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Acute embryonic exposure to corticosterone alters physiology, behaviour and growth in nestlings of a wild passerine

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ABSTRACT

Maternally-derived glucocorticoids can modify the normal development of young animals. To date, little is known about maternal effects that are mediated by acute embryonic exposure to glucocorticoids. In birds, elevated maternal transmission of corticosterone (CORT) to egg albumen is mainly dependent on acute stress. In this study, we increased CORT levels in the egg albumen of a wild passerine, the great tit (*Parus major*), breeding in favourable deciduous and less suitable coniferous habitat. Subsequently we measured the somatic growth, baseline and acute glucocorticoid responses, immunity and behaviour of prenatally manipulated offspring with respect to control siblings. We found that prenatally CORT-exposed nestlings had lower baseline CORT levels, a more rapid decline in CORT during recovery from a standardized stressor, and a reduced heterophil/lymphocyte ratio compared with controls. Although stress-induced total CORT levels remained unchanged, free CORT levels were significantly lower and the levels of corticosteroid binding globulins (CBG) significantly higher in experimental offspring. Prenatally CORT-exposed offspring begged longer after hatching than controls. Stress-induced behavioural activity of fledglings did not differ between treatments, while its association with baseline CORT levels was significant in the control group only. The body mass and tarsus length of fledglings was positively affected by manipulation in unfavourable coniferous habitat only. We conclude that maternal effects related to elevated levels of albumen CORT modify diverse aspects of offspring phenotype and potentially increase offspring performance in resource poor environments. Moreover, our results indicate that maternal glucocorticoids may suppress the effect of hormones on behavioural responses.

1. Introduction

An animal's phenotype is the product of complex interactions between its genotype and the environment in which it develops. Unpredictable environmental conditions can disrupt normal developmental processes, leading to modified phenotypes (Love et al., 2013). In vertebrates, one fundamental physiological system that links phenotypes to environmental changes is the hypothalamic-pituitary-adrenal (HPA) axis. Exposure to various stressors results in activation of the HPA axis with consequent elevation of circulating plasma glucocorticoids. At acute levels these hormones act to modify behaviour and physiology as individuals attempt to cope with unpredictable events (Sapolsky et al., 2000; Boonstra, 2013). Once homeostasis has been restored, glucocorticoids (GC) rapidly return to their baseline levels. At lower concentrations glucocorticoid hormones primarily have metabolic functions, such as regulating energy intake, -storage and -mobilization (Sapolsky et al., 2000). However, exposure to repeated or prolonged stressors gives rise to chronic elevation of baseline levels (Boonstra et al., 1998; Clinchy et al., 2004; but see Rich and Romero, 2005; Cyr and Romero, 2007) that has been shown to have detrimental effects on offspring performance (Zanette et al., 2011; Boonstra, 2013; Clinchy et al., 2013).

Breeding animals that encounter stressful events may transfer elevated levels of glucocorticoids to their embryos (e.g., Saino et al., 2005; Sheriff et al., 2010; Pitk et al., 2012) thereby modifying offspring neonatal size, growth rate, immunity and oxidative status (Love et al., 2005; Haussmann et al., 2011). Many of these effects appear to be mediated by permanent changes in HPA axis functioning (Hayward et al., 2006; Love and Williams, 2008; Sheriff et al., 2010; Zimmer et al., 2013), mainly through perturbation of the negative feedback systems that regulate glucocorticoid secretion, such as those involving intracellular glucocorticoid and mineralocorticoid receptors (Banerjee et al., 2012).

From an evolutionary perspective, maternally-derived stress can induce both adaptive and maladaptive fitness outcomes depending on the predictability of the future environment (Sheriff and Love, 2013).

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In a high-predation environment, in which the hormonal response itself facilitates escape from the stressor, a stronger response should be adaptive, promoting offspring survival through enhanced antipredator behaviour (Cockrem, 2013; Sheriff and Love, 2013). In a low-predation environment, the negative impacts of elevated corticosteroids on health and reproduction may tip the balance towards a reduced HPA axis responsiveness (Macri et al., 2004). Moreover, increases in maternal GCs allow offspring to adapt to unpredictable environments, mediated through increased maintenance-activities, such as foraging and exploration (Meylan and Clobert, 2005; Schultz and Kitayski, 2008).

To date, the majority of vertebrate studies have explored maternal effects induced by chronic embryonic exposure to glucocorticoids (reviewed in Henriksen et al., 2011). In birds, maternal stress has usually been mimicked by injecting corticosterone (CORT) into egg yolk (Love and Williams, 2008; Haussmann et al., 2011; Zimmer et al., 2013). Given that yolk deposition takes 4–5 days in small passerines (Ojanen, 1983a), increased CORT levels in the yolk should mainly simulate the effect of extended stressors such as food limitation, social competition or unfavourable weather conditions, while ACTH-injection that presumably mimicked short-term stressor, did not seem to elevate CORT level in the yolk (Rettenbacher et al., 2005). In addition, short-term encounters with a predator are a common source of stress in the wild, and may have a strong influence on egg quality via hormone transmission from the mother to egg albumen (Saino et al., 2005; Pitk et al., 2012). In birds, egg albumen is produced in less than a day (Burley and Vadehra, 1989), indicating that the levels of CORT in this egg compartment may reliably reflect short-term changes in the plasma levels of hormones. Although CORT concentration in the albumen is about 1/4–1/2 of that in the yolk in wild birds (De Baere et al., 2015), the total amount of CORT in the albumen of small passerines can be nearly equal to that in the yolk because the albumen of passerine eggs is about 2.4 to 4 times heavier than the yolk (Ojanen, 1983b; Burley and Vadehra, 1989). However, little is known about maternal effects that are mediated by acute embryonic exposure to corticosterone. Only two studies on wild birds have measured the effect of albumen CORT on offspring phenotypic traits (Rubolini et al., 2005; Saino et al., 2005) and neither of these studies looked at alterations in the offspring HPA axis responsiveness.

At present, it is still poorly understood when and how exactly steroids in the egg albumen influence the HPA axis responsiveness. Available evidence from chickens suggests that CORT injected into the albumen influences embryonic mortality and hatch weight in a dose-dependent manner, supraphysiological doses causing detrimental effects (Heiblum et al., 2001). Embryonic glucocorticoids may affect the negative feedback mechanisms of the HPA axis in various ways by modulating neurotransmitter systems, influencing gene transcription and down- or upregulating glucocorticoid receptors (von Engelhardt et al., 2009; Henriksen et al., 2011). Given that albumen is metabolized by the developing embryo at a higher rate than yolk (Romanoff, 1960), the effects of elevated albumen and yolk CORT on offspring performance may differ.

In our experiment, we increased the level of CORT in egg albumen of a small passerine bird, the great tit (*Parus major*) to mimic maternal effects induced by acute embryonic exposure to CORT, such as maternal exposure to a predator during egg-laying period (Pitk et al., 2012). Previous studies have shown that CORT injections into egg may have diverse (either positive or negative) effects on different aspects of offspring phenotype (e.g., Hayward and Wingfield, 2004; Rubolini et al., 2005, Zimmer et al., 2013). First, we predicted that embryonic exposure to CORT increases the offspring HPA axis responsiveness and reduces somatic growth (Hayward and Wingfield, 2004; Janczak et al., 2006; Coslovski and Richner, 2011). The elevated adrenocortical response has also been associated with increased fear behaviour (Davis et al., 2008a) and activation of cellular immunity (Sapolsky et al., 2000) as reflected by elevated heterophil/leukocytes (H/L) ratio (Bishop et al., 1968). Romero (2004) has suggested that increased CORT response can also be related to more rapid recovery from the stress through enhanced negative feedback. Second, if prenatal exposure to CORT reduces the HPA axis responsiveness (Hayward et al., 2006; Love and Williams, 2008) no negative effects on offspring growth and cellular immunity can be expected. Third, offspring phenotype can also be affected by spatial differences in food availability, or via synergistic effects of food limitation and predation risk on maternal GCs (Clinchy et al., 2004; Sheriff et al., 2010). In our study system, suitability of deciduous versus coniferous forests for breeding varies remarkably in year-specific way (Tilgar et al., 2002; Mänd et al., 2005). Our previous studies have shown that in good breeding years great tits start egg-laying earlier and produce larger clutches, eggs and nestlings in deciduous when compared to coniferous forests (Mänd et al., 2005, Tilgar et al., 2005). On the other hand, the number and quality of offspring have a tendency to be higher in coniferous forests in bad breeding years (Tilgar et al., 2002), which is paradoxical given that deciduous habitat is clearly preferred over coniferous habitat in our study area as well as in other areas (Mänd et al., 2005). Hence, we hypothesized that nestlings in the low quality postnatal habitat (either deciduous or coniferous) in that particular season would exhibit increased hormonal and behavioural stress responses to balance energetic deficit and would therefore have a relatively lower body condition irrespective of the treatment effects when compared to nestlings in the high quality postnatal habitat.

2. Material and methods

The study was carried out with free-living great tits near the town of Kilingi-Nõmme (south-western Estonia; 58° 7′N, 25° 5′E) in April–June 2011. The study area (ca. 50 km^2) consists of a mosaic of deciduous and coniferous forest patches. Deciduous forests occur either as isolated patches within agricultural landscapes or as 250–500-m-wide riparian strips along stream valleys. They are mostly secondary forests growing on fertile soils and contain a rich deciduous understorey. The most common deciduous species are grey alder (*Alnus incana*) and silver birch (*Betula pendula*). Coniferous forests are typically managed and grow on nutrient-poor sandy or peat soils, where the dominant tree species is Scots pine (*Pinus sylvestris*), which sometimes forms mixed stands with Norway spruce (*Picea abies*) or downy birch (*Betula pubescens*). Previous studies in this area have found consistent habitat-related variation in reproductive patterns of great tits. Great tits breeding in deciduous forests (the preferred habitat) had larger clutches than those breeding in coniferous forests, while the number of fledglings and recruits per pair varies between habitats in year-specific way (Mänd et al., 2005, Lodjak et al., 2015). In the study year of 2011, great tits started egg-laying earlier and they produced larger broods in deciduous forests when compared to those in coniferous forests (Table A). Nest-boxes were mounted on tree trunks at a height of 1.5–1.8 m and checked regularly to obtain clutch initiation, laying sequence and clutch completion dates.

We manipulated egg CORT concentration in 47 nests. Four eggs (egg laying position from 3rd to 7th) were removed from each clutch, and two were randomly assigned to each of the sham- and CORT-injected groups. These eggs were removed from the clutch for injection before clutch completion and returned to the nest after manipulation on the same day. Eggs in the experimental group were injected with 0.5 ng CORT dissolved in 5 μl sterile sesame oil. Our previous study on great tits showed that this amount of CORT corresponds to about 1.5 standard deviations of the amount of CORT estimated for the entire albumen (Pitk et al., 2012). Thus, the CORT concentration of the injected eggs was elevated to a level within the natural range of variation. Eggs in the control group were injected with 5 μl sterile sesame oil only. Injections were made into the albumen from the acute pole of the egg using 25 μl syringes (series N – LT glass luer lock, ILS) with a 26 gauge needle (Terumo Neolus, NN-2613R, for single use). The hole was sealed