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Physiology

Developmental stress can uncouple relationships between physiology and behaviour

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Phenotypic correlations (r_P) have frequently been observed between physiological and behavioural traits, and the nature of these associations has been shown to be modulated by a range of environmental stressors. Studies to date have examined the effects of acute stressors on physiology–behaviour interrelations, but the potential for permanent changes induced by exposure to stress during development remains unexplored. We exposed female zebra finches to dietary restriction during the nestling stage and tested how this affected r_P among a variety of physiological traits (haematocrit, stress-induced corticosterone level and basal metabolic rate (BMR)) and behavioural traits (activity and feeding rates in novel and familiar environments). Developmental stress completely uncoupled the relationship between activity in a novel environment and two physiological traits: haematocrit and BMR. This suggests that nutritionally based developmental stress has provoked changes in the energy budget that alleviate the trade-off between maintenance (BMR) and locomotor activities.

1. Introduction

Physiology and behaviour are so inextricably linked that they have long been viewed as complementary in the fields of ecology and evolution [1]. The relationships between physiological and behavioural traits, however, are commonly modulated by exposure to environmental stressors such as hypoxia [2], food deprivation [3], unpredictable food supply [4], conspecific density [5], water velocity [6], predators [7] and ambient temperature [8]. Stressors can challenge an individual's homeostasis and oblige it to adjust physiologically or behaviourally to cope, therefore either demanding higher performance or constraining the expression of traits at the population level [9]. Previous studies testing the modulating effect of stressors on relationships between physiology and behaviour have examined acute exposure to stress [2–9], implying that the changes caused by stressors are temporary. By contrast, many stressors experienced during development induce long-term phenotypic changes [10], such that any modulating effects on the physiology–behaviour relationship should last throughout adulthood. For example, experiencing dietary restriction early during development usually leads to compensatory growth, which in turn affects a whole suite of physiological and behavioural traits expressed in later life [11–14].

We have recently shown in female zebra finches (*Taeniopygia guttata*) that food restriction early in the developmental phase leads to a variety of phenotypic changes expressed at adulthood [15]. Compared to control adult females, birds experiencing early food restriction had higher haematocrit (ratio of packed red blood cell volume to total blood volume) and basal metabolic rate (BMR; the minimal energy expenditure of a post-absorptive animal at rest when measured under standardized conditions), but unaltered stress response to capture and handling (plasma corticosterone levels) [15]. Developmental food restriction also led to a

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higher feeding rate in a novel environment, but had no effect on activity level [15]. Such phenotypic alterations of some traits, but not others, may allow individuals to adaptively 'match' the environment that they are most likely to encounter as adults [10]. Indeed, individual variation in haematocrit, BMR, corticosterone level, feeding rate and activity have high evolutionary significance in light of body condition, energy metabolism, stress response, resource acquisition and 'person-ality', respectively, and the type of relationship between these traits has profound evolutionary consequences [9,16]. Thus, our objective was to test whether early food restriction alters the phenotypic correlation (r_P) between these physiological and behavioural traits.

2. Material and methods

(a) Animals and treatments

A detailed description of the material and methods used in this study can be found in a previous publication [15] and in the electronic supplementary material. Briefly, breeding pairs of zebra finches were moved into assigned cages that contained a nest-box and nesting material. On completion of a clutch, each pair was randomly assigned to one of two treatment groups: (i) control pairs that received seed ad libitum throughout breeding or (ii) food-restricted pairs that received a limited amount of food daily, approximating their daily requirements, mixed in a 3 : 1 volumetric ratio of milled rice husk : seed [11], which forced parents to search through the husks to locate whole seeds. These treatment groups were maintained on these diets until day 30 post-hatch, at which time all pairs received ad libitum seed and fresh greens daily. All offspring stayed within their sibling groups and within visual and acoustic contact of their parents until day 60 post-hatch, at which time they were moved to another cage and housed with same-sex individuals from other nests. The restricted diet treatment group had lower nestling growth rates than the control group [15]. All females raised under food-restricted diets are termed 'stressed females' ($n = 10$) and those raised on ad libitum diets are referred to as 'control females' ($n = 9$). At day 100 post-hatch, birds were moved into single-sex cages containing two control and two stressed birds, and maintained on an ad libitum diet. From August to December 2013, we took three to four repeated sets of physiological and behavioural measurements (see the electronic supplementary material).

(b) Statistical analysis

Analyses were conducted in ASReml-R. Given that we collected repeated measures, we first used a multivariate mixed model to estimate the correlations at the among- and within-individual levels [17], but our low sample size led to convergence problems. Thus, we averaged all measures for each individual and estimated the r_P between z-transformed traits (mean = 0, variance = 1) by fitting a multivariate model that allowed an unstructured correlation matrix between the residual variance of each trait. Our r_P estimates are conditioned on body mass because we included a fixed effect of body mass fitted separately to each trait. Using a multivariate model allowed us to calculate mass-conditioned r_P estimates in a one-step process, which is preferable to a two-step analysis such as when residuals are first calculated and then used for testing correlations. We first ran a model using data from all trials, and then separately for control and stressed birds. We tested for the statistical significance of each r_P using a likelihood ratio test comparing the log-likelihoods of a full model to a reduced model that restrained the r_P of interest to 1×10^{-8}. Twice the difference in the
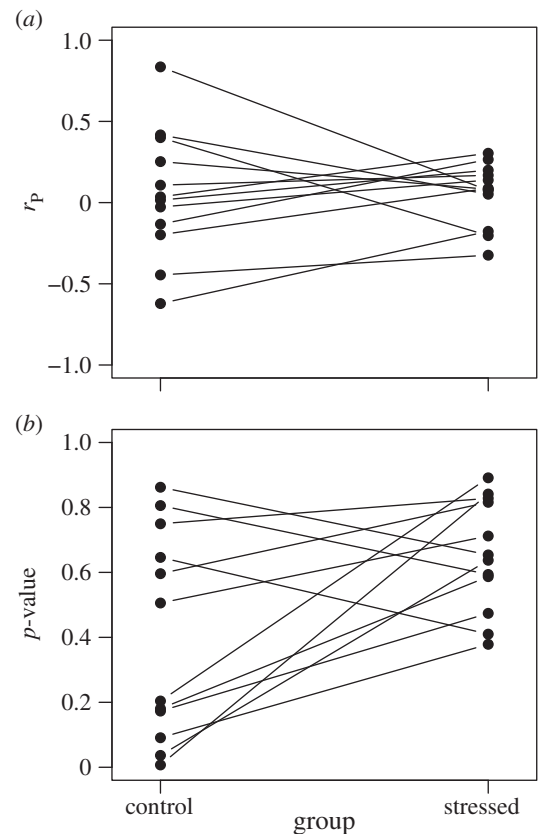

Figure 1. (a) Phenotypic correlations (r_P) between physiological and behavioural traits in control and stressed female zebra finches that experienced dietary restriction during their development, and (b) p-values. Lines connect the same r_P or p-value estimate in both treatment groups.

log-likelihoods between the two nested models is assumed to follow a χ^2-distribution with d.f. equal to the difference in the number of parameters estimated.

3. Results

Using pooled data for control and stressed birds, only the r_P between feeding in the novel and familiar environments was significantly different from zero (electronic supplementary material, table S2a). However, splitting the dataset between control and stressed birds revealed striking differences in the r_P estimates among physiological and behavioural variables (electronic supplementary material, table S2b,c). While the r_P estimates ranged from -0.62 to 0.84 in control females, these ranged from only -0.32 to 0.30 in stressed females (figure 1a) and the p-values were generally lower in the control than stressed females (paired t-test, $t_{11} = -2.42$, $p = 0.03$; figure 1b). This is also illustrated by sharper and darker ellipses in figure 2a compared with 2b.

While the r_P between haematocrit and activity in a novel environment was high and significant in control birds ($r_P = 0.84 \pm 0.11$, $p = 0.007$; electronic supplementary material, table S2b; figure 2a), it was low and non-significant in birds that experienced developmental stress ($r_P = 0.08 \pm 0.38$, $p = 0.84$; electronic supplementary material, table S2c; figure 2b). Similarly, the r_P between BMR and activity in a novel environment was negative and significant in control birds ($r_P = -0.62 \pm 0.23$, $p = 0.037$; electronic supplementary material, table S2b; figure 2a), but was non-significant in birds experiencing developmental stress ($r_P = -0.18 \pm 0.37$,
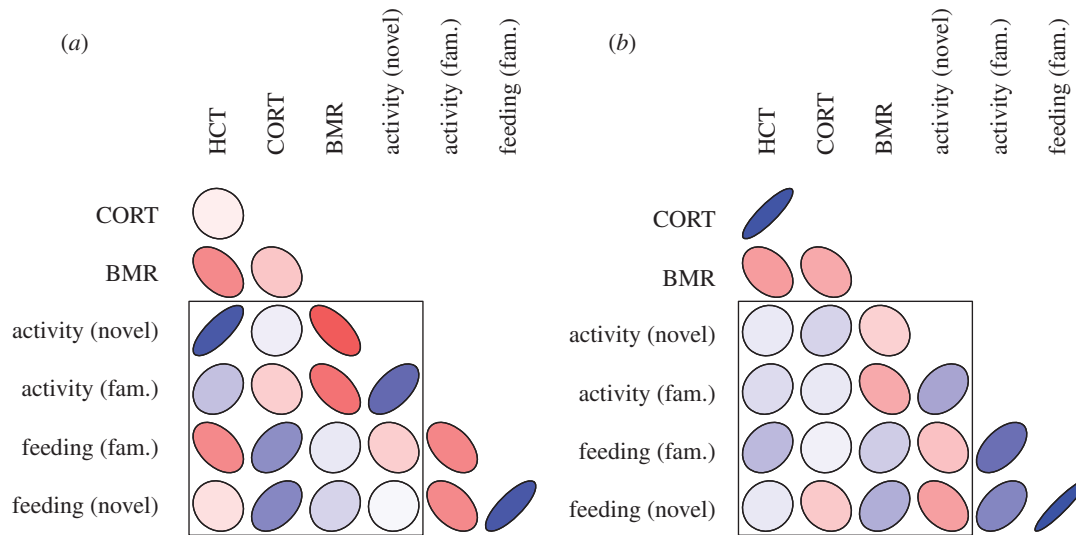
Figure 2. Ellipse representation of the phenotypic correlations between physiological and behavioural traits in adult female zebra finches in (a) control and (b) stressed birds that experienced dietary restriction during their development. The physiological traits include haematocrit (HCT), stress-induced corticosterone level (CORT), and basal metabolic rate (BMR). The behavioural traits include activity and feeding rates in a novel and familiar (fam.) environments. Ellipses within the squares are those showing the relationships between physiology and behaviour. The shape of the ellipse represents a level curve of the density of a bivariate normal with the matching correlation. Blue ellipse tilted rightwards, positive. Red ellipse tilted leftwards, negative. (Online version in colour.)

$p = 0.64$; electronic supplementary material, table S2c; figure 2b). Note that in both control and stressed females, the r_Ps between physiology (haematocrit and BMR) and activity in a *familiar* environment were non-significant ($p > 0.09$; electronic supplementary material, table S2b).

4. Discussion

It is intuitive to expect that higher levels of physical activity and exploration should be associated with higher maintenance metabolism [16]. This is because higher daily energy expenditure requires greater daily food assimilation which, in turn, leads to increases in the size and activity of digestive organs and a consequent rise in BMR [18]. It is also conceivable that inherently higher BMR will necessitate increased rates of feeding. In control females, however, we obtained a negative r_P between BMR and activity in a novel environment, as has also been found in free-living female great tits (*Parus major*) [19]. This negative r_P may be due to constraints imposed by the principle of allocation, whereby limits on the amount of food an animal can process result in trade-offs in its energy budget. Thus, maintaining a high BMR may monopolize energy that would otherwise be available for other energy-demanding processes, such as locomotor activity [16]. The reverse causality is also possible, such that high activity levels can only be achieved by a concomitant reduction in maintenance costs (BMR). To the extent that the trade-off between activity and BMR is unavoidable, then variability in these traits can be maintained by environmental variability favouring either high maintenance (e.g. survival) or high productivity (e.g. reproductive activity).

Our results also show that, in control birds, the associations between physiology (haematocrit and BMR) and activity were significant in a novel environment, but not in a familiar environment. Thus, exposure to novelty revealed a relationship between physiology and behaviour that disappeared once birds had become familiarized to the situation. This revealing effect of exposure to a novel environment is similar to previously described effects of other acute stressors, such as hypoxia [2], food deprivation [3], unpredictable food supply [4], conspecific density [5] and water velocity [6]. By contrast, other acute stressors have an uncoupling effect, such as the absence of a protective cover [7] and decreasing ambient temperature [8]. Thus, the most common impact of acute stressors seems to be a revealing effect (six cases, including this study) rather than an attenuating effect (two cases), but more studies on this topic are needed to clarify how particular situations influence the extent of physiological/behavioural coupling [9].

Our most important finding was showing that food restriction during development changed physiological–behavioural relationships at adulthood. That is, correlations between activity level in a novel environment and two physiological traits (haematocrit and BMR) in control birds were not present in stressed birds. The uncoupling effect of early food restriction may have come about by affecting the phenotypic variance [9], because stressed females showed increased phenotypic variance in haematocrit and BMR, but reduced phenotypic variance in activity in a novel environment [15]. Alternatively, the relationship between physiology and behaviour might have been altered by differential trait sensitivity to early food restriction [9]. Indeed, early food restriction caused an increase in haematocrit, BMR and feeding rate, but not in activity [15]. If we assume that the negative r_P found in control birds is caused by allocation of restricted energy availability to competing resources (see above), then it implies that nutritionally based developmental stress has provoked changes in the energy budget, via increased feeding rate, that alleviate trade-offs between costs of maintenance (BMR) and locomotor activities (see also [14]).

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