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Developmental post-natal stress can alter the effects of pre-natal stress on the adult redox balance

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ABSTRACT

Across diverse vertebrate taxa, stressful environmental conditions during development can shape phenotypic trajectories of developing individuals, which, while adaptive in the short-term, may impair health and survival in adulthood. Regardless, the long-lasting benefits or costs of early life stress are likely to depend on the conditions experienced across differing stages of development. Here, we used the Japanese quail (*Coturnix coturnix japonica*) to experimentally manipulate exposure to stress hormones in developing individuals. We tested the hypothesis that interactions occurring between pre- and post-natal developmental periods can induce long-term shifts on the adult oxidant phenotype in non-breeding sexually mature individuals. We showed that early life stress can induce long-term alterations in the basal antioxidant defences. The magnitude of these effects depended upon the timing of glucocorticoid exposure and upon interactions between the pre- and post-natal stressful stimuli. We also found differences among tissues with stronger effects in the erythrocytes than in the brain in which the long-term effects of glucocorticoids on antioxidant biomarkers appeared to be region-specific. Recent experimental work has demonstrated that early life exposure to stress hormones can markedly reduce adult survival (Monaghan et al., 2012). Our results suggest that long-term shifts in basal antioxidant defences might be one of the potential mechanisms driving such accelerated ageing processes and that post-natal interventions during development may be a potential tool to shape the effects induced by pre-natally glucococorticoid-exposed phenotypes.

1. Introduction

Early life events can drive phenotypic traits of developing individuals (Monaghan, 2008; Mousseau and Fox, 1998). A growing scientific interest focuses on furthering our understanding of the long-term effects associated with poor quality developmental environments on important phenotypic traits that can impact health and adult survival. Pioneering studies in mammals have identified a variety of perinatal stressors (e.g. intrauterine growth restriction, maternal separation, reduced maternal care and child abuse) and persistent metabolic changes in the developing individuals that are thought to be relevant in determining adult health outcomes (for recent reviews see Cottrell and Seckl, 2009; Meaney et al., 2007; Laviola and Macrì, 2013). Changes in adult phenotypes in response to stressful developmental conditions have now been reported in a broader range of vertebrate taxa (e.g. De Fraipont et al., 2000; Monaghan et al., 2012; Roche et al., 2012). It is now widely believed that "developmental programming" may reflect a conserved biological phenomenon across vertebrate species, with significant consequences for a range of health indicators in later life (Love et al., 2013).

Glucocorticoid stress hormones are the main candidates as mediators of developmental stress programming (Seckl, 2004). Growing individuals are exposed to glucocorticoids during their pre-natal development, primarily via maternal routes (e.g. Hayward and Wingfield, 2004; McCormick, 1999; see also review by Henriksen et al., 2011) or during post-natal development, for instance via the direct effects of environmental stressors on their own physiological systems (e.g. Meylan et al., 2002; Spencer et al., 2009). It has been proposed that maternal stress hormones induce anticipatory responses in the embryo that could prime its phenotype to better cope with the future post-natal environmental stimuli (Bateson et al., 2004; Gluckman and Hanson, 2004). However, delayed costs may arise because of inevitable physiological constraints, for example those associated with poor maternal conditions (Grafen, 1988), or because of a mismatch between the predicted and the encountered post-natal environmental conditions (Monaghan, 2008). Acute and persistent exposure to stress hormones can be damaging

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for key self-maintenance processes, such as energetic metabolism, cellular differentiation, myelination, apoptosis or neurogenesis (de Kloet et al., 2005; Sapolsky et al., 1990). Oxidative stress may play a key role in mediating these long-term costs as it results from the production of biomolecular oxidative damage to cells (Costantini and Verhulst, 2009; Halliwell and Gutteridge, 2007), and it has been implicated in cell senescence and neurodegenerative disorders (Finkel and Holbrook, 2000). Prolonged exposure to exogenous glucocorticoids promotes cellular oxidative stress in the body (e.g., Costantini et al., 2011a; McIntosh and Sapolsky, 1996; McIntosh et al., 1998). The magnitude of these effects differs among tissues, with brain and blood showing respectively high and moderately high effect sizes, and, importantly, across individuals' life cycle, with juvenile stressed individuals been more vulnerable than adults (Costantini et al., 2011a).

The hypothesised links between early life stress and shifts in an individual's basal oxidative balance is beginning to be explored (Haussmann and Marchetto, 2010; Haussmann et al., 2012). Recent evidence shows that embryonic exposure to exogenous glucocorticoids can permanently increase gene expression of antioxidant enzymatic defences (Atanasova et al., 2009) or produce increases in oxidative damage and cell senescence in later life (Haussmann et al., 2012). However, the "molecular imprinting" initiated in the stressed embryos is likely to be plastic, mediated by the environment encountered at birth and throughout post-natal development (Monaghan, 2008). Therefore, the degree of developmental plasticity and the long-term consequences arising from these potential adjustments may depend on the nature of both pre- and early post-natal cues. For instance, pre- and post-natal stressful developmental conditions may have cumulative effects on cellular energetic state, exacerbating oxidative insults to tissues as a result of additive physiological constraints or stimulating investment in antioxidants to prevent damage to biomolecules. The potential long-lasting effects of such interactions have hitherto been untested.

In this study we analysed for the first time the long-term potential interactive effects of pre- and post-natal physiological overexposure to glucocorticoids on adult oxidative status. By using a precocial bird species (Japanese quail, *Coturnix coturnix japonica*) as our model, we were able to independently manipulate corticosterone concentrations (B, primary glucocorticoid in birds) in the egg yolk and/or in the endogenous circulation of the hatchlings during the accelerated phase of growth. Our experiment was specifically designed to study the effects of pre-natal conditions under differing post-natal developmental environments in the absence of the potential confounding factors of maternal care. Upon adulthood, we analysed the effects of these early life stressful manipulations on oxidative damage and antioxidant biomarkers in the brain and in the blood.

2. Materials and methods

Birds in this study were also used in a previous study to determine short- and long-term effects of the early life exposure to glucocorticoids on the hormonal phenotype (see Marasco et al., 2012 for full details). The experiment was conducted on a captive population of outbred Japanese quail kept at the University of Glasgow (Cochno Research Centre and Farm, Scotland, UK). The temperature was maintained at 19 °C and the photoperiod was kept at 12:12 h light:dark. Experimental birds were obtained from a breeding stock of 20 females and 10 males. Breeding quail were housed in trios (2 females:1 male) in 79 × 48 × 58 cm enclosures that were maintained throughout the experimental period. Fresh-laid eggs were collected, identified by color and pattern, and marked according to maternal identity. Four groups of experimental birds were established and treated as follows (see paragraph below for details): (1) pre-natal and post-natal untreated birds (CC); (2) pre-natal B-treated and post-natal untreated birds (BC); (3) pre-natal untreated and post-natal B-treated birds (CB); (4) pre- and post-natal B-treated birds (BB). Treatment order was counterbalanced across females. The experiment was repeated in two batches (batch 1: September 2010-December 2010; batch 2: December 2010-March 2011). All procedures were carried out under Home Office Project Licence 60/4068 and Personal Licence 60/12436.

2.1. Pre-natal and post-natal hormonal manipulation

The day on which the eggs were placed in the incubator (Ova-Easy 190A, Brinsea Products Ltd., UK) was designated as embryonic day 0 (E0). At day E5 (the earliest time to determine egg fertility), fertile eggs were identified and selected for the yolk hormonal manipulation. Our injection protocol at day E5 insured that corticosterone levels were elevated once yolk layers in the eggs had ceased to exist; pilot dye studies were carried out prior to the experiment to determine the depth of injection required to place the hormone into the yolk (K.A.S., unpublished). Eggs were injected at the conical tip with 10 μl of a sterile solution of B (Sigma Aldrich, Poole, UK; concentration B: 850 ng/ml) dissolved in peanut oil (B-eggs) or with 10 μl of sterile peanut oil alone (C-eggs) using a SGE syringe (Fisher Scientific, Loughborough, UK). Each egg puncture was sealed using a transparent and breathable wound dressing (Germolene New Skin, Devon, UK) before the egg was returned to the incubator. The dose of B injected (8.5 ng) was designated to increase concentrations of B within the yolk by 1.8 Standard Deviation, similarly as previous studies in birds (Hayward et al., 2006; Saino et al., 2005). This physiological increase was confirmed to be within the relevant biological levels by previous pilot work that quantified yolk B concentrations in a sample of eggs ($n = 8$) taken from a previous generation of our breeding females using both radioimmunoassay and liquid chromatography-mass spectroscopy (Boogert et al., 2013). At day E14 eggs were transferred into hatchers and separated according to maternal identity with plastic dividers in order to identify the maternity of the birds after hatching. Upon hatching (between days E17 and E19), hatchlings were individually marked with unique color combination using nail varnish (this was replaced with a unique numbered plastic leg band at 1-week-old) and placed back into the hatchers to allow the plumage to dry. Then, chicks hatched from B-eggs were randomly allocated either to the BC or the BB treatment groups; chicks hatched from C-eggs were assigned either to the CC or the CB treatment groups. After 24–36 h post-hatching (day PN1), the hatchlings ($n = 2–7$) were housed in 4 age- and treatment-specific brooding enclosures in one single room until day PN19. They were provided with food (turkey starter crumbs, Dodson and Horrell, Northamptonshire, UK) and water *ad libitum*. Between days PN5 and 19, birds in the CB and BB treatments were subjected to post-natal oral manipulation with B, while birds in the CC and BC groups were given carrier alone. Doses were delivered using 1 single mealworm (*Tenebrio molitor*, size 13–18 mm) injected with 10 μl of B dissolved in peanut oil (concentration B: 4.5 mg/ml between post-natal day 5–15; concentration B: 9 mg/ml between post-natal day 16 and 19) or with 10 μl of peanut oil alone. This gave a daily hormone amount of 0.045 mg between day PN5 and 15 and 0.09 mg between days PN16 and 19. Previous pilot work, and data obtained from a sub-set of birds from the present experiment, confirmed that this dosing schedule mimicked a 10-min peak (maximum adrenocortical activity in the majority of birds, see Cockrem, 2013 for a recent review) in circulating plasma B levels that was within the physiological range of quail chick responses to a standardised capture-restraint protocol at day PN8 and PN16 (Marasco et al., 2012). According to previous work in birds, our post-natal treatment is likely to have elevated endogenous plasma B concentrations in