Hormones and Behavior 64 (2013) 494–500

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

# Developmental programming: Cumulative effects of increased pre-hatching corticosterone levels and post-hatching unpredictable food availability on physiology and behaviour in adulthood ☆

Cédric Zimmer, Neeltje J. Boogert, Karen A. Spencer *

*School of Psychology and Neuroscience, University of St. Andrews, St. Andrews, United Kingdom*

ARTICLE INFO

*Article history:*
Received 7 March 2013
Revised 24 June 2013
Accepted 17 July 2013
Available online 26 July 2013

*Keywords:*
Developmental programming
Environmental matching
Exploration
HPA axis
Neophobia
Pre-natal stress
Post-natal stress
Risk-taking
Quail

ABSTRACT

Prolonged exposure to stress during development can have long-term detrimental effects on health and wellbeing. However, the environmental matching hypothesis proposes that developmental stress programs physiology and behaviour in an adaptive way that can enhance fitness if early environments match those experienced later in life. Most research has focused on the harmful effects that stress during a single period in early life may exert in adulthood. In this study, we tested the potential additive and beneficial effects that stress experienced during both pre- and post-hatching development may have on adult physiology and behaviour. Japanese quail experienced different stress-related treatments across two developmental life stages: pre-hatching corticosterone (CORT) injection, post-hatching unpredictable food availability, both pre- and post-hatching treatments, or control. In adulthood, we determined quails' acute stress response, neophobia and novel environment exploration. The pre-hatching CORT treatment resulted in attenuated physiological responses to an acute stressor, increased activity levels and exploration in a novel environment. Post-hatching unpredictable food availability decreased adults' latency to feed. Furthermore, there were cumulative effects of these treatments across the two developmental stages: quail subjected to both pre- and post-hatching treatments were the most explorative and risk-taking of all treatment groups. Such responses to novel environments could enhance survival in unpredictable environments in later life. Our data also suggest that these behavioural responses may have been mediated by long-term physiological programming of the adrenocortical stress response, creating phenotypes that could exhibit fitness-enhancing behaviours in a changing environment.



## Introduction

Prolonged exposure to adverse conditions during development can have serious long-term effects on an individuals' physiology and behaviour, leading to significantly higher risks of many health pathologies and behavioural disorders later in life (Cottrell and Seckl, 2009; Lupien et al., 2009; Sachser et al., 2011; Welberg and Seckl, 2001). For this reason the role of developmental environments in shaping adult phenotypes has received a lot of attention in the last decade (Lindstrom, 1999; Monaghan, 2008). In contrast, one recent view proposes an adaptive framework of developmental programming, where shaping of physiology and behaviour by early-life conditions can enhance fitness if early environmental conditions match those experienced across life stages. According to this 'environmental matching hypothesis', negative effects of developmental adversity may occur due to a mismatch between environmental conditions at different life stages (Bateson et al., 2004; Gluckman et al., 2005; Monaghan, 2008). Whilst this is an intriguing hypothesis that has prompted theoretical studies (Monaghan, 2008), there is currently a lack of studies that have tested it empirically (Gluckman et al., 2005; Monaghan et al., 2012). No study to date has explored the interaction between different developmental stages in shaping these potentially adaptive responses in adulthood. In addition, we have little information about the effects of developmental conditions on behaviours, such as risk-taking behaviour, that may be related to coping with adverse conditions.

One fundamental physiological system that links an individual to changes in the environment is the hypothalamic–pituitary–adrenal (HPA) axis or stress axis (Cottrell and Seckl, 2009; Monaghan, 2008). This axis is activated during adverse conditions in both development and adulthood and, in vertebrates, results in the release of glucocorticoids (Weinstock, 2008; Welberg and Seckl, 2001). This stress response facilitates a switch of physiological processes and behaviours from non-essential activities to those that promote short-term survival, such as increased locomotion and mobilisation of energy stores (Wingfield and



* Corresponding author at: School of Psychology and Neuroscience, University of St. Andrews, South Street, St. Andrews, KY16 9JP, United Kingdom.
*E-mail addresses:* cz6@st-andrews.ac.uk (C. Zimmer), nb40@st-andrews.ac.uk (N.J. Boogert), karen.spencer@st-andrews.ac.uk (K.A. Spencer).

0018-506X/$ – see front matter © 2013 The Authors. Published by Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.yhbeh.2013.07.002

Romero, 2001). This axis is therefore a prime candidate for a mechanism by which adversity over different developmental stages could program an individual's behaviour in an adaptive manner. Indeed, many studies have already shown a link between stress during development and later behavioural effects, such as sexual signalling, longevity, breeding behaviour, learning and memory (Henriksen et al., 2011; Lupien et al., 2009; Monaghan, 2008; Monaghan et al., 2012; Sachser et al., 2011; Spencer and Verhulst, 2007; Weinstock, 2008). Stress during the last days of pregnancy can also result in alterations to behaviours linked to risk-taking, such as higher fearfulness (Henriksen et al., 2011) and reduction in exploration levels (Champagne and Meaney, 2006) in rats (*Rattus norvegicus*).

Many of these effects appear to be mediated by permanent changes in HPA axis functioning, mainly through perturbation of the negative feedback systems that regulate glucocorticoid secretion, such as the intracellular glucocorticoid receptors (GR) and mineralocorticoid receptors (MR) (Banerjee et al., 2012; Cottrell and Seckl, 2009; Lupien et al., 2009; Welberg and Seckl, 2001). In mammals, pre-natal stress through immobilisation during six hours from day 8.5 of pregnancy to birth in mice (*Mus musculus*) resulted in a prolonged response to an acute stress (Chung et al., 2005). However, post-natal glucocorticoid treatment in rats led to a decreased stress response later in life (Vázquez et al., 2012). In birds, injection of glucocorticoids into starling (*Sturnus vulgaris*) eggs resulted in a reduced stress response (Love and Williams, 2008). Conversely, post-hatching deprivation of maternal care in zebra finches (*Taeniopygia guttata*) resulted in a prolonged response to an acute stressor (Banerjee et al., 2012). In both mammals and birds, the prolonged response to acute stress is linked to a decreased expression of glucocorticoid receptors in areas of the brain linked with HPA negative feedback (Banerjee et al., 2012; Chung et al., 2005). When living in an environment with frequent exposure to stressful stimuli, an attenuated stress response could allow an individual to better cope with these conditions; repeated elevated levels of glucocorticoids have detrimental effects, and a quicker return to baseline levels may facilitate more adaptive behaviours (Love and Williams, 2008; Weinstock, 2008). Moreover, in stressful situations increased exploration and risk-taking associated with an attenuated stress response could also be adaptive (Blas et al., 2007; Cavigelli and McClintock, 2003; Dingemanse et al., 2004; Love and Williams, 2008; Martins et al., 2007; Smith and Blumstein, 2008). The overwhelming majority of experimental studies conducted to date have focused on possible detrimental effects that developmental stress during a single developmental stage, either pre- or post-natal, may exert on adult traits. However, to test the environmental matching hypothesis, empirical data are required that address how a combination of pre- and post-natal stressors affects phenotypic traits.

In this study, we exposed test subjects to stress-related treatments during pre- and/or post-hatching development and examined the effects on behavioural traits related to risk-taking behaviour and HPA axis functioning in adulthood. We used a precocial avian species (Japanese quail, *Coturnix japonica*) as it allowed us to easily manipulate pre-hatching glucocorticoid levels by injecting eggs and avoid the confounding effects of post-hatching/-natal maternal care as present in altricial birds and mammals, respectively (Henriksen et al., 2011; Spencer et al., 2009). To test the effects of environmental matching between developmental and adult conditions, and the importance of the developmental timing and nature of the stressor(s) in programming adult physiology and behaviour, we created four treatment groups: we treated one group in the pre-hatching phase only by injecting corticosterone (CORT) into the egg yolks, thereby simulating the transfer of elevated CORT from the mother into her eggs, which is known to occur in several bird species, including Japanese quail (Henriksen et al., 2011). We treated another group in the post-hatching phase only by exposing chicks to an unpredictable food availability paradigm (Buchanan et al., 2003; Cuthill et al., 2000), thereby simulating a stressful foraging environment. A third group was exposed to both treatments and the final group was maintained as a control. We hypothesized that both pre- and post-hatching treatments would affect physiology and behaviour as seen in previous studies. If the environmental matching hypothesis is correct, we predicted that birds in the pre- or post-hatching treatment groups would exhibit behaviours that could potentially enhance fitness under stressful adult conditions, such as reduced neophobia and more exploratory or risk-taking behaviours in novel environments (Blas et al., 2007; Cavigelli and McClintock, 2003; Dingemanse et al., 2004; Smith and Blumstein, 2008). Additionally, if stress experienced during early life matched across both developmental stages and with conditions later in life, we also predicted that birds in the pre- and post-hatching treatment group would show stronger behavioural responses when exposed to stressful conditions as adults than birds exposed to only one of the treatments. Finally, we expected that these behavioural changes would be associated with changes to the acute CORT response to stress (Cavigelli and McClintock, 2003; Love and Williams, 2008; Spencer and Verhulst, 2007).

## Methods

### *Pre- and post-hatching treatments and stress response measurements*

Unrelated Japanese quail eggs ($n = 76$) were obtained from Moonridge Farm, Exeter, UK and placed in an incubator (Ova-Easy 190A, Brinsea Products Ltd, UK) at 37.5 °C and 55% humidity. After 5 days of incubation, half of these eggs (CORT: $n = 38$) were injected with 10 μl CORT (Sigma Aldrich, Poole, UK; concentration CORT: 850 ng/ml) dissolved in sterile peanut oil at the egg apex under sterile conditions. This gave a dose of 8.5 ng of CORT, which increases endogenous CORT concentrations in the yolk within 1.8 SD above control yolks, which is similar to previous studies that have increased CORT levels within physiologically relevant ranges (e.g. Hayward et al., 2006; Love and Williams, 2008). CORT levels were quantified in a sample of yolks from eggs from the same mothers used in this study ($n = 8$) and basal levels were validated using both radioimmunoassay and liquid chromatography-mass spectroscopy (LC–MS–MS). Experimental injection of 8.5 ng of CORT increased whole yolk CORT levels to 17.1 ± 8.3 (SD) ng/ml (RIA analysis, for details see section below). Control eggs were injected with peanut oil alone (Ctrl: $n = 38$). Punctures were sealed with a transparent wound dressing (Germolene New Skin, UK) and each egg was given a unique mark. On day 14 of incubation, eggs were moved to two treatment-specific hatchers (Hatchmaker, Brinsea Ltd, UK), where they were maintained at 37 °C and 75% humidity until hatching on day 18. Fifty-nine eggs hatched, with hatching success for control eggs 74% and for CORT injected eggs 82%. Upon hatching, each chick was given a unique nail polish mark and was returned to the hatcher for 24 h to allow feathers to dry. Chicks of each pre-hatching treatment were subsequently randomly allocated to four pens (114 × 114 × 58 cm) with *ad libitum* food (minced Turkey crumb, BOCM, UK), water and a heat lamp. Pens were maintained at 30 °C for the first 4 days post-hatching, followed by a reduction of 2 °C per day until chicks were 10 days of age, when all additional heat sources were removed and birds were moved to treatment-specific enclosures ($n = 8$; 2 pens per treatment, 100 cm × 86 cm) that were maintained at 20–22 °C throughout the rest of the experiment. The photoperiod was 14 L:10D at all times. When chicks were 4 days old, one pen of each pre-hatching treatment (CORT or Ctrl) was assigned to one of two post-hatching food treatments: either food removal for 25% of daylight hours (3.5 h) on a random daily schedule for 15 days (Food −: $n = 28$) or *ad libitum* food at all times (Ctrl: $n = 31$). Random removal of food has been shown to increase peak CORT levels in starlings (Buchanan et al., 2003), without causing food restriction (Buchanan et al., 2003; Cuthill et al., 2000). Moreover, this treatment is ecologically relevant since precocial birds are not fed by their parents and have to find food by themselves. Thus, while the CORT treatment simulated maternal 'programming' of the offspring regarding a future