



Royal Netherlands Academy of Arts and Sciences (KNAW) KONINKLIJKE NEDERLANDSE AKADEMIE VAN WETENSCHAPPEN

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

Baugh, A.T.; Senft, R.A.; Firke, M.; Lauder, A.; Schroeder, J.; Meddle, S.L. ; van Oers, K.; Hau, Michaela

published in

Hormones and Behavior
2017

DOI (link to publisher)

[10.1016/j.yhbeh.2017.05.011](https://doi.org/10.1016/j.yhbeh.2017.05.011)

document version

Peer reviewed version

[Link to publication in KNAW Research Portal](#)

citation for published version (APA)

Baugh, A. T., Senft, R. A., Firke, M., Lauder, A., Schroeder, J., Meddle, S. L., van Oers, K., & Hau, M. (2017). Risk-averse personalities have a systemically potentiated neuroendocrine stress axis: a multilevel experiment in *Parus major*. *Hormones and Behavior*, 93(July), 99-108. <https://doi.org/10.1016/j.yhbeh.2017.05.011>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the KNAW public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain.
- You may freely distribute the URL identifying the publication in the KNAW public portal.

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

E-mail address:

pure@knaw.nl

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

3
4 Alexander T. Baugh^{a*}, Rebecca A. Senft^a, Marian Firke^a, Abigail Lauder^a, Julia Schroeder^b,
5 Simone L. Meddle^c, Kees van Oers^d, and Michaela Hau^{e,f}

6
7 ^a Department of Biology, 500 College Avenue, Swarthmore College, Swarthmore, PA 19081,
8 USA;

9
10 ^b Department of Life Sciences, Imperial College London, Silwood Park Campus, Buckhurst
11 Road, SL5 7PY Ascot, Berks, United Kingdom; julia.schroeder@imperial.ac.uk

12
13 ^c The Roslin Institute, The Royal (Dick) School of Veterinary Studies, The University of
14 Edinburgh, Easter Bush, Midlothian, EH25 9RG, United Kingdom;
15 simone.meddle@roslin.ed.ac.uk

16
17 ^d Department of Animal Ecology, Netherlands Institute of Ecology (NIOO-KNAW),
18 Droevendaalsesteeg 10, 6708 PB Wageningen, The Netherlands; k.vanoers@nioo.knaw.nl

19
20 ^e Evolutionary Physiology Group, Max Planck Institute for Ornithology, Eberhard-Gwinner-Str.,
21 82319 Seewiesen, Germany; mhau@orn.mpg.de

22
23 ^f Department of Biology, Universitätsstrasse 10, University of Konstanz, Konstanz, Germany

24
25
26 Running title: HPA axis underlies avian personalities

27
28
29 *Corresponding author:
30 Swarthmore College
31 500 College Avenue
32 Martin Biological Laboratory
33 Swarthmore PA 19081
34 USA
35 Tel. 610-690-5243
36 Fax. 610-328-8663
37 Email: abaugh1@swarthmore.edu
38

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

55

56 *Keywords*

57 ACTH, behavioral syndromes, corticosterone, dexamethasone, glucocorticoid receptor, HPA
58 axis, mineralocorticoid receptor, negative feedback, personality, stress.

59

60

61

62 INTRODUCTION

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

73 The hypothesis that hormones serve as mechanisms underpinning animal personality has
74 been the subject of growing interest (Williams, 2008; Koolhaas et al., 2010). Glucocorticoids
75 (hereafter CORT) are proposed to be key steroids involved in one of the major axes of
76 personality: the shy-bold continuum (Øverli et al., 2007; Carere et al., 2010). In part, this
77 hypothesis rests on the pleiotropic nature of steroids—these endocrine products circulate
78 throughout the organism and bind to multiple receptor types across diverse tissues. Hence, a
79 single hormone can simultaneously affect multiple targets, thereby precisely modulating the
80 expression of several behaviors (Ketterson and Nolan, 2009).

81 As the end products of the hypothalamic-pituitary-adrenal (HPA) axis, CORT facilitate
82 critical functions in vertebrates: coping metabolically with the fluctuating demands of normal
83 life, such as day-night rhythmicity, locomotor activity and predictable daily and life-history
84 events (Landys et al., 2006). Further, the HPA axis is essential for coping with unpredictable,

85 acutely challenging events, such as exposure to unfamiliar environments or objects (Lendvai et
86 al., 2011), inclement weather (Breuner and Hahn, 2003), predators (Cockrem and Silverin,
87 2002), but also sexual behaviors and social victory (Koolhaas et al., 2011). The regulation of the
88 HPA axis consists of several components: First, low baseline concentrations fluctuate according
89 to diel rhythms and metabolic demands and are known to promote feeding behavior (Dallman et
90 al., 1993). Second, within a few minutes after an acute challenge is perceived, CORT (following
91 an elevation of their upstream secretagogues such as adrenocorticotrophic hormone, ACTH)
92 becomes elevated and continues to rise in the blood until it reaches a peak, typically within 30–
93 90 min (Baugh et al., 2013; Droste et al., 2011). At these stress-induced concentrations, CORT
94 facilitates a metabolic shift from protein and fat synthesis towards gluconeogenesis by altering
95 transcription in target cells (Gray et al., 1990; Hasselgren, 1999; Sapolsky et al., 2000; Oakley
96 and Cidlowski, 2013). Third, negative feedback reduces circulating levels, allowing baseline
97 concentrations to be re-achieved (Romero, 2004).

98 Regulation of circulating CORT concentrations is made possible by two intracellular
99 receptors in the brain that bind CORT. The mineralocorticoid receptor (MR) has a high affinity
100 and low capacity for CORT and is therefore thought to be principally active at baseline CORT
101 concentrations (Romero, 2004; Landys et al. 2006). In contrast, the low affinity and high
102 capacity glucocorticoid receptor (GR) exhibits increased binding at stress-induced concentrations
103 (de Kloet et al., 1998; Funder, 1997) and is also thought to play a critical role in regulating
104 negative feedback through binding to receptors located in the pituitary and hypothalamus,
105 thereby inhibiting the secretagogues that lead to further elevations in CORT (De Kloet 1991;
106 Ronchi et al. 1998; Romero 2004). Moreover, because of its upstream location in the HPA axis,
107 receptor expression in the brain has the potential to explain intraspecific variation in stress

108 physiology and behavior. Here we examine MR and GR expression in the hypothalamus and
109 hippocampus, two brain regions known for their involvement in HPA regulation and roles in
110 mediating behavior (Nelson 2005). Higher GR expression in these regions, for example, might
111 result in stronger negative feedback and thus a systemically less potentiated HPA axis (i.e. lower
112 CORT at all post-stressor time-points).

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

126 In the present study we tested for the presence of among-individual variance in both
127 behavioral traits and functional aspects of the HPA axis and then tested the hypothesis that
128 variance in HPA axis function explains behavioral variance. Because environmental context can
129 drive considerable acute variation in plasma glucocorticoids, we sought to control experimentally
130 certain aspects of the environment—nutrition and exposure to conspecifics—but allowed

131 physical aspects of the environment to vary naturally (e.g. weather). Using semi-natural
132 enclosures, we studied wild-caught great tits (*Parus major*), a species that has been the subject of
133 extensive investigation in animal personality (van Oers and Naguib, 2013) and, more recently, of
134 intra-population variation in glucocorticoid physiology (Hau et al., 2016). We predicted that: (1)
135 risk-taking behaviors expressed in the context of a foraging task will both vary at the among-
136 individual level (i.e. exhibit repeatability) and covary at the among-individual level (i.e. exhibit
137 syndromes); (2) four functional aspects of the HPA axis—baseline CORT, the stress response,
138 negative feedback strength and adrenal sensitivity—will likewise vary and covary at the among-
139 individual level; (3) the expression patterns of MR and GR in two regions of the brain that
140 regulate the HPA axis (hippocampus and hypothalamus) will be correlated with HPA function,
141 with higher GR expression predicted to strengthen negative feedback; and thus GR expression in
142 these regions is predicted to correlate negatively with a systemically potentiated HPA axis
143 (Romero, 2004); and (4) repeatable elements of the behavioral phenotype are correlated with
144 repeatable elements of the endocrine phenotype; specifically, that birds with lower GR
145 expression would express a consistently potentiated HPA stress axis and more risk-averse
146 personalities.

147

148 MATERIALS AND METHODS

149 *Animals*

150 We used a repeated measures study design that included behavioral testing (N=27; 15 females),
151 plasma hormone assessment (N=25; 13 females) and neural hormone receptor mRNA
152 quantification (N=25; 13 females; unequal sample sizes reflect the fact that two birds died of
153 unknown causes between behavioral and hormonal assessments; Fig. 1). In 2009, we collected

154 eggs from 14 nests (7 nests had clutch sizes of 1; 1 nest had a clutch size of 2; 6 nests had clutch
155 sizes of 3) from an established nest box population (Westerheide, NL). Eggs were then
156 distributed to unique and random wild foster parents to decouple nestling experience and
157 relatedness among siblings. Because other maternal effects prior to hatching (e.g. yolk
158 hormones) might influence the adult phenotype, we call this a ‘nest of origin’ effect (hereafter
159 NestID) rather than strictly genetic relatedness. Ten days after hatching, fledglings were
160 transported to the Netherlands Institute for Ecology (NIOO-KNAW, Heteren, NL) and hand-
161 raised in captivity until nutritional independence.

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

173

174 *Behavioral Testing*

175 Twenty-seven birds were tested in a behavioral assay for object neophobia and risk-taking on
176 three repeated occasions (RTA₁₋₃; Fig. 1). Testing order was randomized with the exception that

177 adjacent aviaries were never sampled on the same day and the two sexes were balanced each
178 day. To ensure motivation and to habituate birds to feeding on the ground, each bird was
179 restricted to three mealworms per day in a bowl centered on the floor of the aviary during a
180 three-day window prior to testing. We tested a maximum of 7 birds per day during the morning
181 (7:30-12:00). To habituate birds to the experimental set-up, we placed a camouflaged blind in
182 front of each aviary at a distance of 3 m beginning 24 h prior to testing. The experimenter
183 occupied the blind during the testing.

184 Our neophobia/risk-taking assessment was modified from a procedure previously
185 validated as a measure of personality in this species (van Oers et al., 2005). Briefly, a small
186 platform (30 cm²) containing live mealworms and a loaded mousetrap was introduced to the
187 aviary floor and monofilament was used to trigger the trap from a distant observation blind (see
188 S2). All birds alighted initially on the aviary floor and then hopped onto the front of the platform
189 near the mealworm dish, at a distance of approximately 20 cm from the mousetrap. No birds
190 were injured during the course of this assay.

191 We recorded (1) *Initial Latency*: time elapsed from the start of the trial until the bird
192 approached the platform and retrieves a mealworm—because this is each bird’s first exposure to
193 the platform, we assume this latency reflects a low risk response to novelty (object neophobia);
194 (2) *Reward Latency*: time elapsed since retrieval of the first worm and the return to the platform
195 for a second mealworm (note: all birds flew to a shrub to consume each mealworm). Upon
196 alighting on the platform for this second mealworm the experimenter triggered the trap, startling
197 the bird (all birds flew away); and (3) *Startle Latency*: time elapsed between triggering the trap
198 and the bird returning to the platform and retrieving a mealworm—we assume this latency
199 reflects a high risk response. Trials in which birds did not return within the maximum trial

200 duration (20 min) were not included in the repeatability, covariance or PCA analyses because
201 assigning a constant value (e.g. 1200 sec) would artificially inflate these estimates. Although,
202 exclusion of these incomplete trials could under-represent the most risk-averse personalities, a
203 pilot study conducted with wild-caught birds showed that extending this time window to longer
204 durations (60 min) resulted in few additional latency data. The number of mealworms on the
205 platform was counted following each trial to ensure that the expected number of worms was
206 retrieved. This testing procedure was repeated three times per bird, with a three-day interval
207 separating repeated trials. Upon completion of the third trial, each bird was measured for tarsus
208 length, flattened wing cord length, body mass, and fat score (Cherry, 1982); body condition was
209 estimated using the scaled mass index method (SMI; Pieg and Green, 2009), and completion of
210 prebasic moult was confirmed.

211

212 *HPA Assessments*

213 *Validation*

214 In March 2012 we validated the HPA assessments following (Dickens et al., 2009a), including
215 the pharmacological dosages, time courses, and the cross reactivity of the pharmacological
216 reagents in the ELISA. We used wild-caught adult great tits—used only for this validation—
217 from a nest box population in Radolfzell, Germany (N=13) (S1).

218

219 *Blood collection*

220 Twenty-five birds were tested for plasma glucocorticoid dynamics using a repeated measures
221 HPA assessment (Fig. 1). The first assessment (HPA₁) was conducted during a one-week period
222 in late August following the third and final set of behavioral trials to facilitate analysis of

223 hormone-behavior relationships. The second assessment (HPA₂) was performed in November
224 preceding the brain collection. Sampling was limited to 0800-1100. Samples were collected by
225 puncturing the brachial vein and collecting the blood using a heparinized microcapillary tube.
226 Birds were restrained in small cotton bags during the intervals between sampling time points.

227 Our method for assessing the HPA axis has been used previously in birds (Dickens et al.,
228 2009a; Hau et al., 2015) and reptiles (Romero and Wikelski, 2010) to simultaneously quantify
229 four aspects of HPA axis function: (1) Baseline CORT (*BaseCORT*): this first blood sample
230 precedes the handling/restraint-induced stress response. *BaseCORT* was collected within 2 min
231 following entry into the aviary to reduce contamination from the stress response (Baugh et al.,
232 2013; Romero and Reed, 2005) and was followed by placing the bird in a small cotton restraint
233 bag for 15 min. (2) Stress response (*StressCORT*): this second blood sample provides an estimate
234 of the early stage of each bird's acute response to handling/restraint and was immediately
235 followed by an intramuscular injection of dexamethasone (DEX; 1000 µg kg⁻¹; diluted to 50 µL
236 in PBS), which stimulates strong negative feedback of the HPA axis, thereby down-regulating
237 subsequent CORT secretion (Dickens et al., 2009a; Hau et al., 2015); this injection was followed
238 by a 90-min restraint period. (3) Negative feedback strength (*DexCORT*): the CORT
239 concentration here reflects the strength of negative feedback following the DEX injection (higher
240 CORT here indicates *weaker* negative feedback); this was followed immediately by an
241 intramuscular injection of adrenocorticotrophic hormone (ACTH; Sigma #A6603; 100 IU kg⁻¹
242 diluted to 50 µL in PBS), followed by a 15-min restraint period. (4) Adrenal sensitivity
243 (*ActhCORT*): finally, birds were bled a fourth time to estimate the capacity of the adrenal glands
244 to produce CORT upon pharmacological stimulation of the HPA axis by the injected
245 secretagogue. Birds were then immediately measured for biometrics, released into their aviary

246 and monitored for health. Blood samples were kept on wet ice during sample collection and then
247 centrifuged (1400 g for 10 min). The plasma fraction was frozen at -80 C until all samples were
248 assayed simultaneously.

249

250 *Enzyme Immunoassay*

251 In July 2013 we estimated plasma CORT concentrations using a commercial enzyme
252 immunoassay kit (Enzo Life Sciences, Cat. No. ADI 900-097; Donkey anti-Sheep IgG). The
253 details of our EIA procedure, including its validation, extraction, recoveries, technical
254 repeatability and preparation of standards are reported in (Baugh et al., 2014; Ouyang et al.,
255 2011). The intra- and inter-assay coefficients of variation (CV; 9 plates)—8.1% and 8.2%,
256 respectively. The assay has a detection limit of 27 pg mL⁻¹. The cross-reactivity of the antiserum
257 is 100% for corticosterone, 28.6% for deoxycorticosterone and 1.7% for progesterone.

258

259 *Neural Receptors Quantification*

260 Following a 14-day recovery from the second HPA assessment, birds (N=25) were captured by
261 hand net and decapitated. Trunk blood was collected and kept on wet ice while whole brains
262 were dissected from the skulls and frozen in aluminum foil on dry ice and maintained at -80 C
263 until cryosectioning. The interval separating entry into the aviary and frozen tissue was < 3 min.
264 We transported brains on dry ice to the Roslin Institute at the University of Edinburgh, mounted
265 them on OCT (TissueTek) and sectioned them coronally at 15 µm onto polysine pretreated
266 slides. Tissue was stored at -80 C. Two slides from each animal (each with six sections) were
267 used for radioactive *in situ* hybridization and a custom Python script and ImageJ were used for
268 silver grain quantification in the paraventricular nucleus of the hypothalamus (PVN) and the

269 hippocampus (HP)—two regions implicated in HPA axis regulation (Dickens et al., 2009b).
270 Details of our methods are described in (Senft et al., 2016).

271

272 *Statistical Analyses*

273 *Behavior: General*

274 We used a repeated measures ANOVA to test for effects of repeat number and latency type,
275 including covariates for sex, SMI, and fat score. This permitted us to evaluate whether birds
276 became habituated to the novel object platform across repeated trials. Effect sizes (partial eta-
277 squared and Cohen's d) were calculated for significant main effects, interactions and pair-wise
278 comparisons.

279

280 *Among-individual variances (i.e. repeatabilities)*

281 All repeatability and covariance analyses were performed in R 3.0.2. We estimated the within-
282 and among-individual variance components using Bayesian general linear mixed models
283 (GLMMs) with a Gaussian error distribution, and used the variance component estimates to
284 calculate the repeatability of each behavioral and hormonal trait (\log_{10} transformed and z-
285 standardized). Our linear mixed models approach (Sokal and Rohlf, 1995; Nakagawa and
286 Schielzeth, 2010) has important advantages over earlier ANOVA-based methods for
287 repeatability estimation (intra-class correlation coefficients; Lessells and Boag, 1987), including
288 the incorporation of environmental covariates and nested terms (i.e. adjusted repeatabilities),
289 robustness to data heterogeneity (missing values, unbalanced designs), and the ability to estimate
290 uncertainty around the repeatability estimate because variances are estimated directly. These
291 models were constructed in MCMCglmm (Hadfield, 2010). We ran models without fixed effects

292 (i.e. agreement repeatabilities), with individual identity as the sole random effect, and used
293 inverse-Wishart priors. A second set of models were conducted to correct our estimates for nest
294 of origin effects and determine whether the nest of origin explained some of the variation in
295 traits. Therefore, we added NestID as a random effect to the models. We then repeated all the
296 above models, this time accounting for fixed effects (i.e., adjusted repeatabilities), by adding
297 variation in body condition (SMI, scalar) and the fat score as fixed covariates (S4 for adjusted
298 repeatabilities), with a similar prior. Sex was not included as a term in any of the models because
299 behavioral, endocrinological and quantitative genetics studies in *P. major* have shown no
300 evidence for sex-dependent expression of exploratory behavior (Dingemanse et al. 2002; van
301 Oers et al. 2004a; Carere et al. 2005), risk-taking behavior (van Oers et al. 2005), or HPA axis
302 function (Stöwe et al. 2010; Baugh et al. 2014).

303 We used the variance component estimates to calculate effects of individual identity and
304 nest of origin. We ran each model for 1,000,000 iterations, used default sampling, and we ran
305 model diagnostics to confirm that the autocorrelation between subsequent stored iterations was
306 not higher than 0.1. We report the repeatabilities, and variance components calculated as a ratio
307 of the total variance, with 95% credible intervals (95CI).

308

309 *Covariances*

310 We performed bivariate GLMMs to estimate within- and among-individual covariation. These
311 models were constructed in MCMCglmm (Hadfield, 2010), where individual identity was fitted
312 as random effect. Covariances in both the random effect and the residual were allowed to take on
313 any value (for a similar analysis and sample size, see Araya-Ajoy and Dingemanse, 2016). We
314 compared the Deviance Information Criterion (DIC, Spiegelhalter et al., 2002) of these models

315 with one from a model where we fixed the covariance within individuals to zero. A difference of
316 >5 in DIC was considered statistically significant (Spiegelhalter et al., 2002). We ran these
317 models with uninformative priors and ran each model for 2,000,000 iterations. Standard model
318 diagnostics confirmed that autocorrelation among sampled iterations was low.

319

320 *Linking neural receptors, HPA dynamics and behavior*

321 HPA₂ was timed to precede brain collection (Fig. 1) to test the hypothesis that MR and GR
322 expression in the HP and PVN predict HPA axis function. To do this we used the average MR
323 and GR expression in the PVN and HP calculated across four coronal sections per bird (N=25).
324 We constructed general linear models to describe how neural receptor expression predicts CORT
325 concentrations for the four HPA components. We also included two fixed variables in these
326 models to represent body condition, SMI and furcular fat score, that have been shown to be
327 correlated with CORT secretion (Wingfield et al., 1994).

328 To test the broader relationships among receptors, hormones and behavior—and in order
329 to reduce family-wise error rates—we reduced the dimensionality for all three of these
330 phenotypic categories using principal components analysis and then used path analyses (SPSS
331 version 21) to test the strength and direction of relationships among phenotypic levels (Fig. 2).
332 We tested two *a priori* models that minimized the number of paths: (1) Full model: MR and GR
333 directly influence both HPA axis function and behavior and the HPA axis also directly influences
334 behavior; and (2) Reduced model: MR and GR only indirectly influence behavior via the HPA
335 axis. We calculated the fit of both models ($1 - \pi(e_{\text{HPA}} * e_{\text{Risk}})$) and a summary statistic ($Q = (1 -$
336 $\text{Fit}_{\text{Full}}) / (1 - \text{Fit}_{\text{Reduced}})$) and then compared the significance of this quotient with a Chi-squared test
337 of significance ($W = -(N-d) * \log_e(Q)$, where N=sample size and d=the number of dropped paths).

338 Measurements from RTA₁ and HPA₁ were used and all datasets were log₁₀-transformed and z-
339 standardized prior to component extraction. For the behavioral data, we excluded trials in which
340 the bird did not return to the platform within the maximum window of time (20 min) because
341 assigning maximum values here would artificially inflate the eigenvalues (final N_{path analysis}=14).
342 All analyses extracted only one component (PC1) with eigenvalues > 1 (which explained 63-
343 82% of the variance) and correlation matrices indicated that all pairwise correlation coefficients
344 varied between 0.1 and 0.9 (S8). Therefore, we performed path analyses using PC1 for each trait
345 category (Fig. 2). Residual error for these analyses did not deviate from Gaussian, visually or
346 statistically (Shapiro-Wilk; all p > 0.20) and observed power for the omnibus path model was
347 adequate (power=0.83).

348

349 RESULTS

350 *Behavior: General*

351 Sex (F₁=0.674, p=0.443), SMI (F₁=0.044, p=0.840) and fat score (F₃=0.503, p=0.694) did not
352 explain significant variance in behavior. The repeated measures ANOVA therefore only included
353 the within-subjects factors (latency type and repeat number; S2). Latencies were generally high
354 for *Initial Latency* and *Startle Latency* (mean ± SD, sec: 347.8 ± 279.9; 283.3 ± 232.0,
355 respectively)—indicating that this assay predictably elicited a neophobic and startle response,
356 respectively—but low for *Reward Latency* (143.5 ± 163.8), suggesting that the first mealworm
357 acted as a food reward. There was a significant main effect of latency type (F_{2,26}=22.82, p<0.001,
358 partial η²=0.74). There was no main effect of repeat number (F_{2,26}=1.54, p=0.233), but there was
359 an interaction between latency type and repeat number (F_{4,52}=3.08, p=0.024, partial η²=0.73) due
360 to a marginal reduction in the *Initial Latency* between RTA₁ and RTA₂ (pairwise comparison:

361 $p=0.01$, cohen's $d=0.92$ (95CI=0.19-1.64); Fig. 1). No other pairwise comparison was significant
362 (S2), suggesting that the novelty of the platform diminishes over repeated exposures.

363

364 *Behavioral variances*

365 Repeatabilities for all three latencies were high (0.41–0.53; see Bell et al. 2009) and statistically
366 significant (i.e. with 95CI that were not zero-bound; Table 1; S5(a–c)). NestID did not explain
367 significant variance in the three behaviors (Table 1), suggesting that risk-taking phenotypes may
368 not be strongly explained by relatedness or pre-hatching maternal effects (e.g. yolk hormones) or
369 both. However, the statistical power to detect NestID effects was not particularly high because
370 half of the nests did not have siblings, so we cannot exclude this possibility. Moreover, results
371 stayed qualitatively the same when accounting for fat score and SMI (S4 for adjusted
372 repeatabilities).

373

374 *Behavioral covariances*

375 There was a significant positive among-individual covariance between *Initial* and *Startle*
376 *Latencies* (Table 3; Fig. 3a; S5(d–m)), demonstrating that birds that are chronically neophobic
377 are chronically more risk-averse (i.e. personality). There was a similar trend between *Initial* and
378 *Reward Latencies* (Table 3; Fig. 3b). The DIC of the model in which the covariance between
379 *Initial Latency* and *Reward Latency* was fixed to zero did not fit the data better than a model in
380 which the covariance was allowed to take on any value (Δ DIC = 13.6). This was also true for the
381 model including covariances between *Initial Latency* and *Startle Latency* (Δ DIC = 23.8). In both
382 models the covariance within-individuals was positive and statistically significant (95CI do not

383 overlap zero), demonstrating that at a given moment in time a bird exhibiting more neophobic
384 behavior will also predictably exhibit more risk-averse behavior (Table 3).

385

386 *Hormones: General*

387 Our validation study indicated that our drug dosages and timeline induced predictable variation
388 in CORT concentrations, with low *BaseCORT*, moderately high *StressCORT*, low *DexCORT* and
389 high *ActhCORT* (S1). In our experimental study we observed a main effect of HPA component
390 ($F_{3,72}=186.8$, $p<0.001$, partial $\eta^2=0.97$), again with low *BaseCORT* and *DexCORT*, and high
391 *StressCORT* and *ActhCORT* (S3). There was also a main effect of season, with higher CORT
392 concentrations in November (HPA₂) compared to August (HPA₁) ($F_{1,24}=24.29$, $p<0.001$, partial
393 $\eta^2=0.51$; S3). Lastly, there was an interaction effect between season and HPA component
394 ($F_{3,72}=25.06$, $p<0.001$, partial $\eta^2=0.71$), driven by weakened negative feedback (higher
395 *DexCORT*) in November and a concomitant increase in *ActhCORT* (S3). The between-subject
396 factors of sex ($F_{1,23}=3.46$, $p=0.08$), SMI ($F_{1,23}=2.5$, $p=0.12$), and fat score ($F_{1,23}=1.99$, $p=0.17$)
397 did not significantly explain variance in the CORT variables and therefore were not included in
398 the repeated measures ANOVAs.

399

400 *Hormonal variances*

401 One bird had a very low CORT concentration for the first *BaseCORT* sample. We ran models
402 that included and excluded this statistical outlier, and the results did not differ qualitatively
403 (direction and proportionality of estimates), thus we report the inclusive results. For all four
404 traits, repeatabilities were statistically significant (i.e. 95CIs were not zero-bound). *DexCORT*
405 and *ActhCORT* exhibited qualitatively higher repeatabilities than *BaseCORT* and *StressCORT*,

406 but the 95CI did overlap so the estimates are not statistically significantly different from each
407 other (Table 2). When accounting for NestID, the amount of variance explained by differences
408 among individuals was qualitatively lower, and a larger part of the variance, especially for
409 *BaseCORT*, was explained by NestID, but again, 95CIs overlapped (Table 2; S6(a–d)). Results
410 stayed qualitatively the same when accounting for fat score and SMI (S4 for adjusted
411 repeatabilities).

412

413 *Hormonal covariances*

414 The difference in DIC between the bivariate model of *BaseCORT* and the *StressCORT* where
415 covariances were fixed to zero and where they were allowed to take on any value was large
416 ($\Delta\text{DIC} = 27$), thus we assumed that covariance modelling better explained the data. There were
417 no statistically significant covariances at the among-individual level (Table 3; 95CI overlap
418 zero), despite a trend for some trait pairs, including high *BaseCORT* being linked with a high
419 *StressCORT* (Fig. 3c), and weak negative feedback (high *DexCORT*) linked to strong adrenal
420 sensitivity (high *ActhCORT*) (Fig. 3d). The covariances within individuals for all trait pairs
421 examined were statistically significant and positive (Table 3; S6(h,l,p)). Similarly, the model that
422 included covariance between the *StressCORT* and *DexCORT* was statistically significant (ΔDIC
423 $= 19.5$) and the covariance within individuals was statistically significant and positive, while the
424 covariance among individuals was not different from zero (Table 3; S6(e–p)). Similarly, the
425 covariances within individuals were statistically significant and positive for the pairs *DexCORT*
426 versus *ActhCORT*, and *StressCORT* versus *ActhCORT*. Again, the differences in DICs confirmed
427 that the models including covariance better explained the data ($\Delta\text{DIC}_{\text{Dex vs Acth}} = 26$ and $\Delta\text{DIC}_{\text{Stress}}$
428 $\text{vs Acth} = 16$).

429

430 *Linking neural receptors, HPA dynamics and behavior*

431 Expression of GR was higher in the PVN than in the HP, and MR exhibited the opposite pattern.
432 Expression of MR was positively correlated across the PVN and the HP and there was a trend for
433 a positive correlation for GR across the two nuclei (Fig. 2 and Senft et al., 2016). In contrast,
434 there were no correlations between the two receptor types (MR versus GR) for any combination
435 of nuclei or PC (all $p > 0.15$).

436 Given the widespread covariance among trait items (e.g. *Initial* and *Startle Latencies*),
437 the principal component analyses allowed us to reduce our dataset into four trait categories (MR
438 phenotype; GR phenotype; HPA potentiation phenotype; risk-aversion phenotype; Fig. 2; see S8
439 for PCA details; Budaev 2010) and minimize family-wise error rates. Using these trait
440 categories, our path analyses indicated that higher MR and lower GR predict a more potentiated
441 HPA axis (higher CORT) and more risk-averse (longer latencies) personalities (Fig. 2). Model fit
442 was significantly higher for the full model (0.785) compared to the reduced model (0.539) with
443 two dropped paths ($\chi^2=9.12$, $df=2$, $p < 0.05$; Fig. 2), indicating support for a direct and an indirect
444 (i.e., via HPA axis) influence of MR and GR on risk aversion. These results were robust to the
445 structural details of the path model—the qualitative outcome did not change with
446 inclusion/exclusion of items in principal components and the relationships among these higher-
447 level trait dimensions mirrored patterns detected at lower levels of analysis. For example,
448 variation in *ActhCORT* positively predicted startle latencies ($R^2=0.41$, $F_{1,12}=8.36$, $p=0.014$); GR
449 expression in the hippocampus negatively predicted startle latencies ($R^2=0.39$, $F_{1,12}=7.76$,
450 $p=0.016$); GR expression in the PVN negatively predicted *ActhCORT* ($R^2=0.24$, $F_{1,23}=6.51$,

451 $p=0.019$); and MR expression in the hippocampus positively predicted *StressCORT* ($R^2=0.20$,
452 $F_{1,23}=5.82$, $p=0.024$; see also S7, S8, S9).

453

454

455 DISCUSSION

456 We found support for the hypothesis that avian personality is correlated with individual
457 differences in HPA axis function. Our repeated measures design allowed us to partition variance
458 in behavioral, endocrine and neuroendocrine traits in the same individuals under semi-natural
459 conditions, providing an integrative picture of trait lability and interaction—to our knowledge
460 this is the first such study to integrate across these levels of organization. The results support our
461 prediction that individuals with consistently more potentiated HPA axes exhibit risk-averse
462 personalities. These results help to unify findings from previous research examining the HPA
463 axis and personality in vertebrates (Ellis et al., 2006; Koolhaas et al., 1999) including great tits
464 (Hau et al., 2016) and provide an important test of the assumption that pharmacological
465 challenges provide a window into upstream neural receptor phenotypes.

466

467 *Personality*

468 Risk-taking behaviors were repeatable—approximately 30-50% of the variation in each can be
469 attributed to individual differences, a relatively high fraction for behavioral traits (Bell et al.,
470 2009). And in contrast to the HPA components, nest of origin explained only a small fraction of
471 the behavioral variation. This finding extends previous work that indicates that these types of
472 risk-taking behaviors are components of a more general personality suite that includes
473 exploration and boldness (van Oers et al., 2004b, 2004c; van Oers et al., 2005; Hall et al. 2015).

474 Moreover, these behaviors are phenotypically correlated, owing to the joint contributions of
475 positive among- and within-individual correlations. The within-individual correlation implies
476 that either dynamic internal (e.g., circadian state) or external variables (e.g., temperature) or both
477 varied across observations of the same individual and that these variables modulated the
478 expression of both traits simultaneously (see Baugh et al., 2014). Given that we controlled the
479 social and nutritional environments, this within-individual correlation further indicates that these
480 two behaviors are codependent on a variety of influences beyond nutritional state and social
481 context (see Drosmann et al. 2014). This within-individual correlation could emerge as a
482 consequence of ultradian cycles (Droste et al., 2011); for example, baseline CORT and stress-
483 induced CORT will both be higher following the periodic and pulsatile release of ACTH. The
484 among-individual correlation between these two behaviors, however, provides evidence for their
485 joint contribution to personality and is only possible given the among-individual variance (i.e.
486 repeatability) of both behaviors (Baugh et al., 2014). This correlation means that birds that are on
487 average more neophobic are also on average more risk-averse, and vice versa. This is consistent
488 with the finding in this species that risk-taking behavior is genetically linked with general aspects
489 of the shy-bold continuum, including spatial and object neophobia (van Oers et al., 2005).

490

491 *HPA function*

492 Our HPA assessments yielded the predicted results (i.e. low BaseCORT, stress-induced
493 increases, decrease after DEX- and increase following ACTH-injection). This is similar to what
494 has been documented in some other species (Romero and Wikelski, 2010; Schmidt et al., 2012;
495 MacDougall-Shackleton et al., 2013; Hau et al., 2015), but differs from the lack of ACTH
496 sensitivity reported in chukar partridge (*Alectoris chukar*; Dickens et al. 2009a). The higher

497 CORT in November compared August suggests a seasonal pattern in DEX sensitivity, with
498 weaker sensitivity in November. There was also a seasonal increase in *ActhCORT*, but this is
499 likely due to the positive correlation between *DexCORT* and *ActhCORT*. Although our birds had
500 recently completed prebasic molt at the time of the August assessment, the enduring
501 physiological consequences of a recent molt or other seasonally variable inputs to the HPA axis
502 might have downregulated axis sensitivity (Romero 2006). Despite this seasonality, all four HPA
503 measures exhibited significant repeatability.

504 Baseline CORT exhibited the lowest repeatability, consistent with previous studies in
505 great tits (Baugh et al., 2014) and other species (Hau et al., 2015; Ouyang et al., 2011). The other
506 three HPA components contained moderate (*StressCORT*) to high (*DexCORT*, *ActhCORT*)
507 amounts of repeatability, consistent with the only other report that has estimated repeatabilities in
508 a subset of these traits (Hau et al., 2015). The repeatabilities of *DexCORT* and *ActhCORT* might
509 be interesting to examine in future functional studies. Variation in negative feedback, for
510 example, has been shown to correlate with nutritional state and mortality in Galapagos marine
511 iguanas (*Amblyrhynchus cristatus*; Romero and Wikelski, 2010).

512 We also demonstrated that there are positive phenotypic correlations between several of
513 these HPA components. Most of the covariance between traits is at the within-individual level. In
514 other words, this suggests that a bird with high baseline CORT at a particular moment will have
515 high stress-induced CORT minutes later; but that same bird a month later might have moderate
516 initial CORT and moderate stress-induced CORT. This hypothetical bird is a relatively high-
517 CORT individual (i.e. there is repeatability in both components), but dynamic variables, not
518 stable individual differences, are driving the correlation between the HPA traits (Baugh et al.,
519 2014). The interdependence of these HPA measures has implications for coping with repeated

520 stressors; for example, a positive trend between *DexCORT* and *ActhCORT* suggests that birds
521 with stronger negative feedback are less able to mount a strong secondary stress response, owing
522 perhaps to a refractory state. To our knowledge, these are the first estimates of combined
523 phenotypic, within- and among-individual covariances in these HPA axis traits. Given trends
524 indicating among-individual correlations between *BaseCORT* and *StressCORT* and between
525 *DexCORT* and *ActhCORT*, we suggest future research examine this question further. However,
526 because *BaseCORT* and *StressCORT* concentrations are likely regulated by separate receptor
527 populations, thereby potentially decoupling them, we would not predict strong correlations
528 between these measures (de Kloet et al., 1993; Romero, 2004).

529

530 *Linking neural receptors, HPA dynamics and behavior*

531 By estimating the expression of MR and GR in two brain regions known to regulate the HPA
532 axis—the PVN and hippocampus—we provide support for the hypothesis that functional aspects
533 of HPA axis dynamics can be predicted on the basis of receptor expression. Overall, we showed
534 that higher MR levels predicted a more potentiated HPA axis (higher CORT) and, more
535 importantly, higher GR levels predicted a less potentiated axis. Previously we showed that the
536 expression of hippocampal MR is positively correlated with the expression of MR in the PVN,
537 and a similar trend was observed for GR across these two nuclei (Senft et al., 2016). This within-
538 individual correlation across nuclei is similar to what has been shown across diverse tissues in
539 songbirds (Lattin et al., 2015) and suggests a neuroendocrine suite that constrains the flexibility
540 of HPA axis function, which might explain individual differences in the HPA components
541 measured in this study. Future research should also quantify receptor protein expression, as
542 transcript and protein levels have been shown to be uncorrelated in house sparrow (*Passer*

543 *domesticus*) brains (Medina et al., 2013), and transcript expression might yield different results.
544 For example, in vivo studies in rodents have demonstrated that GR under-expression, which
545 leads to reduced negative feedback (i.e. more potentiated HPA axis) can be compensated for by
546 MR over-expression (Harris et al., 2013), suggesting that higher MR densities in rodent brains
547 might be associated with increased negative feedback (i.e. less potentiated HPA axis). The
548 classic model that MR strictly controls basal HPA drive whereas GR controls negative feedback
549 is being revisited (Kolber et al., 2009), and there is empirical evidence that it is the ratio of MR
550 and GR that might be critical to understanding HPA axis function (Harris et al., 2013). Lastly,
551 because MR and GR are widely distributed throughout the brain, including in *P. major* (Senft et
552 al., 2016), links between receptor expression and behavior need to be more thoroughly explored.

553 We found support for the hypothesis that variation in the neuroendocrine stress axis is
554 correlated with personality. Specifically, more neophobic and risk-averse birds exhibited a more
555 potentiated stress axis. This finding was confirmed with data reduction methods showing that the
556 three behavioral measures were largely explained by a single principal component, and likewise
557 for the four HPA components. PC1 for the behavioral measures might represent behavioral
558 inhibition under conditions of risk and PC1 for the HPA components might represent endocrine
559 potentiation. These two principal components were significantly and positively correlated: birds
560 exhibiting more risk-averse personalities also exhibited more potentiated HPA axes. It is
561 remarkable that the expression patterns of two receptors in the brain can explain such a large
562 amount of variation in HPA axis function and animal personality. We agree with Ball and
563 Balthazart (2008) that identifying functional interrelationships between behavior and hormones
564 at the individual level, which has occasionally failed (Crews 1998; Adkins-Regan 2005), is
565 greatly facilitated by inclusion of target tissue variables such as neural receptor expression. We

566 would further propose that understanding the behavior and hormonal sides of the equation are
567 greatly enhanced by multilevel approaches, including the characterization of syndromes. Lastly,
568 because recent work has demonstrated links between immune function and risk-taking behavior
569 (Jacques-Hamilton et al. 2017) and because glucocorticoids are known to suppress the immune
570 system, it will be important for future work to integrate among all three of these phenotypic
571 categories in wild animals.

572 These patterns expand on earlier studies in this species; we previously demonstrated a
573 genetic correlation between spatial/object neophobia and glucocorticoid reactivity, with shy
574 selection line birds exhibiting stronger stress responses (Baugh et al., 2012). Likewise, in a study
575 of wild birds we showed that slower explorers exhibited faster and more enduring glucocorticoid
576 responses (Baugh et al., 2013). The present study, which used pharmacological challenges in
577 addition to the more conventional stress series, confirms that the more enduring stress response
578 in more risk-averse personalities is due to weaker negative feedback *per se*. These results are
579 generally consistent with studies of stress and personality in other vertebrates (Baugh et al.,
580 2012). What is still unclear, however, is the directionality and causality of these hormone-
581 behavior relationships (Koolhaas et al., 2010). Further study is needed to examine the
582 developmental-organizational programming of HPA and behavior phenotypes (Schmidt et al.,
583 2013; Marasco et al., 2016) and new techniques are needed to acutely manipulate endocrine
584 function in a physiologically relevant manner.

585

586 CONCLUSIONS

587 Our multilevel and integrative approach—from neural receptor expression to plasma hormone
588 dynamics and behavior—provides new insight into the network of trait (co)variances that are

589 associated with animal personality. Our results support the hypothesis that two hormone
590 receptors in the brain play an integral role in HPA axis function, which is in turn associated with
591 predictable variation in personality. Further, our findings support the hypothesis that pleiotropic
592 effects of steroid hormones can act as proximate mechanisms that integrate behavioral traits into
593 personality suites. Overall, these results unify earlier research documenting the relationship
594 between endocrine stress reactivity and the shy-bold continuum in songbirds. Lastly, if the
595 widespread trait covariances shown here are the consequences of genetic correlations, as has
596 been demonstrated previously for specific HPA-behavior trait pairs (Baugh et al., 2012), this
597 would imply that selection targeting any one of these levels might affect the evolution of suites
598 of concerted traits (Ketterson and Nolan, 1999; McGlothlin and Ketterson, 2008).

599

600 ETHICS

601 Our protocols were approved under permit 35-9185.81/G-10/76 by District Administration
602 Freiburg Department of Agriculture, Rural Areas, Veterinary and Food Administration, Baden-
603 Wuerttemberg, Germany at the Max Planck Institute for Ornithology, Radolfzell, Germany (MH,
604 ATB) and permit NIOO.10-06 of the Royal Dutch Academy of Sciences (DEC-KNAW) to
605 KVO.

606

607 COMPETING INTERESTS

608 We have no competing interests.

609

610 FUNDING

611 This work was supported by the Alexander von Humboldt Foundation grant number 1141248
612 (ATB), the Max Planck Society (MH), The Netherlands Institute of Ecology (NIOO-KNAW;

613 KVO), The Roslin Institute at the University of Edinburgh (SLM), HHMI and the Biology
614 Department at Swarthmore College (ATB, RAS, MF, AL).

615

616 ACKNOWLEDGEMENTS

617 We thank Sarah C. Davidson and Michael Quetting for assistance with data collection in the field
618 and two anonymous reviewers for insightful comments that improved this manuscript.

619

620 REFERENCES

621

622 Adkins-Regan, E., 2005. Hormones and animal social behavior. Princeton University Press,
623 Princeton, NJ.

624

625 Araya-Ajoy, Y.G., Dingemanse, N.J., 2016. Repeatability, heritability, and age-dependence of
626 aggressiveness in a wild passerine bird. *J. Anim. Ecol.* 86, 227–238.

627

628 Ball, G.F., Balthazart, J., 2008. Individual variation and the endocrine regulation of behaviour
629 and physiology in birds: a cellular/molecular perspective. *Philos. Trans. R Soc. Lond. B Biol.*

630 *Sci.* 363, 1699-1710.

631

632 Baugh, A.T., Schaper, S.V., Hau, M., Cockrem, J.F., de Goede, P., van Oers, K., 2012.

633 Corticosterone responses differ between lines of great tits (*Parus major*) selected for divergent
634 personalities. *Gen. Comp. Endocrinol.* 175, 488–494.

635

636 Baugh, A.T., van Oers, K., Naguib, M., Hau, M., 2013. Initial reactivity and magnitude of the
637 acute stress response associated with personality in wild great tits (*Parus major*). Gen. Comp.
638 Endocrinol. 189, 96–104.

639

640 Baugh, A.T., van Oers, K., Dingemanse, N., Hau, M., 2014. Baseline and stress-induced
641 glucocorticoid concentrations are not repeatable but covary within individual great tits (*Parus*
642 *major*). Gen. Comp. Endocrinol. 208, 154–163.

643

644 Bell, A.M., Hankison, S.J., Laskowski, K.L., 2009. The repeatability of behaviour: a meta-
645 analysis. Anim. Behav. 77, 771–783.

646

647 Breuner, C.W., Hahn, T.P., 2003. Integrating stress physiology, environmental change, and
648 behavior in free-living sparrows. Horm. Behav. 43, 115–123.

649

650 Budaev, S.V., 2010. Using principal components and factor analysis in animal behaviour
651 research: caveats and guidelines. Ethology 116, 472–480.

652

653 Carere, C., Drent, P.J., Privitera, L., Koolhaas, J.M., Groothuis, T.G.G., 2005. Personalities in
654 great tits *Parus major*: stability and consistency. Anim. Behav. 70, 795–805.

655

656 Carere, C., Caramaschi, D., Fawcett, T.W., 2010. Covariation between personalities and
657 individual differences in coping with stress: Converging evidence and hypotheses. Curr. Zool.
658 56, 728–740.

659

660 Cherry, J.D., 1982. Fat deposition and length of stopover of migrant white crowned sparrows.
661 Auk 99, 725–732.

662

663 Cockrem, J.F., Silverin, B., 2002. Sight of a predator can stimulate a corticosterone response in
664 the great tit (*Parus major*). Gen. Comp. Endocrinol. 125, 248–255.

665

666 Crews, D. 1998. On the organization of individual difference in sexual behavior. Am. Zool. 38,
667 118–132.

668

669 Dall, S.R.X., Bell, A.M., Bolnick, D.I., Ratnieks, F.L.W., 2012. An evolutionary ecology of
670 individual differences. Ecol. Lett. 15, 1189–1198.

671

672 Dallman, M.R., Strack, A.M., Akana, S.F., Bradbury, M.J., Hanson, E.S., Scribner, K.A., Smith,
673 M., 1993. Feast or famine: critical role of glucocorticoids with insulin in daily energy flow.
674 Front. Neuroendocrinol. 14, 303–347.

675

676 De Kloet, E.R., 1991. Brain corticosteroid receptor balance and homeostatic control. Front.
677 Neuroendocr. 12, 95–164.

678

679 De Kloet, E.R., 1998. Brain corticosteroid receptor balance in health and disease. Endocr. Rev.
680 19, 269–301.

681

682 de Kloet, E.R., Oitzl, M.S., Joëls, M., 1993. Functional implications of brain corticosteroid
683 receptor diversity. *Cell. Mol. Neurobiol.* 13, 433–455.
684

685 Dickens, M.J., Delehanty, D.J., Romero, L.M., 2009a. Stress and translocation: alterations in the
686 stress physiology of translocated birds. *Proc. Roy. Soc. B* 276, 2051–2056.
687

688 Dickens, M., Romero, L.M., Cyr, N.E., Dunn, I.C., Meddle, S.L., 2009b. Chronic stress alters
689 glucocorticoid receptor and mineralocorticoid receptor mRNA expression in the European
690 starling (*Sturnus vulgaris*) brain. *J. Neuroendocrinol.* 21, 832–840.
691

692 Drosman, A.J., Brooks, K.C., Mateo, J.M., 2014. Within-individual correlations reveal link
693 between a behavioral syndrome, condition, and cortisol in free-ranging Beldings ground
694 squirrels. *Ethology* 121, 125–134.
695

696 Droste, S.K., de Groote, L., Atkinson, H.C., Lightman, S.L., Reul, J.M.H.M., Linthorst, A.C.,
697 2011. Corticosterone levels in the brain show a distinct ultradian rhythm but a delayed response
698 to forced swim stress. *Endocrinology* 149, 3244–3253.
699

700 Ellis, B.J., Jackson, J.J., Boyce, W.T., 2006. The stress response systems: universality and
701 adaptive individual differences. *Develop. Rev.* 26, 175–212.
702

703 Funder, J.W., 1997. Glucocorticoid and mineralocorticoid receptors: biology and clinical
704 relevance. *Annu. Rev. Med.* 48, 231–240.

705

706 Gray, J.M., Yarian, D., Ramenofsky, M., 1990. Corticosterone, foraging behavior, and
707 metabolism in dark-eyed juncos, *Junco hyemalis*. *Gen. Comp. Endocrinol.*, 79, 375–384.

708

709 Groothuis, T.G.G., Carere, C., 2005. Avian personalities: characterizations and epigenesis.
710 *Neurosci. Biobehav. Rev.* 29, 137–150.

711

712 Hadfield, J.D., 2010. MCMC methods for multi-response generalized linear mixed models: The
713 MCMCglmm R package. *J. Stat. Softw.* 33, 1–22.

714

715 Hall, M.L., van Asten, T., Katsis, A.C., Dingemanse, N.J., Magrath, M.J.L, Mulder, R.A., 2015.
716 Animal personality and pace-of-life syndromes: do fast-exploring fairy wrens die young? *Front.*
717 *Ecol .Evol.* 25. doi.org/10.3389/fevo.2015.00028

718

719 Harris, A.P., Holmes, M.C., de Kloet, E.R., Chapman, K.E., Seckl, J.R., 2013. Mineralocorticoid
720 and glucocorticoid receptor balance in control of HPA axis and behaviour.
721 *Psychoneuroendocrinol.* 38, 648–658.

722

723 Hasselgren, P.O., 1999. Glucocorticoids and muscle catabolism. *Curr. Opin. Clin. Nutr. Metab.*
724 *Care* 2, 201–205.

725

726 Hau, M., Haussmann, M.F., Greives, T.J., Matlack, C., Costantini, D., Quetting, M., Adelman,
727 J.S., Miranda, A.C., Partecke, J., 2015. Repeated stressors in adulthood increase the rate of
728 biological ageing. *Front. Zool.* 12, 4.
729
730 Hau, M., Casagrande, S., Ouyang, J.Q., Baugh, A.T., 2016. Glucocorticoid-mediated phenotypes
731 in vertebrates: multilevel variation and evolution. In M. Naguib, J.C. Mitani, L.W. Simmons, L.
732 Barrett, S. Healy, M. Zuk (Eds.), *Adv. Study Behav.* 48, 41–115.
733
734 Ketterson, E.D., Nolan, Jr., V., 1999. Adaptation, exaptation, and constraint: a hormonal
735 perspective. *Am. Nat.* 154, S4–S25.
736
737 Kolber, B.J., Wieczorek, L., Muglia, L.J., 2009. HPA axis dysregulation and behavioral analysis
738 of mouse mutants with altered GR or MR function. *Stress* 11, 321–338.
739
740 Koolhaas, J.M., Korte, S.M., De Boer, S.F., van Der Vegt, B.J., van Reenen, C.G., Hopster, H.,
741 De Jong, I.C., Ruis, M.A.W., Blokhuis, H.J., 1999. Coping styles in animals: current status in
742 behavior and stress-physiology. *Neurosci. Biobehav. Rev.* 23, 925–935.
743
744 Koolhaas, J.M., de Boer, S.F., Buwalda, B., van Reenen, K., 2007. Individual variation in coping
745 with stress: a multidimensional approach of ultimate and proximate mechanisms. *Brain Behav.*
746 *Evol.* 70, 218–226.
747

748 Koolhaas, J.M., de Boer, S.F., Coppens, C.M., Buwalda, B., 2010. Neuroendocrinology of
749 coping styles: towards understanding the biology of individual variation. *Front.*
750 *Neuroendocrinol.* 31, 307–321.

751

752 Koolhaas, J.M., Bartolomucci, A., Buwalda, B., de Boer, S.F., Flugge, G., Korte, S.M., Meerlo,
753 P., Murison, R., Olivier, B., Palanza, P., Richter-Levin, G., Sgoifo, A., Steimer, T., Stiedl, O.,
754 van Dijk, G., Wöhr, M., Fuchs, E., 2011. Stress revisited: a critical evaluation of the stress
755 concept. *Neurosci. Biobehav. Rev.* 35, 1291–1301.

756

757 Landys, M.M., Ramenofsky, M., Wingfield, J.C., 2006. Actions of glucocorticoids at a seasonal
758 baseline as compared to stress-related levels in the regulation of periodic life processes. *Gen.*
759 *Comp. Endocrinol.* 148, 132–149.

760

761 Lattin, C.R., Keniston, D.E., Reed, J.M., Romero, L.M., 2015. Are receptor concentrations
762 correlated across tissues within individuals? A case study examining glucocorticoid and
763 mineralocorticoid receptor binding. *Endocrinology* 156, 1354–1361.

764

765 Lendvai, A.Z., Bókony, V., Chastel, O., 2011. Coping with novelty and stress in free-living
766 sparrows. *J. Exp. Biol.* 214, 821–828.

767

768 Lessells, C.M., Boag, P.T., 1987. Unrepeatable repeatabilities: a common mistake. *Auk* 104,
769 116–121.

770

771 MacDougall-Shackleton, S.A., Schmidt, K.L., Furlonger, A.A., MacDougall-Shackleton, E.A.,
772 2013. HPA axis regulation, survival, and reproduction in free-living sparrows: Functional
773 relationships or developmental correlations? *Gen. Comp. Endocrinol.* 190, 188–193.
774

775 Marasco, V., Herzyk, P., Robinson, J., Spencer, K.A., 2016. Pre- and post-natal stress
776 programming: developmental exposure to glucocorticoids causes long-term brain-region specific
777 changes to transcriptome in the precocial Japanese quail. *J. Neuroendocrinol.* 28,
778 doi:10.1111/jne.12387.
779

780 McGlothlin, J.W., Ketterson, E.D., 2008. Hormone-mediated suites as adaptations and
781 evolutionary constraints. *Philos. Trans. R. Soc. Lond. B* 363, 1611–1620.
782

783 Medina, C.O., Lattin, C.R., McVey, M., Romero, L.M., 2013. There is no correlation between
784 glucocorticoid receptor mRNA expression and protein binding in the brains of house sparrows
785 (*Passer domesticus*). *Gen Comp Endocrinol.* 193, 27–36.
786

787 Nakagawa, S., Schielzeth, H., 2010. Repeatability for Gaussian and non-Gaussian data: a
788 practical guide for biologists. *Biol. Rev.* 85, 935–956.
789

790 Nelson, R.J., 2005. *An introduction to behavioral endocrinology*. 4th ed. Sunderland: Sinauer
791 Associates.
792

793 Oakley, R.H., Cidlowski, J.A., 2013. The biology of the glucocorticoid receptor: new signaling
794 mechanisms in health and disease. *J. Allergy Clin. Immunol.* 132, 1033–1044.
795

796 Ouyang, J.Q., Hau, M., Bonier, F., 2011. Within seasons and among years: When are
797 corticosterone levels repeatable? *Horm. Behav.* 60, 559–564.
798

799 Øverli, Ø., Sorensen, C., Pulman, K.G.T., Pottinger, T.G., Korzan, W.J., Summers, C.H.,
800 Nilsson, G.E., 2007. Evolutionary background for stress-coping styles: relationships between
801 physiological, behavioral, and cognitive traits in non-mammalian vertebrates. *Neurosci.*
802 *Biobehav. Rev.* 31, 396–412.
803

804 Pieg, J., Green, A.J., 2009. New perspectives for estimating body condition from mass/length
805 data: the scaled mass index as an alternative method. *Oikos* 118, 1883–1891.
806

807 Réale, D., Reader, S.M., Sol, D., McDougall, P.T., Dingemanse, N.J., 2007. Integrating animal
808 temperament within ecology and evolution. *Biol. Rev.* 82, 291–318.
809

810 Romero, L.M., 2004. Physiological stress in ecology: lessons from biomedical research. *Trends*
811 *Ecol. Evol.* 19, 249–255.
812

813 Romero, L.M., 2006. Seasonal changes in hypothalamic-pituitary-adrenal axis sensitivity in free-
814 living house sparrows (*Passer domesticus*). *Gen. Comp. Endocrinol.* 149, 66–71.
815

816 Romero, L.M., Reed, J.M., 2005. Collecting baseline corticosterone samples in the field: is under
817 3 min good enough? *Comp. Biochem. Physiol.* 140, 73–79.
818

819 Romero, L.M., Wikelski, M., 2010. Stress physiology as a predictor of survival in Galapagos
820 marine iguanas. *Proc. Roy. Soc. B* 277, 3157–3162.
821

822 Ronchi, E., Spencer, R.L., Krey, L.C., McEwen, B.S., 1998. Effects of photoperiod on brain
823 corticosteroid receptors and the stress response in the golden hamster (*Mesocricetus auratus*).
824 *Brain Res.* 780, 348–351.
825

826 Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids influence stress
827 responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr.*
828 *Rev.* 21, 55-89.
829

830 Schmidt K.L., Furlonger, A.A., Lapierre, J.M., MacDougall-Shackleton, E.A., MacDougall-
831 Shackleton, S.A., 2012. Regulation of the HPA axis is related to song complexity and measures
832 of phenotypic quality in song sparrows. *Horm. Behav.* 61, 652–659.
833

834 Schmidt, K.L., Moore, S.D., MacDougall-Shackleton, S.A., MacDougall-Shackleton, E.A.,
835 2013. Early-life stress affects song complexity, song learning and volume of the brain nucleus
836 RA in adult male song sparrows. *Anim. Behav.* 86, 25–35.
837

838 Senft, R.A., Meddle, S.L., Baugh, A.T., 2016. Distribution and abundance of glucocorticoid and
839 mineralocorticoid receptors throughout the brain of the great tit (*Parus major*). PLoS ONE 11,
840 e0148516.

841

842 Spiegelhalter, D.J., Best, N.G., Carlin, B.P., van der Linde, A., 2002. Bayesian measures of
843 model complexity and fit. J. Roy. Statist. Soc. B 64, 583–639.

844

845 Sokal, R.R., Rohlf, F.J., 1995. Biometry: the principles and practice of statistics in biological
846 research. San Francisco.: W.H. Freeman.

847

848 Stöwe, M, Rosivall, B., Drent, P.J., Möstl, E., 2010. Selection for fast and slow exploration
849 affects baseline and stress-induced corticosterone excretion in great tit nestlings *Parus major*.
850 Horm. Behav. 58, 864–871.

851

852 Van Oers, K., Drent, P.J., de Jong, G., van Noordwijk, A.J., 2004a. Additive and nonadditive
853 genetic variation in avian personality traits. Heredity 93, 496–503.

854

855 Van Oers, K., Drent, P.J., De Goede, P., Van Noordwijk, A.J., 2004b. Realized heritability and
856 repeatability of risk-taking behaviour in relation to avian personalities. Proc. Roy. Soc. B 271,
857 65–73.

858

859 Van Oers, K., de Jong, G., Drent, P.J., Van Noordwijk, A.J., 2004c. A genetic analysis of avian
860 personality traits: correlated, response to artificial selection. Behav. Genet. 34, 611–619.

861

862 Van Oers, K., Naguib, M., 2013. Avian personality, in: Carere, C., Maestriperi, D.
863 (Eds.), *Animal Personalities: Behavior, Physiology, and Evolution*. University of Chicago Press,
864 Chicago, pp. 66–95.

865

866 Van Oers, K., Klunder, M., Drent, P., 2005. Context dependence of personalities: risk-taking
867 behavior in a social and a nonsocial situation. *Behav. Ecol.* 16, 716–723.

868

869 Williams, T.D., 2008. Individual variation in endocrine systems: moving beyond the ‘tyranny of
870 the Golden Mean’. *Phil. Trans. Roy. Soc.* 363, 1687–1698.

871

872 Wingfield, J.C., Suydam, R., Hunt, K., 1994. The adrenocortical responses to stress in snow
873 buntings (*Plectrophenax nivalis*) and Lapland longspurs (*Calcarius lapponicus*) at Barrow,
874 Alaska. *Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol.* 108, 299–306.

875

876

877 **Figure Legends**

878

879 **Figure 1.** Experimental timeline. Birds were tested on a risk-taking assay on three occasions
880 (RTA₁₋₃) with 6-day intervals. Following a 9-day recovery period, they were sampled using a
881 four component HPA assessment (A-D), once in August (HPA₁) and again in November (HPA₂)
882 with a 52 day interval separating these two assessments. Following a 14-day recovery period,
883 brains and trunk blood were harvested.

884

885 **Figure 2.** Relationships among traits depicted in a path model. For each level of organization
886 (neuroendocrine: MR and GR expression; endocrine: HPA₁ components; behavior: Initial and
887 Startle Latencies), traits were reduced to the first principal component (PC1). For traits measured
888 repeatedly per bird, agreement repeatabilities (R; subject as random factor) are indicated inside
889 circular arrows. Estimates of covariance between traits within each category are indicated with
890 bi-directional black arrows at the among- (Cov-A) and the within-individual levels (Cov-W).
891 The phenotypic correlations (Pearson's r) for MR and GR across the two nuclei are indicated
892 with bi-directional grey arrows. The path model yields beta coefficients (β) describing the
893 direction and magnitude of effect of independent variables (GR, MR, HPA) on dependent
894 variables (HPA, Risk-taking). Error estimates for dependent variables in the path model are
895 indicated in grey ellipses. The model indicates that higher MR expression in the hippocampus and
896 hypothalamus and lower GR expression in these two brain areas predicts a more potentiated
897 HPA axis (higher CORT) and more risk-averse personalities (higher latencies). Inset: the
898 reduced model has two dropped paths. * denotes statistical significance ($p < 0.05$) for repeatability
899 (R), covariance (Cov) and correlation (r) estimates.

900

901 **Figure 3.** Graphical representations of the among-individual correlations for the three
902 behavioural traits (a,b) and four HPA components (c,d). All values are \log_{10} -transformed and
903 plotted as standardized (z) scores and best fit lines are linear regressions. Plots show the
904 correlation between the average (per bird) values of the three behavioral traits (a,b) and two HPA
905 assessments (c,d). A statistically significant positive correlation in (a) is graphical evidence of
906 among-individual correlation (i.e. syndrome) between Initial Latency and Startle Latency (i.e.
907 consistently neophilic birds are also consistently more risk-taking). The other trait pairs (b-d)

908 were not statistically significant but positive trends here suggest the possibility of among-
909 individual correlations.