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Glucocorticoid receptor expression in blood, but not across brain regions, reveals long-term effects of early life adversity in zebra finches

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ABSTRACT

Early-life environment can affect organisms for life on many levels. The glucocorticoid receptor (GR) gene has a pivotal role mediating organismal physiological and behavioral responses to environmental change, and is sensitive to early-life environmental conditions and epigenetic programming. Longitudinal studies require non-lethal sampling of peripheral tissues (e.g. blood), but this approach is dependent on the extent to which GR expression in peripheral tissues covaries with GR expression in central tissues. To test for the long-term effects of early life adversity on GR expression across brain and peripheral tissues, we manipulated developmental conditions of captive zebra finches ($n = 45$), rearing them in either benign or harsh conditions through manipulation of parental foraging costs. We measured relative GR mRNA expression in blood and five brain regions in adulthood: hippocampus, hypothalamus, amygdala, ventral striatum, and the nidopallium caudolaterale (analogous to the mammalian prefrontal cortex), using qPCR. We further tested whether GR expression was modulated by natal brood size (which affected growth), age at sampling, and sex. GR expression correlations among tissues varied widely in magnitude and direction, ranging from -0.27 to +0.80, indicating that our understanding of developmental effects on GR expression and associated phenotypes needs to be region specific rather than organism wide. A more consistent pattern was that GR expression increased with age in blood, ventral striatum and hippocampus; GR expression was independent of age in other tissues. Developmental treatment did not affect GR expression in any of the tissues measured directly, but in blood and ventral striatum of adult females we found a positive correlation between nestling mass and GR expression. Thus, GR expression in blood was affected by early life conditions as reflected in growth in adult females, a pattern also found in one brain tissue, but not ubiquitous across brain regions. These results point at sex-dependent physiological constraints during development, shaping early life effects on GR expression in females only. Further study is required to investigate whether these tissue-dependent effects more generally reflect tissue-dependent long-term effects of early life adversity. This, together with investigating the physiological consequences of GR expression levels on individual performance and coping abilities, will be fundamental towards understanding the mechanisms mediating long-term impacts of early life, and the extent to which these can be quantified through non-lethal sampling.

1. Introduction

Environmental conditions experienced during development can induce long-term phenotypic changes [1-4]. In particular, early life adversity can shape organisms for life, affecting many traits through changes in physiological systems, and determining organismal performance and the ability to overcome environmental challenges [5,6]. This 'developmental phenotypic plasticity' is defined as irreversible changes in the phenotype resulting from environmentally-induced alterations in development [7], as early life effects tend to be more permanent compared to environmental effects later in life [8]. One way in which environmental variation can lead to changes in the phenotype is via epigenetic mechanisms, which alter gene expression by affecting either transcription or translation without changes in the primary nucleotide sequence of the genome [9]. These mechanisms are triggered by a variety of internal and external factors across taxa [10-14], and have been suggested as key mediators of long-term developmental effects on offspring phenotype [15,16].

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The glucocorticoid receptor has a pivotal role mediating organismal coping, including physiological and behavioral responses to environmental change, and is sensitive to early-life environmental conditions and epigenetic programming [5,14,17,18]. Glucocorticoids (GCs, i.e. cortisol, corticosterone) are steroid hormones produced by the hypothalamus-pituitary-adrenal (HPA) axis, and facilitate behavioral and physiological processes that are instrumental for environmental coping, in particular when it affects energy expenditure [19-21]. GC actions are mediated by binding to two intracellular GC receptors: the mineralocorticoid (MR) and the glucocorticoid (GR) receptor. MR has higher affinity for GCs and mainly regulates traits associated with metabolism, foraging and activity-rest cycles, whereas GR mediates most phenotypic changes associated with daily peaks and acute (e.g. 'stress-induced') increases in GC levels, and the negative feedback to terminate them [22-27]. Recent evidence further suggests MR and MR/GR ratio to also play a role in mediating negative feedback dynamics, acute GC responses and tissue sensitivity to GCs [25,28,29,30,31], increasing the complexity of the system. GR is expressed in most tissues and cell types, including bird nucleated blood cells [27,32,33]. Because receptors bind to the hormone and induce the cell to respond in a target-specific manner, receptor number is highly correlated with the magnitude of the GC-mediated responses and downstream effects [34]. Furthermore, variation in GR expression levels attributed to early life conditions across taxa may lead to lifelong consequences in organismal physiology and behavior [5,14,17]. For instance, epigenetically-induced reduction in GR expression in mice following maternal care deprivation has been related to disruption of the homeostatic mechanisms that regulate the activity of the HPA axis [5,14]. In fact, many studies in humans and lab rodents point at individuals suffering early life adversity typically showing lower number of GRs associated with attenuated coping capacity [5,17]. These associations, however, have barely been explored in other taxa and in an ecological context [35-37]. GR in mammals and birds is predominately expressed in certain brain areas, such as the hypothalamus, amygdala or hippocampus [25,38–42]. The relative abundance of GR across brain regions such as the hippocampus, however, seems to differ among species, at least in birds [40,43]. GR expression includes brain regions influencing HPA activity (e.g. hypothalamus) such that the binding of GCs can inhibit HPA-activity (i.e., negative feedback) regardless of the continued presence of a stressor or stimulus [25,38,41,43,44].

Longitudinal studies allow for detection of trait changes over time within individuals, providing further insights into causes and consequences of variation and trait plasticity compared to cross-sectional studies, but require multiple samples from the same individuals through time. Since it is often not feasible to repeatedly sample tissues of biological relevance from the same individual, most studies investigating the associations between environmental variation, GR expression and phenotypic traits are cross-sectional. As an alternative to destructive sampling, assessment of GR expression in peripheral tissues (e.g. blood, saliva) is widely used in humans and lab rodents, and has been encouraged by some studies showing that expression of some genes is correlated between peripheral and central regions [45,46]. This is supported by additional evidence pointing at GR expression in bird blood (which has nucleated red blood cells, making them a potential target for assessing changes in gene expression) being sensitive to environmental changes, including early life [36,47] and prenatal period [48], with notable consequences for chick phenotype. In an ecological context, where sample sizes are often limited and there is a large amount of uncontrolled environmental variation, it becomes especially relevant to investigate whether (early life) environmental changes affect GR expression in different tissues in a comparable way, and in particular when comparing central and peripheral tissues. This question becomes fundamental towards the use of peripheral samples as non-terminal method to infer changes in gene expression in relevant brain regions and at an organismal level, and obtain information on how environmental factors affect HPA regulation in longitudinal experiments. Thus, investigating the use of non-lethal samples to quantify changes in GR expression in central tissues becomes a key step towards further studying the mechanisms driving early life adversity's effects, and their plasticity.

In this cross-sectional study, we manipulated early life environment of captive zebra finches (*Taeniopygia guttata*) living in semi-natural conditions, by rearing them in either benign or harsh environment via experimental manipulation of parental foraging costs [49]. In altricial birds like zebra finches, a fundamental part of the early developmental conditions is determined by the parents, because nestlings completely rely on their parents for nutrition, warmth and protection. In adulthood, these offspring were sacrificed to measure GR expression in blood and five brain regions, including regions previously showed to be involved in HPA regulation in mammals (hypothalamus, amygdala, hippocampus, and nidopallium caudolaterale – analogous to the mammalian prefrontal cortex; [50,51]) and early life effects (amygdala, ventral striatum – also hippocampus -). We tested for the effects of developmental treatment on GR expression across tissues, to investigate whether harsher conditions during early life led to reduced GR expression in this species. We also investigated whether such effects were shaped by factors like brood size, growth (as early life determinants of body condition in our population; [52]), age at sampling, and sex. We further tested for correlations among GR expression across tissues, and compared the magnitude of the change in GR expression that developmental treatment caused across tissues.

2. Methods

2.1. Birds, housing and manipulation of early life environment

We investigated the gene expression of 45 zebra finches (25 females, 20 males), born in an experimental population [49] that consisted of a manipulation of the foraging environment during the breeding stages. Birds bred in four outdoor aviaries (310 × 210 × 150 cm), where each aviary held 18 or 19 adult individuals, with a sex ratio of 1:1. Two aviaries had high foraging costs, and two had low foraging costs. We manipulated foraging costs as described in Koetsier & Verhulst [53], by providing each aviary with a single food box (120 × 10 × 60 cm), suspended from the ceiling, which was filled with commercial tropical seeds. This food box had ten holes in total (i.e. five on each side), from which the birds could access the food. In the low foraging cost environment, there was a perch attached to each hole. In the high foraging cost environment, these perches were absent, forcing the bird to fly back and forth to the hole to obtain food. We previously showed that increasing foraging costs doubled the time spent foraging [53].

Each of the four aviaries was equipped with fourteen nest-boxes and nest material ad libitum from April to November each year (2018–2019) to facilitate breeding. Offspring mass and biometry (i.e. tarsus, wing, head-bill length) were measured at days 15 and 30 (details in [49]). Once offspring reached 31 ± 1 days, they were moved from their parental aviary to an equal-sized aviary with ad libitum food in a silo on the floor where four adults, two of each sex, were present for sexual imprinting. Once offspring reached 65 ± 4 days, we could identify their sex and subsequently moved them to single-sex aviaries, again with two adults of each sex. Once offspring reached the age of 100 days, they were housed in single-sex indoor aviaries, without adults present. The harsh foraging treatment negatively affected offspring growth (i.e. body mass and size at day 15, [49]). We therefore consider this experimental treatment to impose early life adversity on chicks. Specifically, at the age of 15 days, offspring growing up under harsh foraging conditions had lower mass and structural size. These differences were attenuated by the age of 30 days, when offspring reared in harsh foraging environment still had significantly lower mass, but effects on structural size had weakened [49].

2.2. Blood and brain samples

We sampled all individuals that survived until the sampling date, and therefore there was no other selection method of the individuals included in the study. This led to some nests having more than one individual sampled. Specifically, the 45 birds included in the study came from 32 nests. We euthanized each individual by decapitation conform Annex IV of European Directive 2010/63/EU. Birds included in the study were sampled on five different dates, between July 2019 and February 2021. Sampling dates were constrained by another study on the same individuals [54] that in turn was delayed by the Covid-19 pandemic. Within each batch, there was natural variation in birth date, causing additional variation in age at sampling, but we performed no sampling selection based on any bird trait (i.e. sex, body mass), as birds were captured in a previously determined, randomized order. Adult birds were euthanized in rapid succession, and blood was subsequently collected from the carotid artery with heparinized capillaries and was directly put into Eppendorf tubes with TRIzol (Sigma-Aldrich #T3809) to facilitate RNA extraction. The brain was isolated from the rest of the head and immediately gradually frozen in aluminum foil on a copper block in presence of liquid nitrogen. Both blood and brain samples were stored at −80 °C until RNA isolation.

RNA isolation was performed on multiple brain regions. To this end, each brain was dissected on a cooled Petri dish underneath a (LED) lighted binocular. Hemispheres were split sagittally with a razor blade. Regions were then isolated in a fixed manner, and for both hemispheres simultaneously, in the following order: 1) hippocampus (HP), 2) (ventral) striatum (VS), 3) hypothalamus (HYP), 4) nidopallium caudolaterale (NCL) – analogous to the mammalian prefrontal cortex [50, 51] and 5) amygdala (AMY). The locations of these brains regions were based on the Zebra finch Expression Brain Atlas (ZEBrA) [55]. The isolated brain regions were directly stored in 100 μL TRI Reagent (Invitrogen #15596018) and ready for subsequent RNA isolation.

2.3. RNA isolation and cDNA conversion

2.3.1. Brain

RNA was isolated following suppliers manual (Invitrogen TRIzol Reagent User Guide MAN0001271) with minor changes in volumes (scaled down) and centrifugation temperatures (2 °C instead of 4 °C). In brief, all solid tissue was manually homogenized with a disposable pestle and 900ul TRIzol added. To facilitate the separation of RNA from DNA and proteins, after 5 min of incubation, 200ul Chloroform and 400ul Isopropanol were added. After 10 min of incubation, layers were formed by centrifuging at 12.000 g at 2 °C for 10 min. The RNA (upper)layer was transferred to a new tube and precipitated with 75% Ethanol, centrifuged, washed twice and dissolved in 12 ul RNAse-free water. Samples were immediately checked on Nanodrop for concentration and contamination.

Isolated RNA of brain tissue was converted to cDNA with a conversion kit (Thermoscientific RevertAid H minus First strand cDNA Synthesis Kit #K1632). Conversion to cDNA was performed following suppliers manual in a normal PCR cycler. After conversion, cDNA was diluted 1:50 and stored at −20 °C until analysis (within weeks).

2.3.2. Blood

Immediately after collection, 75ul of whole blood was stored in 300 μL TRI reagent BD (Sigma-Aldrich #T3809) at −80 °C and thawed prior to RNA isolation following manufacturer's protocols. Isolated RNA of blood was converted to cDNA with a different conversion kit (SuperScript III Reverse Transcriptase-kit Fisher #18,080–044). Following supplier's manual, Random Primers (Fisher #N8080127) and RNAse OUT (Fisher #10,777–019) were used in the protocol. Conversion was performed in a PCR cycler. Following the conversion, the samples were centrifuged briefly and stored at −20 °C.

2.4. Gene expression analyses

Quantitative real-time PCR was carried out on cDNA using previously published zebra finch gene-specific primer pairs for the GR mRNA (Forward: 5′ TGA AGA GCC AGT CCC TGT TCG AG, Reverse: 5′ CAA CCA CAT GCA TAG AGT CCA GCA; [35]) and the reference gene ß-actin (Forward: 5′ CTG GCA CCA CTC CTT CTA CA, Reverse: 5′ ATA CAT GGC TGG GGT GTT GA; [56]). Quantitative PCR was performed on a Bio-Rad CFX96 using 96-well PCR plates (Bio-Rad #HSP9601). cDNA samples were diluted 1:50 (18μL cDNA and 982μL MilliQ). The mastermix for the qPCR contained per reaction: 12,5μL Bio-Rad SsoAdvanced Universal SYBR® Green Supermix, 6μL MilliQ and 0,25μL forward & reverse primer (100 nM in reaction). Reaction volume was 25 μL (6μL cDNA, 19μL master mix), and each sample was included in triplicate on the plate. On each plate there was also a golden sample (pool of cDNA of multiple individuals), a negative template control (NTC) from the RNA isolation, and a negative control (NC), a sample including only master mix. Reference gene was measured for each tissue in each bird, whereas the golden sample was a pool of multiple tissues from multiple birds. Samples were randomized across plates.

The ratio of gene expression (i.e. relative GR expression) was calculated as follows [57]:

$$Ratio = \frac{(E_{target})\Delta CP_{target}^{(control-sample)}}{(E_{ref})\Delta CP_{ref}^{(control-sample)}}$$

where E refers to the qPCR efficiency. The target gene was GR gene (i.e. NR3c1), and the reference gene was ß-actin. ΔCP refers to the difference in quantification cycles (CQ) between the golden sample and the gene of interest.

2.5. Statistical analyses

To test for the dependency of relative GR expression among the six tissues sampled, we ran a principal component analysis (PCA) using the individuals for which GR expression in all tissues was obtained ($N = 42$). This analysis integrates all correlations in one overall estimate (Table S1). The first principle component explained 39% of variance, which we did not consider sufficiently informative to use this instead of the raw GR expression data. Pearson correlations were also obtained to test for the magnitude and direction of specific correlations, with and without adjustment for age effects (Table S2).

We ran a general linear mixed model using the lme4 package in R [58] to test for the effects on GR expression (i.e. ratio of gene expression, hereby 'GR expression') across tissues, i.e. including all tissues simultaneously. Developmental treatment (harsh vs. benign), sex and tissue were included as categorical factors; age at sampling and brood size were included as covariates, and individual identity was included as random intercept to account for the non-independence of different tissue measurements within individuals. We also included two-way interactions containing developmental treatment to explore whether the effect of early life adversity depended on sex, brood size, tissue or age at sampling, and the three-way interaction containing developmental treatment, sex and tissue to test for a sex-dependent effect of early life environment across tissues. The latter interaction was included because earlier studies reported sex dependent effects of developmental conditions [59–61] and such effect may be tissue dependent. To enable comparison of treatment effects between tissues we calculated standardized mean-difference effect sizes (Cohen's D) of the developmental treatment effect (harsh vs. benign) on GR expression for each tissue.

The developmental treatment impaired growth [49], and we therefore ran an additional general linear mixed model replacing developmental treatment with nestling body mass at day 15, along with the variables included in the model described above. Because the three-way interaction between tissue, sex and nestling body mass was close to significance, we ran follow-up models per tissue to facilitate the

interpretation of results, including the variables as previously, and the two-way interactions containing nestling mass. We also ran equivalent models in VS and Blood to test for the effect of individual mass in adulthood (i.e. day 100), instead of nestling mass, on GR expression to investigate whether the association between nestling mass and GR expression was due to physical condition only – i.e. independently on early life conditions (see results section). We are aware that this procedure entails multiple tests, increasing the probability of making Type I errors. We limited this risk by sequential testing from higher order to lower order effects, where low order effects are tested post-hoc contingent on the statistical significance of the higher order effects. Applying multiple comparison corrections to the post-hoc tests would not be appropriate because they are guided by significant findings.

All statistical analyses were performed using R version 4.0.1 [62]. PCA analyses were performed using the prcomp function. We used Type III sum of squares with the Anova function in the car package [63]. GR expression values were logarithmically (ln) transformed. To facilitate the interpretation of the model estimates, all variables included were mean-centered, except for Tissue, because it is a factor with more than two levels. Aviary ID and Nest ID explained a very small proportion of variance in GR expression (<1%) and therefore were not included in the models. All models fitted assumptions of normality of residuals, normality of random effects, multicollinearity and homogeneity of variance (*check_model* function in R package *performance* package, [64]).

3. Results

Correlations of GR expression across tissues were weak in general and highly variable, with their magnitude and direction differing strongly across tissues (r=−0.27–0.80; Tables S1 and S2, Fig. 1). GR expression in the amygdala was significantly and positively correlated with GR expression in the nidopallium caudolaterale ($r = 0.52$, $p < 0.001$) and the hypothalamus ($r = 0.41$, $p = 0.01$), and showed a non-significant trend for a negative correlation with GR expression in the ventral striatum ($r = -0.27$, $p = 0.09$). GR expression levels in the ventral striatum were strongly correlated with GR expression levels in the hippocampus ($r = 0.80$, $p < 0.001$; Table S2). Among these correlations, however, only the one existing between the ventral striatum and the hippocampus held when controlling for age effects on GR expression ($r = 0.66$, $p<0.001$; Table S2).

GR expression differed among tissues (Table 1), being significantly higher in blood and ventral striatum (Tukey contrast for multiple comparisons of means; $p<0.001$; Fig. 2). Developmental treatment did not significantly affect GR expression in adulthood, alone or in interaction with sex, age, brood size or tissue (Table 1). We calculated the effect sizes (Cohen's D, [65]) of the developmental treatment effect (harsh vs. benign) by tissue, to visualize whether the effect of developmental treatment on GR expression was comparable among tissues (Fig. 3). None of these effect sizes significantly differed from zero, with positive values in Amygdala, Blood and Nidopallium Caudolaterale (i.e. higher GR expression when reared in harsh conditions), and negative values in Hippocampus, Hypothalamus and Ventral Striatum (i.e. lower GR expression when reared in harsh conditions). GR expression increased with age (Table 1, Fig. 4). Data visualization (Fig. 4) suggested that the magnitude of this effect differed among tissues. Follow-up models revealed this interaction was caused by GR expression in VS (χ^2_1=25.86, $p<0.001$), blood (χ^2_1=12.14, $p<0.001$) and HP (χ^2_1=14.65, $p<0.001$) being strongly associated with age, while GR expression in Nidopallium Caudolaterale, Amygdala and Hypothalamus were independent of age (all $p > 0.15$).

Including nestling mass at day 15 as predictor of GR expression instead of developmental treatment revealed the three-way interaction between sex, tissue and Mass15 - testing whether the tissue-dependent association between Mass15 and GR statistically differed between sexes - was close to significance ($F_{5,\ 198.38}$= 2.02, $p = 0.07$; Table S3). We considered support of $p = 0.07$ for this three-way-interaction (for which statistical power was low) sufficiently strong to warrant follow-up analyses. Follow-up models revealed an interaction between nestling mass and sex in both blood and VS (Table 2), but not in other tissues (all $P>0.1$, Table S4). This sex-dependent effect was due to a positive association between nestling mass and GR expression in these two tissues only in females, but not in males (Tissue x Mass15 interaction: $F_{5,\ 109.78}$= 3.12, $p = 0.01$ in females; $F_{5,\ 88.88}$= 0.09, $p = 0.99$ in males; Fig. 5). None of the previous effects was present if including body mass in adulthood (Table S5) or compensatory growth (Table S6) instead of nestling mass in the models.

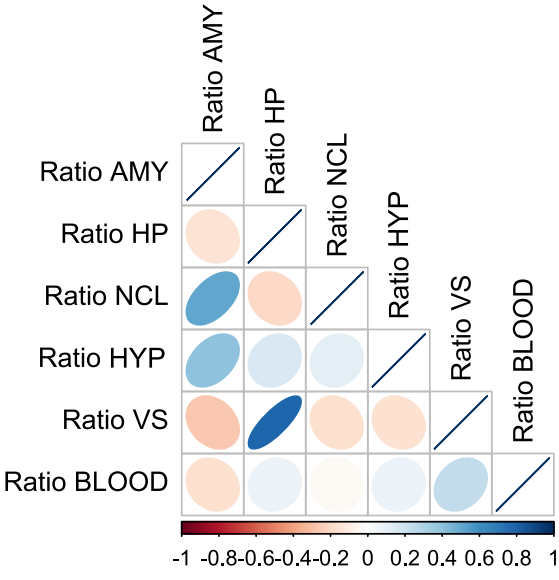

Fig. 1. Pearson correlations among tissue-specific relative GR expressions in all birds pooled ($N = 42$). AMY: amygdala, HP: Hippocampus, NCL: Nidopallium Caudolaterale, HYP: Hypothalamus, VS: Ventral striatum.

Table 1
Main model testing the effects of benign vs. harsh developmental treatment (i.e. low vs. high parental foraging costs) on relative GR expression. AMY: amygdala, HP: Hippocampus, NCL: Nidopallium Caudolaterale, HYP: Hypothalamus, VS: Ventral striatum. Note that all variables except Tissue were mean-centered for the analysis.

	Estimate	s.e.	d.f.	F	P
Intercept	−0.681	0.134			
Sex	−0.127	0.275	1, 232.61	0.213	0.644
Dev. Treatment	−0.054	0.270	1, 37.980	0.221	0.649
Age	0.002	0.000	1, 37.628	14.38	**<0.001**
Brood size	0.021	0.074	1, 38.49	0.078	0.780
Tissue	–	–	5, 202.60	20.24	**<0.001**
Blood	1.284	0.186			
HP	0.602	0.187			
HYP	0.214	0.189			
NCL	0.431	0.186			
VS	1.524	0.186			
Dev. Treat: Age	0.000	0.001	1, 38.017	0.049	0.823
Dev. Treat.: Sex	−0.050	0.261	1, 37.695	0.000	0.988
Dev. Treat.: Brood size	−0.032	0.153	1, 39.027	0.047	0.828
Dev. Treat.: Tissue	–	–	5, 202.754	0.309	0.902
Sex: Tissue	–	–	5, 202.623	0.475	0.784
Tissue: Sex: Dev. Treat.	–	–	5, 202.805	0.330	0.895
Random effects			Variance		s.d.
BirdID			0.022		0.148
Residual			0.797		0.893

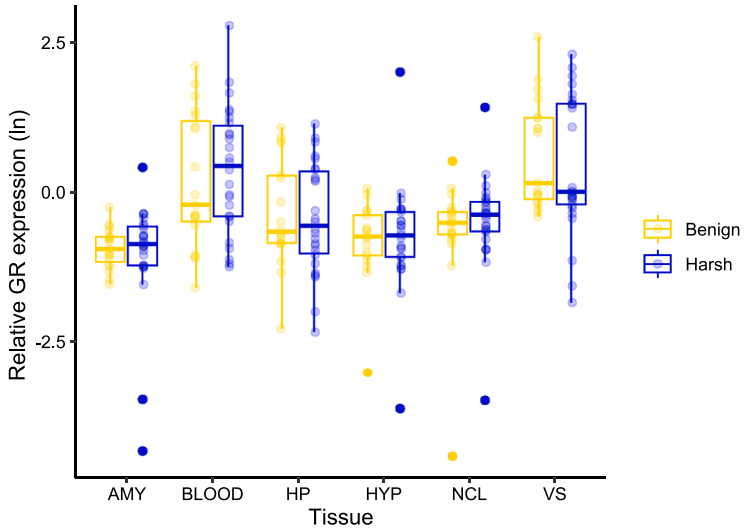

Fig. 2. Relative GR expression by developmental treatment (i.e. benign vs harsh parental foraging conditions) across the six tissues measured: AMY: amygdala, HP: Hippocampus, NCL: Nidopallium Caudolaterale, HYP: Hypothalamus, VS: Ventral striatum. Lower and upper hinges represent inter quartile range, and the median is represented in between. Whiskers represent scores outside the lower and upper quartile.

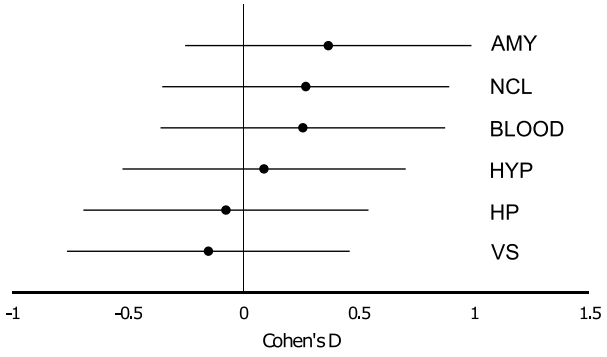

Fig. 3. Effect sizes (Cohen's $D \pm 95\%$ CI) of the effect of developmental treatment (i.e. benign vs. harsh parental foraging conditions) on relative GR expression by tissue in adulthood. AMY: amygdala, HP: Hippocampus, NCL: Nidopallium Caudolaterale, HYP: Hypothalamus, VS: Ventral striatum. Cohen's $D > 0$ show higher GR expression levels in harsh developmental conditions, whereas Cohen's $D < 0$ show higher GR expression levels in benign developmental conditions.

4. Discussion

Our experimental manipulation of parental foraging costs led to impaired chick growth [49], but did not directly affect GR expression in brain or blood in adulthood. Our developmental treatment evidently increased early life adversity, given its consequences for chick growth, but the magnitude of this effect may vary strongly among individuals, depending on e.g. parental compensation and the level of within-nest competition. We assume it is because of this (large) variation relative to the magnitude of the treatment effect we did find effects of individual nestling mass on GR expression in adulthood in two out of the six tissues analyzed: blood and ventral striatum. Females with lower body mass as nestlings showed lower levels of GR expression in these two tissues, whereas there was no such relationship in males. This association reflects a long-term impact of early life conditions on adult GR expression rather than mass in adulthood, which was unrelated to GR expression. This is not surprising, because nestling body mass differences due to parental foraging treatment gradually disappeared with age as chicks started to forage for themselves, allowing their growth to 'catch up' [49]. An association between early life conditions and GR expression is consistent throughout the mammal literature [5,12,14,17,18] , but this association has rarely been tested in birds [37,48].

The fact that nestling mass at day 15 was a predictor of GR expression in females only, suggests females are more susceptible to adverse developmental conditions when compared to males. This pattern is consistent with other studies on the same species showing females being more susceptible to early-life stressors than males [59,60,61]. Sex differences in resource allocation to different physiological systems may lie at the base of sex-specific effects of early life adversity [60,66]. Such a form of canalization is to be expected when the fitness consequences of changes in GR expression differ between the sexes [67], but the fitness consequences of GR expression remain to be elucidated. It is also possible that males and females responded differently to the level of nest competition [68]. The results of a previous study in the same population pointed at GR expression in adulthood being more affected by environmental conditions during adulthood than by early life conditions [36]. This would support the idea that the benign environment that all birds included in this study shared during adulthood may have attenuated phenotypic differences arising during early life. Alternatively, early life effects could have manifested at a regulation step different than transcription (i.e. translation), leading to differences in protein synthesis. Further studies are needed to explore the generality of this pattern, its reversibility, and the epigenetic processes involved.

We found an effect of body mass in early life on GR expression in two out of six tissues (five brain, one peripheral), as illustrated by the interaction between tissue and nestling mass at day 15 (see results). We see several explanations that could underlie this difference among tissues. First, there may be a tissue-specific sensitivity to environmental effects and GC secretion experienced during early life, depending for instance on relative importance on GC function. Tissues may differ extensively in the number of GRs they express so that, when exposed to environmental adversity, some cell types and species will probably be exceptionally plastic, whereas others will not. Thus, the magnitude of epigenetic mechanisms that further alter the GC function on these tissues may differ, modulated further by evolutionary history, life history strategy, and ecology of the species [47]. A second explanation builds on potential temporal variation in tissue development, with different tissues having sensitivity periods at different stages. Indeed, the timing and nature of early life adversity is a critical factor to consider with respect to the relationship between changes in tissue function, early environment and subsequent development of coping abilities and behavior [69].

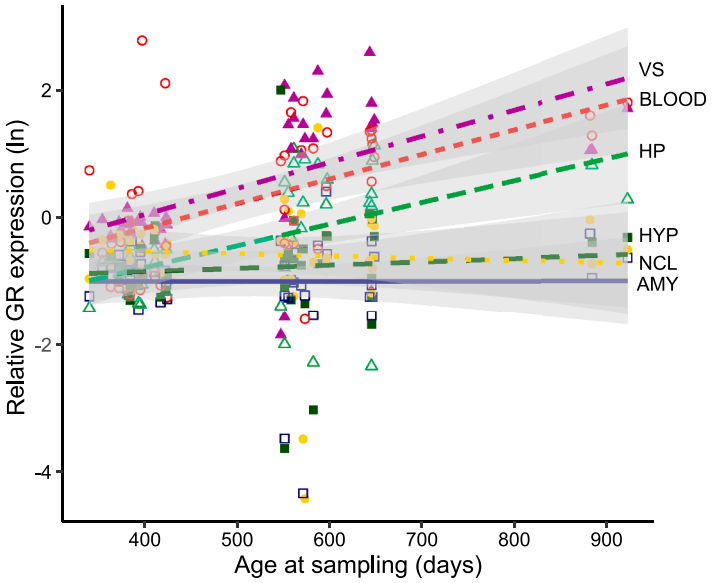

Fig. 4. Relationship between relative GR expression and age at sampling across the six tissues measured. AMY: amygdala, HP: Hippocampus, NCL: Nidopallium Caudolaterale, HYP: Hypothalamus, VS: Ventral striatum.

Table 2
Main model testing the effects of nestling mass on relative GR expression (ln) in adulthood in Blood (A) and Ventral Striatum (B).

A	Estimate	s.e.	χ^2_1	P
Intercept	−0.026	0.150		
Sex	0.107	0.304	0.179	0.672
Mass 15	0.063	0.120	0.083	0.773
Age	0.003	0.001	10.250	**0.001**
Brood size	−0.277	0.165	2.770	0.096
Mass 15: Sex	−0.437	0.222	3.863	**0.049**
Mass 15: Age	−0.000	0.001	0.040	0.842
Mass 15: Brood size	0.032	0.132	0.060	0.807
B	**Estimate**	**s.e.**	χ^2_1	**P**
Intercept	−0.028	0.143		
Sex	−0.184	0.289	0.319	0.572
Mass 15	0.124	0.114	0.615	0.433
Age	0.004	0.001	13.504	**<0.001**
Brood size	−0.103	0.157	0.382	0.536
Mass 15: Sex	−0.417	0.211	3.898	**0.048**
Mass 15: Age	0.000	0.001	0.001	0.981
Mass 15: Brood size	0.060	0.126	0.224	0.636

Another explanation could lie on our experimental manipulation during early life not being 'severe' enough as to inflict long-term changes in GR expression of detectable magnitude at an organismal level (i.e. across most tissues). Finally, effects of early life conditions may initially have been stronger and present throughout the organism, but that these effects have gradually reversed, or eclipsed by other processes occurring later in life (see above). All these processes may also potentially underlie the highly variable and overall weak GR expression correlations among tissues that we found, which are consistent with previous results in birds [70]. Interestingly, the two tissues showing a long-lasting effect of early life conditions on GR expression (Blood and Ventral Striatum) were also the tissues most affected by age, and those with the highest GR expression. The latter suggests blood and ventral striatum are more sensitive to GR secretion, and thus potentially GR expression in these tissues being more sensitive to environmental conditions. Although very few studies up to date have investigated the associations between age at sampling and GR expression, and only cross-sectionally (i.e. in brain tissue) there is some evidence for GR expression being higher in human adults and adolescents when compared to infants [71], and in older vs. younger depressed female rats [72], in line with our results. Further research testing for the associations between GR expression in peripheral tissues (i.e. longitudinal studies) and age are needed to investigate the generality of the above patterns.

Given that birds have nucleated erythrocytes, and these represent ±99% of the blood cells, we assume that gene expression levels of the GR gene quantified in whole blood samples correspond to this cell type mostly. This contrasts to the majority of previous results reporting effects of early life environment on blood GR expression being performed in white blood cells in mammals. This may be important, because GR functionality in avian red blood cells remains mostly unknown, begging the question whether expression variation can be interpreted in the same way functionally in the two taxa. Furthermore, gene expression levels (i.e. mRNA) are not necessarily proportional to protein levels, and these associations may also be tissue or taxa dependent. In addition, some studies in free-living birds have also revealed different patterns of GR binding in peripheral and central tissues [70,73]. Interestingly, our results show that avian blood cells show long-term effects of body mass during early life on GR gene expression, with patterns that are common to at least one brain region: the ventral striatum. The ventral striatum is a key region supporting decision-making - and the capacity to appropriately respond to rewarding/aversive stimuli -, social interactions and learning [74,75]. Altered developmental trajectories in this tissue have important implications for cognitive, behavioral and socioemotional development [75]. While the ventral striatum is active and functional already during very early stages, its functionality shows plasticity throughout development, making it highly susceptible to environmental influence during this period [76]. It remains to be tested what the pattern would be in other tissues not sampled in our study, especially tissues directly related to GC regulation (e.g. adrenals) or function (i.e. liver).

The fact that earlier studies investigating GR expression were done mostly on laboratory rodents (experimental) or humans (observational) prevents us from drawing conclusions on the generality or consistency of our findings, or on their potential implications for GC responses and HPA activity. An emerging field of avian studies has used an ecological approach to investigate changes in MR or GR mRNA expression in wild birds with regard to particular behavioral, ecological or physiological contexts [40,44,48,77]. Despite known similarities between structure and function of the focal brain regions in birds and mammals, these studies are not yet easily interpreted in terms of known patterns in

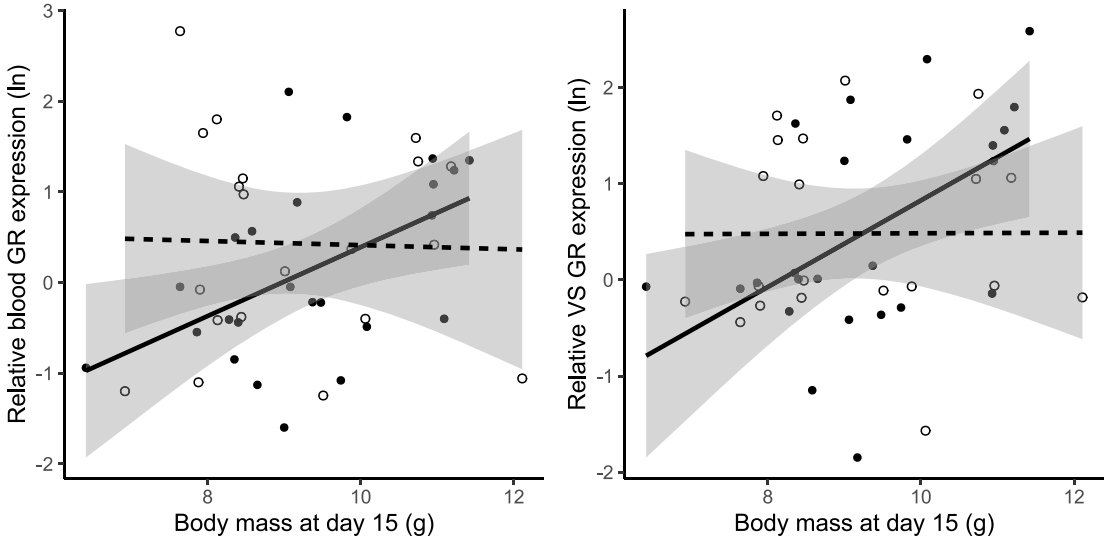

Fig. 5. Relationship between relative GR expression and nestling body mass in blood (left panel) and ventral striatum (right panels) in females (black dots, continuous line) and males (open dots, dashed line).

mammals. For example, arctic breeding passerines transitioning into parental phase typically show a strong reduction in HPA sensitivity to standardized stress [40,78,79,80]. The mammalian literature would predict this pattern to be a consequence of increased MR and GR expression in the hippocampus and hypothalamus, respectively [12,14]. However, MR expression decreased and GR expression remained static in these birds [40]. This result could be partly due to MR and GR protein increasing without an increase in gene expression, as mRNA and protein can be regulated independently. There is additional evidence supporting the association between higher levels of GR and/or enhanced stress responses (i.e. GC increases in response to an standardized stressor), in brain ([43,47], 2022) and blood [36,47,48], however to what extent this association depends on the ecological context and is comparable among tissues remains unexplored. Additionally, there is some evidence suggesting that the ratio between MR and GR is important to HPA reactivity, which may also account for the differences observed between mammalian and avian studies [38,81], but more research is needed to establish links between MR and GR expression and HPA activity in birds.

Our study shows that GR expression in blood can trace early life effects in the long term through body mass, showing patterns also found in one brain region, but not in the other four brain regions sampled. These effects differed between males and females, which points at sex-dependent physiological constraints during development shaping early life outcomes on GR expression. This environmental sensitivity may change through developmental stages and be tissue-dependent; thus, further research is required to confirm our results and find out whether these long-term effects reflect a more profound impact of early life adversity on these tissues (i.e. higher sensitivity), or else a lower plasticity to attenuate such impact (i.e. lower resilience). This, together with investigating the physiological consequences of GR expression levels on individual performance and coping abilities, will be key steps forward towards understanding the mechanisms mediating long-term impacts of early life, and whether we can measure them in a non-lethal way.

Ethics statement

This research was performed under license AVD1050020174344, approved by the Central Committee for Animal Experiments of the Netherlands

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Declaration of Competing Interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.physbeh.2023.114310.

References

[1] M. Briga, E. Koetsier, J.J. Boonekamp, B. Jimeno, S. VerhµLst, Food availability affects adµLt survival trajectories depending on early developmental conditions, Proc. Biol. Sci. 284 (1846) (2017), https://doi.org/10.1098/rspb.2016.2287.
[2] T. Burton, N.B. Metcalfe, Can environmental conditions experienced in early life influence future generations? Proc. R. Soc. B. Biol. Sci. 281 (1785) (2014), 20140311.
[3] V. Lummaa, T. Clutton-Brock, Early development, survival and reproduction in humans, Trend. Ecol. Evol. (Amst.) 17 (3) (2002) 141–147.
[4] P. Monaghan, M.F. Haussmann, The positive and negative consequences of stressors during early life, Early Hum. Dev. 91 (11) (2015) 643–647.
[5] P.Z. Liu, R. Nusslock, How Stress Gets Under the Skin: early Life Adversity and Glucocorticoid Receptor Epigenetic Regulation, Curr. Genom. 19 (8) (2017) 653–664, https://doi.org/10.2174/1389202919666171228164350.

[6] A.M. Vaiserman, A.K. Koliada, Early-life adversity and long-term neurobehavioral outcomes: epigenome as a bridge? Hum. Genom. 11 (1) (2017) 1–15.
[7] A. Forsman, Rethinking phenotypic plasticity and its consequences for individuals, populations and species, Heredity (Edinb) 115 (4) (2015) 276–284.
[8] B. Sepers, J.A. Erven, F. Gawehns, V.N. Laine, K. van Oers, Epigenetics and early life stress: experimental brood size affects DNA methylation in great tits (Parus major), Front. Ecol. Evol. 9 (2021) 124.
[9] E.J. Richards, Inherited epigenetic variation—Revisiting soft inheritance, Nat. Rev. Genet. 7 (5) (2006) 395–401.
[10] M. Kundakovic, K. Gudsnuk, J.B. Herbstman, D. Tang, F.P. Perera, F. A. Champagne, DNA methylation of BDNF as a biomarker of early-life adversity, Proc. Natl Acad. Sci. 112 (22) (2015) 6807–6813.
[11] J.A. Law, S.E. Jacobsen, Establishing, maintaining and modifying DNA methylation patterns in plants and animals, Nat. Rev. Genet. 11 (3) (2010) 204–220.
[12] D. Liu, J. Diorio, B. Tannenbaum, C. Caldji, D. Francis, A. Freedman, M.J. Meaney, Maternal care, hippocampal glucocorticoid receptors, and hypothalamic- pituitary-adrenal responses to stress, Science 277 (5332) (1997) 1659–1662, https://doi.org/10.1126/science.277.5332.1659.
[13] F. Pértille, M. Brantsæter, J. Nordgreen, L.L. Coutinho, A.M. Janczak, P. Jensen, C. Guerrero-Bosagna, DNA methylation profiles in red blood cells of adult hens correlate with their rearing conditions, J. Exp. Biol. 220 (19) (2017) 3579–3587.
[14] I.C. Weaver, N. Cervoni, F.A. Champagne, A.C. D'Alessio, S. Sharma, J.R. Seckl, M. J. Meaney, Epigenetic programming by maternal behavior, Nat. Neurosci. 7 (8) (2004) 847–854.
[15] F.A. Champagne, J.P. Curley, Epigenetic mechanisms mediating the long-term effects of maternal care on development, Neurosci. Biobehav. Rev. 33 (4) (2009) 593–600.
[16] R. Jaenisch, A. Bird, Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals, Nat. Genet. 33 (3) (2003) 245–254.
[17] P.O. McGowan, A. Sasaki, A.C. D'alessio, S. Dymov, B. Labonté, M. Szyf, M. J Meaney, Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse, Nat. Neurosci. 12 (3) (2009) 342–348.
[18] R. Yehuda, N.P. Daskalakis, A. Lehrner, F. Desarnaud, H.N. Bader, I. Makotkine, J. D. Flory, L.M. Bierer, M.J. Meaney, Influences of maternal and paternal PTSD on epigenetic regulation of the glucocorticoid receptor gene in Holocaust survivor offspring, Am. J. Psychiatry 171 (8) (2014) 872–880.
[19] W. Beerling, J.M. Koolhaas, A. Ahnaou, J.A. Bouwknecht, S.F. de Boer, P. Meerlo, W.H.I.M. Drinkenburg, Physiological and hormonal responses to novelty exposure in rats are mainly related to ongoing behavioral activity, Physiol. Behav. 103 (3–4) (2011) 412–420.
[20] B. Jimeno, M. Hau, S. Verhulst, Corticosterone levels reflect variation in metabolic rate, independent of 'stress, Sci. Rep. 8 (1) (2018) 1–8.
[21] J.M. Koolhaas, A. Bartolomucci, B. Buwalda, S.F. de Boer, G. Flügge, S.M. Korte, E. Fuchs, Stress revisited: a critical evaluation of the stress concept, Neurosci. Biobehav. Rev. 35 (5) (2011) 1291–1301.
[22] E.R. De Kloet, E. Vreugdenhil, M.S. Oitzl, M. Joëls, Brain corticosteroid receptor balance in health and disease, Endocr. Rev. 19 (3) (1998) 269–301.
[23] E.R. De Kloet, Hormones and the stressed brain, Ann. N. Y. Acad. Sci. 1018 (1) (2004) 1–15.
[24] F. Holsboer, The corticosteroid receptor hypothesis of depression, Neuropsychopharmacology 23 (5) (2000) 477–501.
[25] M. Joëls, H. Karst, R. DeRijk, E.R. de Kloet, The coming out of the brain mineralocorticoid receptor, Trend. Neurosci. 31 (1) (2008) 1–7.
[26] L.M. Romero, Physiological stress in ecology: lessons from biomedical research, Trend. Ecol. Evol. (Amst.) 19 (5) (2004) 249–255.
[27] R.L. Spencer, L.E. Chun, M.J. Hartsock, E.R. Woodruff, Glucocorticoid hormones are both a major circadian signal and major stress signal: how this shared signal contributes to a dynamic relationship between the circadian and stress systems, Front. Neuroendocrinol. 49 (2018) 52–71.
[28] E.R. de Kloet, M. Joëls, Mineralocorticoid receptors and glucocorticoid receptors in HPA stress responses during coping and adaptation. Oxford Research Encyclopedia of Neuroscience, 2020.
[29] F. ter Heegde, R.H. De Rijk, C.H. Vinkers, The brain mineralocorticoid receptor and stress resilience, Psychoneuroendocrinology 52 (2015) 92–110.
[30] M. Joëls, E.R. de Kloet, 30 years of the mineralocorticoid receptor: the brain mineralocorticoid receptor: a saga in three episodes, J. Endocrinol. 234 (1) (2017) T49–T66.
[31] H. Karst, F.S. den Boon, N. Vervoort, M. Adrian, L.C. Kapitein, M. Joëls, Non-genomic steroid signaling through the mineralocorticoid receptor: involvement of a membrane-associated receptor? Mol. Cell. Endocrinol. 541 (2022), 111501.
[32] D.M. Cohen, D.J. Steger, Nuclear receptor function through genomics: lessons from the glucocorticoid receptor, Trend. Endocrinol. Metabol. 28 (7) (2017) 531–540.
[33] L.M. Romero, J.C. Wingfield, Tempests, poxes, predators, and people: Stress in Wild Animals and How They Cope, Oxford University Press, 2015.
[34] J.N. Vanderbilt, R. Miesfeld, B.A. Maler, K.R. Yamamoto, Intracellular receptor concentration limits glucocorticoid-dependent enhancer activity, Mol. Endocrinol. 1 (1) (1987) 68–74.
[35] S.B. Banerjee, A.S. Arterbery, D.J. Fergus, E. Adkins-Regan, Deprivation of maternal care has long-lasting consequences for the hypothalamic–pituitary–adrenal axis of zebra finches, Proc. R. Soc. B. Biol. Sci. 279 (1729) (2012) 759–766.
[36] B. Jimeno, M. Hau, E. Gómez-Díaz, S. Verhulst, Developmental conditions modulate DNA methylation at the glucocorticoid receptor gene with cascading effects on expression and corticosterone levels in zebra finches, Sci. Rep. 9 (1) (2019) 1–11.
[37] C. Zimmer, K.A. Spencer, Modifications of glucocorticoid receptors mRNA expression in the hypothalamic-pituitary-adrenal axis in response to early-life stress in female Japanese quail, J. Neuroendocrinol. 26 (12) (2014) 853–860.
[38] E.R. de Kloet, From receptor balance to rational glucocorticoid therapy, Endocrinology 155 (8) (2014) 2754–2769.
[39] Z.G. Hodgson, S.L. Meddle, M.L. Roberts, K.L. Buchanan, M.R. Evans, R. Metzdorf, S.D. Healy, Spatial ability is impaired and hippocampal mineralocorticoid receptor mRNA expression reduced in zebra finches (Taeniopygia guttata) selected for acute high corticosterone response to stress, Proc. R. Soc. B. Biol. Sci. 274 (1607) (2007) 239–245.
[40] J.S. Krause, M.A. McGuigan, V.R. Bishop, J.C. Wingfield, S.L. Meddle, Decreases in Mineralocorticoid but not Glucocorticoid Receptor mRNA Expression During the Short Arctic Breeding Season in Free-Living G ambel's W hite-Crowned Sparrow (Z onotrichia leucophrys gambelii), J. Neuroendocrinol. 27 (1) (2015) 66–75.
[41] R.M. Sapolsky, L.M. Romero, A.U. Munck, How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions, Endocr. Rev. 21 (1) (2000) 55–89.
[42] R.A. Senft, S.L. Meddle, A.T. Baugh, Distribution and abundance of glucocorticoid and mineralocorticoid receptors throughout the brain of the great tit (Parus major), PLoS One 11 (2) (2016), e0148516.
[43] M. Dickens, L.M. Romero, N.E. Cyr, I.C. Dunn, S.L. Meddle, Chronic stress alters glucocorticoid receptor and mineralocorticoid receptor mRNA expression in the European starling (Sturnus vulgaris) brain, J. Neuroendocrinol. 21 (10) (2009) 832–840.
[44] J.M. Cornelius, G. Perreau, V.R. Bishop, J.S. Krause, R. Smith, T.P. Hahn, S. L. Meddle, Social information changes stress hormone receptor expression in the songbird brain, Horm. Behav. 97 (2018) 31–38.
[45] N.P. Daskalakis, H. Cohen, G. Cai, J.D. Buxbaum, R. Yehuda, Expression profiling associates blood and brain glucocorticoid receptor signaling with trauma-related individual differences in both sexes, Proc. Natl Acad. Sci. 111 (37) (2014) 13529–13534.
[46] D.S. Tylee, D.M. Kawaguchi, S.J. Glatt, On the outside, looking in: a review and evaluation of the comparability of blood and brain "-omes, American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 162 (7) (2013) 595–603.
[47] C. Zimmer, H.E. Hanson, L.B. Martin, FKBP5 expression is related to HPA flexibility and the capacity to cope with stressors in female and male house sparrows, Horm. Behav. 135 (2021), 105038.
[48] F. Ruiz-Raya, J.C. Noguera, A. Velando, Covariation between glucocorticoid levels and receptor expression modulates embryo development and postnatal phenotypes in gulls, Horm. Behav. 149 (2023), 105316.
[49] Y.H. Gerritsma, M.M. Driessen, M. Tangili, S.F. de Boer, S. Verhulst, Experimentally manipulated food availability affects offspring quality but not quantity in zebra finch meso-populations, Oecologia (2022) 1–15.
[50] O. Güntürkün, The Avian 'prefrontal cortex' and Cognition, 2005, https://doi.org/10.1016/j.conb.2005.10.003.
[51] Herold et al., 2011: The receptor architecture of the pigeons' nidopallium caudolaterale: an avian analogue to the mammalian prefrontal cortex. DOI: 10.1007/s00429-011-0301-5.
[52] M. Briga, B. Jimeno, S. Verhulst, Coupling lifespan and aging? The age at onset of body mass decline associates positively with sex-specific lifespan but negatively with environment-specific lifespan, Exp. Gerontol. 119 (2019) 111–119.
[53] E. Koetsier, S. Verhulst, A simple technique to manipμLate foraging costs in seed-eating birds, J. Exp. Biol. 214 (Pt 8) (2011) 1225–1229, https://doi.org/10.1242/jeb.050336.
[54] Y.G. Gerritsma, M.M. Driessen, S Verhulst, Zebra finch behaviour differed consistently between individuals but was not affected by early-life adversity, Anim. Behav. (2023) in press.
[55] P.V. Lovell, M. Wirthlin, T. Kaser, A.A. Buckner, J.B. Carleton, B.R. Snider, C. V. Mello, ZEBrA: Zebra finch expression brain atlas—a resource for comparative molecμLar neuroanatomy and brain evolution studies, J. Comp. Neurol. 528 (12) (2020) 2099–2131, https://doi.org/10.1002/cne.24879.
[56] M. Tagirov, J. Rutkowska, Sexual dimorphism in the early embryogenesis in zebra finches, PLoS One 9 (12) (2014), e114625.
[57] M.W. Pfaffl, A new mathematical model for relative quantification in real-time RT–PCR, Nucleic Acids Res. 29 (9) (2001) e45.
[58] D. Bates, M. Maechler, B. Bolker, S. Walker, R.H.B. Christensen, H. Singmann, G. Grothendieck, Package 'lme4', Linear Mixed-Effect. Model. Using S4 Class. 1 (6) (2011). R package version.
[59] C.H. De Kogel, Long-Term Effects of Brood Size ManipμLation on Morphological Development and Sex-Specific Mortality of Offspring, J. Anim. Ecol. 66 (2) (1997) 167, https://doi.org/10.2307/6019.
[60] K.L. Schmidt, E.A. MacDougall-Shackleton, K.K. Soma, S.A. MacDougall-Shackleton, Developmental programming of the HPA and HPG axes by early-life stress in male and female song sparrows, Gen. Comp. Endocrinol. 196 (2014) 72–80.
[61] S. Verhulst, M.J. Holveck, K. Riebel, Long-term effects of manipulated natal brood size on metabolic rate in zebra finches, Biol. Lett. 2 (3) (2006) 478–480.
[62] R Core Team, R: A language and Environment For Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2020. URL, https://www.R-project.org/.
[63] J Fox, S Weisberg. An R Companion to Applied Regression, Third edition, Sage, Thousand Oaks CA, 2019. https://socialsciences.mcmaster.ca/jfox/Books/Companion/.
[64] D. Lüdecke, M.S. Ben-Shachar, I. Patil, P. Waggoner, D. Makowski, performance: an R package for assessment, comparison and testing of statistical models, J. Open Source Software 6 (60) (2021), 3139, https://doi.org/10.21105/joss.03139.

[65] J. Cohen, Statistical Power Analysis For the Behavioral Sciences, Erlbaum, Hillsdale, NJ, 1988.
[66] T.A. Wilkin, L.E. King, B.C. Sheldon, Habitat quality, nestling diet, and provisioning behaviour in great tits Parus major, J. Avian Biol. 40 (2) (2009) 135–145.
[67] J.J. Boonekamp, E. Mulder, S. Verhulst, Canalisation in the wild: effects of developmental conditions on physiological traits are inversely linked to their association with fitness, Ecol. Lett. 21 (2018) 857–864.
[68] M. Briga, Growing Up and Growing old. A longitudinal Study On Aging in Zebra Finches, University of Groningen, 2016.
[69] D.G. Gee, B.J. Casey, The impact of developmental timing for stress and recovery, Neurobiol. Stress 1 (2015) 184–194.
[70] C.R. Lattin, D.E. Keniston, J.M. Reed, L.M. Romero, Are receptor concentrations correlated across tissues within individuals? A case study examining glucocorticoid and mineralocorticoid receptor binding, Endocrinology 156 (4) (2015) 1354–1361.
[71] W.R. Perlman, M.J. Webster, M.M. Herman, J.E. Kleinman, C.S. Weickert, Age-related differences in glucocorticoid receptor mRNA levels in the human brain, Neurobiol. of Aging 28 (3) (2007) 447–458.
[72] Q. Wang, M. Joels, D.F. Swaab, P.J. Lucassen, Hippocampal GR expression is increased in elderly depressed females, Neuropharmacology 62 (1) (2012) 527–533.
[73] C.R. Lattin, L.M. Romero, Seasonal variation in glucocorticoid and mineralocorticoid receptors in metabolic tissues of the house sparrow (Passer domesticus), Gen. Comp. Endocrinol. 214 (2015) 95–102.
[74] R.N. Cardinal, J.A. Parkinson, J. Hall, B.J. Everitt, Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex, Neurosci. Biobehav. Rev. 26 (3) (2002) 321–352.
[75] B. Goff, N. Tottenham, Early-life adversity and adolescent depression: mechanisms involving the ventral striatum, CNS Spectr. 20 (4) (2015) 337–345.
[76] D.S. Fareri, N. Tottenham, Effects of early life stress on amygdala and striatal development, Dev. Cogn. Neurosci. 19 (2016) 233–247.
[77] C. Zimmer, C.C. Taff, D.R. Ardia, K.A. Rosvall, C. Kallenberg, A.B. Bentz, A. R. Taylor, L.S. Johnson, M.N. Vitousek, Gene expression in the female tree swallow brain is associated with inter-and intra-population variation in glucocorticoid levels, Horm Behav 147 (2023) 105280.
[78] R.L. Holberton, J.C. Wingfield, Modulating the corticosterone stress response: a mechanism for balancing individual risk and reproductive success in arctic-breeding sparrows? Auk 120 (4) (2003) 1140–1150.
[79] S.L. Meddle, N.T. Owen–Ashley, M.I. Richardson, J.C Wingfield, Modulation of the hypothalamic–pituitary–adrenal axis of an Arctic–breeding polygynandrous songbird, the Smith's longspur, Calcarius pictus, Proc. R. Soc. Lond. B Biol. Sci. 270 (1526) (2003) 1849–1856.
[80] J.C. Wingfield, K.M. O'REILLY, L.B. Astheimer, Modulation of the adrenocortical responses to acute stress in arctic birds: a possible ecological basis, Am. Zool. 35 (3) (1995) 285–294.
[81] A.P. Harris, M.C. Holmes, E.R. De Kloet, K.E. Chapman, J.R. Seckl, Mineralocorticoid and glucocorticoid receptor balance in control of HPA axis and behaviour, Psychoneuroendocrinology 38 (5) (2013) 648–658.