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Delayed behavioral effects of postnatal exposure to corticosterone in the zebra finch (*Taeniopygia guttata*)

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Abstract

Early developmental conditions can significantly influence the growth and survival of many animal species. We studied the consequences of exposure to corticosterone (CORT), a stress hormone, during the nestling stage on two behavioral traits (neophobia, social dominance) measured when the birds had reached independence. Nestling zebra finches (*Taeniopygia guttata*) were exposed twice daily to exogenous CORT via oral administration for a 12-day period up until fledging. Experimental CORT administration depressed nestling growth rates, confirming results previously obtained in this species. Our data on neophobic behavior revealed a significant interaction between sex and treatment, with CORT-dosed males showing reduced latencies to approach a novel object, while there was little effect of corticosterone treatment on female neophobia. There was no significant effect of age (30 or 50 days), however, there was a non-significant trend towards an interaction between treatment and age, with neophobia increasing with age in the CORT-dosed birds, but decreasing in controls. At 50 days of age previous exposure to corticosterone resulted in reduced success in competitions for a non-food-based resource (a perch) in both sexes. There were no effects of brood size on any behavioral traits measured here, but this may be due to the small range in brood size used. Our results show that elevated levels of stress hormones during postnatal development can have significant effects on important behavioral traits, i.e., neophobia and dominance. Moreover, they confirm the importance of rearing conditions in shaping adult phenotypes.

Keywords: Stress hormones; Behavioral development; Evolution; Developmental stress; Phenotypic plasticity; Social dominance

Introduction

Developmental conditions can have significant effects on the expression of adult phenotypes (Bateson, 2001; Birkhead et al., 1999; Blount et al., 2003; Buchanan et al., 2003; Gil, 2003; Ketterson et al., 1996; Metcalfe and Monaghan, 2001; Moussaeu and Fox, 1998; Nowicki et al., 2002; Spencer et al., 2003, 2005; Verhulst et al., 2006). Such effects are not restricted to morphology, but extend to highly flexible traits such as behavior. For example, adverse environments during growth and development have been linked to adjustments in fear-related behavior (Gregus et al., 2005; Penke et al., 2001), sexual signal expression (Blount et al., 2003; Buchanan et al., 2003; Gustafsson et al., 1995; Nowicki et al., 1998, 2002; Ohlsson et al., 2002; Spencer et al., 2003, 2004, 2005) and cognitive abilities (de Kloet et al., 2002; Kitaysky and Wingfield, 2003; Sandstrom and Hart, 2005) in adult animals. Many studies in adult birds have shown that plasma concentrations of corticosteroids can rise rapidly in response to a range of environmental perturbations (Pereyra and Wingfield, 2003; Silverin et al., 1997; Wingfield et al., 1998; Wingfield, 1994), however, until recently equivalent data in growing birds have been lacking. Recent studies have now shown that during postnatal development, a range of natural and experimental stressors, such as food restriction, aggression and handling can induce elevated circulating concentrations of stress hormones in nestlings of many altricial avian species (Blas et al., 2005; Bowerman et al., 2002; Kitaysky et al., 2001a, 1999; Love et al., 2003a,b; Saino et al., 2003; Sims and Holberton, 2000;

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Sockman and Schwabl, 2001). The existence of this physiological response in growing individuals poses the question whether exposure to stress hormones during development influences an individual's phenotype in the longer term, after the stressful circumstances have subsided.

Exposure to elevated levels of stress hormones during development has been shown to modify the physiological response to experimentally induced stress in adulthood, in many cases elevating maximal levels or prolonging levels over time (Anisman et al., 1998; Hayward and Wingfield, 2004; Penke et al., 2001; Parfitt et al., 2004). It is thought that this effect may be linked to a change in glucocorticoid receptor expression during development and thus regulation of stress hormone levels later in life, as well as the development of other components of the underlying neuroendocrine stress axis (Anisman et al., 1998; Sapolsky and Meaney, 1986). Early exposure to stress hormones may therefore play a role in 'programming' the neuroendocrine stress axis, shaping the 'hormonal phenotype' of the animal, with consequences for both behavioral and physiological responses to a variety of stressors in later life. In support of this hypothesis, developmentally mediated variation in the stress response has been linked to adjustments in some aspects of fear-related behavior and cognitive abilities in adult animals (de Kloet et al., 2002; Gregus et al., 2005; Kitaysky and Wingfield, 2003). Fear-related behaviors, which can have significant fitness effects, can be quantified in a standardized manner, by recording the behavioral responses of an animal to a novel object or environment.

Many birds are social foragers that live in large flocks, allowing more efficient foraging and greater individual energetic gains (Powell, 1974) and this is especially true during the non-breeding season and during early independence in many species including the zebra finch (Zann, 1996). During this activity dominance hierarchies are established and maintained through repeated pairwise interactions, where a dominant bird displaces a subordinate. These aggressive interactions can provoke large increases in individual stress hormones such as corticosterone in birds (Wingfield, 1994), in both the dominant and subordinate birds (Creel, 2001). Dominant birds often gain an advantage over subordinates through increased access to better or safer food or safe perching places, however, elevated social status can also carry significant costs (Verhulst and Salomons, 2004). Being able to compete for a range of resource types is therefore vital to ensure survival and maximize fitness, making competitive ability an essential part of an individuals behavioral phenotype.

In this study we investigated the effects of experimental exposure to the stress hormone corticosterone (CORT) during the latter half of the nestling period on neophobic behavior and competitive ability in later life in the altricial bird species zebra finch (*Taeniopygia guttata*). The study utilized genetically related sibling pairs of birds, where one received oral administration of small amounts of CORT between the ages of 7 and 18 days post-hatching and the other received only the carrier solution (peanut oil) during this period. Birds were then tested after nutritional independence to examine two aspects of their behavioral phenotype: their response to novel objects and competitive ability.

Methods

Corticosterone manipulation

Adult zebra finches were randomly paired ($n=20$ pairs) and housed at the Biological Centre at the University of Groningen in 80×40×40-cm cages equipped with nestboxes and nesting material (hay). The birds were maintained at a temperature of 20–24°C and a photoperiod of 14L:10D. Breeding pairs were provided with a commercial tropical seed mixture (Teurlings, Dordrecht, The Netherlands), water, shell grit, and cuttlefish bone *ad libitum*. Pairs were also given egg food 3 times per week, until their offspring hatched when they received supplementary egg food daily. Upon hatching first and second hatched nestlings in each nest were individually marked by colored nail polish to each bird's toes. Brood sizes were standardized to either 3 or 5 nestlings (population range, 2–6 nestlings) by adding or removing nestlings at 2 days of age (additional nestlings in a nest were aged within 1 day of foster siblings), ensuring that two siblings remained in each nest that were genetically related to the parents. When nestlings reached 7 days of age experimental treatments began. First and second hatched chicks were assigned to one of two treatments: corticosterone administration or control, counterbalancing across broods for chick hatch order to control for any potential differences in basal corticosterone levels (Schwabl, 1999). Following Spencer et al. (2003), birds in the corticosterone group were dosed 0.0125 mg of corticosterone daily, dissolved in peanut oil (concentration 0.25 mg/ml) via two 25 μl doses (at least 6 h apart) from the age of 7–18 days of age. This dosing schedule would have meant that birds experienced a reduced physiological dose with age as they increased in mass; a mass-scaled approach would have allowed the effective dose to remain constant. Control birds were dosed with the same amount of the peanut oil carrier solution at the same time as their sibling. All manipulations ceased at the age of 18 days. All birds had their mass recorded at 7, 12, 18 and 30 days of age. The sex of each bird was determined via the presence of sexually dimorphic adult plumage, which begins to appear after 35 days of age. All procedures were carried out following peer review and approval from the ethical committee for animal experiments of the University of Groningen (under license D4299).

Hormonal assay

To assess the effects of stress hormone administration on circulating levels of corticosterone over time blood samples (<100 μl) were collected from 12-day-old birds at 0, 10, 30 or 120 min after administration of either corticosterone or peanut oil. Blood was collected in heparinized capillary tubes after puncture of the brachial vein with a 25-gauge needle, centrifuged and the plasma stored at −20°C for later hormone assay. Due to the small size of juvenile zebra finches each bird was bled once only. Samples were useable from 4 birds in each time period and each treatment (total $n=32$), this reduction in sample size was due to the difficulty in obtaining enough blood from such small birds. Corticosterone concentrations were measured after extraction of 20 μl aliquots of plasma in diethyl ether, by radioimmunoassay (Maddocks et al., 2001; Buchanan et al., 2003) using anti-corticosterone antiserum code B21–42 (Endocrine Sciences, Tarzana, CA) and [1,2,6,7-3H]-corticosterone label (Amersham, UK). All birds showed corticosterone levels above the detection limit for this assay. The extraction efficiency was 70–91%. The assay was run with 50% binding at 1.48 ng/ml, and the detection limit for 7.3 μl aliquots of extracted plasma was 0.31 ng/ml. All samples were run in duplicate in a single assay.

Behavioral measurements

Neophobic behavior

Neophobic behavior was investigated twice, once around the time of nutritional independence (27–35 days of age) and again when birds were fully independent from their parents (50–60 days of age). The same methodology was used for each trial. Each bird was removed from their home cage, which they