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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 life-long 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.

1. 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

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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, 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 (Cameron et al., 2005; Flinn, 2006; Del Guidice et al., 2011; Glover, 2011; Del Giudice, 2014). Maladaptive outcomes may be most likely to occur when there are mismatches between predicted and actual environmental conditions (Low et al., 2012; Del Guidice, 2014). 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 TNFα (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 years 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?

2. 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 g using a digital scale, and individually marked on the day it was laid. All analyses are for the offspring resulting from replacement clutches.