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Pre and post-natal antigen exposure can program the stress axis of adult zebra finches: evidence for environment matching

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Abstract

Both maternal exposure to stressors and exposure of offspring to stressors during early life can have lifelong effects on the physiology and behavior of offspring. Stress exposure can permanently shape an individual's phenotype by influencing the development of the hypothalamic-pituitary-adrenal (HPA) axis, which is responsible for the production and regulation of glucocorticoids such as corticosterone (CORT). In this study we used captive zebra finches (*Taeniopygia guttata*) to examine the effects of matching and mismatching maternal and early post-natal exposure to one of two types of antigens or a control on HPA axis reactivity in adult offspring. Prior to breeding, adult females were injected with lipopolysaccharide (LPS), keyhole limpet hemocyanin (KLH) or a control. Offspring of females in each of the three treatments were themselves exposed to LPS, KLH or a control injection at 5 and 28 days post-hatch. When offspring were at least 18 months of age, standardized capture and restraint stress tests were conducted to determine the impact of the treatments on adult stress responsiveness. We found significant interaction effects between maternal and offspring treatments on stress-induced CORT levels, and evidence in support of the environment matching hypothesis for KLH-treated birds not LPS-treated birds. KLH-treated offspring of KLH-treated mothers exhibited reduced stress-induced CORT levels, whereas LPS-treated or control offspring of KLH-treated mothers exhibited elevated stress-induced CORT levels. Although the treatment effects on baseline CORT were non-significant, the overall pattern was similar to the effects observed on stress-induced CORT levels. Our results highlight the complex nature of HPA axis programming, and to our knowledge, provide the first evidence that a match or mismatch between pre and post-natal antigen exposure can have life-long consequences for HPA axis function.

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Keywords

Corticosterone; development; environment matching; hypothalamic-pituitary-adrenal axis; keyhole limpet hemocyanin; lipopolysaccharide; maternal effects; maternal immune activation; stressors

Introduction

Adverse early life environments in humans can elevate the risk of both physical and psychiatric disorders in adulthood (e.g., Batten et al., 2004; Goodwin and Stein, 2004; Phillips et al., 2005; Read et al., 2005; Anda et al., 2006). The developmental origins of health and disease hypothesis proposes that pre-natal and post-natal environments play unique roles in influencing adult health and the risk of disease development (Godfrey, 2006). Early life experiences can permanently alter the organizational structure and sensitivity of physiological systems, thereby affecting the manner in which an individual responds to both intrinsic and extrinsic changes. For example, there is evidence that pre-natal and post-natal environments can program the sensitivity of the hypothalamic-pituitary-adrenal (HPA) axis (Shanks et al., 1995; Meaney, 2001; Karrow, 2006; Champagne et al., 2008; Love et al., 2013). The HPA axis is responsible for the production of glucocorticoids (GCs), which are involved in a suite of physiological and behavioral processes. Baseline levels of GCs are important for the maintenance of homeostasis, whereas the release of elevated levels of GCs in response to stressors initiates a cascade of physiological and behavioral changes to help organisms cope with environmental challenges (Breuner et al., 2008).

The sensitivity of the HPA axis to pre-natal and post-natal experiences is thought to be an adaptive mechanism that allows individuals to cope with current environmental conditions (Karrow, 2006; Love et al., 2013), and to potentially prepare for expected future conditions (Gluckman et al., 2005; Love and Williams 2008a; Zimmer et al., 2013). This programming effect may represent a “predictive adaptive response” (PAR- Gluckman et al., 2005). PARs are thought to prepare an individual to cope with anticipated high or low levels of stressors (based on conditions experienced during development) via attenuated or heightened sensitivity, respectively, to those stress levels (*sensu* Love et al., 2013). This “environmental matching” (Monaghan, 2008) or “maternal matching” (Love et al., 2005) strategy may become maladaptive if the early developmental environment does not match later environments (*sensu* Hales and Barker, 1992; Bateson et al., 2004; Sheriff et al., 2009).

Although previous studies have documented programming effects of the maternal (Takahashi and Kalin, 1991; Reul et al., 1994; Welberg and Seckl, 2001; Yehuda et al., 2007; Perroud et al., 2014) or neonatal (Plotsky and Meaney, 1993; Shanks et al., 1995; Shanks et al., 2000) environments on HPA axis sensitivity, few studies have examined the combined long-term impact of both pre-natal and early post-natal exposure to stressors on HPA axis function in mammals (e.g., Vallée et al., 1999; Koo et al., 2003) or birds (e.g., Love and Williams 2008b; Zimmer et al., 2013), and fewer still have examined the effects of the same stressor in both pre-natal and post-natal environments (e.g., in rats; Koo et al., 2003). Early life adversity is linked to psychopathological disorders such as depression,

conduct disorders, autism, and schizophrenia (Glover, 2011; Pechtel and Pizzagalli, 2011; Carr et al., 2013), but some of the effects of early life stress exposure that appear maladaptive, such as anxiety, aggression, and risk-taking, may improve fitness in adverse environments. Adaptive models of early stress exposure propose that early life adversity adaptively modulates developmental trajectories in ways that enhance survival and reproductive success in adverse environments (Flinn, 2006; Glover, 2011; Del Giudice et al., 2011; Cameron et al., 2005; Del Giudice, 2014). Maladaptive outcomes may be most likely to occur when there are mismatches between predicted and actual environmental conditions (Del Giudice, 2014; Low et al., 2012). Recognition of the adaptive effects of early life stress exposure provides a theoretical framework to understand interactions between maternal and post-natal environmental conditions and opens new areas of inquiry to test interventions that can mitigate the maladaptive effects of early life stress exposure and the adaptive, but psychologically and socially undesirable effects.

Both mothers and offspring may be exposed to pathogens or parasites, and maternal and neonatal immune activation have documented effects on HPA axis sensitivity (Shanks et al., 1995; Howerton and Bale, 2012). Activation of the immune system can also have long-lasting effects on numerous other traits, including learning and behavior (Bilbo and Schwarz, 2009; Meyer et al., 2009; Grindstaff et al., 2012), growth rates (Klasing and Leshchinsky, 1999; Grindstaff, 2008), immune function (Lemke and Lange, 1999; Grindstaff et al., 2006; Pihlaja et al., 2006; Merrill and Grindstaff, 2014), and endocrine function (Karrow, 2006; Hsiao and Patterson, 2011). One way in which immune activation can affect endocrine function is through the release of cytokines such as IL-1, IL-6, and TNF (Turnbull and Rivier, 1995). These cytokines have demonstrated effects on HPA axis activity in adults, as well as on HPA axis programming in neonates (Karrow, 2006; Pechnick et al., 2006), and in the fetuses of immune challenged mothers (Hsiao and Patterson, 2011; Howerton and Bale, 2012).

In this study we were interested in the long-term effects of matching and mismatching between pre-natal and post-natal immune challenges on HPA axis functioning. We manipulated antigen exposure by injecting breeding female zebra finches (*Taeniopygia guttata* Vieillot) and their offspring with one of two antigen treatments (lipopolysaccharide (LPS) or keyhole limpet hemocyanin (KLH)) or a control (phosphate buffered saline (PBS)). We then measured baseline and stress-induced levels of the GC stress hormone corticosterone (CORT) at approximately 1.5 y of age to examine the long-term effects of the treatments on stress reactivity. LPS is a major component of gram-negative bacteria cell walls and is targeted by the host immune system. When LPS is injected into a vertebrate, it induces a systemic, febrile response (Hart, 1988), which activates the HPA axis and results in elevated CORT levels, as well as increases in pro-inflammatory cytokines (e.g., IL-6) (Karrow, 2006). The organism also produces anti-LPS antibodies as part of an adaptive, secondary response to the infection (Grindstaff, 2008). KLH is a large, immunogenic oxygen-binding protein that results in the production of anti-KLH antibodies but should not activate the HPA axis when injected without dinitrophenyl (DNP) or adjuvants in small to moderate doses (Stenzel-Poore et al., 1993), although this has not yet been examined in birds. There is some evidence, however, that KLH may have a larger impact on HPA axis

functioning (*sensu* Gaillard et al., 1998). LPS is a thymus-independent type 1 antigen, meaning that antibody production is not dependent upon T-cell assistance (Janeway et al., 2001), whereas KLH is a thymus-dependent antigen and production of KLH-reactive antibodies requires the presence of armed helper T-cells (Janeway et al., 2001). T-cells can synthesize adrenocorticotropic hormone (Gaillard, 1994), which is involved in the production and release of CORT, and these immune cells are thought to play a role in regulating the HPA axis. It is not clear whether production of T-cells as part of a primary or secondary response to KLH would be sufficient to activate the HPA axis (Stenzel-Poore et al., 1993). Nonetheless, the use of LPS and KLH offers insight into how activation of T-cells in females during the egg-laying period, and in the offspring during the early post-natal stage, might impact HPA axis functioning in adult offspring.

We were interested in four questions for this study: 1) is there an effect of maternal antigen exposure on adult offspring CORT levels, 2) is there an effect of offspring antigen exposure on adult offspring CORT levels, 3) are there environment matching effects of maternal and offspring antigen exposure on adult offspring CORT levels, and if so, 4) are these effects found for both T-dependent and T-independent antigens?

Methods

For a detailed description of the experimental set-up and design, see Grindstaff et al., (2012). In short, captive adult zebra finches were housed as breeding pairs in cages visually isolated from one another in an indoor aviary facility. Twenty females were randomly assigned to each treatment group to receive LPS, KLH, or phosphate buffered saline (PBS) as the control. Females in the KLH treatment were injected with 50 μg of KLH (EMD Millipore Bioscience, Billerica, MA, USA, 374817) in 50 μl of sterilized PBS (Sigma-Aldrich, St. Louis, MO, USA, P5368) (n = 20) (Hasselquist et al., 1999). Birds in the LPS treatment were injected with 1.0 mg of LPS/kg body weight (Sigma L7261) in 50 μl of sterilized PBS (n = 20) (Owen-Ashley et al., 2006). These doses have been demonstrated to elicit robust immune responses without leading to prolonged morbidity or mortality (Grindstaff et al, 2012; Merrill and Grindstaff, 2014). Birds in the control treatment were injected with 50 μl of sterilized PBS only. All birds were injected intra-abdominally after cleaning the site of injection with a 70% isopropyl alcohol swab. Females were injected once prior to the production of their first clutch, and given a booster injection at least 35 days after the first injection (Fig. 1). This was long enough to ensure that the primary immune response had occurred and subsided. First clutch eggs were collected within 24 h of laying and replaced with dummy eggs. Once enough birds for each treatment had completed their first clutch, birds were given their booster injection and the dummy eggs were removed 7 days later. Females then laid replacement clutches and each egg was weighed to the nearest 0.01 grams using a digital scale, and individually marked on the day it was laid. All analyses are for the offspring resulting from replacement clutches.

Young from a natal nest were divided across the three treatments, and within 72 h of hatching were cross-fostered into age, clutch and brood size-matched nests (+/− 1) to that of the natal nest. On day 5, nestlings received a primary injection of KLH, LPS, or PBS. Young within a foster nest received the same treatment as the foster mother. Doses of KLH, LPS

and PBS were half those in the adult treatments so as to not overwhelm the chicks' immune systems and reduce the risk of mortality. On day 28, the young received a booster injection with the adult doses.

Stress Test

Once the birds were old enough to live independently (approximately 40 days), they were housed in same-sex group cages (n=2-10 birds/cage) until the stress test. When the birds were at least 18 months old, they were subjected to a standardized capture and restraint stress protocol to assess baseline CORT and stress-induced levels of CORT at two time points; 10 and 40 min post-stress initiation. By examining CORT at 10 and 40 min post-stressor, we were able to acquire information on the magnitude of the stress response as well as some insight into the duration of the stress response, thus providing a more robust picture of the CORT profile. Same-sex pairs were placed in cages (45W × 45D × 40H cm) and allowed to acclimate for 24 h prior to the stress test. Birds had *ad libitum* access to food and water during this acclimation period. Stress tests occurred between 1035 and 1400 h to control for diel variation in CORT levels (Breuner et al., 1999). On the day of the test, we captured both birds from the cage at the same time and collected approximately 50 μl of blood from the jugular vein using a sterile syringe within 3 min of capture (Romero and Reed, 2005). The bird was then placed in a cloth bag for 10 min, at which point another blood sample was collected. The bird was then returned to the cloth bag for another 30 min before a final blood sample was collected. Following completion of the stress test, blood was spun down in a centrifuge at 1,845 g for 7 min. Plasma was pulled off the sample using a Hamilton syringe and stored at −80° C until assay. All work was approved by the Oklahoma State University Institutional Animal Care and Use Committee, under protocol AS107.

Plasma corticosterone assessment

We used a commercially available EIA kit (catalog no. ADI-901-097, Enzo Life Sciences International Inc., Plymouth Meeting, PA, USA) to measure plasma CORT. The kit was validated for use with zebra finches by testing the parallelism of a series of plasma dilutions against the standard curve. We also optimized the assay in our lab for zebra finches following Wada et al., (2007) prior to sample quantification. In short, we prepared a 1:40 sample dilution by adding 1% steroid displacement reagent (15 μl) to 10 μl of plasma, and then adding 375 μl of assay buffer to each sample 5 min later. Samples were then vortexed and aliquoted in duplicate (100 μl per well) to assay plates. A standard curve ranging from 20,000 to 32 pg/mL was run in triplicate for each plate. Samples and standards were then incubated with 50 μl conjugated CORT and 50 μl antibody for 2 h at room temperature while being shaken at 0.84 g. Following incubation, wells were washed, filled with 200 μl of substrate, and then incubated for 1 h at room temperature without shaking. 50 μl of stop solution was then added to each well, and each plate was read on a spectrophotometer at 405 nm. The detection limit for the assay is 27 pg (Enzo Life Sciences), and one sample fell below this detection limit and was removed from analyses. Intra-plate variation ranged from 4.54% to 11.14%, and inter-plate variation was 8.33%. There is some cross-reactivity for detection of other steroid hormones, namely deoxycorticosterone (cross-reactivity=28.6%).

All other steroid hormones have less than 2% cross-reactivity with the kit components (Enzo Life Sciences).

Statistics

To determine whether antigen challenge affected egg mass, we constructed a GLMM with maternal ID as a random effect, maternal treatment as a fixed effect, and egg mass as the dependent variable. We did not find an effect of maternal challenge ($F_{2,34.99} = 2.46$, $P = 0.1$), so we did not include egg mass in the models examining CORT production. We also looked for an effect of maternal treatment on latency to lay, but found no effect ($F_{2,38} = 0.07$, $P = 0.93$) so did not include it in any models.

To examine the impact of early life experience on baseline and stress-induced levels of CORT, we ran mixed models with six or seven independent variables of interest: maternal treatment, young treatment, maternal ID, time (for repeated measures), sex, hatch order, and foster nest hatch order. Hatch order coincided with lay order in over 95% of the eggs laid (unpublished data). Foster nest hatch order is the age of the cross-fostered young relative to other young in the foster nest. All variables were checked for normality of residuals and homogeneity of variance prior to analyses. CORT data were log transformed to achieve normality prior to analysis. Antigen challenge was successful in stimulating the production of antigen-reactive antibody in females and transmission of KLH and LPS specific antibodies to offspring. Furthermore, maternal antigen challenge significantly impacted antibody production by offspring (Merrill and Grindstaff, 2014).

Baseline levels of CORT are important for modulating homeostatic processes and were analyzed independently and as part of the stress-response. To examine baseline CORT independently, we used linear mixed models (Proc Mixed) in which maternal ID was included as a random effect, and the denominator degrees of freedom were approximated using the containment method (Littell et al., 2006). For stress-induced levels of CORT we used repeated measures mixed models analyses incorporating baseline CORT, CORT 10, and CORT 40 values. Maternal ID and time by maternal ID were included as random factors, and maternal treatment, young treatment, time, sex, hatch order, foster nest hatch order, the interaction between maternal treatment and young treatment, the interaction between time and young treatment as well as the interaction between time and maternal treatment were included as fixed effects. The covariance structures between repeated measurements of the same individuals were explicitly modeled. A spatial power law covariance model was used to account for unequally spaced longitudinal measurements in which correlations were expected to decline as a function of time (Littell et al., 2006). The spatial power law covariance model is a generalization of an autoregressive error model (Littell et al., 2006), which allows for accurate assessment of within subject slopes (Schielzeth and Forstmeier, 2009). Denominator degrees of freedom were approximated using the Kenward-Rodgers method (Littell et al., 2006). We used post-hoc pair-wise *t*-tests of least squares means to compare significant terms generated in the full models and alpha was set at 0.05 for all analyses. Correcting for the number of pairwise comparisons would result in very small alpha levels, so we present raw p-values for each post-hoc pairwise comparison. Finally, we examined the relationships among CORT levels for each sampling

period (baseline, CORT at 10 min post-stressor (CORT 10), and CORT at 40 min post-stressor (CORT 40)) using linear mixed models with maternal ID and young ID as random effects. Analyses were run in SAS (v. 9.3, SAS Institute, Cary, North Carolina, 2010) or JMP (v. 10.0, SAS Institute, Cary, North Carolina, 2010).

Results

Baseline CORT

None of the model parameters were significant in explaining baseline CORT levels (Table 1). There was a non-significant trend for an interaction between maternal and young treatment, suggesting that the interplay between maternal treatment and offspring treatment may have moderately impacted baseline levels of CORT (Figure 2A).

Stress-induced CORT levels

There was a significant effect of time in which all treatments exhibited an increase from baseline to stress-induced CORT levels (Table 2). There was no effect of maternal or young treatment independently on stress-induced CORT levels, but there was a significant interaction effect between maternal and offspring treatments (Table 2). KLH-treated offspring of KLH-treated mothers had significantly lower stress-induced CORT levels than control offspring of KLH-treated mothers or KLH-treated offspring of control mothers. KLH-treated offspring of KLH-treated mothers also had significantly lower stress-induced CORT levels than LPS-treated offspring of KLH-treated mothers or control offspring of LPS-treated mothers. Additionally, control offspring of control mothers had significantly lower stress-induced CORT levels than both LPS and control offspring of KLH-treated mothers and borderline lower levels than KLH-treated offspring of control-treated mothers (Figure 2B).

Two of the treatment group combinations exhibited a decline in mean CORT from 10 to 40 min post stressor and these were both KLH-treated offspring of antigen-treated mothers (Figure 3). In contrast, KLH-treated offspring of control mothers continued to increase from 10 to 40 min post-stressor and were the only group to exhibit a significant increase over this time (Figure 3).

To further examine the significant interaction effect between maternal and offspring treatments on sensitivity of the offspring HPA axis, we broke the analyses down by maternal treatment. Within the maternal KLH treatment group, there was a significant effect of time on offspring stress-induced CORT levels ($F_{2,38.4} = 77.78$, $P < 0.001$) and a significant effect of young treatment ($F_{2,15.2} = 4.97$, $P = 0.02$). Post-hoc analyses showed that KLH-treated offspring had significantly lower stress-induced CORT levels than LPS-treated offspring ($t_{1,15.1} = 2.6$, $P = 0.02$) as well as control offspring ($t_{1,15.2} = 3.0$, $P < 0.01$) (Figure 2B). Within the maternal LPS treatment group, there was a significant effect of time ($F_{2,56.6} = 135.48$, $P < 0.001$) on offspring CORT levels, but no other significant effects ($P > 0.28$). Within the control maternal group, restraint time again impacted offspring CORT levels ($F_{2,53.9} = 88.18$, $P < 0.001$) and there was a non-significant trend for an effect of

young treatment on CORT levels ($F_{2,21.2} = 1.83$, $P = 0.19$) with KLH-treated young having higher CORT levels.

Relationships among CORT measurements

Baseline CORT and CORT 10 min were significantly positively related (estimate ± s.e.m. = 0.37 ± 0.05, $F_{1,93} = 62.36$, $P < 0.001$), as were baseline CORT and CORT 40 min (estimate ± s.e.m. = 0.28 ± 0.07, $F_{1,93} = 14.4$, $P < 0.001$) and CORT 10 min and CORT 40 min (estimate ± s.e.m. = 0.44 ± 0.12, $F_{1,95} = 13.05$, $P < 0.001$). CORT 10 and 40 were both significantly higher than baseline CORT (CORT 10 and baseline; $t_{1,93} = 22.86$, $P < 0.001$; CORT 40 and baseline; $t_{1,93} = 18.51$, $P < 0.001$), but did not differ significantly from each other ($t_{2,95} = 1.18$, $P = 0.23$).

Discussion

We manipulated both the pre-natal and post-natal antigenic environments to determine if mismatches between the environments can program the sensitivity of the offspring HPA axis. We found a significant interaction effect between maternal and neonatal antigen treatments on stress-induced levels of CORT in adult zebra finches, which provides evidence for a programming effect of mismatches in the antigenic environment. Zebra finches produced elevated levels of CORT at 10 and 40 minutes post-restraint during adulthood. This supports prior work on avian species that has documented increases in circulating CORT levels as a result of restraint stress over this time period (e.g., Romero and Reed, 2005). In our study, neither maternal nor young treatment alone resulted in significant differences in baseline or stress-induced CORT levels, but the interaction between the two may have led to distinct strategies for coping with different levels and/or types of stressors. Baseline CORT levels appear to have been less impacted by the treatments than stress-induced CORT, but the trends generally mirror those seen in stress-induced levels with the exception of LPS-treated offspring of KLH-treated mothers (Figure 2). Treatment with KLH had a more pronounced effect on shaping the HPA axis than treatment with LPS in which birds exposed to KLH exhibited an environment matching relationship between pre-natal and post-natal treatments. When both mothers and offspring were challenged with KLH, the offspring had lower HPA axis reactivity than when either only the mother or only the offspring were challenged with KLH. Similarly, control offspring of control mothers exhibited lower HPA axis reactivity than control offspring of KLH-challenged mothers. These data indicate that the combination of pre-natal and early post-natal exposure to stressors such as pathogens and parasites can program the HPA axis, but that programming may be specific to the type of antigen.

Stress-induced CORT

The adrenocortical stress response is an adaptive mechanism for coping with challenging stimuli, and affects numerous physiological and behavioral processes (Breuner et al., 2008). Early life experiences are known to impact the short and long-term functioning of the HPA axis, but previous work on the organizational effects of exposure to glucocorticoids during development has yielded mixed findings. Some work indicates that exposure to elevated levels of glucocorticoids during development results in heightened response to stressors in

adulthood (e.g., Hayward and Wingfield, 2004; Chung et al., 2005; Banerjee et al., 2012), whereas other work has found that exposure to elevated glucocorticoids during development results in an attenuated stress response later in adulthood (e.g., Lingas and Matthews, 2001; Love and Williams, 2008a; Vázquez et al., 2012). In this study, exposure to KLH during both pre-natal and post-natal development resulted in relatively low stress-induced CORT levels, and a more rapid cessation of CORT production in the 40 min following the stressor, both of which may be beneficial traits for an individual exposed to many such stressors (*sensu* Love and Williams, 2008a; Weinstock, 2008; Love et al., 2013). In contrast, birds exposed to KLH during the pre-natal *or* post-natal environment experienced higher levels of stress-induced CORT, suggesting that the match or mismatch between pre-natal and post-natal environments was critical for shaping HPA axis function in adulthood (*sensu* Hales and Barker, 1992; Bateson et al., 2004), but only for KLH-treated birds.

Exposure to stressors during the hypo-responsive period early in life may induce altered sensitivity of the HPA axis in adulthood through altered expression of glucocorticoid receptors (GR) and mineralocorticoid receptors (MR) in key regions of the brain (Meaney et al., 1996; Banerjee et al., 2012). Zebra finches do not produce a detectable increase in CORT levels as a result of restraint stress during at least the first 10 days post-hatch and have been suggested to exhibit a stress hypo-responsive period early in life (Wada et al., 2009). Thus, we hypothesize that the immune challenge of nestlings on day 5 post-hatch was responsible for the effect we found on the adult response to capture and restraint stress. Both GR and MR are found in the zebra finch brain and loss of maternal care early in life alters adult sensitivity to stressors and decreases receptor expression (Banerjee et al., 2012). However, the mechanism linking the loss of maternal care in zebra finches with effects on the HPA axis has not been identified. In Long Evans rats, offspring of mothers with high levels of maternal care have decreased methylation in the promoter region of the GR (Weaver et al., 2004) and elevated levels of GR mRNA in the hippocampus (Liu et al., 1997). Thus, epigenetic mechanisms may be responsible for the persistent effects of maternal and developmental immune challenges on the stress responsiveness of zebra finch offspring. Epigenetic modifications as a result of mismatches between environmental conditions in the maternal and offspring generations have been implicated as a mechanism underlying the link between the early life environment and adult risk of disease (Godfrey et al., 2007).

Baseline CORT

We did not find significant effects of maternal or young treatment, or a significant interaction effect between the two on baseline CORT levels. There was a significant positive correlation between baseline and stress-induced CORT levels, however, and the interaction effect between maternal and young treatments was nearly significant. As was observed for stress-induced CORT levels, KLH treatment appeared to have a larger impact on baseline CORT levels than LPS treatment. Also control offspring of control mothers had lower baseline CORT levels than control offspring of KLH-challenged mothers as we found for stress-induced CORT levels. Both KLH and LPS challenged offspring of KLH challenged mothers, had lower baseline CORT levels than control offspring of KLH challenged mothers. This suggests that the interaction between pre-natal and post-natal environments

influenced baseline CORT levels, although not to the extent that it affected stress-induced responses.

Baseline CORT levels are thought to act in a permissive manner on physiological and behavioral processes via Type I receptors located in the brain (Sapolsky et al., 2000; Romero, 2004). These receptors likely regulate daily and seasonal CORT cycles and the organism's energetic state (McEwen and Wingfield, 2003; Romero, 2004; Crespi et al., 2013). Elevated baseline CORT may be indicative of an individual that lives in a state of high preparedness for occasional exposure to stressors, whereas low baseline CORT may be adaptive for an individual that is likely to experience either many or very few such events during its life (*sensu* Love et al., 2013). There is some indication that baseline CORT levels may exhibit less repeatability than stress-induced CORT levels (Rensel and Schoech, 2011 and references therein), and it is possible that with a larger sample size, we would have been able to capture a stronger effect of antigen treatment on baseline CORT levels.

KLH versus LPS injections

As described above, KLH is a thymus-dependent antigen and requires activation of helper T-cells, and LPS is a thymus-independent antigen and does not require helper T-cells (Janeway et al., 2001). It is likely that the differences we have documented between KLH and LPS are related to the manner in which the antigens activate the immune system. In a related study, Grindstaff et al., (2012) found significantly reduced growth rates in KLH-treated offspring compared to LPS-treated or control zebra finch offspring. Taken together with the data here, these results suggest that the costs of a specific immune response to KLH were high for developing zebra finches (Demas et al., 1997; Klasing and Leshchinksy, 1999). T-cells have the capacity to impact HPA axis functioning through the release of adrenocorticotropic hormone (Gaillard, 1994), and it is possible that activation of helper T-cells in response to KLH exposure resulted in increased production of CORT. The match or mismatch of high CORT production in KLH-treated birds may have led to the attenuated response of baseline and stress-induced CORT in KLH-KLH treated birds, and a heightened response in control-KLH and KLH-control birds. To our knowledge, no studies have examined the long-term effects of T-cell activity during development on HPA-axis programming, but this could be an important avenue to explore.

Another possibility is that the differences between LPS and KLH are related to the timing of immunological response relative to egg production. There is good evidence that exposure to LPS induces an energetically costly immune response (Hart, 1988), but the duration of the response may be significantly shorter than that of the response to KLH (Jurincic-Winkler et al., 1995). This difference in duration could explain some of our results. Females were challenged with a booster injection 7 days prior to the removal of the dummy egg clutch, meaning that females would not have initiated clutch-replacement until at least 7 days post-injection. If females shape their offspring's development via maternal effects, they may do so by manipulating the relative and total abundance of resources and hormones in the egg. Consequently, if mothers exposed to LPS were no longer in the energetically expensive immune response phase by the time they were allocating resources to their eggs, the impact of LPS may have been lessened compared to KLH. Similarly, because the offspring

response to KLH was likely of longer duration than the response to LPS the two injections of KLH on days 5 and 28 post-hatch may have mimicked chronic inflammatory conditions, rather than acute exposure to inflammation.

Conclusions

Changes in HPA axis activity may be adaptive responses for anticipating future events (Mousseau and Fox, 1998; Gluckman et al., 2005; Love et al., 2008a), or may be maladaptive if early environmental conditions are not predictive of later conditions (Hales and Barker, 1992; Bateson et al. 2004). In many species, there is great variation in HPA axis sensitivity to stress, both among individuals and within individuals over time. This variation may be a result of early developmental experiences, because exposure to pre-natal and post-natal stressors can permanently shape HPA axis functioning (Welberg and Seckl, 2001; Champagne and Curley, 2008; Love et al., 2013). These changes may shift the range of potential developmental pathways the offspring can then take. In this study, we examined the long-term effects of maternal and early life exposure to different antigens and documented a significant interaction effect between maternal and offspring treatment on stress-induced levels of CORT. We were explicitly interested in whether exposure to any antigen, the same antigen, or a mismatch between antigens or between control treatment and antigen treatment would result in differential HPA axis programming in the adult bird. It is unclear in this situation whether HPA axis programming due to antigen exposure is beneficial for the adult birds (*sensu* Gluckman et al., 2005), but it is likely context dependent. For example, low levels of maternal care in rats are linked to increased corticosterone levels in pups and decreased spine density in hippocampal neurons (Bagot et al., 2009). These effects seem maladaptive but the phenotype is associated with enhanced learning and memory in stressful conditions (Champagne et al., 2008). The changes in stress physiology and brain morphology induced by low levels of maternal care, therefore, are adaptive responses to an adverse early life environment if later life conditions are also adverse (Meaney, 2010; Del Giudice et al., 2011).

Environment matching effects mediated through interactions between the immune and endocrine systems have clinical relevance for humans by highlighting novel interventions to reduce maladaptive effects of early life adversity and any potentially adaptive, but psychologically undesirable effects (Del Giudice, 2014). Although mismatches between the maternal and developmental environments may induce increased sensitivity in the HPA axis to adaptively regulate the inflammatory response early in life, hyper-sensitivity in the long-term may increase the risk of developing stress-related diseases, particularly if individuals are also genetically more susceptible to metabolic or psychiatric illnesses (Shanks and Lightman, 2001; Meyer et al., 2009). This work suggests that interventions aimed at decreasing the prevalence and impact of stress-related diseases may be achieved by reducing the risk of infection, and by providing anti-inflammatory treatment after infection early in life, especially in susceptible populations (Meyer et al., 2009).

Our results highlight the complex nature of HPA axis programming, and that differences in the type of antigen an individual is exposed to during development can have life-long consequences for HPA axis function. The data also underscore the need to examine both

maternal and neonatal experiences in understanding the long-term impacts of early life stressors.

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Highlights

- We examined effects of pre and postnatal antigen exposure on HPA axis function
- Treatments resulted in a match or mismatch between pre and postnatal environments
- Prenatal exposure to KLH (but not LPS) led to environment-matching effects
- Pre and postnatal KLH exposure resulted in an attenuated stress response
- Prenatal KLH and postnatal LPS or control exposure led to elevated stress response

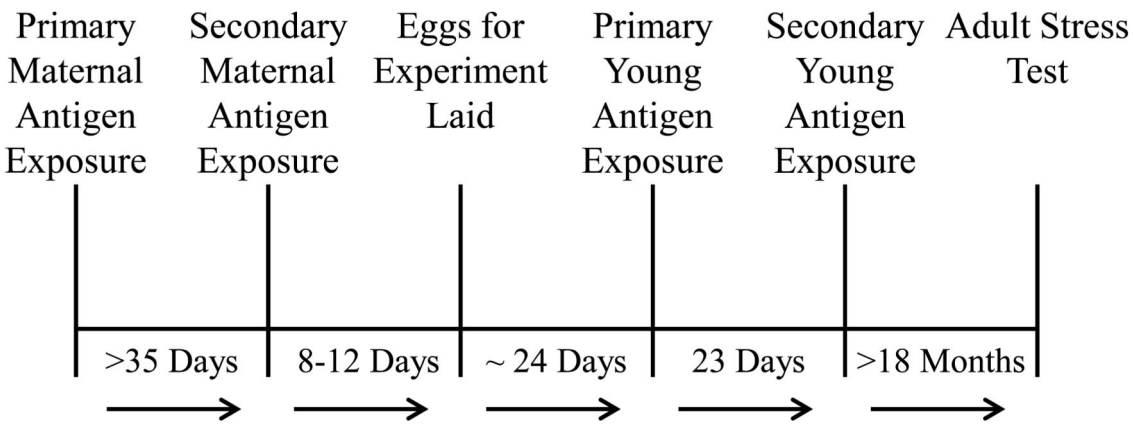

Figure 1.

(from *Merrill and Grindstaff, 2014*). Timeline for pre-natal and post-natal experimental procedures. Adult female zebra finches were exposed to one of three experimental treatments (keyhole limpet hemocyanin (KLH), lipopolysaccharide (LPS), or phosphate buffered saline (control)) prior to egg laying. Females were then given a secondary injection of the same treatment and allowed to lay a clutch of eggs that were replaced with dummy eggs. Dummy egg clutches were removed 7 days after secondary injection and females laid replacement clutches over the subsequent 4-5 days. Eggs hatched approx. 19 days later, and the offspring were injected with a primary injection on day 5, and secondary injection on day 28. Adult stress tests were conducted when birds were at least 18 months of age.

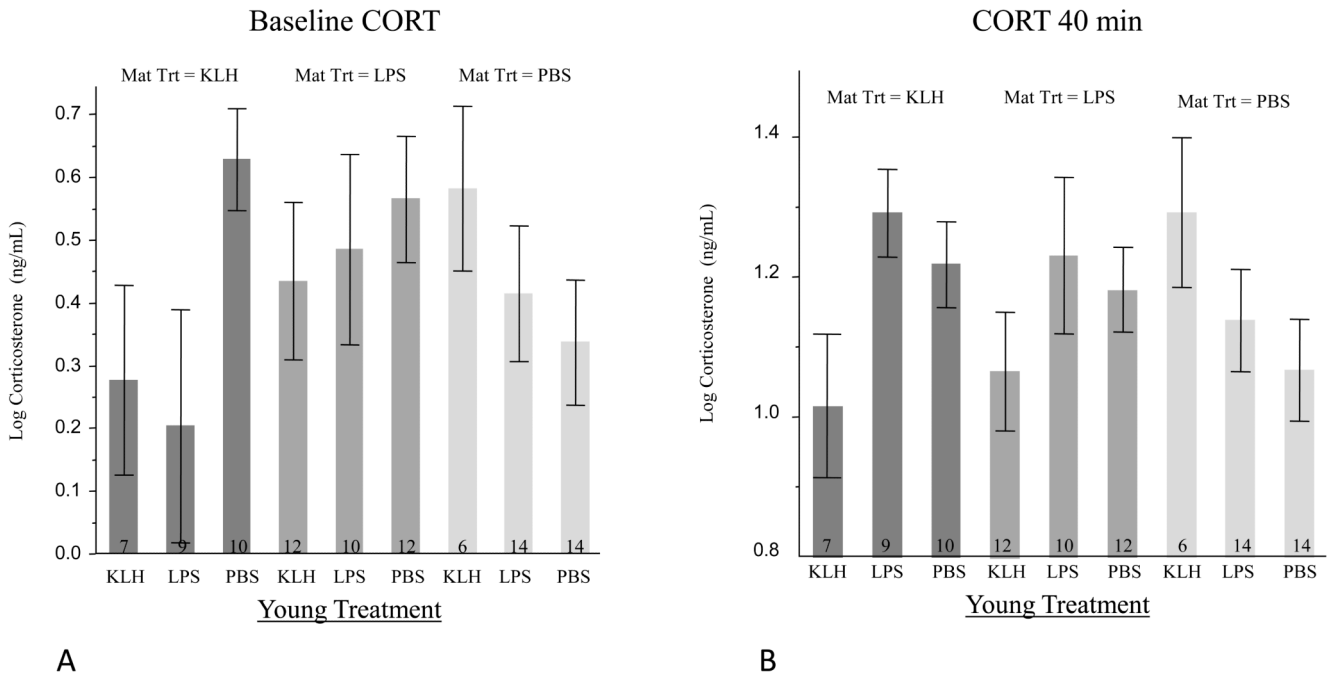

Figure 2.

Baseline (A) and stress-induced (B) corticosterone (CORT) levels by young and maternal treatment group. Numbers at the bottom of the bars represent the sample size for each young and maternal treatment group, and bars represent mean ± s.e.m. Darkest colored bars are for offspring of females exposed to KLH, intermediate bars are for offspring of females exposed to LPS, and lightest bars are for offspring of females given a control injection.

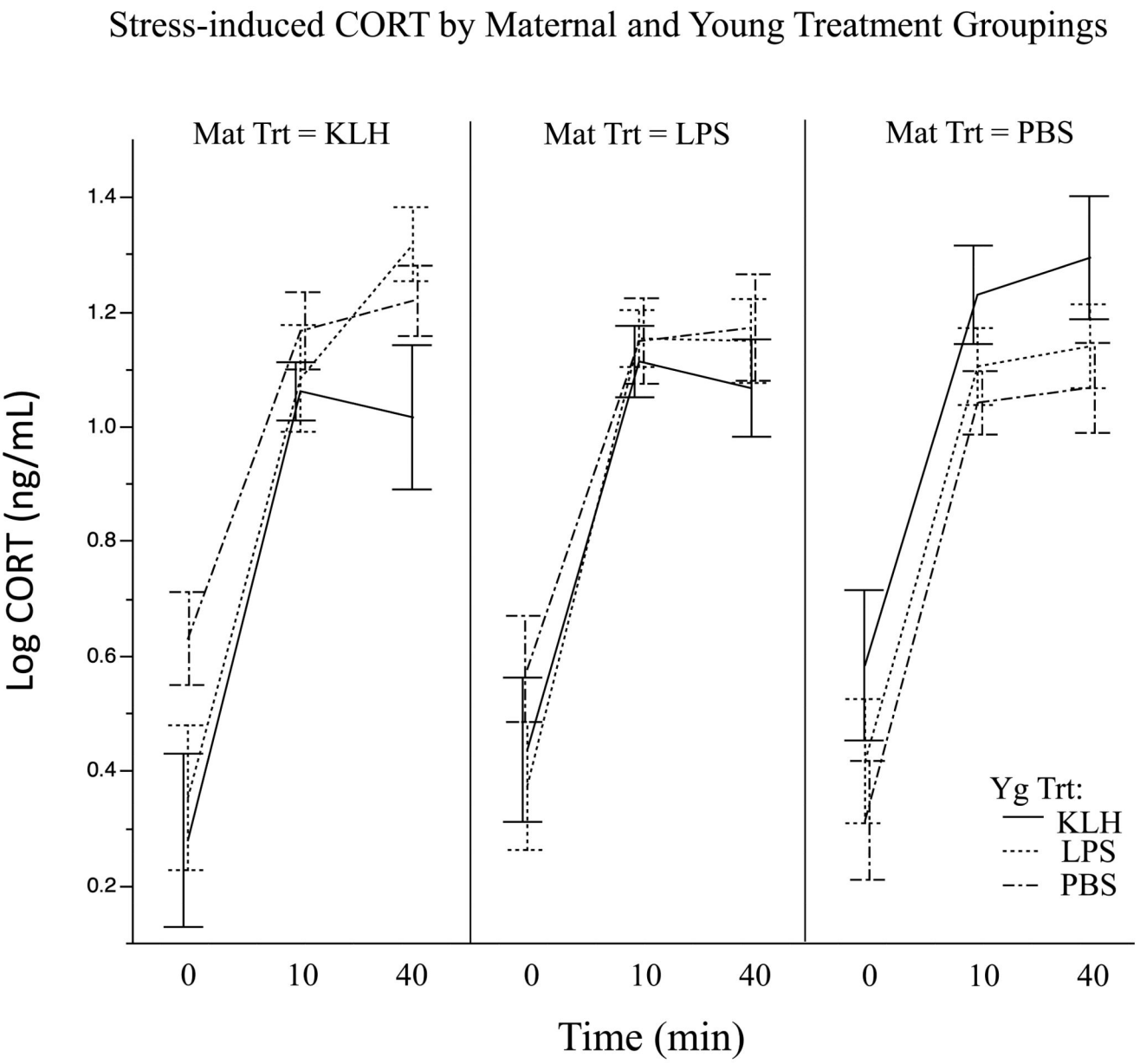

Figure 3.
CORT levels over time for each treatment pairing, grouped by maternal treatment. Bars represent mean ± s.e.m.

Table 1

Results of the mixed model analyses for baseline CORT. The model included maternal ID as a random effect. Bolded values are for fixed effects that were significant at alpha < 0.05.

Variable	DF	F-value	P
Mat Trt	2, 33	0.22	0.8
Yng Trt	2, 33	0.42	0.66
Mat Trt × Yng Trt	4, 33	2.45	0.07
Sex	1, 33	0.11	0.74
Foster Nest Hatch Order	5, 33	1.75	0.15
Hatch Order	5, 33	1.11	0.37

Table 2

Results of the repeated measures mixed model for stress-induced CORT. The model included maternal ID and the interaction between time and maternal ID as random effects. Bolded values are for fixed effects that were significant at alpha < 0.05.

Variable	DF	F Value	P
Mat Trt	2. 68.4	0.17	0.85
Yng Trt	2, 68.5	0.4	0.67
Time	**2,70.1**	**264.13**	**<.0001**
Mat Trt × Yng Trt	**4, 68.9**	**3.04**	**0.02**
Sex	1, 69.4	0.32	0.57
Foster Nest Hatch Order	5, 69.6	1.66	0.15
Hatch Order	5, 69	1.98	0.11
Mat Trt × Time	4, 71.8	0.37	0.83
Yng Trt × Time	4, 147	0.61	0.66