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Research paper

Interactions of body temperature and nutritional status on hypothalamo-pituitary-adrenal axis activity in pre-thermoregulatory eastern bluebird chicks (*Sialia sialis*)

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A R T I C L E I N F O

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A B S T R A C T

Early life experiences can affect the function of the hypothalamo-pituitary-adrenal (HPA) axis of vertebrates, with potential fitness consequences later in life. In altricial species, for example, variation in parental behavior, e.g. brooding or feeding, can modify the activity of the HPA axis of the young by altering their exposure to noxious stimuli as the young develop in the nest. We have shown that a drop in the body temperature of eastern bluebird (*Sialia sialis*) chicks, such as occurs when females are away from the nest, elevates their blood corticosterone levels. If repeated during the early days of their development, cooling bouts also reduce the chicks' later corticosterone secretion in response to handling. Thus, variation in maternal behavior has the capacity to shape the function of the chicks' HPA axis. To better understand how maternal absence from the nest activates the HPA axis of bluebird chicks, we experimentally mimicked the cooling that occurs when the female is away from the nest, and investigated a) the age at which the HPA axis becomes capable of responding to cooling by increasing corticosterone secretion, b) whether corticosterone secretion remains elevated throughout long periods of cooling, and c) whether fasting (also potentially associated with maternal absence) interacts with cooling to affect corticosterone secretion. Cooling for 18 min significantly elevated circulating corticosterone levels of chicks as young as 4 days post-hatch, indicating that their HPA axis is sensitive to cooling very early in life. Corticosterone levels remained elevated throughout longer bouts of cooling. However, a 1-hr period of fasting had no effect on corticosterone secretion, regardless of whether chicks were cooled or not. Collectively, these data demonstrate that variation in maternal brooding behavior can substantially modify the corticosterone profiles of chicks during early postnatal development, and that chick temperature is likely the main driver of this.

1. Introduction

In vertebrate animals, exposure of developing young to noxious or threatening stimuli can profoundly shape their morphology, physiology, and behavior throughout life, with the potential to impact fitness. Many of these effects are mediated in part by activation of the hypothalamo-pituitary-adrenal (HPA) axis, which produces a substantial rise in glucocorticoids following exposure to such stimuli during critical periods of development (Hennessy and Weinberg, 1990; Lynn and Kern, 2014; Pravosudov and Kitaysky, 2006; Rensel et al., 2010; Schoech et al., 2011). Elevations of glucocorticoids early in life can alter a variety of phenotypic characteristics in developing young (Lindström, 1999; Love et al., 2013; Monaghan, 2008; Schoech et al., 2011). Such characteristics include the development of the HPA axis itself, which may then in turn determine how individuals respond to noxious stimuli later in life (Banerjee et al., 2012; Lynn and Kern, 2017; van Oers et al., 1997, 1998; Rots et al., 1996; Spencer et al., 2009; ter Wolbeek et al., 2015).

For altricial young, which complete much of their development after birth or hatching, parental behavior can minimize or exacerbate exposure to environmental stimuli. For example, parental behavior can alter the offspring's nutritional status, body temperature, parasite load, and vulnerability to attack by predators, thereby producing variability in circulating glucocorticoid levels during critical periods of development (Harriman et al., 2014; Ibáñez-Álamo et al., 2011; Kitaysky et al., 2006; Lynn and Kern, 2014; Pravosudov and Kitaysky, 2006; Rensel et al., 2010; Schoech et al., 2011; Sears and Hatch, 2008). Consequently, parenting behavior may affect the young's exposure to glucocorticoids during early development, which may in turn affect the offspring's responses to environmental cues later in life (Lindström, 1999;

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Love and Williams, 2008).

Parental care is widespread among birds (Clutton-Brock, 1991; Lack, 1968), making this vertebrate class an excellent group for studying how parental behavior can modify the phenotype of chicks (Auer and Martin, 2017). Many studies have demonstrated that post-hatching parental care influences the glucocorticoid secretion of nestlings, often permanently (partially reviewed in Lynn and Kern, 2014). However, surprisingly little experimental work in this area has focused on free-living populations.

In addition, temperature is a developmentally important variable across a wide array of vertebrates (Cossins and Bowler, 1987; Gillooly et al., 2002). For example, exposure of nestlings to both high and low temperatures after hatching has been consistently linked to growth and survival in free-living populations (Mainwaring and Hartley, 2016; Rodríguez et al., 2016; Rodriguez and Barba, 2016a,b; Salaberria et al., 2014). Chick temperature is tightly controlled by the brooding behavior of parents in species with altricial young (Dawson and Hudson, 1970; Webb, 1993), which can thus buffer the nestlings' exposure to temperature variation and its developmental consequences. We have previously demonstrated clear links among maternal care, the body temperature of chicks, and both the development and activation of the HPA axis in free-living eastern bluebirds (*Sialia sialis*). For example, variation in maternal brooding behavior is sufficient to produce variation in the chicks' surface temperatures during the first week of life (Lynn and Kern, 2014). If we experimentally mimic the drops in surface temperature that occur when females are absent from the nest, we see immediate and significant elevations in circulating levels of corticosterone in chicks as young as 5 days old (Lynn and Kern, 2014). Moreover, repeated cooling of this kind during the first week of life produces changes in the function of the HPA axis of these young birds such that they later respond to a novel stressor with lower corticosterone secretion than control chicks (Lynn and Kern, 2017). Thus, we have shown that modifications of maternal brooding behavior, by way of resulting variations in nestling body temperature, can result in differential corticosterone profiles among chicks in the short term, and altered HPA function in the longer term. Sensitivity of the developing HPA axis to early, periodic corticosterone exposure has also been demonstrated in other avian species (reviewed in Lynn and Kern, 2017), suggesting that such relationships among maternal care, temperature, and HPA function are likely widespread.

Maternal absence from the nest can clearly affect the development of the HPA axis in chicks by lowering their body temperature, and chicks that were repeatedly cooled for brief (18-min) periods early in life responded to a novel stressor later in life with lower corticosterone secretion than control chicks, as illustrated above (Lynn and Kern, 2014, 2017). But, more prolonged absences (i.e., > 18 min) may also influence the activity and development of this axis by affecting other variables, such as reducing how often chicks are fed. When females leave the nest repeatedly or for long periods, the chicks not only cool, but may also be fed less often, depending on paternal contributions to provisioning. Food restriction is well known to robustly activate corticosterone secretion in adult birds both in the laboratory (Fokidis, 2013; Lynn et al., 2003, 2010; , 2015; Richardson, 1997) and in the field (Astheimer et al., 1995; Smith et al., 1994; Wingfield et al., 1983; Wingfield, 1985). In addition, reducing the rate or amount of food intake of chicks in several avian species not only elevates their corticosterone secretion in an immediate timeframe, but also alters their physiology, morphology, and behavior later in life (Kitaysky et al., 2006; Pravosudov and Kitaysky, 2006; Sears and Hatch, 2008; Rensel et al., 2010; Schoech et al., 2011). Furthermore, variation in the quality or nutritional value of food provided by the parents could potentially produce differences in corticosterone secretion among their chicks. Thus, the possibility exists that chicks may experience periodic elevations of corticosterone as a result of a combination of factors associated with maternal absence from the nest.

Nevertheless, the immediate and long-lasting effects of parental behavior on the HPA axis of nestlings in free living birds are still not well characterized. For example, surprisingly little is known about how early in life chicks are able to respond to stimuli associated with maternal absence by increasing glucocorticoid secretion; the dynamics of glucocorticoid secretion in young chicks during a prolonged period of maternal absence; and, how stimuli associated with maternal absence (cooling, nutritional status, absence of tactile cues, etc.) may interact to affect the activity and development of the HPA axis.

To better understand how these variables may contribute to the cumulative glucocorticoid exposure of young birds early in their development, we experimentally mimicked the cooling induced in eastern bluebird chicks when females are absent from the nest (Lynn and Kern, 2014, 2017), and investigated 1) the age at which the HPA axis becomes capable of responding to cooling by increasing corticosterone secretion, 2) whether corticosterone secretion is elevated continuously during long periods of cooling, and 3) whether fasting interacts with cooling to affect circulating corticosterone. We predicted that (1) chicks exposed to short-duration cooling treatments would have elevated circulating corticosterone earlier than 5 days post-hatch; (2) circulating corticosterone would rise during short bouts of cooling, but would return to baseline levels during longer cooling bouts due to the negative feedback effects of the hormone on the HPA axis; and (3) both fasting and cooling that chicks would experience during periods of maternal absence would increase corticosterone secretion, with the highest levels expected in chicks that were both fasted and cooled.

2. Methods

2.1. Birds and study area

We studied a total of 196 eastern bluebird chicks in 64 broods in Wayne (40°45′N, 81°W) and Ashland (40°55′N, 82°W) Counties, Ohio, during May-August 2015 and May-June 2016. We monitored nest boxes with sufficient regularity to predict hatch date, and visited each nest box on the day of expected hatch whenever possible to confirm this. We also verified the nestlings' ages by comparing their morphological measurements to previously established growth curves for body mass (± 0.01 g), wing chord (± 0.5 mm) and tarsus length (± 0.1 mm) that were developed for nestlings in this population (see Lynn and Kern, 2014 for details). Chicks that hatched more than 24 h later than their nestmates, or that did not fall on the established growth curves for body mass and/or structural measures were not included in the study.

2.2. Nestling temperature treatments

For each of the studies described below, chicks were exposed to one of two experimental temperature treatments, following methods described in detail in Lynn and Kern (2014). Hereafter, these temperature treatments are referred to as 'Cool' and 'Control'. Briefly, in the Cool treatment, the chick was placed in a weighing boat within a 5.7-liter capacity cooler (Igloo) containing two ice blocks, one on each side of the weighing boat. In the Control treatment, which maintained chicks at brooding temperatures (Lynn and Kern, 2014), the nestling was placed in a weighing boat within a second Igloo cooler near a single activated hand warmer (HeatMax). Chambers were always set up 40–60 min before testing to ensure that the temperatures within them had equilibrated at the time of testing. We have shown previously that the equilibrium temperatures of chambers set up in this way were as follows: Cool: 9.08 ± 0.04 °C, Control: 23.3 ± 0.06 °C, and that chicks placed in these chambers for up to 18 min achieve surface temperatures (T_s) that mimic those that occur between brooding bouts (Cool chambers) or during brooding bouts (Control chambers) in nests in this study population (Lynn and Kern, 2014).