axis response (De Bellis, 2001; Seckl, 2004), and generally negative changes to reproductive physiology and behavior (Guzmán et al., 2006; Kapoor and Matthews, 2008; Rhind et al., 2001). To date, most non-mammalian studies investigating the long-term effects of early-life stressor or glucocorticoid exposure on endocrine regulation have focused on the HPA axis. In captivity, early-life stressors including post-natal corticosterone (the primary avian glucocorticoid) exposure in song sparrows (*Melospiza melodia*) and zebra finches (*Taeniopygia guttata*), food restriction in Western scrub-jays (*Aphelocoma californica*), and maternal deprivation in zebra finches all hypersensitize the adult glucocorticoid stress response (Banerjee et al., 2012; Pravosudov and Kitaysky, 2006; Schmidt

et al., 2014; Spencer et al., 2009). Consistent with captive findings, in the wild, early life maltreatment experience is correlated with sex-specific long-term changes to the HPA axis in a seabird, the Nazca booby (*Sula granti*; Grace and Anderson, 2018). However, much less is known regarding the effects of early-life stress on other endocrine systems in non-mammals, such as the hypothalamic-pituitary-gonadal (HPG) axis (but see Farrell et al., 2015; Schmidt et al., 2014).

The HPG axis directly affects fitness by regulating reproduction. In female birds, the onset of breeding involves the increased expression of gonadotropin releasing hormone (GnRH) that triggers the release of two pituitary hormones: luteinizing hormone (LH) and follicle stimulating hormone (FSH). These two hormones stimulate release of sex steroids (e.g., estradiol and testosterone) and gonadal development (reviewed in Dawson, 2008). A suite of reproductive behaviors are then expressed, including courtship, mating, and nest-building (Ball, 1993). The HPG axis interacts with the HPA axis; for example an acute release of glucocorticoids is thought to shift energy investment away from reproduction and to suppress the HPG axis (Wingfield and Sapolsky, 2003) with potential effects on the onset of breeding in wild vertebrates (Lattin et al., 2016). Accordingly, elevated circulating glucocorticoid

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concentrations have been shown to affect the HPG axis and to delay or even suppress territoriality, mating behavior, and laying (e.g. Goutte et al., 2011, 2010). Despite these connections between glucocorticoids and the HPG axis in adults, only a few studies have examined long-term effects of early-life glucocorticoid elevation on the HPG axis in birds, and these have found mixed, sex-specific results. Developmental, post-natal corticosterone exposure in song sparrows increases male testosterone concentrations, but decreases female estradiol concentrations (Schmidt et al., 2014), and in zebra finches reduces female incubation effort (Spencer et al., 2010), but increases male parental care (Crino et al., 2014). Developmental stressor experience (i.e., unpredictable food availability) also alters the HPG axis in male European starlings (*Sturnus vulgaris*) in the form of lower androgen levels, but has an indirect effect on female androgen levels operating through growth rate (Farrell et al., 2015).

A number of studies have suggested that life history trade-offs may allow apparently detrimental effects of early-life stress observed under controlled conditions to be adaptive under natural conditions (Crino and Breuner, 2015). For example, barn swallows (*Hirundo rustica*) increase glucocorticoid deposition in eggs following exposure to a predator cue, which results in smaller nestlings (Saino et al., 2005). While small size at fledging is often correlated with decreased survival (e.g., De Kogel, 1997), it may be beneficial to offspring in a high predator environment by increasing flight performance (Crino and Breuner, 2015). Changes to HPG axis regulation in response to early-life stressor experience may mediate some life history trade-offs to mitigate negative effects of stressor exposure. Male zebra finches exposed to elevated developmental corticosterone sing lower quality songs as adults (Spencer et al., 2003), and are less attractive to females (Spencer et al., 2005). However, they appear to compensate for these poor sexual signals by increasing parental investment, resulting in higher single-season reproductive success (Crino et al., 2014), possibly through changes to HPG regulation. An increase in reproductive success may mitigate fitness costs of decreased longevity (Crino et al., 2014) because early-life stress is often associated with poor adult survival in birds (Grace et al., 2017a; Monaghan et al., 2012).

Here, we evaluate long-term effects of post-natal corticosterone exposure on early breeding season reproductive condition in the female house sparrow (*Passer domesticus*). We have previously shown in this species that exposure to elevated corticosterone at the nestling stage negatively impacts adult anti-predator behavior (Grace et al., 2017b) and decreases adult longevity (Grace et al., 2017a). House sparrows in this population will produce two to three broods per breeding season, and those that initiate breeding earlier in the season have more breeding attempts, lay more eggs, and produce over double the number of fledglings than later breeders (Chastel et al., 2003). Life-history theory predicts that reproductive investment should increase with decreasing future reproductive prospects (e.g., the “terminal investment hypothesis”) (Clutton-Brock, 1984; Pianka and Parker, 1975; Trivers, 1972). Thus sparrows exposed to early-life corticosterone may invest more heavily in reproduction and/or shift reproduction to earlier in the breeding season to offset an increased mortality risk. To test this, we elevated circulating corticosterone non-invasively in wild nestling house sparrows between 8 and 11 days post-hatching. Altricial nestlings increase corticosterone in response to disturbance three- to 16-fold (reviewed in Crino et al., 2014). Our treatment increased circulating concentrations of corticosterone on average 8.6-fold (Grace et al., 2017b), well within the range observed in response to natural stressors and within the biologically relevant range for house sparrows (Angelier et al., 2016). Birds were brought into captivity at fledging, and females were given a GnRH challenge at the start of their first potential breeding season. A GnRH challenge is often used to assess the reproductive condition of individuals and involves intravenous or intramuscular administration of GnRH and measurement of LH response (reviewed in Jawor et al., 2006). If females exposed to post-natal corticosterone early in life are ready to reproduce earlier in the breeding

season, we expect an increased LH response to a GnRH challenge compared to control females.

2. Methods

All experimental procedures were approved by the French government (DREAL Poitou-Charentes, R45GRETA1-10) the Muséum National d'Histoires Naturelles, and the Centre National de la Recherche Scientifique, and conform to guidelines set forth by the French Ministry of Agriculture and Fisheries, and the Ministry of Higher Education and Research.

2.1. Study population and nestling manipulation

As nestlings, all house sparrows in this study were part of a free-living, wild population in the vicinity of the rural agricultural town of Prissé la Charrière, Deux-Sèvres, France (46°09'12"N 0°28'59"W). All nest boxes in the study area were monitored for clutch initiation (first laid egg) and hatching dates. At six days post-hatching all nestlings were color banded and at nine days were banded with a uniquely numbered permanent aluminum ring. At eight days post-hatching, nestling hormonal manipulation began. The HPA axis may develop slowly in altricial species (Wada, 2008) such as house sparrows, thus treatment began in the mid-late nestling period, when eyes are open and nestlings of a related species, the white-crowned sparrow, are able to mount a strong HPA axis stress response (Wada et al., 2007). All nests in the study area with more than one nestling were used. Half of the nestlings in each nest were assigned to corticosterone-fed and control groups, respectively; assignment was alternated between nestlings (corticosterone-fed first, control second, etc...) as they were blindly removed from the nest. Assignment order was alternated between nests (corticosterone-fed first, control second; control first, corticosterone-fed second), and corticosterone-fed and control nestlings did not differ in mass at the start of treatment ($n = 131$ nestlings) (Grace et al., 2017a).

Corticosterone was delivered non-invasively following the method of Breuner et al. (1998) and used successfully in subsequent studies (Breuner and Wingfield, 2000; Lohmus et al., 2006; Saldanha et al., 2000). Meal worms were injected with 20 μ L of 0.6 mg/mL corticosterone (for nestlings aged 8–9 d) or 0.9 mg/mL corticosterone (for nestlings aged 11 d) in dimethyl sulfoxide (DMSO). Control nestlings were fed worms injected with 20 μ L of DMSO. Corticosterone concentrations were determined based on previous work with house sparrow nestlings (Loiseau et al., 2008) and Gambel's white-crowned sparrows (*Zonotrichia leucophrys*, Breuner and Wingfield, 2000; Breuner et al., 1998), scaled to the average mass of house sparrows during the ages of treatment. Worms were chilled at -20°C prior to injection to limit movement, and were injected ventrally, into the central abdomen, between exoskeletal segments. If any fluid leaked from the mealworm it was discarded.

Nestlings were fed corticosterone- and vehicle-injected worms with blunt-end forceps, at their nest box, on days 8 (once – morning), 9 (twice – morning and evening), and 11 post-hatching (once – morning). The schedule of feedings was designed to be unpredictable, to discourage habituation by nestlings. Treatment was stopped after 11 d post-hatching to prevent premature fledging due to nest disturbance.

2.2. Captive housing

At and after twelve days post-hatching, nests were checked daily and nestlings that showed signs of flight were taken into captivity. Two (one control, one corticosterone-fed) to four (two control, two corticosterone-fed) nestlings were removed from each nest, resulting in a sample size of 97 birds from 23 nests in captivity. In three instances only three small fledglings were in the nest, thus, we brought all fledglings into captivity due to survival concerns (one of these died within one week of captive housing). Birds were kept on natural

daylight schedules and caretakers were blind to treatment group. Fledglings were housed in wire bird cages (Vision S01, 45.5 × 35.5 × 51 cm) with siblings (2–4 birds per cage) until birds reached basic plumage (i.e., adult stage), after which (and for the rest of the experiment) age- and sex-matched pairs consisting of one corticosterone-fed and one control bird were housed together. When possible these pairs were siblings. Fledglings were hand-fed until they were capable of feeding on their own (mean ± s.e.m = 26.8 ± 4.8 d, max = 41d, min = 18 d). Once birds were self-feeding, they were supplied with mixed seeds ad libitum, vitamin and mineral soaked cat food, salt/mineral blocks, water (changed daily), and millet on the stalk. Grit was supplied three times per week and cages were equipped with perches of varying heights. After reaching basic plumage, cages were arranged so that all females were in visual and auditory, but not physical contact with several unrelated males. No females were allowed physical contact with males until after testing, described below. All birds were kept on natural daylight schedules provided by large windows and overhead lights.

2.3. Gonadotropin-releasing hormone challenge

On May 12–13, 2016 (i.e., early breeding season, Chastel et al., 2003) we conducted a gonadotropin-releasing hormone (GnRH) challenge on adult females (ages 285–353 d post-hatching, $n = 20$). Females were captured in their home cage between 1030 and 1700 h, at least four hours after sunrise and before sunset. An initial blood sample was taken within three minutes of disturbance. Birds were then injected via the jugular vein with 25 μ L of 25 ng/ μ L GnRH (American Peptide, Chicken LH-RH, 54–8-23) dissolved in phosphate-buffered saline (PBS). Similar doses of GnRH have been shown to elicit an LH response and a subsequent increase in testosterone levels in other bird and passerine species (Goutte et al., 2010; Needham et al., 2017; Schoech et al., 1996; Wingfield et al., 1979). A second blood sample was obtained an average of 11.8 min post-injection (min = 9.8 min., max = 14.0 min., sd = 1.19 min.). A subset of females ($n = 4$, 2 control and 2 corticosterone-fed) were injected with 25 μ L of phosphate-buffered saline and sampled on the same schedule to control for changes in hormones due to injection, alone. All blood samples were obtained via brachial venipuncture with 27 gauge needles and heparinized capillary tubes. After blood sampling, females were weighed (electronic balance: ± 0.1 g), muscle and fat were scored (0–5 scale with 0 being extremely emaciated muscle or no visible fat), and culmen, ulna, and tarsus were measured (caliper: ± 0.1 mm).

2.4. Luteinizing hormone assay

Plasma was separated from the cellular fraction by centrifugation at 2000g for 7 min, and then frozen at -20°C until further analysis. Laboratory analysis was conducted at the Centre d'Etudes Biologiques de Chizé. Plasma luteinizing hormone (LH) concentration was determined by radioimmunoassay as described in Goutte et al. (2010). All samples were run in a single assay. The lowest detectable concentration of LH was 1.38 ng/mL and the intra-assay coefficient of variation was 14.70% ($n = 4$).

2.5. Growth Rate

Birds were weighed (electronic balance: ± 0.1 g) at the nestling stage prior to beginning treatment (8 d post-hatching) and immediately following cessation of treatment (12 d post-hatching), juvenile stage (68–89 d post-hatching, juvenile plumage), and adult stage (285–353 d post-hatching). Previous work has shown that house sparrows in this population respond to corticosterone treatment with depressed growth during treatment, followed by catch-up growth through the juvenile stage (Grace et al., 2017a). Thus, we calculated three growth rates: (1) mass change during treatment (i.e., the difference between mass at 12 d

post-hatching and 8 d post-hatching, “Nestling Growth Rate”); (2) mass change post-treatment when most catch-up growth occurs (i.e., the difference between mass at the juvenile stage and 12 d post-hatching, “Juvenile Growth Rate”); and (3) mass change between juvenile and adult stages (“Adult Growth Rate”).

2.6. Statistical analyses

To determine the effectiveness of the GnRH challenge, changes in circulating LH concentration pre- and post-injection was analyzed for GnRH-injected females using a paired-samples *t*-test, and for PBS-injected females using a paired-samples Wilcoxon test because of low sample size.

The effect of treatment on LH concentration was analyzed via linear mixed models (R v. 3.4.0, package ‘nlme’; Pinheiro et al., 2017), using females injected with GnRH. Because our sample size limited the number of parameters we could include in the analysis, we conducted a series of preliminary linear mixed models evaluating the independent effects of predictors on LH concentration. This preliminary analysis suggested that hour of sampling, age of bird, fat score, and linear measures (tarsus, culmen, wing) did not predict LH concentration ($p > 0.18$), and thus these predictors were not included in subsequent analyses. Mass and muscle score were not significantly correlated (Pearson's $r = -0.16$, $p = 0.34$) and preliminary analyses indicated both were potentially important predictors of LH, so both variables were included in analyses. Because we were limited by sample size, but were interested in both the effect of treatment and possible indirect or interactive effects of treatment on LH through growth rate, we built several global models and evaluated derivatives of these global models via model selection. All models included a random intercept for bird ID. Nest of origin ($n = 14$) was not included as a random effect because preliminary analyses indicated it was not important in predicting LH concentration. Our first global model consisted of the predictors: Treatment (corticosterone-fed, control), Nestling Growth Rate, Sample Number (1 = pre-injection, 2 = post-injection), Mass (Z-scored), and Muscle Score (0–5 scale). All two way interactions were allowed between Sample Number, Treatment and all other predictors. Thus, in nlme syntax, the first global models was: LH (ng/mL) \sim Nestling Growth Rate * Sample Number + Nestling Growth Rate * Treatment + Treatment * Mass + Treatment * Sample Number + Mass * Sample Number + Muscle Score * Sample Number, $random = \sim 1 | Ring2$. Our second, and third global models substituted Nestling Growth Rate for Juvenile Growth Rate, and Adult Growth Rate, respectively.

Regression models were evaluated within a multimodel inference framework using Akaike's Information Criterion corrected for small sample size (AICc), which has the benefit of evaluating the relative strength of multiple alternative hypotheses and balances information gained with parsimony. Models were derived from subsets of the global models using package MuMIn (Barton, 2015). To limit the possibility of overfitting, we only considered models with ≤ 10 degrees of freedom because these had at least four samples per degree of freedom. Thus, our global model was too large to be considered for the top model set and was only a tool from which to derive simpler models. No model with greater than 10 degrees of freedom was within $\Delta 3$ AICc of the top model, so this evaluation criterion did not affect our top model set. Estimates and standard errors for coefficients in our top models were also evaluated for signs of overfitting (e.g., large errors), none of which were found. The top model set and model evaluation parameters are provided in Table 1. Estimates were first chosen to optimize the log likelihood for AICc model comparison, then to optimize the restricted maximum likelihood for final beta coefficients of top models. We only report results for models whose AICc value was less than that of the null (i.e., no effect of treatment). For our top model we report both marginal r-squared values (i.e., proportion of variance explained by fixed factors alone) and conditional r-squared values (i.e., the proportion of variance explained by both random and fixed factors) obtained using the

Table 1

Model evaluation parameters for models that are within $\Delta 3$ of the top model, and the first model that does not include treatment. Fixed model predictors are listed in the first column, followed by AIC model evaluation parameters. All models included the random effect of bird ID. A * indicates an interaction, “df” is degrees of freedom, “ Δ ” indicates the difference between the AICc values of the top model and the model in question. The smallest AICc value is 275.9.

Model	df	Δ	Log Likelihood
Treatment * Mass + Muscle Score * Sample Number	9	0	-125.93
Treatment * Mass + Muscle Score * Sample Number + Mass * Sample Number	10	2.60	-125.44
Treatment * Mass + Muscle Score * Sample Number + Juvenile Growth Rate	10	2.82	-125.55
Treatment * Mass + Muscle Score * Sample Number + Nestling Growth Rate	10	2.87	-125.57
Mass + Muscle Score * Sample Number	7	4.72	-131.55

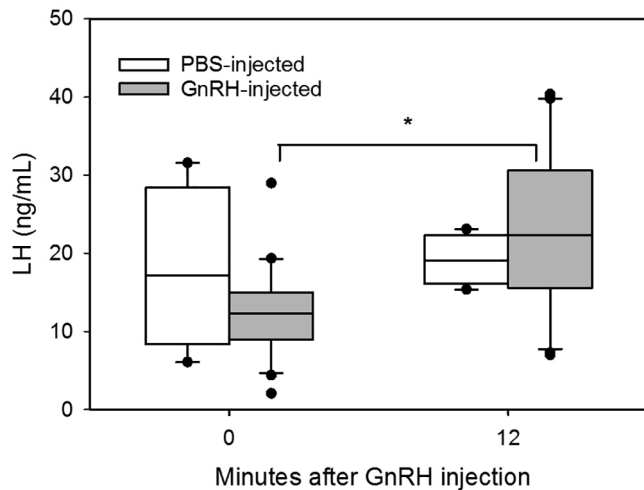


Fig. 1. LH concentration for PBS-injected and GnRH-injected females immediately before injection (0 min), and an average of 12 min after injection. Boxes represent 25th and 75th percentiles, error bars represent 10th and 90th percentiles, dots are outliers, and central line is the median line. LH was significantly higher post-injection for GnRH-injected females but not for PBS-injected females (indicated by a *).

r.squaredGLMM function in R (Johnson, 2014; Nakagawa and Schielzeth, 2013).

3. Results

GnRH injection, but not PBS injection increased circulating LH concentration in sparrow females (GnRH, paired-samples *t*-test: $t(19) = -4.38, p < 0.001$; PBS, paired-samples Wilcoxon test $v(3) = 5, p > 0.99$; Fig. 1). Treatment did have an important effect on LH concentration, but this effect was mediated by mass. The top model predicting LH concentration included the interaction between mass and treatment, and muscle score and sample number (Δ AICc of the closest model excluding treatment = 4.72; marginal $r^2 = 0.62$; conditional $r^2 = 0.62$; $N = 40, 20$ individuals; Table 1). Circulating LH in both initial and GnRh-induced samples increased with mass for treated females, but mass had little influence on control females (treatment \times mass: $p = 0.01$, Table 2). Thus, corticosterone-fed females with high mass had higher initial and induced LH than control females (Fig. 2). Induced LH concentration was higher than initial LH concentration for all females but that difference was smaller for females with high muscle scores (sample number \times muscle score: $p = 0.02$, Fig. 3).

4. Discussion

Our results indicate that current state interacts with early-life glucocorticoid exposure to influence endocrine breeding condition in female house sparrows. As predicted, post-natal early-life corticosterone exposure resulted in elevated baseline and GnRH-induced luteinizing

Table 2

Beta coefficients and associated 95% confidence intervals (CIs), *t*-values, and *p*-values for parameters in the top model predicting LH secretion. 95% CIs are calculated from adjusted standard error. This model also included the random effect of bird ID. For fixed factors, the non-reference factor is indicated in parenthesis. A: indicates an interaction term. Beta coefficients of predictors for which 95% CI did not overlap zero are highlighted in bold. Mass is z-scored to facilitate comparison of beta coefficients.

Predictor	Beta	\pm 95% CI	<i>t</i> -value	<i>p</i> -value
Mass	1.65	2.88	1.12	0.28
Treatment (corticosterone-fed)	2.87	3.98	1.41	0.18
Muscle Score	-0.47	4.51	-0.20	0.84
Sample Number (post-injection)	32.68	17.00	3.77	< 0.01
Mass: Treatment	7.84	5.31	2.89	0.01
Muscle Score: Sample Number	-8.54	6.31	-2.65	0.02

hormone (LH) concentrations compared to control females, but only for females with high mass. Corticosterone-fed females with low mass exhibited no difference in LH concentrations compared to controls. LH concentration and responsivity to GnRH is a positive correlate of reproductive condition (Jawor et al., 2006) and life history theory predicts that investment in current reproduction should increase as future reproductive opportunities decline (Clutton-Brock, 1984). Thus, our results support the hypothesis that females may mitigate negative impacts of early-life corticosterone exposure on longevity by investing in early reproduction, but only when current energetic condition allows. Energetic requirements for early breeding in this population are 10–40% higher than for late breeding (Chastel et al., 2003) and female sparrows with low mass may be constrained in their ability to invest in early reproduction by limited energetic reserves. An energetic constraint on reproduction is unexpected for this study because all birds were provided ad libitum food and water. However, study mortality averaged 36.7% during captivity (14 d – one year of age), and mortality events were associated with acute changes in mass (Grace et al., 2017a). Thus, while mass in our study likely does not reflect available food resources or ability to obtain food, it may reflect differences in health, metabolism, or energetic demands.

Our results indicate that mass and muscle score had opposite relationships with LH concentration (and were not correlated with each other). Induced GnRH concentration increased with mass and decreased with muscle score. This suggests that these two “condition” measures reflect different aspects of sparrow body condition. Mass may positively correlate with reproductive condition due to the energetic requirements of breeding (Chastel et al., 2003), while muscle score may negatively reflect reproductive condition because female birds can catabolize protein in muscle tissue during egg development (Houston et al., 1995). Thus, female sparrows with high GnRH responsivity in our study may have low muscle scores due to egg production. However, no females produced eggs within a week following GnRH injections, making it unlikely that egg production, alone, was responsible for this relationship. Alternatively, muscle score and mass may be decoupled in our study due to captive living. Flight muscle exercise is not required to obtain food in captivity and birds with poor keel muscle condition can

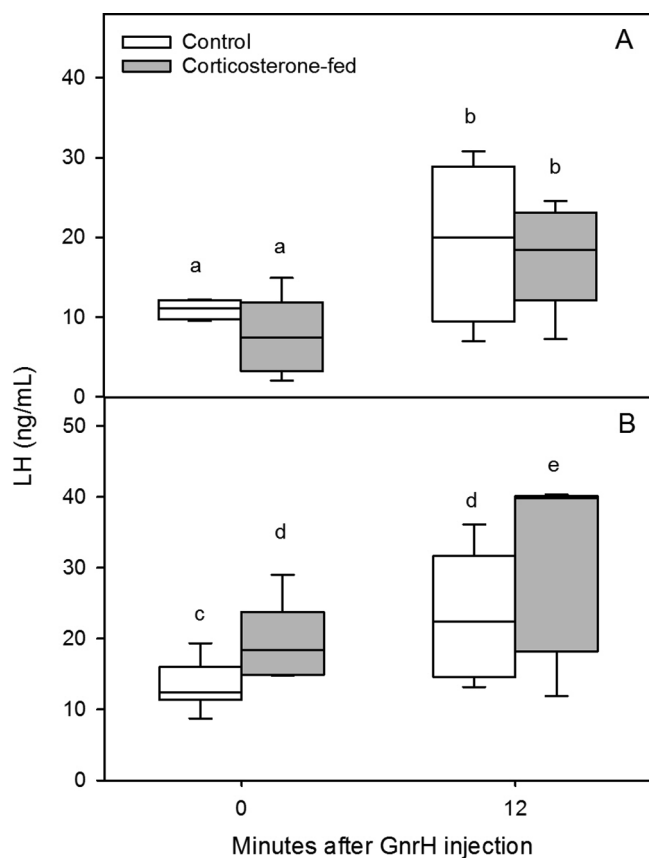


Fig. 2. Influence of treatment on LH concentration pre-GnRH injection (0 min) and an average of 12 min post-GnRH injection for females with (A) low mass and (B) high mass. Raw data is binned by mass values below or above the mean for display, only. Boxes represent 25th and 75th percentiles, error bars represent 10th and 90th percentiles, and central line is the median line. Lowercase letters indicate significant differences. There was no difference in initial or induced LH concentration between control and corticosterone-fed females with low mass. For those with high mass, corticosterone-fed females had higher initial and induced LH concentration than control females. LH concentration increased from pre- to post-injection for all females.

have high mass. In light of this, our results for muscle score suggest that highly active birds may be less responsive to GnRH, and thus less ready to commence breeding, than less active birds.

Early-life corticosterone exposure increased average baseline LH by 30% and average GnRH-induced LH by 26%, for females with greater than average mass. Thus, exposure to elevated corticosterone during the mid-late nestling period had strong organizing effects on regulation of the HPG axis of female house sparrows, but that effect was moderated by current mass. Early-life corticosterone exposure was a better predictor of LH than growth rate at any life stage. No other avian study to our knowledge has evaluated the effect of early-life glucocorticoid or stressor exposure on luteinizing hormone production in adulthood, and only a few avian studies have investigated other HPG axis responses (e.g., estradiol, testosterone) or reproductive biology (Crino et al., 2014; Farrell et al., 2015; Schmidt et al., 2014; Spencer et al., 2010). Similar to our results, male zebra finches exposed to developmental stress increase behavioral investment in reproduction (Crino et al., 2014). However, our results contrast with those of song sparrows, for which elevated early-life corticosterone resulted in a reduction in circulating estradiol (a downstream hormone positively correlated with LH), and thus, presumably a decrease in reproductive condition (Schmidt et al., 2014). One explanation for this difference is that song sparrows exposed to developmental stress do not exhibit the decline in survival (Schmidt et al., 2014), that is observed in house sparrows

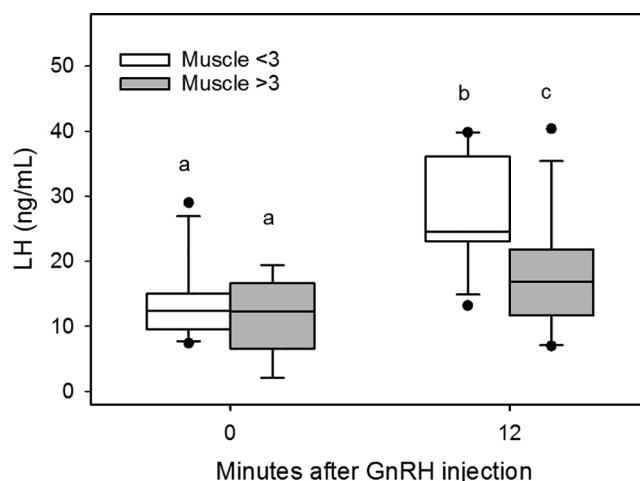


Fig. 3. LH concentration as a function of muscle score pre-GnRH injection (0 min) and an average of 12 min post-injection. Raw data is combined for both control and corticosterone-fed females because the effect did not differ by treatment. Muscle scores are binned as below or above/equal to three for display, only. Boxes represent 25th and 75th percentiles, error bars represent 10th and 90th percentiles, and central line is the median line. Lowercase letters indicate significant differences. LH concentration increased across time for all females, but that increase was higher for females with low muscle scores.

(Grace et al., 2017a) and zebra finches (Monaghan et al., 2012). Thus, increased investment in reproduction may not be beneficial for song sparrows, or they may respond to early-life stress with mechanisms that promote longevity over fecundity. Methodological differences may also explain the disparity between these results. Experimental song sparrows were treated with corticosterone for a much longer period of time (7–60 d post-hatching) than house sparrows in our study (7–11 d post-hatching). The prolonged exposure to corticosterone during development in song sparrows may have had negative long-term effects on areas of the brain that regulate female reproductive hormones and behavior (Schmidt et al., 2014), that did not occur in our study. Our lack of findings regarding growth rate also contrasts with results for female European starlings, for which accelerated growth rate and not developmental stressor exposure is associated with depressed integrated androgen concentrations (Farrell et al., 2015). This disparity could be a function of species differences, methodological differences (corticosterone exposure vs. unpredictable food manipulation), or endpoint measurements (LH vs. androgens).

Future studies are needed to determine if these differences in circulating and GnRH-induced LH concentrations translate to differences in fitness between control and early-life corticosterone exposed females. If LH responsiveness reflects breeding readiness, then we would expect that females exposed to elevated corticosterone as nestlings would initiate egg laying earlier in the season than control females. Although early fledging date is associated with lower fledgling survival in this species (Husby et al., 2006; Ringsby et al., 1998), early nest initiation is associated with increased nesting attempts and higher overall fledgling production per season in this population (Chastel et al., 2003). However, early breeding is energetically costly and correlated with a 42% increase in basal metabolic rate compared to later breeding (Chastel et al., 2003). Thus, future research must evaluate the relative costs and benefits of early breeding on fitness following early-life corticosterone elevation, and the relationship between LH responsiveness and lay date. Furthermore, it is unknown if the HPG axis of males exposed to elevated early-life corticosterone would be affected in a similar way to that of females. If so, early-life corticosterone exposure could result in assortative mating indirectly by altering the breeding timeline of affected individuals. Early-life stressor exposure also frequently induces long-term programming changes to the HPA axis (Banerjee et al., 2012;

Pravosudov and Kitaysky, 2006; Schmidt et al., 2014; Spencer et al., 2009), and further research is needed to illuminate the relationships between early-life corticosterone exposure, the HPG axis, and the HPA axis in this species.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ygcen.2019.113238>.

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