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Risk-averse personalities have a systemically potentiated neuroendocrine stress axis: A multilevel experiment in *Parus major*

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ABSTRACT

Hormonal pleiotropy—the simultaneous influence of a single hormone on multiple traits—has been hypothesized as an important mechanism underlying personality, and circulating glucocorticoids are central to this idea. A major gap in our understanding is the neural basis for this link. Here we examine the stability and structure of behavioral, endocrine and neuroendocrine traits in a population of songbirds (*Parus major*). Upon identifying stable and covarying behavioral and endocrine traits, we test the hypothesis that risk-averse personalities exhibit a neuroendocrine stress axis that is systemically potentiated—characterized by stronger glucocorticoid reactivity and weaker negative feedback. We show high among-individual variation and covariation (i.e. personality) in risk-taking behaviors and demonstrate that four aspects of glucocorticoid physiology (baseline, stress response, negative feedback strength and adrenal sensitivity) are also repeatable and covary. Further, we establish that high expression of mineralocorticoid and low expression of glucocorticoid receptor in the brain are linked with systemically elevated plasma glucocorticoid levels and more risk-averse personalities. Our findings support the hypothesis that steroid hormones can exert pleiotropic effects that organize behavioral phenotypes and provide novel evidence that neuroendocrine factors robustly explain a large fraction of endocrine and personality variation.

1. Introduction

Upon exposure to a social or environmental challenge, individuals within a population often differ consistently in their behavioral response (reviewed in Réale et al., 2007; Bell et al., 2009; Dall et al., 2012). Moreover, single behaviors (e.g. aggressiveness) are often linked within an individual with other behaviors (e.g. exploration; reviewed in Groothuis and Carere, 2005). These consistent individual differences and trait correlations are the basis for the concept of animal personality (similar to 'coping styles', 'behavioral syndromes'), which has now been demonstrated in a wide variety of species (van Oers and Naguib, 2013). This research highlights the constraints on behavioral flexibility, on the independent evolvability of traits, and suggests that the mechanisms that underlie one particular behavior might subserve other behaviors (Réale et al., 2007).

The hypothesis that hormones serve as mechanisms underpinning animal personality has been the subject of growing interest (Williams, 2008; Koolhaas et al., 2010). Glucocorticoids (hereafter CORT) are proposed to be key steroids involved in one of the major axes of personality: the shy-bold continuum (Øverli et al., 2007; Carere et al., 2010). In part, this hypothesis rests on the pleiotropic nature of steroids—these endocrine products circulate throughout the organism and bind to multiple receptor types across diverse tissues. Hence, a single hormone can simultaneously affect multiple targets, thereby precisely modulating the expression of several behaviors (Ketterson and Nolan, 1999).

As the end products of the hypothalamic-pituitary-adrenal (HPA) axis, CORT facilitate critical functions in vertebrates: coping metabolically with the fluctuating demands of normal life, such as day-night rhythmicity, locomotor activity and predictable daily and life-history events (Landys et al., 2006). Further, the HPA axis is essential for coping with unpredictable, acutely challenging events, such as exposure to unfamiliar environments or objects (Lendvai et al., 2011), inclement

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weather (Breuner and Hahn, 2003), predators (Cockrem and Silverin, 2002), but also sexual behaviors and social victory (Koolhaas et al., 2011). The regulation of the HPA axis consists of several components: First, low baseline concentrations fluctuate according to diel rhythms and metabolic demands and are known to promote feeding behavior (Dallman et al., 1993). Second, within a few minutes after an acute challenge is perceived, CORT (following an elevation of their upstream secretagogues such as adrenocorticotropic hormone, ACTH) becomes elevated and continues to rise in the blood until it reaches a peak, typically within 30–90 min (Baugh et al., 2013; Droste et al., 2011). At these stress-induced concentrations, CORT facilitates a metabolic shift from protein and fat synthesis towards gluconeogenesis by altering transcription in target cells (Gray et al., 1990; Hasselgren, 1999; Sapolsky et al., 2000; Oakley and Cidlowski, 2013). Third, negative feedback reduces circulating levels, allowing baseline concentrations to be re-achieved (Romero, 2004).

Regulation of circulating CORT concentrations is made possible by two intracellular receptors in the brain that bind CORT. The mineralocorticoid receptor (MR) has a high affinity and low capacity for CORT and is therefore thought to be principally active at baseline CORT concentrations (Romero, 2004; Landys et al., 2006). In contrast, the low affinity and high capacity glucocorticoid receptor (GR) exhibits increased binding at stress-induced concentrations (de Kloet, 1998; Funder, 1997) and is also thought to play a critical role in regulating negative feedback through binding to receptors located in the pituitary and hypothalamus, thereby inhibiting the secretagogues that lead to further elevations in CORT (de Kloet, 1991; Ronchi et al., 1998; Romero, 2004). Moreover, because of its upstream location in the HPA axis, receptor expression in the brain has the potential to explain intraspecific variation in stress physiology and behavior. Here we examine MR and GR expression in the hypothalamus and hippocampus, two brain regions known for their involvement in HPA regulation and roles in mediating behavior (Nelson, 2005). Higher GR expression in these regions, for example, might result in stronger negative feedback and thus a systemically less potentiated HPA axis (i.e. lower CORT at all post-stressor time-points).

Beyond single behaviors, the ways in which individuals respond hormonally to stressors may underlie several of the correlated behaviors that often characterize personality (Koolhaas et al., 2007). Further, if individuals vary consistently in functional aspects of the HPA axis—the circulating concentrations of glucocorticoids (CORT) and the expression patterns of receptors in behaviorally relevant tissues (e.g. nervous system)—this could give rise to variation in personality. Indeed, there is often remarkable intra-population variation in concentrations of baseline and stress-induced CORT (Hau et al., 2016). The fraction of this variation that represents among-individual variance has been studied in recent years and has yielded mixed results, reflecting in part the fact that only a subset of these studies used repeated measures designs (Baugh et al., 2014). However, understanding the endocrine basis of animal personality requires repeatedly characterizing behavioral, endocrine and neuroendocrine traits in the same individuals (reviewed in Ball and Balthazart, 2008)—a step that, to our knowledge, has not been undertaken until now.

In the present study we tested for the presence of among-individual variance in both behavioral traits and functional aspects of the HPA axis and then tested the hypothesis that variance in HPA axis function explains behavioral variance. Because environmental context can drive considerable acute variation in plasma glucocorticoids, we sought to control experimentally certain aspects of the environment—nutrition and exposure to conspecifics—but allowed physical aspects of the environment to vary naturally (e.g. weather). Using semi-natural enclosures, we studied wild-caught great tits (*Parus major*), a species that has been the subject of extensive investigation in animal personality (van Oers and Naguib, 2013) and, more recently, of intra-population variation in glucocorticoid physiology (Hau et al., 2016). We predicted that: (1) risk-taking behaviors expressed in the context of a foraging task will both vary at the among-individual level (i.e. exhibit repeatability) and covary at the among-individual level (i.e. exhibit syndromes); (2) four functional aspects of the HPA axis—baseline CORT, the stress response, negative feedback strength and adrenal sensitivity—will likewise vary and covary at the among-individual level; (3) the expression patterns of MR and GR in two regions of the brain that regulate the HPA axis (hippocampus and hypothalamus) will be correlated with HPA function, with higher GR expression predicted to strengthen negative feedback; and thus GR expression in these regions is predicted to correlate negatively with a systemically potentiated HPA axis (Romero, 2004); and (4) repeatable elements of the behavioral phenotype are correlated with repeatable elements of the endocrine phenotype; specifically, that birds with lower GR expression would express a consistently potentiated HPA stress axis and more risk-averse personalities.

2. Materials and methods

2.1. Animals

We used a repeated measures study design that included behavioral testing (N = 27; 15 females), plasma hormone assessment (N = 25; 13 females) and neural hormone receptor mRNA quantification (N = 25; 13 females; unequal sample sizes reflect the fact that two birds died of unknown causes between behavioral and hormonal assessments; Fig. 1). In 2009, we collected eggs from 14 nests (7 nests had clutch sizes of 1; 1 nest had a clutch size of 2; 6 nests had clutch sizes of 3) from an established nest box population (Westerheide, NL). Eggs were then distributed to unique and random wild foster parents to decouple nestling experience and relatedness among siblings. Because other maternal effects prior to hatching (e.g. yolk hormones) might influence the adult phenotype, we call this a 'nest of origin' effect (hereafter NestID) rather than strictly genetic relatedness. Ten days after hatching, fledglings were transported to the Netherlands Institute for Ecology (NIOO-KNAW, Heteren, NL) and hand-raised in captivity until nutritional independence.

In November 2010, the birds were transported by automobile to the Max Planck Institute for Ornithology-Radolfzell, where all experimental and laboratory work was conducted. After two weeks of quarantine, birds were housed singly in large outdoor aviaries (3 × 3 × 2 m high) in alternating male-female adjacencies (birds had audible but not visible contact). These captive conditions facilitated control of the social and nutritional environments—singly housed birds were fed an ad libitum diet and fresh water. Each aviary contained an elevated feeding platform, a nest box, hanging perches and live shrubs. Birds were acclimatized to these housing conditions for three months before testing began. We first characterized behavioral traits using three repeated samplings, and then characterized HPA axis function using two repeated measures sampling events, and lastly we sacrificed the birds to estimate the expression of hormone receptors in the brain (Fig. 1).

2.2. Behavioral testing

Twenty-seven birds were tested in a behavioral assay for object neophobia and risk-taking on three repeated occasions (RTA_{1-3}; Fig. 1). Testing order was randomized with the exception that adjacent aviaries were never sampled on the same day and the two sexes were balanced each day. To ensure motivation and to habituate birds to feeding on the ground, each bird was restricted to three mealworms per day in a bowl centered on the floor of the aviary during a three-day window prior to testing. We tested a maximum of 7 birds per day during the morning (7:30–12:00). To habituate birds to the experimental set-up, we placed a camouflaged blind in front of each aviary at a distance of 3 m beginning 24 h prior to testing. The experimenter occupied the blind during the testing.

Our neophobia/risk-taking assessment was modified from a procedure previously validated as a measure of personality in this species