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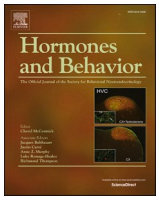

Glucocorticoid receptor expression as an integrative measure to assess glucocorticoid plasticity and efficiency in evolutionary endocrinology: A perspective

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ABSTRACT

Organisms have to cope with the changes that take place in their environment in order to keep their physical and psychological stability. In vertebrates, the hypothalamic-pituitary-adrenal (HPA) axis plays a key role in mediating phenotypic adjustments to environmental changes, primarily by regulating glucocorticoids (GCs). Although circulating GCs have widely been used as proxy for individual health and fitness, our understanding of HPA regulation is still very limited, especially in free-living animals. Circulating GCs only exert their actions when they are bound to receptors, and therefore, GC receptors play a pivotal role mediating HPA regulation and GC downstream phenotypic changes. Because under challenging conditions GC actions (as well as negative feedback activation) occur mainly through binding to low-affinity glucocorticoid receptors (GR), we propose that GR activity, and in particular GR expression, may play a crucial role in GC regulation and dynamics, and be ultimately related to organismal capacity to appropriately respond to environmental changes. Thus, we suggest that GR expression will provide more comprehensive information of GC variation and function. To support this idea, we compile previous evidence demonstrating the fundamental role of GR on GC responses and the fine-tuning of circulating GCs. We also make predictions about the phenotypic differences in GC responsiveness - and ultimately HPA regulation capacity - associated with differences in GR expression, focusing on *GC plasticity* and *efficiency*. Finally, we discuss current priorities and limitations of integrating measures of GR expression into evolutionary endocrinology and ecology studies, and propose further research directions towards the use of GR expression and the study of the mechanisms regulating GR activity to gather information on coping strategies and stress resilience. Our goals are to provide an integrative perspective that will prompt reconsideration on the ecological and physiological interpretation of current GC measurements, and motivate further research on the role of GR in tuning individual responses to dynamic environments.

1. Introduction and aims

Organisms have to cope daily with the changes that take place in their environment in order to keep their physical and psychological stability (i.e. homeostasis). Hormones are crucially involved in this link between organisms and their environment, as endocrine systems integrate environmental variation to produce a range of phenotypes from the same genotype (i.e. *phenotypic plasticity*; Dufty et al., 2002). In vertebrates, one hormonal system that plays a key role in transducing environmental signals into organismal phenotypic variation is the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis acts as an interface between an individual and changes in its internal and external environments, and mediates relevant body processes (e.g. digestion, immune function, energy metabolism, behavior), primarily by regulating the secretion and release of glucocorticoids (i.e. GCs; Hau et al., 2016; Romero, 2004; Sapolsky et al., 2000). HPA axis activity helps organisms adjust their phenotypes to predictable and unpredictable changes in their environment. Thus, differences in GC signaling among individuals or populations are expected to reflect differences in coping capacity (i.e. the ability to successfully and efficiently overcome an internal or external unpredictable challenge). As a consequence, circulating GC concentrations have been frequently used as proxies for

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individual health, welfare or fitness in disciplines from biomedicine to conservation biology (Schoenle et al., 2018, Caulfield and Cavigelli, 2020, Schoenle et al., 2021). This reliance on GCs, however, contrasts with our still modest understanding on HPA function and regulation, especially in free-living species.

During the past decades, it has been suggested several times that circulating GCs are only one component of a complex process from the regulation of their secretion to their phenotypic effects, and that focusing on temporal dynamics of the HPA axis (Sapolsky, 1983, De Kloet, 2004, McDonald et al., 1986, Bradley, 1990, Boonstra and Singleton, 1993) or key regulatory components (e.g. GC receptors, GC transport proteins; Landys et al., 2006, Lattin and Romero, 2014, Breuner & Orchinik, 2002, Krause et al., 2015, Krause et al., 2021) could be a more accurate way to capture individual condition and physiological status. However, the insightful but still limited knowledge on the mechanistic underpinnings of GC variation and regulation provided by these and other studies, contrasts with a wide body of research in the field of evolutionary endocrinology still only relying on GC concentrations. In fact, scepticism around the use of circulating GC concentrations to capture individual performance and fitness has prevailed over time and remains a topic of debate (e.g. Buwalda et al., 2012; Dantzer et al., 2014; MacDougall-Shackleton et al., 2019; Zimmer et al., 2021; Romero and Beattie, 2022; Zimmer et al., 2022). This illustrates the need for a deeper focus on the mechanisms driving GC variation and regulation in order to fully comprehend their ecological relevance.

Because GCs under challenging conditions primarily exert their actions when bound to the glucocorticoid receptor (GR), we propose that an increased focus on GR activity is a necessary step to achieve a better understanding of GC function and regulation, and ultimately of organisms' capacities to regulate effective physiological and behavioral responses to cope with environmental challenges. Although the central role of GR on GC regulation has been highlighted by several studies during the past decades (De Kloet et al., 1998; Landys et al., 2006; Krause et al., 2015), and its downstream effects on health are relatively well established in the biomedical literature (Kadmiel and Cidlowski, 2013; De Kloet et al., 1998; Liu et al., 1997), these ideas remain often overlooked in evolutionary endocrinology and ecology especially in free-living populations (but see e.g. Landys et al., 2006, Lattin and Romero, 2014). This may be partly due to the challenges associated with the required sample in free living populations requiring terminal sampling (see Section 5.2). In this perspective article, we suggest that focusing on GR actions, while taking into consideration the physiological steps involved in GCs function (secretion, transport and integration), will provide a more comprehensive understanding of GC variation and eventually of HPA regulation. Previous studies, although frequently mentioning these same mechanistic principles and well aware of the importance of GR on GC dynamics (e.g. Sapolsky, 1983, De Kloet et al., 1998, Landys et al., 2006, Liebl and Martin, 2013, Lattin and Romero, 2014, Lattin et al., 2015), most often measured only GR expression - without linking it to GC concentrations - or only GC concentrations. Indeed, studies linking among and within individual differences in GR levels with differences in GC concentrations and responses remain rare, which currently limits our capacity to address the relative importance of GR function on GC regulation and dynamics in natural environments. Thus, we are primarily interested in the importance that GR may have within an eco-evolutionary context of response to environmental challenges by better understanding the relationship between GR variation and GC regulation.

First, we compile previous evidence demonstrating the pivotal role of GR on GC physiological actions and the fine-tuning of circulating GCs, and elaborate predictions on the differences in GC regulation and dynamics associated with differences in GR signaling (i.e. GR expression levels). Most of the evidence and examples compiled here specifically refer to GR actions in central regulatory regions of the HPA axis (i.e., hypothalamus, hippocampus and pituitary gland). However, as GR is expressed in nearly every cell type of the body, we also highlight the interest of further investigating whether regulation in central tissues is related to regulation in peripheral tissues, as a fundamental step towards longitudinal studies of GC dynamics. Finally, we emphasize current priorities and limitations of integrating measures of GR function and regulation in evolutionary endocrinology, and we propose further research directions towards the use of this trait to gather information on environmental coping, stress resilience and evolution of the HPA axis. While several pioneering studies have emphasized the importance of studying GR function, we feel this approach has not yet been fully integrated or incorporated in the field of evolutionary endocrinology (see above), and thus we aim our work to serve as a bridge linking previous knowledge, novel ideas, current limitations and alternatives, and future research directions on the study of GC regulation. We hope this article will prompt reconsideration on the ecological and physiological information provided by current GC measurements, and motivate further research on the role of GR and other regulatory components tuning individual responses to dynamic environments.

2. Glucocorticoid measurements and the importance of HPA axis regulation

Glucocorticoids are considered as mediators of homeostasis in vertebrates (Sapolsky et al., 2000; McEwen and Wingfield, 2003; Romero et al., 2009), as they fluctuate with energetic needs (McEwen and Wingfield, 2003; Eikenaar et al., 2014; Jimeno et al., 2018) and regulate physiological and behavioral responses to environmental challenges (McEwen and Wingfield, 2003; Romero et al., 2009; Koolhaas et al., 2011). Circulating GCs fluctuate daily and seasonally following activity/rest cycles and predictable environmental changes (Sapolsky et al., 2000; Romero, 2002; Landys et al., 2006). These concentrations also increase (i.e., acute stress response) within a few minutes of exposure to unpredictable challenges (Sapolsky, 1983; Romero and Reed, 2005; O'Reilly and Wingfield, 2001), helping the organism cope with these challenges and mitigate their effects. However, GCs can impose costs at very high or prolonged levels, such as dysregulation of metabolic processes, increased oxidative stress, telomere shortening, cognitive impairment, or suppression of reproductive and parental behaviors (Wingfield and Sapolsky, 2003; Haussmann et al., 2012; Monaghan, 2014). For instance, GC exposure during early development often has long-term health consequences in humans and lab rodents (e.g. impaired kidney function, increased risk of diabetes; Moritz et al., 2005). In mammals, high GC levels have suppressive effects on male and female reproductive physiology and behavior (reviewed in Wingfield and Sapolsky, 2003). In snowshoe hares, high levels of plasma GCs were associated with reduced leucocyte counts, increased glucose mobilization, and higher body-weight loss during winter (Boonstra & Singleton 1993). Thus, GC concentrations are tightly regulated by negative feedback (MacDougall-Shackleton et al., 2013; Lattin and Kelly, 2020; Vitousek et al., 2019), and the capacity to elevate and down-regulate GCs quickly is fundamental for coping with environmental challenges by optimizing GC exposure (Romero and Wikelski, 2010; Zimmer et al., 2019; Blas et al., 2006), and potentially crucial for fitness (Taff and Vitousek, 2016; McCormick and Romero, 2017; Caulfield and Cavigelli, 2020; Lattin and Kelly, 2020). For example, female tree swallows (*Tachycineta bicolor*) showing weaker negative feedback responses were more likely to abandon their nests after disturbances (Zimmer et al., 2019). Because negative feedback determines the magnitude of GC exposure, individual variation in negative feedback ability may underlie trade-offs between survival and reproduction (reviewed in Lattin and Kelly, 2020).

A wide amount of research has focused on trying to unveil relationships between GC concentrations and several phenotypic traits, often with the intention of use GCs as proxies of fitness, physical status or animal welfare, or of the capacity of organisms to cope with challenges (Caulfield and Cavigelli, 2020; Dantzer et al., 2014; Madliger et al., 2015; Madliger and Love, 2016; Schoenle et al., 2018; Schoenle

et al., 2021). However, the associations between GC levels and measures of health or fitness are not ubiquitous, and highly context dependent (Caulfield and Cavigelli, 2020; Schoenle et al., 2018; Schoenle et al., 2021). These inconsistencies may result from the complexity of HPA axis regulation that cannot be captured by single GC measures (Sapolsky et al., 2000, Krause et al., 2015, Zimmer et al., 2020; see below). Additionally, it has been shown that the effect of some selective pressures on shaping GCs are relatively consistent across broad taxonomic scales, but that selection seems to act differently on different GC levels (baseline vs. stress-induced; Vitousek et al., 2019). At macroevolutionary level, baseline and stress-induced GC levels were not associated with coping capacity. Thus, it is conceivable that variation in other elements of the HPA axis such as upstream or downstream regulatory components are better associated with this capacity (Vitousek et al., 2019). However, it is still unknown whether variation in GC levels is linked with variation in upstream and downstream regulatory components, or the degree to which selection operates on different components of the HPA axis (Hau, 2007; Ketterson et al., 2009; Zimmer et al. submitted). This currently prevents us from getting conclusions on how GCs mediate adaptive responses to environmental challenges and HPA axis evolution. Therefore, it appears necessary to investigate traits that mediate the propensity to effectively regulate GC concentrations (and/or the speed of the response) in order to explore the capacity of organisms to adjust their phenotype to prevailing environmental conditions. However, research on how to properly quantify these complex traits and the kind of information they may provide is currently at its early stage.

3. What are we really measuring when we measure circulating GCs? From the gland to the cell

GCs are produced in the adrenal gland and released in systemic circulation following a cascade of reactions in the hypothalamus and pituitary gland (Sapolsky et al., 2000; *GC secretion*, Fig.1). Thus, the amount of circulating GCs at a single time point is only one step of a dynamic process. After entering the blood stream, GCs circulate towards target tissues (i.e. *GC transport*, Fig.1), where the amount of GCs available to receptors will be determined by CBG and other binding proteins (i.e. albumin; Breuner et al., 2013). Upon reaching tissues and cells (i.e. *GC integration*, Fig. 1), GCs exert physiological actions by binding to two receptors: the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR) (De Kloet et al., 1998; Sapolsky et al., 2000; Romero & Wingfield, 2016; Landys et al., 2006; Lattin and Romero, 2014). MR is expressed in specific tissues (e.g. hippocampus, liver, kidney, heart, colon), while GR is expressed in nearly every cell type, including nucleated blood cells, which are found in all taxa but which accessibility and abundance is limited in some taxa like mammals (see Section 5.3; Romero & Wingfield, 2016; Spencer et al., 2018; Cohen and Steger, 2017). Because MR has a 10-fold higher affinity for GCs than GR, it is saturated at lower circulating concentrations, and consequently MR mainly regulates traits associated with metabolism, foraging and

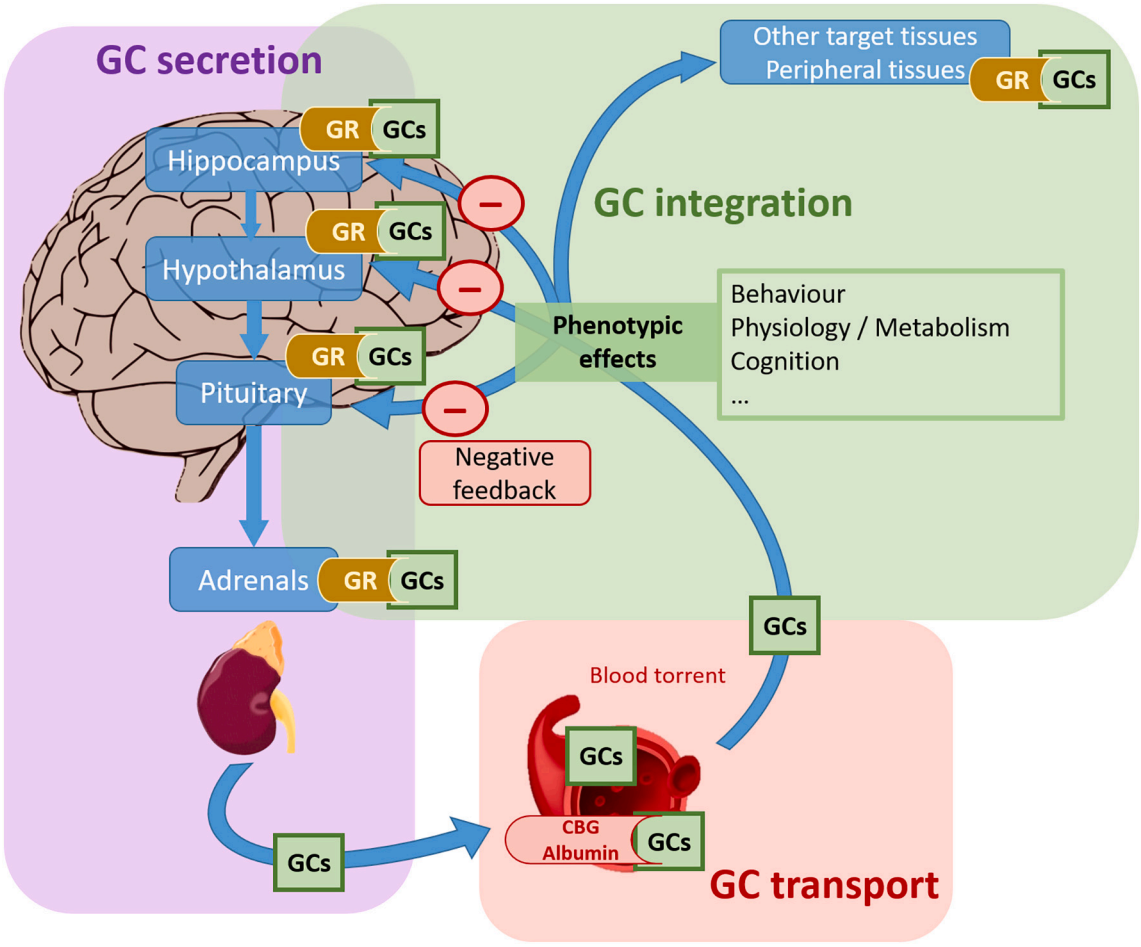

Fig. 1. Graphical representation of the different steps taking place until GCs exert physiological actions: secretion, transport and integration, the latter determined by binding to GRs. GCs are secreted by the adrenal gland into the circulation following a cascade of reactions in the hypothalamus and pituitary gland. After entering the blood stream, GCs circulate towards target tissues (i.e. *transport*), where the percentage of GCs available to receptors will be determined by CBG and other binding proteins. When reaching target tissues and cells (i.e. *integration*), GCs exert physiological actions by binding to intracellular GR. GRs mediate most phenotypic modifications associated with acute responses, and the negative feedback to terminate them. GC binding to GR results in widespread phenotypic effects.

activity level. GRs are additionally recruited with increasing level of GCs at daily peaks and with acute (e.g. 'stress-induced') increases in GC levels (De Kloet et al., 1998; Holsboer, 2000). Thus, GR mediates most phenotypic modifications associated with the acute stress response, and the negative feedback to terminate it (De Kloet et al., 1998; De Kloet, 2004; Romero, 2004; Spencer et al., 2018). Activation of GRs by GCs results in widespread effects (Wingfield et al., 1998; Sapolsky et al., 2000; Datson et al., 2008; Tasker et al., 2006) that will culminate in phenotypic adjustments in metabolism, immune system function, vascular tone, behavior and central nervous system function, among others (Revollo and Cidlowski, 2009; Fig. 1).

We could assume the amount of circulating GCs to be a measure of the amount of GCs potentially available for target tissues. However, because GCs only act after binding to their receptors, similar concentrations of circulating GCs or of GCs available to receptors (Fig. 1) may result in very different physiological states and functional outcomes for individuals differing in receptor density. For instance, although it remains to be determined whether GR levels in peripheral tissues reflect GR levels in the HPA axis (see Section 5.2), zebra finches with low GR expression in blood cells had baseline GC levels that fell within the range of stress-induced levels of individuals with high GR expression (Jimeno et al., 2019). Thus, we can quantify the amount of circulating GCs during a stress response, but only a fraction will interact with GR and be functional for HPA regulation and phenotypic outcomes in order to cope with the challenge. What ecologists and evolutionary endocrinologists are interested in is the information from GCs that can be interpreted and integrated through binding to GC receptors (i.e. *semiotic information*; O'Connor et al., 2019; Zimmer et al., 2020; Zimmer et al., 2022). However, currently mostly *syntactic information* has been obtained (i.e. the reduction in uncertainty of a system on the basis of the difference between two states of the system; Box 1). From this syntactic information, only the fraction corresponding to semiotic information – that may differ among and within individuals – will affect HPA axis regulation, organismal function and eventually fitness (Zimmer et al., 2020, 2022). As GC concentrations measured in most studies contain no information on receptor activity or other aspects of the HPA axis, ecological studies need to turn the attention to the GC integration step and the semiotic content of GCs, instead of the GC concentrations only (Fig. 1; Zimmer et al., 2021). In the context of coping with unpredictable challenges, a critical point for GC functional responses is the action of GRs expressed within the HPA axis, along with other components (e.g. CBG and 11β-HSDs) that modulate the amount of GCs that may bind to the receptors (Krause et al., 2015, 2021, Zimmer et al., 2020, Fig.1). Despite the functional importance of GR activity and regulation, there are surprisingly few studies experimentally testing for the associations between non-manipulated GR expression (i.e. non pharmacologically or surgically – induced) and variation in plasma GCs in evolutionary endocrinology (but see Krause et al., 2015; Baugh et al., 2017; Culbert et al., 2018; Madison et al., 2018, Zimmer et al. submitted). This is also common to the biomedical literature, where very few studies report relationships between GR expression and natural variation in GC levels (e.g. Yehuda et al., 2009; González'Ramírez et al., 2020), as most studies in this field investigating GR function and activity mostly focused on the effects of exogenous GC treatments or chronic stress on GR expression (Checkley, 1996; Han et al., 2017; Dickens et al., 2009; Lee et al., 2010). These studies, however, have been fundamental towards a better understanding of GR physiological effects and plasticity. Furthermore, information content of circulating GCs during a stress response is only extracted and interpreted when GCs bind to GRs, and the phenotypic changes in response should be related to the number of GR activated. Thus, data linking GC concentrations, GR expression and phenotypic changes are necessary to make this conclusion, but too few currently exist (Zimmer et al., 2022). We propose that, in addition to GC levels, concentrating on GR and its function would allow us to focus on the integration step and to get closer to the GCs semiotic information content.

4. Implications of GR expression in GC regulation: plasticity and efficiency

Several studies have pointed out the need for better descriptions of GC dynamics, and more recently quantification of the semiotic information of GCs, in order to measure organismal resilience to environmental challenges, namely 'stress resilience' (e.g. Sapolsky, 1983; Boonstra and Singleton, 1993; Romero et al., 2009; Zimmer et al., 2020, 2022). This resilience likely involves plasticity in GC function in response to environmental change, which would entail having access to a broader range of circulating GCs during the response in both elevation and negative feedback steps (i.e. *GC plasticity*; see below), and / or rapid integration of hormones to match environmental conditions (i.e. *GC efficiency*, see below), including the ability of individuals to return to baseline levels and restore homeostasis (e.g. via negative feedback mechanisms). These traits may mediate stress resilience and eventually contribute to *HPA axis flexibility* (see Box 1; Zimmer et al., 2020, 2021), ultimately connecting individual phenotypes to performance and eventually fitness.

Box 1
Glossary

- **Glucocorticoid (GC) efficiency:** Amount of circulating GCs integrated per time unit within target tissues.
- **Glucocorticoid integration:** Incorporation into target tissues cells of – functional - circulating GCs that will exert physiological actions through intracellular receptor binding.
- **Glucocorticoid integration potential** (of a cell/tissue): Amount of GCs that can be integrated through intracellular receptor binding and exert physiological actions.
- **Glucocorticoid plasticity:** Extent of the within-individual range of plasma GC concentrations (i.e. elevation and recovery) that can be integrated via receptor binding in response to an unpredictable challenge.
- **HPA axis flexibility:** Rapid and reversible change in HPA axis regulation that occurs within individuals in response to unpredictable challenges. Measured as the extent of the range and/or speed of changes in individuals' GC concentrations across diverse conditions (Taff and Vitousek, 2016; Zimmer et al., 2020).
- **Phenotypic plasticity:** The extent to which an organism can adjust its physiology, behavior, morphology and/or development in response to environmental cues (Dufty et al., 2002).
- **Semiotic information:** The meaning and quality of information carried by syntactic information. Semiotic information represents the part of syntactic information that is interpreted (i.e., it is the functional information encoded in a signal; Zimmer et al., 2020, 2022).
- **Stress resilience:** propensity of an individual to use GCs to cope with adversity (Zimmer et al., 2020)
- **Syntactic information:** Reduction in uncertainty of a system on the basis of the difference between two states of the system. Syntactic differs from semiotic information in that the former does not involve the meaning or interpretation of the signal (Zimmer et al., 2020, 2022).

If we follow the assumption that the number of GRs in target tissues determines the amount of GCs that may be integrated via receptor binding (Fig. 1), we expect that a higher number of GRs will be associated with a higher GC *integration potential*, and thus with access to a wider range of circulating GC concentrations that could exert physiological actions in target tissues. This would result in higher *GC plasticity* (Fig. 2; Box 1), providing individuals with greater capacity to adjust phenotypically to prevailing conditions via greater range of achievable phenotypes. Furthermore, under equal circulating GC concentrations, we would also expect a higher number of GRs in a tissue to result in a higher amount of GCs integrated per time unit (i.e. higher amount of GRs available at one time point). The latter should be related to the speed of the GC response and derived physiological effects (which would also include negative feedback in the specific tissues involved; see below), which can be termed *GC efficiency* (Fig. 2; Box 1). Both *GC plasticity* and *efficiency* will be related to higher semiotic information transduction, which makes GR activity and regulation fundamental to understanding HPA axis regulation, especially in the context of response to challenges that can be mitigated by GCs. Therefore, we can make the following predictions for individuals with low GR expression, when compared with individuals with higher GR expression (Fig. 2):

- Reduced *GC integration* per time unit when circulating levels increase, resulting in lower *GC efficiency*, and thus leading to slower and weaker physiological and behavioral responses induced by elevated GCs.
- Faster receptor saturation with increasing circulating GC concentrations (i.e. higher slope of the relationship between % GR bound and GC concentrations), resulting in lower information transduction and thus lower *GC plasticity*. This would lead to narrower range of GC concentrations exerting physiological actions. Thus, individuals with low GR expression would reach their endocrine ceiling faster, limiting their ability to adjust their phenotype in response to challenges when compared to individuals with higher receptor density.

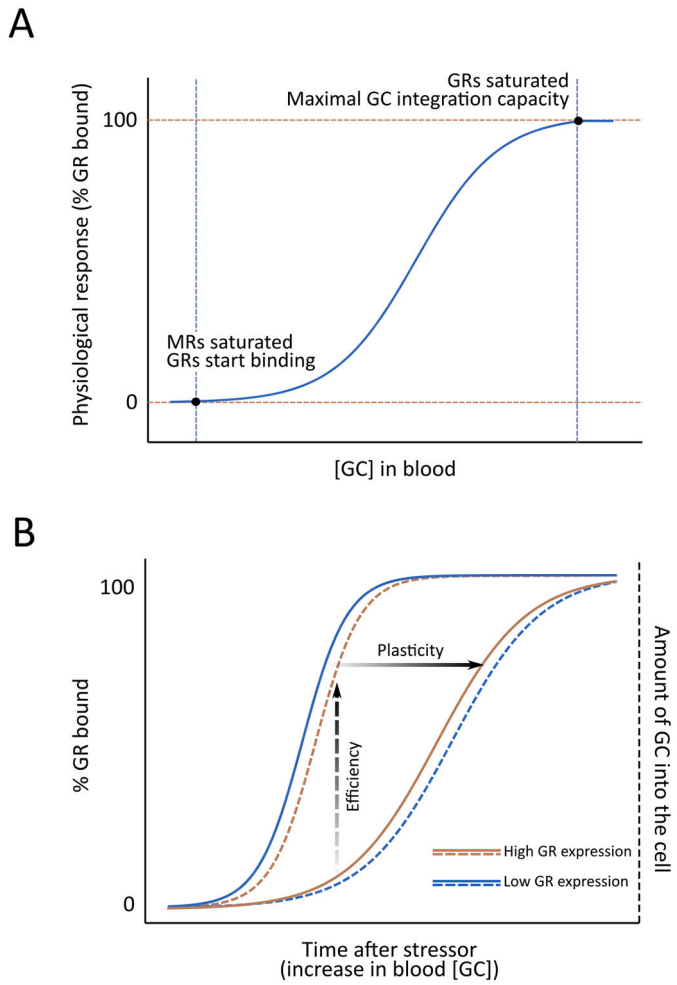

Fig. 2. Graphical representation of (A) the expected relationship between GC concentrations in blood and GR binding (i.e. *GC integration*) in a target tissue, and (B) the expected differences (pointed with arrows) in GC plasticity and efficiency between individuals with high (orange) or low (blue) GR expression. Differences in GC plasticity (i.e. extent of the range of plasma GC concentrations and their change an individual may exhibit within an acute GC response) will be determined by GR expression via total binding capacity (continuous lines, left axis). Differences in GC efficiency (i.e. the amount of GCs integrated in the cell or tissue per time unit) will depend on the number of GRs bound at a certain time point (dashed lines and right axis). When GC concentrations in blood increase, individuals with high GR levels will have a lower percentage of their GRs bound at one time point, and will be integrating more GCs, when compared to individuals with low GR expression.

4.1. GR expression and GC responses: GC profile, acute increases and negative feedback

According to the above framework, high GR levels would enable individuals to mobilize GCs rapidly to match environmental challenges, as well as efficiently dampen the response via negative feedback (see below). Thus, individuals with high GR expression would increase their capacity to respond adaptively to challenges by achieving an appropriate phenotype, whereas those with low GR expression may not be able to adjust their phenotypes, or do it less efficiently or effectively. Negative feedback is mainly coordinated by GCs binding to GR in the hippocampus, hypothalamus and pituitary gland, resulting in a decrease in GC secretion (Breuner and Orchinik, 2001; Zimmer et al., 2019; Romero, 2004), and is crucial to cope with environmental challenges and for health and fitness (Holsboer, 2000; Boyle et al., 2005; Romero and Wikelski, 2010; Anacker et al., 2011; Zimmer et al., 2019; Vitousek et al., 2019; Lattin and Kelly, 2020). Most previous research reporting associations between GR expression and negative feedback stems from biomedical field, and usually shows that lower GR expression is associated with reduced negative feedback efficacy and negative health outcomes. For instance, patients with depression usually have decreased GR expression resulting in lower feedback efficiency (Holsboer, 2000), and people with history of early life stress typically show attenuated number of GRs associated with reduced negative feedback efficacy (Liu and Nusslock, 2017). Furthermore, patients with post-traumatic stress disorder usually show low baseline GC levels and enhanced negative feedback response associated with a greater number of GRs in white blood cells (Yehuda, 2009; Yehuda et al., 2015). The above results are in agreement with some recent examples in more ecologically relevant literature showing positive associations between GR expression in HPA tissues and enhanced negative feedback responses (Zimmer and Spencer, 2014; Zimmer et al. 2017). Therefore, GR expression may be the mechanism allowing effective regulation of GCs by turning on and then off the HPA axis efficiently (Zimmer et al., 2019; Zimmer et al., 2020). Likewise, higher GC plasticity and efficiency mediated by high level of GR may allow better fine-tuning of the GC stress response.

In line with these predictions, a recent study on wild breeding female tree swallows from three different populations suggests that individuals with higher GR expression in the hypothalamus showed stronger negative feedback (i.e. more efficient GC regulation; Zimmer et al. submitted). Furthermore, the population breeding in the most unpredictable environment showed an enhanced stress-induced GC response (Zimmer et al., 2020), suggesting that higher HPA responsiveness (i.e. stronger acute response coupled with strong negative feedback) could be more adaptive in challenging environments. A consistent pattern has also been found for the relationships between plasma GC levels and GR expression in blood: Jimeno et al., 2019 found that individual zebra

finches (*Taeniopygia guttata*) with higher GR expression showed enhanced plasma GC responses associated with greater negative feedback efficacy in response to a standard stressor. These associations point to a less responsive HPA axis ("flatter" GC profile) in individuals with lower GR expression, although whether GR expression in blood mirrors expression in the HPA axis remains to be tested (see Section 5.2). Taken together, these results would be in accordance with high GC plasticity and efficiency in individuals expressing more GRs. Indeed, it has been previously proposed that a strong negative feedback coupled with a high stress response may mitigate the cost of mounting a strong stress response while getting its phenotypic benefits especially when facing frequent unpredictable short-term challenges (Zimmer et al., 2019; Zimmer et al., 2020). This adaptive interaction between acute increases and recovery has also been proposed for hypothalamus-pituitary-gonadal responses in birds (McGlothlin et al., 2007, McGlothlin et al., 2010).

While the associations between higher GR expression and enhanced negative feedback responses are consistent across studies (see above), previous research has reported positive and negative associations between the magnitude of the acute stress response and GR expression. Adult rats deprived from maternal care during development showed increased hippocampal GR expression and enhanced feedback sensitivity, but an attenuated stress response (Liu et al., 1997). Also in rats, tactile stimulation induced a decrease in plasma GCs and an increase in brain GR in pups during the first days of life, as compared to unstimulated pups (Jutapakdeegul et al., 2003). In line with this, white-rumped snow finches (*Onychostruthus taczanowskii*) wintering in the Qinghai-Tibet Plateau exhibited suppression of the stress response to an acute stressor, but increased GR expression in the hypothalamus, when compared to other times of the year (Li et al., 2020). This contrasts with findings from other bird studies reporting positive associations between the magnitude of the stress response and GR expression in HPA axis tissues (Zimmer et al. submitted) and in blood (Jimeno et al., 2019). The apparent discrepancy for stress-induced levels may result from differences in the timing of samples, in the speed of negative feedback activation among species, and/or in GR expression plasticity (see Section 5). Using dexamethasone injection to measure negative feedback efficacy allows for measurements that are standardized across individuals and independent of the stress response (Zimmer et al., 2019). In contrast, stress-induced GC concentrations, measured after the same time interval in all individuals, do not allow controlling for differences in the timing of reaching the peak concentrations in response to that specific stimulus, or of triggering negative feedback. Thus, the speed of the response will determine the measurements of stress-induced GCs, whereas negative feedback is often chemically induced and represents the maximal physiological response exhibited by the organism. Because individuals with elevated GR (in the brain regulatory regions) are likely to trigger negative feedback earlier than those with reduced number, GC levels at stress-induced sampling may already be decreasing in individuals with higher GR expression, leading to the wrong conclusion that they have a lower stress response (see Section 5). Thus, as currently there is no perfect way to measure peak GCs level we suggest, when possible, that researchers measure chemically induced negative feedback when measuring individual stress response. This measure provides more information on HPA axis regulation, is more standardized, and is mainly determined by GR expression (Zimmer et al., 2019, 2020; Vitousek et al., 2019; Lattin and Kelly, 2020). Inferring GR activity through these chemically-induced measurements, however, is not enough to fully understand the role of GR activity on GC regulation. For instance, dexamethasone is actively transported away from the brain at the level of blood brain barrier, which results in dexamethasone mainly binding to GRs in the pituitary gland when compared to binding in the hypothalamus and hippocampus (De Kloet et al., 1975; Meijer et al., 1998; Cole et al., 2000).

Whereas the above evidence highlights the importance of GR actions on GC regulation, an additional step in our understanding of this regulation would be investigating the mechanisms that underlie variation in GR activity. Most recent research has suggested a physiological mediator of GR affinity and activity at a cellular level, i.e. FKBP5 protein, as a reliable indicator of HPA axis flexibility and stress resilience (Zimmer et al., 2020). FKBP5 is a cochaperone in the GR complex with an inhibitory effect on GR signaling and activity that may allow determining an individual's capacity for GR-mediated modifications of gene expression. Additionally, other mechanisms can also modulate the intensity of GC actions after GR binding, potentially shaping the associations between GC concentrations, GR expression, phenotype and fitness (e.g. post-transcriptional control, epigenetic modifications; see Section 5). Although this work is focused on the role of GR and aims at motivating researchers to consider GR activity when investigating GC variation and dynamics, we acknowledge the complexity of the system (e.g. pre-binding regulatory components, MR/GR interactions, or post-transcription modulators), and further hope that our considerations will also increase the interest of researchers on a more systemic approach in order to link HPA activity and phenotypic responses.

5. Outstanding questions and further directions

Despite the existing evidence supporting the central role of GR on GC dynamics, empirical data on the associations between GR expression and GC variation are still limited, especially in free-living organisms. Improving our understanding on GC variation and regulation in the wild may provide us with fundamental knowledge regarding the relative importance of specific environmental or intrinsic factors on both GC and GR function. Compared to research in captivity, working with free-living individuals may reveal ecologically relevant differences in the link between GC regulation and GR activity, derived from variation in factors such as early life environment, food availability, abiotic environment or social context, which may underlie context-dependent associations with fitness components. It will also help to answer an enduring question in evolutionary endocrinology: whether selection acts equivalently on circulating hormone levels and receptors or whether one type of trait may be the primary target of selection. In this section, we compile promising research avenues and unanswered questions that we hope will help researchers interpret and delineate the ecological relevance and evolutionary implications of GR and in particular of GR expression. We also summarize the main physiological mechanisms and ecological factors that may underlie variation in GR activity and expression and therefore should be considered in upcoming studies, along with methodological aspects to consider.

5.1. Environmental effects, GR plasticity and within- vs. among-individual variation

Understanding the extent to which environment experienced throughout different life stages can influence variation in GR expression becomes relevant towards accurately investigating the role of GR in mediating GC phenotype. However, plasticity in GR expression remains poorly understood, in part because within-individual changes in HPA regulation are rarely quantified in an ecological context. Previous evidence – observational and experimental - has reported dynamic within-individual changes in GR expression triggered by environmental and intrinsic factors such as seasonality (Breuner and Orchinik, 2001), increased energy expenditure (Lu et al., 2017), increased foraging costs (Jimeno et al., 2019), social environment (Cornelius et al., 2018) or exposure to exogenous GCs (Spencer et al., 1991). Interestingly, all these studies point at GR expression being lower in more challenging environments. This would entail lower GC plasticity and efficiency, which might come as a consequence of the need to maintain higher overall GC levels as a response to increased, sustained energetic demands. Whether this pattern occurs in both the short- and long-term remains to be investigated. Moreover, although the effects of those GR responses on individual phenotype and coping capacity remain poorly investigated,

recent work on wild individuals kept in captivity has started to shed light on the magnitude and context-dependence of the associations between GC concentrations or responses, and GR expression under manipulated environmental conditions (Krause et al., 2021; Cornelius et al., 2018; Krause et al., 2015). Social information from food restricted individuals led to reduced GR expression in HPA tissues in Red crossbills (*Loxia curvirostra*), but the association between these changes and changes in GC concentrations was shaped by the foraging conditions experienced by target individuals (Cornelius et al., 2018). Krause et al. (2021) found no rapid change in GR expression in White-crowned sparrows (*Zonotrichia leucophrys*) during a standard stress response, nor a correlation between receptor expression and GC concentrations at different time points of this response. These results point at environmentally-triggered changes in GR expression to be context- or tissue- dependent (see Section 5.2), and highlight the importance of studying the role of GR on the fine-tuning of GC responses and their plasticity.

Enduring effects of environmental conditions on gene expression can be mediated by epigenetic regulation of gene transcription. Epigenetic mechanisms allow the integration of intrinsic and environmental signals in the genome and can lead to activation or suppression of gene expression (Jaenisch and Bird, 2003). Previous research has pointed to GR being a primary target for long-term epigenetic programming of HPA function, modulating HPA axis regulation depending on the environments experienced by individuals (Meaney et al., 2007; Bartlett et al., 2019). The GR gene (*NR3C1*) in particular has been shown to be sensitive to early-life environmental conditions and this effect has been attributed to epigenetic processes (Yehuda et al., 2014; Weaver et al., 2004; Hompes et al., 2013; Zhang et al., 2013). In rats, offspring of mothers that naturally show high pup licking/grooming behaviors exhibit increased hippocampal GR expression and enhanced negative-feedback efficacy in adulthood. This increased GR expression was associated with lower DNA methylation in GR promoter region (Liu et al., 1997; Weaver et al., 2004). Research on epigenetic regulation of GR in an ecological context is on its infant stage, with to our best knowledge only one study focusing on environmentally-induced epigenetic processes in the GR gene in the wild (Rubenstein et al., 2016). These studies, along with further data on the associations among environmental variation, epigenetic processes, GR expression and phenotypic outcomes, are crucial towards understanding the role of GR in adaptation to changing environments.

An enduring question in evolutionary endocrinology is whether selection acts equivalently on circulating hormone levels and receptors or whether one type of trait (e.g., receptors) is the principal target of selection. In this case, GR expression should be the trait that predicts fitness, but GC concentrations could also predict fitness because of phenotypic correlations with the trait under selection (i.e. GR expression; Hau, 2007, Ketterson et al., 2009). To resolve this question, we need to measure both GR expression and GC concentrations. This may also involve measuring heritability of this traits, their genetic vs. phenotypic correlations, and their association with fitness. We are starting to get some of this measures for GCs (Bairos-Novak et al., 2018; Béziers et al., 2019; Breuner and Berk, 2019; Jenkins et al., 2014; Schoenle et al., 2021; Schoenle et al., 2018; Stedman et al., 2017). However, such data for GR (and other regulatory components of the HPA axis) do not exist. As already mentioned, this likely stems from the requirement of terminal sampling to measure GR expression in the brain but also from logistical constraints associated with the required sample sizes to get meaningful estimates of heritability and genetic correlations.

5.2. Is GR expression correlated among tissues?

In ecological studies, a fundamental aspect to consider when using GR expression to infer GC regulation at an organismal level is whether GR expression levels in regulatory regions of the HPA axis (hypothalamus, hippocampus, pituitary) and in other tissues (e.g. blood) are comparable. This is key towards establishing reliable indicators of variation in GC function, and more specifically towards the use of non-terminal sampling such as blood samples to infer GR expression in HPA axis regulatory regions, or at an organismal level. Indeed, in wildlife, work on GR expression in the HPA axis is rare as it involves terminal sampling (Hau et al., 2016). If such correlations exist, it would allow estimating GR function in the HPA axis in species where terminal sampling is not possible, as well as obtaining longitudinal data. For species where terminal sampling is possible for a limited number of individuals, this would involve establishing the existence of these relationships by measuring GR expression in the different regions of the HPA axis and in some peripheral tissues (e.g. blood, saliva) within individuals. If terminal sampling is not possible at all, a possibility would be to determine whether these relationships exist in one or more related species exposed to similar environmental pressures. Assessment of GR expression in peripheral tissues is widely used in humans and lab rodents, and has been encouraged by some studies showing that expression of some genes are correlated between peripheral and central regions (Tylee et al., 2013). Nevertheless, whether these correlations are restricted to a limited number of genes or tissues remains to be investigated (Tylee et al., 2013), and evidences for GR are mixed. Some data suggest that GR signaling is correlated among brain regions integral to the regulation of GCs and blood (Daskalakis et al., 2014), while studies in wildlife have also revealed distinct patterns of GR expression in peripheral and central tissues (Lattin et al., 2015; Lattin and Romero, 2014). If upcoming studies would not find support for correlations between GR expression in peripheral tissues and HPA axis regions, research may still benefit from investigating the information that we can obtain from GR expression in peripheral tissues (i.e. non-lethal sampling) and its dynamics to improve our understanding of stress coping capacity, as it is already often done in human studies. Besides, we expect upcoming advances on disciplines such as transcriptomics to bring promising research venues towards the study of GR expression and its variation without requiring lethal sampling.

An interesting possibility that remains widely unexplored is that what characterizes individuals and is comparable among tissues is not absolute GR expression levels at one time-point, but the plasticity in GR expression in response to external (e.g. weather conditions, social environment, predator pressure, etc.) and internal (e.g. energy expenditure, illnesses, etc.) factors. Thus, it may occur that central and peripheral tissues within individuals show different levels of GR expression, but that the degree of change in GR expression in response to a challenge is similar among tissues. Further experiments testing for the effects of environmental manipulations on GR expression across central and peripheral tissues simultaneously are needed to test this idea and shed light on the potential use of peripheral tissues to infer GR expression plasticity at an organismal level.

5.3. The importance of the study species

Most studies to date that have provided information on the interplay between GR expression and GC variation have been carried out in the context of biomedical research (e.g. Meijer and de Kloet, 1998; Jutapakdeegul et al., 2003; Ridder et al., 2005). Biomedical studies are mostly performed in captivity on domesticated species, actively or passively selected for reduced stress responsivity. Thus, the measures of GR expression or plasma GC levels that they report are often pharmacologically induced (e.g. adrenalectomy, chemical suppression of receptor binding), or beyond the upper thresholds of natural GC levels (e.g. by administration of exogenous GCs), and thus – although mechanistically revealing - likely ecologically irrelevant. These shortcomings lead to a gap between the outcomes of these studies, and the mechanistic insights that they provide, and a deeper understanding of the interplay between non-manipulated GR expression and endogenous GC regulation, which is almost absent in an ecological context. Differences between model and non-model organisms may also lead to discrepancy among results. For instance, lab rodent lines have been artificially

selected for hundreds of generations in most of the cases, which may reduce the genetic diversity among individuals - and potentially trait plasticity and phenotypic variance – or favour fixed alternative variants that may difficult the replication of results among colonies (Brekke et al., 2018). This could entail limitations in the capacity of these studies to detect variation in correlation patterns (e.g. between GR expression and plasma GCs) among individuals.

Another potentially relevant difference among study species, such as mammals and birds, lies in birds having nucleated erythrocytes (which are also found in reptiles and amphibians). Thus, in studies using peripheral blood as target tissue, RNA used for gene expression analyses will come only from white blood cells in mammals, and mostly from red blood cells (which entail >95 % of cell counts) in birds. Although the functional role of GR in red blood cells remains to be investigated, this difference may have relevant consequences for the study of correlations between gene expression and phenotype.

6. Conclusions

Because GCs only exert their actions when bound to receptors, glucocorticoid receptors play a fundamental role on mediating GC function and downstream phenotypic changes. Here, we propose studies in ecology and evolutionary endocrinology should focus on GR in order to obtain information on GC function and regulation, organismal capacity to respond appropriately to environmental changes, and HPA axis evolution. We base this prediction on the fact that GR binding is the pivotal step towards GC integration into cells and tissues – and therefore their physiological and behavioral outcomes –, and the trigger of negative feedback within the HPA axis. Thus, GR expression may provide crucial and integrative information on how vertebrates species cope with environmental challenges through HPA axis activity.

The potential of GR expression as a tool to assess GC plasticity and efficiency may be particularly relevant for ecological studies when measures of GR expression in peripheral tissues (i.e. blood) mirror expression in HPA axis tissues. This illustrates the importance of further research testing to what extent GR expression and dynamics in HPA axis and peripheral tissues are comparable, as well as whether they are affected by environmental variability and challenges in a comparable way. Answering this and other questions on GR expression dynamics becomes fundamental towards an integrative, easily-quantified and least-invasive measure of HPA regulation capacity.

The extent to which variation in circulating hormones reflects variation in other important regulators of the HPA axis, and whether circulating GCs, receptors, or other regulators are the primary targets of selection, remain poorly understood. We expect GC plasticity and efficiency, as well as plasticity in GR expression, to facilitate dynamic and rapid responses to environmental fluctuations. Through its role on GC function and regulation, GR expression and its mediators thus rise as potential targets of selection and key sources of adjustment to changing environments, which becomes particularly relevant in the current context of global change.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

No data was used for the research described in the article.

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References

Anacker, C., Zunszain, P.A., Carvalho, L.A., Pariante, C.M., 2011. The glucocorticoid receptor: pivot of depression and of antidepressant treatment? Psychoneuroendocrinology 36, 415–425. https://doi.org/10.1016/j.psyneuen.2010.03.007.

Bairos-Novak, K.R., Ryan, C.P., Freeman, A.R., Anderson, W.G., Hare, J.F., 2018. Like mother, like daughter: heritability of female Richardson's ground squirrel Urocitellus richardsonii cortisol stress responses. Curr.Zool. 64 (2), 153–163.

Bartlett, A.A., Lapp, H.E., Hunter, R.G., 2019. Epigenetic mechanisms of the glucocorticoid receptor. Trends Endocrinol.Metab. 30 (11), 807–818.

Baugh, A.T., Senft, R.A., Firke, M., Lauder, A., Schroeder, J., Meddle, S.L., Hau, M., 2017. Risk-averse personalities have a systemically potentiated neuroendocrine stress axis: a multilevel experiment in Parus major. Horm. Behav. 93, 99–108. https://doi.org/10.1016/j.yhbeh.2017.05.011.

Béziers, P., San-Jose, L.M., Almasi, B., Jenni, L., Roulin, A., 2019. Baseline and stress-induced corticosterone levels are heritable and genetically correlated in a barn owl population. Heredity 123 (3), 337–348.

Blas, J., Baos, R., Bortolotti, G.R., Marchant, T.A., Hiraldo, F., 2006. Age-related variation in the adrenocortical response to stress in nestling white storks (Ciconia ciconia) supports the developmental hypothesis. Gen. Comp. Endocrinol. 148 (2), 172–180.

Boonstra, R., Singleton, G.R., 1993. Population declines in the snowshoe hare and the role of stress. Gen. Comp. Endocrinol. 91 (2), 126–143.

Boyle, M.P., Brewer, J.A., Funatsu, M., Wozniak, D.F., Tsien, J.Z., Izumi, Y., Muglia, L.J., 2005. Acquired deficit of forebrain glucocorticoid receptor produces depression-like changes in adrenal axis regulation and behavior. Proc. Natl. Acad. Sci. U. S. A. 102 (2), 473–478. https://doi.org/10.1073/pnas.0406458102.

Bradley, A.J., 1990. Failure of glucocorticoid feedback during breeding in the male red-tailed phascogale Phascogale calura (Marsupialia: Dasyuridae). J. Steroid Biochem. Mol. Biol. 37 (1), 155–163.

Brekke, T.D., Steele, K.A., Mulley, J.F., 2018. Inbred or outbred? Genetic diversity in laboratory rodent colonies. G3: genes, genomesGenetics 8 (2), 679–686.

Breuner, C.W., Berk, S.A., 2019. Using the van Noordwijk and de Jong resource framework to evaluate glucocorticoid-fitness hypotheses. Integr. Comp. Biol. 59 (2), 243–250.

Breuner, C.W., Orchinik, M., 2001. Seasonal regulation of membrane and intracellular corticosteroid receptors in the house sparrow brain. J. Neuroendocrinol. 13 (5), 412–420. https://doi.org/10.1046/j.1365-2826.2001.00646.x.

Breuner, C.W., Orchinik, M., 2002. Beyond carrier proteins: plasma binding proteins as mediators of corticosteroid action in vertebrates. J. Endocrinol. 175, 99–112.

Breuner, C.W., Delehanty, B., Boonstra, R., 2013. Evaluating stress in natural populations of vertebrates: total CORT is not good enough. Funct. Ecol. 27 (1), 24–36.

Buwalda, B., Scholte, J., de Boer, S.F., Coppens, C.M., Koolhaas, J.M., 2012. The acute glucocorticoid stress response does not differentiate between rewarding and aversive social stimuli in rats. Horm. Behav. 61 (2), 218–226.

Caulfield, J.I., Cavigelli, S.A., 2020. Individual differences in glucocorticoid regulation: does it relate to disease risk and resilience? Front. Neuroendocrinol. 56, 100803.

Checkley, S., 1996. The neuroendocrinology of depression and chronic stress. Br. Med. Bull. 52 (3), 597–617. https://doi.org/10.1093/oxfordjournals.bmb.a011570.

Cohen, D.M., Steger, D.J., 2017. Nuclear receptor function through genomics: lessons from the glucocorticoid receptor. Trends Endocrinol. Metab. 28, 531–540. https://doi.org/10.1016/j.tem.2017.04.001.

Cole, M.A., Kim, P.J., Kalman, B.A., Spencer, R.L., 2000. Dexamethasone suppression of corticosteroid secretion: evaluation of the site of action by receptor measures and functional studies. Psychoneuroendocrinology 25 (2), 151–167.

Cornelius, J.M., Perreau, G., Bishop, V.R., Krause, J.S., Smith, R., Hahn, T.P., Meddle, S. L., 2018. Social information changes stress hormone receptor expression in the songbird brain. Horm. Behav. 97, 31–38. https://doi.org/10.1016/j.yhbeh.2017.10.002.

Culbert, B.M., Gilmour, K.M., Balshine, S., 2018. Stress axis regulation during social Ascension in a group-living cichlid fish. Horm. Behav. 103, 121–128.

Dantzer, B., Fletcher, Q.E., Boonstra, R., Sheriff, M.J., 2014. In: Themed Issue Article: Stress in Vertebrates Measures of Physiological Stress: A Transparent or Opaque Window Into the Status, Management and Conservation of Species ?, 2(May), pp. 1–18. https://doi.org/10.1093/conphys/cou023.

Daskalakis, N.P., Cohen, H., Cai, G., Buxbaum, J.D., Yehuda, R., 2014. Expression profiling associates blood and brain glucocorticoid receptor signaling with trauma-related individual differences in both sexes. Proc. Natl. Acad. Sci. 111 (37), 13529–13534.

Datson, N.A., Morsink, M.C., Meijer, O.C., de Kloet, E.R., 2008. Central corticosteroid actions: search for gene targets. Eur. J. Pharmacol. 583, 272–289. https://doi.org/10.1016/j.ejphar.2007.11.070.

De Kloet, E.R., 2004. Hormones and the stressed brain. Ann. N.YAcad. Sci. 1018, 1–15. https://doi.org/10.1196/annals.1296.001.

De Kloet, R., Wallach, G., McEwen, B.S., 1975. Differences in corticosterone and dexamethasone binding to rat brain and pituitary. Endocrinology 96 (3), 598–609.

De Kloet, E.R., Vreugdenhil, E., Oitzl, M.S., Joëls, M., 1998. Brain corticosteroid receptor balance in health and disease. Endocr. Rev. 19 (3), 269–301.

Dickens, M., Romero, L.M., Cyr, N.E., Dunn, I.C., Meddle, S.L., 2009. Chronic stress alters glucocorticoid receptor and mineralocorticoid receptor mRNA expression in the European Starling (Sturnus vulgaris) brain. J. Neuroendocrinol. 21 (10), 832–840. https://doi.org/10.1111/j.1365-2826.2009.01908.x.

Dufty Jr., A.M., Clobert, J., Møller, A.P., 2002. Hormones, developmental plasticity and adaptation. Trends Ecol. Evol. 17 (4), 190–196.

Eikenaar, C., Klinner, T., Stöwe, M., 2014. Corticosterone predicts nocturnal restlessness in a long-distance migrant. Horm. Behav. 66 (2), 324–329. https://doi.org/10.1016/j.yhbeh.2014.06.013.

González'Ramírez, C., Villavicencio Queijeiro, A., Jiménez Morales, S., Bárcenas López, D., Hidalgo Miranda, A., Ruiz Chow, A., Guardado Estrada, M., 2020. The NR3C1 gene expression is a potential surrogate biomarker for risk and diagnosis of posttraumatic stress disorder. Psychiatry Res. 284, 112797 https://doi.org/10.1016/j.psychres.2020.112797.

Han, Q.-Q., Yang, L., Huang, H.-J., Wang, Y.-L., Yu, R., Wang, J., Yu, J., 2017. Differential GR expression and translocation in the hippocampus mediates susceptibility vs. resilience to chronic social defeat stress. Front. Neurosci. 11 (MAY), 287. https://doi.org/10.3389/fnins.2017.00287.

Hau, M., 2007. Regulation of male traits by testosterone: implications for the evolution of vertebrate life histories. BioEssays 29 (2), 133–144.

Hau, F., Casagrande, M., Hau, M., Casagrande, S., Ouyang, J.Q., Baugh, A.T., Baugh, 2016. Glucocorticoid-Mediated Phenotypes in Vertebrates: Multilevel Variation and Evolution. https://doi.org/10.1016/bs.asb.2016.01.002.

Haussmann, M.F., Longenecker, A.S., Marchetto, N.M., Juliano, S.A., Bowden, R.M., 2012. Embryonic exposure to corticosterone modifies the juvenile stress response, oxidative stress and telomere length. Proc. R. Soc. B Biol. Sci. 279 (1732), 1447–1456.

Holsboer, F., 2000. The corticosteroid receptor hypothesis of depression. Neuropsychopharmacology 23 (5), 477–501. https://doi.org/10.1016/S0893-133X(00)00159-7.

Hompes, T., Izzi, B., Gellens, E., Morreels, M., Fieuws, S., Pexsters, A., Claes, S., 2013. Investigating the influence of maternal cortisol and emotional state during pregnancy on the DNA methylation status of the glucocorticoid receptor gene (NR3C1) promoter region in cord blood. J. Psychiatr. Res. 47 (7), 880–891. https://doi.org/10.1016/j.jpsychires.2013.03.009.

Jaenisch, R., Bird, A., 2003. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat. Genet. 33 (3), 245–254.

Jenkins, B.R., Vitousek, M.N., Hubbard, J.K., Safran, R.J., 2014. An experimental analysis of the heritability of variation in glucocorticoid concentrations in a wild avian population. Proc. R. Soc. B 281, 20141302.

Jimeno, B., Hau, M., Verhulst, S., 2018. Corticosterone levels reflect variation in metabolic rate, independent of 'stress'. Sci. Rep. 8 (1) https://doi.org/10.1038/s41598-018-31258-z.

Jimeno, B., Hau, M., Gómez-Díaz, E., Verhulst, S., 2019. 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), 1–11. https://doi.org/10.1038/s41598-019-52203-8.

Jutapakdeegul, N., Casalotti, S.O., Govitrapong, P., Kotchabhakdi, N., 2003. Postnatal touch stimulation acutely alters corticosterone levels and glucocorticoid receptor gene expression in the neonatal rat. Dev. Neurosci. 25 (1), 26–33.

Kadmiel, M., Cidlowski, J.A., 2013. Glucocorticoid receptor signaling in health and disease. Trends Pharmacol. Sci. 34 (9), 518–530.

Ketterson, E.D., Atwell, J.W., McGlothlin, J.W., 2009. Phenotypic integration and independence: hormones, performance, and response to environmental change. Integr. Comp. Biol. 49 (4), 365–379.

Koolhaas, J.M., Bartolomucci, A., Buwalda, B., de Boer, S.F., Flügge, G., Korte, S.M., Fuchs, E., 2011. Stress revisited: a critical evaluation of the stress concept. Neurosci. Biobehav. Rev. 35 (5), 1291–1301.

Krause, J.S., McGuigan, M.A., Bishop, V.R., Wingfield, J.C., Meddle, S.L., 2015. Decreases in mineralocorticoid but not glucocorticoid receptor mRNA expression during the short Arctic breeding season in free-living Gambel's white-crowned sparrow (Zonotrichia leucophrys gambelii). J. Neuroendocrinol. 27 (1), 66–75. https://doi.org/10.1111/jne.12237.

Krause, J.S., Pérez, J.H., Reid, A.M.A., Cheah, J., Bishop, V., Wingfield, J.C., Meddle, S. L., 2021. Acute restraint stress does not alter corticosteroid receptors or 11β-hydroxysteroid dehydrogenase gene expression at hypothalamic–pituitary-adrenal axis regulatory sites in captive male white-crowned sparrows (Zonotrichia leucophrys gambelii). Gen. Comp. Endocrinol. 303, 113701 https://doi.org/10.1016/j.ygcen.2020.113701.

Landys, M.M., Ramenofsky, M., Wingfield, J.C., 2006. Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. Gen. Comp. Endocrinol. 148 (2), 132–149.

Lattin, C.R., Kelly, T.R., 2020. Glucocorticoid negative feedback as a potential mediator of trade-offs between reproduction and survival. Gen. Comp. Endocrinol. 286, 113301 https://doi.org/10.1016/j.ygcen.2019.113301.

Lattin, C.R., Romero, L.M., 2014. Chronic stress alters concentrations of corticosterone receptors in a tissue-specific manner in wild house sparrows (Passer domesticus). J. Exp. Biol. 217 (14), 2601–2608. https://doi.org/10.1242/jeb.103788.

Lattin, C.R., Keniston, D.E., Reed, J.M., Romero, L.M., 2015. Are receptor concentrations correlated across tissues within individuals? A case study examining glucocorticoid and mineralocorticoid receptor binding. Endocrinology 156 (4), 1354–1361. https://doi.org/10.1210/en.2014-1811.

Lee, R.S., Tamashiro, K.L.K., Yang, X., Purcell, R.H., Harvey, A., Willour, V.L., Potash, J. B., 2010. Chronic corticosterone exposure increases expression and decreases deoxyribonucleic acid methylation of Fkbp5 in mice. Endocrinology 151 (9), 4332–4343. https://doi.org/10.1210/en.2010-0225.

Li, D., Davis, J.E., Wang, G., Nabi, G., Bishop, V.R., Sun, Y., Lei, F., 2020. Coping with extremes: remarkably blunt adrenocortical responses to acute stress in two sympatric snow finches on the Qinghai-Tibet Plateau during winter relative to other seasons. Gen. Comp. Endocrinol. 291, 113434 https://doi.org/10.1016/j.ygcen.2020.113434.

Liebl, A.L., Martin, L.B., 2013. Stress hormone receptors change as range expansion progresses in house sparrows. Biol. Lett. 9 (3), 20130181.

Liu, P.Z., Nusslock, R., 2017. How stress gets under the skin: early life adversity and glucocorticoid receptor epigenetic regulation. Curr.Genom. 19 (8), 653–664. https://doi.org/10.2174/1389202919666171228164350.

Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., Meaney, M.J., 1997. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. Science 277 (5332), 1659–1662. https://doi.org/10.1126/science.277.5332.1659.

Lu, K.D., Cooper, D., Haddad, F., Zaldivar, F., Kraft, M., Radom-Aizik, S., 2017. Glucocorticoid receptor expression on circulating leukocytes in healthy and asthmatic adolescents in response to exercise. Pediatr. Res. 82 (2), 261–271. https://doi.org/10.1038/pr.2017.66.

MacDougall-Shackleton, S.A., Schmidt, K.L., Furlonger, A.A., MacDougall-Shackleton, E. A., 2013. HPA axis regulation, survival, and reproduction in free-living sparrows: functional relationships or developmental correlations? Gen. Comp. Endocrinol. 190, 188–193. https://doi.org/10.1016/j.ygcen.2013.05.026.

MacDougall-Shackleton, S.A., Bonier, F., Romero, L.M., Moore, I.T., 2019. Glucocorticoids and "stress" are not synonymous. Integr.Org.Biol. 1 (1), obz017.

Madison, F.N., Kesner, A.J., Alward, B.A., Ball, G.F., 2018. Sex differences in hippocampal mineralocorticoid and glucocorticoid receptor mRNA expression in response to acute mate pair separation in zebra finches (Taeniopygia guttata). Hippocampus 28 (10), 698–706. https://doi.org/10.1002/hipo.22952.

Madliger, C.L., Love, O.P., 2016. Employing individual measures of baseline glucocorticoids as population-level conservation biomarkers: considering within-individual variation in a breeding passerine. ConservationPhysiology 4 (1), cow048. https://doi.org/10.1093/conphys/cow048.

Madliger, C.L., Semeniuk, C.A.D., Harris, C.M., Love, O.P., 2015. Assessing baseline stress physiology as an integrator of environmental quality in a wild avian population: implications for use as a conservation biomarker. Biol. Conserv. 192, 409–417. https://doi.org/10.1016/j.biocon.2015.10.021.

McGlothlin, J.W., Jawor, J.M., Ketterson, E.D., 2007. Natural variation in a testosterone-mediated trade-off between mating effort and parental effort. Am. Nat. 170 (6), 864–875.

McGlothlin, J.W., Whittaker, D.J., Schrock, S.E., Gerlach, N.M., Jawor, J.M., Snajdr, E.A., Ketterson, E.D., 2010. Natural selection on testosterone production in a wild songbird population. Am. Nat. 175 (6), 687–701.

Monaghan, P., 2014. Organismal stress, telomeres and life histories. J. Exp. Biol. 217 (1), 57–66.

McCormick, S.D., Romero, L.M., 2017. Conservation endocrinology. Bioscience 67 (5), 429–442.

McDonald, I.R., Lee, A.K., Than, K.A., Martin, R.W., 1986. Failure of glucocorticoid feedback in males of a population of small marsupials (Antechinus swainsonii) during the period of mating. J. Endocrinol. 108 (1), 63–68.

McEwen, B.S., Wingfield, J.C., 2003. The concept of allostasis in biology and biomedicine. Horm. Behav. 43 (1), 2–15. https://doi.org/10.1016/S0018-506X(02)00024-7.

Meaney, M.J., Szyf, M., Seckl, J.R., 2007. Epigenetic mechanisms of perinatal programming of hypothalamic-pituitary-adrenal function and health. Trends Mol. Med. 13 (7), 269–277.

Meijer, O.C., de Kloet, E.R., 1998. Corticosterone and serotonergic neurotransmission in the hippocampus: functional implications of central corticosteroid receptor diversity. Critical Reviews™Neurobiology 12 (1–2).

Meijer, O.C., De Lange, E.C.M., Breimer, D.D., De Boer, A.G., Workel, J.O., De Kloet, E.R., 1998. Penetration of dexamethasone into brain glucocorticoid targets is enhanced in mdr1A P-glycoprotein knockout mice. Endocrinology 139 (4), 1789–1793.

Moritz, K.M., Boon, W.M., Wintour, E.M., 2005. Glucocorticoid programming of adult disease. Cell Tissue Res. 322 (1), 81–88.

O'Reilly, K.M., Wingfield, J.C., 2001. Ecological factors underlying the adrenocortical response to capture stress in Arctic-breeding shorebirds. Gen. Comp. Endocrinol. 124 (1), 1–11. https://doi.org/10.1006/gcen.2001.7676.

O'Connor, M.I., Pennell, M.W., Altermatt, F., Matthews, B., Melián, C.J., Gonzalez, A., 2019. Principles of ecology revisited: integrating information and ecological theories for a more unified science. Front. Ecol. Evol. 7, 219.

Revollo, J.R., Cidlowski, J.A., 2009. Mechanisms generating diversity in glucocorticoid receptor signaling. Ann. N. Y. Acad. Sci. 1179 (1), 167–178. https://doi.org/10.1111/j.1749-6632.2009.04986.x.

Ridder, S., Chourbaji, S., Hellweg, R., Urani, A., Zacher, C., Schmid, W., Gass, P., 2005. Mice with genetically altered glucocorticoid receptor expression show altered sensitivity for stress-induced depressive reactions. J. Neurosci. 25 (26), 6243–6250. https://doi.org/10.1523/JNEUROSCI.0736-05.2005.

Romero, L.M., 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. Gen. Comp. Endocrinol. 128 (1), 1–24.

Romero, L.M., 2004. Physiological stress in ecology: lessons from biomedical research. Trends Ecol.Evol. 19 (5), 249–255. https://doi.org/10.1016/j.tree.2004.03.008.

Romero, L.M., Beattie, U.K., 2022. Common myths of glucocorticoid function in ecology and conservation. J.Exp.Zool.Part A: Ecol.Integr.Physiol. 337 (1), 7–14.

Romero, L.M., Reed, J.M., 2005. Collecting baseline corticosterone samples in the field: is under 3 min good enough? Comp. Biochem. Physiol. A Mol. Integr. Physiol. 140 (1), 73–79. https://doi.org/10.1016/j.cbpb.2004.11.004.

Romero, L.M., Wikelski, M., 2010. Stress physiology as a predictor of survival in Galapagos marine iguanas. Proc. R. Soc. B Biol. Sci. 277 (1697), 3157–3162. https://doi.org/10.1098/rspb.2010.0678.
Romero, L.M., Wingfield, J.C., 2016. Tempests, Poxes, Predators, and People: Stress in Wild Animals and How They Cope. Oxford University Press.
Romero, L.M., Dickens, M.J., Cyr, N.E., 2009. The reactive scope model—a new model integrating homeostasis, allostasis, and stress. Horm. Behav. 55 (3), 375–389.
Rubenstein, D.R., Skolnik, H., Berrio, A., Champagne, F.A., Phelps, S., Solomon, J., 2016. Sex-specific fitness effects of unpredictable early life conditions are associated with DNA methylation in the avian glucocorticoid receptor. Mol. Ecol. 25 (8), 1714–1728.
Sapolsky, R.M., 1983. Endocrine aspects of social instability in the olive baboon (Papio anubis). Am. J. Primatol. 5 (4), 365–379.
Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids influence stress responses? Preparative actions*. Endocr. Rev. 21 (April), 55–89. https://doi.org/10.1210/er.21.1.55.
Schoenle, L.A., Zimmer, C., Vitousek, M.N., 2018. Understanding context dependence in glucocorticoid–fitness relationships: the role of the nature of the challenge, the intensity and frequency of stressors, and life history. Integr. Comp. Biol. 58 (4), 777–789.
Schoenle, L.A., Zimmer, C., Miller, E.T., Vitousek, M.N., 2021. Does variation in glucocorticoid concentrations predict fitness? A phylogenetic meta-analysis. Gen. Comp. Endocrinol. 300, 113611.
Spencer, R.L., Miller, A.H., Stein, M., McEwen, B.S., 1991. Corticosterone regulation of type I and type II adrenal steroid receptors in brain, pituitary, and immune tissue. Brain Res. 549 (2), 236–246. https://doi.org/10.1016/0006-8993(91)90463-6.
Spencer, R.L., Chun, L.E., Hartsock, M.J., Woodruff, E.R., 2018. 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, 52–71. https://doi.org/10.1016/j.yfrne.2017.12.005.
Stedman, J.M., Hallinger, K.K., Winkler, D.W., Vitousek, M.N., 2017. Heritable variation in circulating glucocorticoids and endocrine flexibility in a free-living songbird. J. Evol. Biol. 30 (9), 1724–1735.
Taff, C.C., Vitousek, M.N., 2016. Endocrine flexibility: optimizing phenotypes in a dynamic world? Trends Ecol. Evol. 31 (6), 476–488.
Tasker, J.G., Di, S., Malcher-Lopes, R., 2006. Rapid glucocorticoid signaling via membrane-associated receptors. Endocrinology 147 (12), 5549–5556. https://doi.org/10.1210/en.2006-0981.
Tylee, D.S., Kawaguchi, D.M., Glatt, S.J., 2013. On the outside, looking in: a review and evaluation of the comparability of blood and brain "-omes". Am. J. Med. Genet. B Neuropsychiatr. Genet. 162 (7), 595–603. https://doi.org/10.1002/ajmg.b.32150.
Vitousek, M.N., Taff, C.C., Ryan, T.A., Zimmer, C., 2019. Stress resilience and the dynamic regulation of glucocorticoids. Integr. Comp. Biol. 59 (2), 251–263. https://doi.org/10.1093/icb/icz087.
Weaver, I.C.G., Cervoni, N., Champagne, F.A., D'Alessio, A.C., Sharma, S., Seckl, J.R., Meaney, M.J., 2004. Epigenetic programming by maternal behavior. Nat. Neurosci. 7 (8), 847–854. https://doi.org/10.1038/nn1276.
Wingfield, J.C., Sapolsky, R.M., 2003. Reproduction and resistance to stress: when and how. J. Neuroendocrinol. 15 (8), 711–724.
Wingfield, J.C., Maney, D.L., Breuner, C.W., Jacobs, J.D., Lynn, S., Ramenofsky, M., Richardson, R.D., 1998. Ecological Bases of Hormone-Behavior Interactions: The "Emergency Life History Stage" 1, vol. 38. academic.oup.com.
Yehuda, R., 2009. Status of glucocorticoid alterations in post-traumatic stress disorder. Ann. N. Y. Acad. Sci. 1179 (1), 56–69. https://doi.org/10.1111/j.1749-6632.2009.04979.x.
Yehuda, R., Cai, G., Golier, J.A., Sarapas, C., Galea, S., Ising, M., Buxbaum, J.D., 2009. Gene expression patterns associated with posttraumatic stress disorder following exposure to the world trade center attacks. Biol. Psychiatry 66 (7), 708–711. https://doi.org/10.1016/j.biopsych.2009.02.034.
Yehuda, R., Daskalakis, N.P., Lehrner, A., Desarnaud, F., Bader, H.N., Makotkine, I., Meaney, M.J., 2014. Influences of maternal and paternal PTSD on epigenetic regulation of the glucocorticoid receptor gene in holocaust survivor offspring. Am. J. Psychiatr. 171 (8), 872–880. https://doi.org/10.1176/appi.ajp.2014.13121571.
Yehuda, R., Hoge, C.W., McFarlane, A.C., Vermetten, E., Lanius, R.A., Nievergelt, C.M., Hyman, S.E., 2015. Post-traumatic stress disorder. Nat.Rev.Dis.Prim. 1, 1–22. https://doi.org/10.1038/nrdp.2015.57.
Zhang, T.Y., Labonté, B., Wen, X.L., Turecki, G., Meaney, M.J., 2013. Epigenetic mechanisms for the early environmental regulation of hippocampal glucocorticoid receptor gene expression in rodents and humans. Neuropsychopharmacology 38, 111–123. https://doi.org/10.1038/npp.2012.149.
Zimmer, C., Spencer, K.A., 2014. 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), 853–860. https://doi.org/10.1111/jne.12228.
Zimmer, C., Taff, C.C., Ardia, D.R., Ryan, T.A., Winkler, D.W., Vitousek, M.N., 2019. On again, off again: acute stress response and negative feedback together predict resilience to experimental challenges. Funct. Ecol. 33 (4), 619–628. https://doi.org/10.1111/1365-2435.13281.
Zimmer, C., Taff, C.C., Ardia, D.R., Rose, A.P., Aborn, D.A., Johnson, L.S., Vitousek, M. N., 2020. Environmental unpredictability shapes glucocorticoid regulation across populations of tree swallows. Sci. Rep. 10 (1), 1–13.
Zimmer, C., Hanson, H.E., Martin, L.B., 2021. FKBP5 expression is related to HPA flexibility and the capacity to cope with stressors in female and male house sparrows. Horm. Behav. 135, 105038.
Zimmer, C., Woods, H.A., Martin, L.B., 2022. Information theory in vertebrate stress physiology. Trends Endocrinol.Metab. 33 (1), 8–17.
Zimmeret al., n.d. C. Zimmer C.C. Taff D. R. Ardia K. A. Rosvall C. Kallenberg A. B. Bentz A.R. Taylor L. Scott Johnson M. Vitousek Gene expression in the female tree swallow brain is associated with among- and within-population variation in glucocorticoid levels. Submitted to this special issue.n.d.