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SYMPOSIUM

How Truly Conserved Is the "Well-Conserved" Vertebrate Stress Response?

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Synopsis The vertebrate stress response is considered to be a highly conserved suite of responses that are evolved to help animals survive noxious environmental stimuli. The two major pathways of the stress response include the catecholamine release that is part of the autonomic nervous system and comprises the immediate fight-or-flight response, and the slower release of corticosteroids from the hypothalamic–pituitary–adrenal axis that help orchestrate longer-term responses. These two pathways are present in every vertebrate yet examined, and the anatomical and physiological architecture underlying these pathways are consistent. Despite these structural similarities, however, recent data indicate substantial temporal and species variation in the actual regulation of these pathways. For example, activation of both pathways varies seasonally in some species but not others, and responses of both pathways can be extensively modulated by an individual's previous experience. Consequently, even though the anatomy of the stress response is highly conserved, the activation and functional output is not highly conserved. Given this variation, it is perhaps not surprising that it is proving difficult to correlate individual stress responses with differences in fitness outcomes. This review summarizes the challenge of making broad generalized assumptions about fitness consequences of the stress response given the functional variation we observe.

Introduction

The concept of stress was first introduced by Selye (1946) nearly 80 years ago, although many of the foundations were laid earlier by Cannon (1932). Selye and Cannon focused on what we now recognize as the two arms of the stress response. Cannon focused on adrenal medulla secretions that helped initiate what has been called the fight-or-flight response, and Selye focused on glucocorticoid release from the adrenal cortex. We now understand these two arms as forming an integrated stress response that can help an individual survive noxious environmental stimuli, but can cause disease, or chronic stress, when activated for too long (Sapolsky et al. 2000).

As work on the stress response progressed over the ensuing years, it became clear that the anatomical and physiological backbones of the two major arms of the stress response were highly conserved across taxa. With often just slight modifications, the anatomy and physiology of adrenal medullary tissue and the hypothalamic–pituitary–adrenal (HPA) axis are nearly identical in fish (Wendalaar Bonga 1997; Reid et al. 1998; Pankhurst 2011), amphibians (Carr 2011), reptiles (Tokarz and Summers 2011), birds (Blas 2015), and mammals (Romero and Butler 2007; Romero 2010). These highly conserved structures, coupled with the importance of the acute stress responses and the well-established role of chronic stress in inducing disease in humans and other animals, led many researchers to conclude that the balance between the positive effects of acute stress responses and the negative effects of chronic stress responses must play a vital role in the Darwinian fitness of the individual animal. In other words, the combined acute (presumed to be positive) and chronic (assumed to be negative) effects of the two preeminent pathways for coping with

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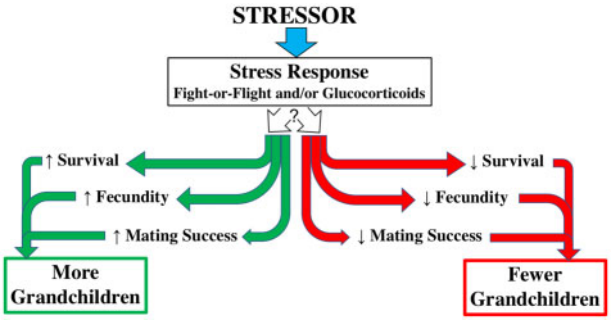

Fig. 1 The two main pathways for coping with a stressor and their potential connections to fitness.

acute noxious environmental events (fight-or-flight and glucocorticoid release) help determine how many grandchildren the individual will produce (Fig. 1)—grandchildren being the ultimate measure of an individual's Darwinian fitness. However, testing this idea had to wait until stress responses could be assessed in wild, freely-behaving animals in their natural habitats. After all, the "fitness" of laboratory rodents depends upon aspects of biology and behavior that are completely separate from the stress response (e.g., caretakers deciding which individuals shall mate).

Starting in the 1970s with studies using telemetry to monitor heart rate as an index of fight-or-flight responses (e.g., Kanwisher et al. 1978) and in the 1980s with studies of glucocorticoid release in free-living animals (Sapolsky 1982; Wingfield et al. 1982), there slowly built a literature documenting the impact of stress responses in connection with either direct (e.g., survival) or indirect (e.g., successful reproduction or clutch sizes) measures of fitness across a number of vertebrate taxa. These studies culminated in two influential review papers (Bonier et al. 2008; Breuner et al. 2008) that finally tested the hypotheses that aspects of the stress response were connected to fitness. The results were underwhelming. Neither review found much support for stress responses being correlated with fitness, but both found substantial interindividual variation in the relationship of glucocorticoids to fitness.

There are many potential sources for the variation, and both reviews spend considerable time discussing the possibilities. One potential source, however, is the fundamental assumption that the activation and function of the vertebrate stress response is highly conserved across individuals and species. Although much of the architecture of the stress response is clearly conserved, the perception of conserved function relies heavily on extrapolating from laboratory studies in domesticated animals. In this article, we revisit evidence that suggests that, as far as function, the vertebrate stress response is not "highly conserved." Rather, there is substantial taxonomic and interindividual variation, and it is this variation that has made it so difficult to test whether or not stress responses do, in fact, help regulate fitness.

Fight-or-flight response

Figure 2A shows the backbone of the fight-or-flight response (Romero and Wingfield 2016). The sympathetic nervous system sends axons to the adrenal medulla, which releases the catecholamines epinephrine and norepinephrine. Glucocorticoids from the adrenal cortex play a permissive role in this response. By increasing production of the catecholamine synthetic enzymes, glucocorticoids both augment and lengthen catecholamine release (Sapolsky et al. 2000). Both epinephrine and norepinephrine then affect numerous tissues throughout the body, with the fight-or-flight response primarily regulated via binding to the beta-adrenergic receptor. This backbone refers to the mammalian system, with minor differences (such as dispersed adrenal medullary tissue instead of a distinct adrenal medulla) in other taxa (Romero and Wingfield 2016). Included in Fig. 2 are starred steps in the pathway where current data indicate that the function of the pathway is not conserved either across time within an individual, between individuals of the same species, or across different species.

Although it is well known that glucocorticoid responses reflect the severity of the stressor, with stronger stressors eliciting greater glucocorticoid release, the same is true for fight-or-flight responses (1 in Fig. 2A). In other words, the fight-or-flight response is not an all-or-nothing response. Heart rate is a good, albeit imperfect, index for catecholamine release, especially when paired with analyses of heart rate variability (Cyr et al. 2009). For example, catecholamine receptor agonists and antagonists can increase and decrease heart rate, respectively (e.g., Cyr et al. 2009; Parker Fischer and Romero 2016), even though other activities, such as exercise, can alter heart rate as well. Using changes in heart rate as an index for catecholamine release in captive European starlings (*Sturnus vulgaris*), stronger stressors elicited greater elevations in heart rate that remained elevated for longer times (Nephew and Romero 2003). This indicates that connecting fight-or-flight responses to fitness will require understanding how the individual animal interprets the strength of the stressor that it is experiencing, which also might have implications to fitness.

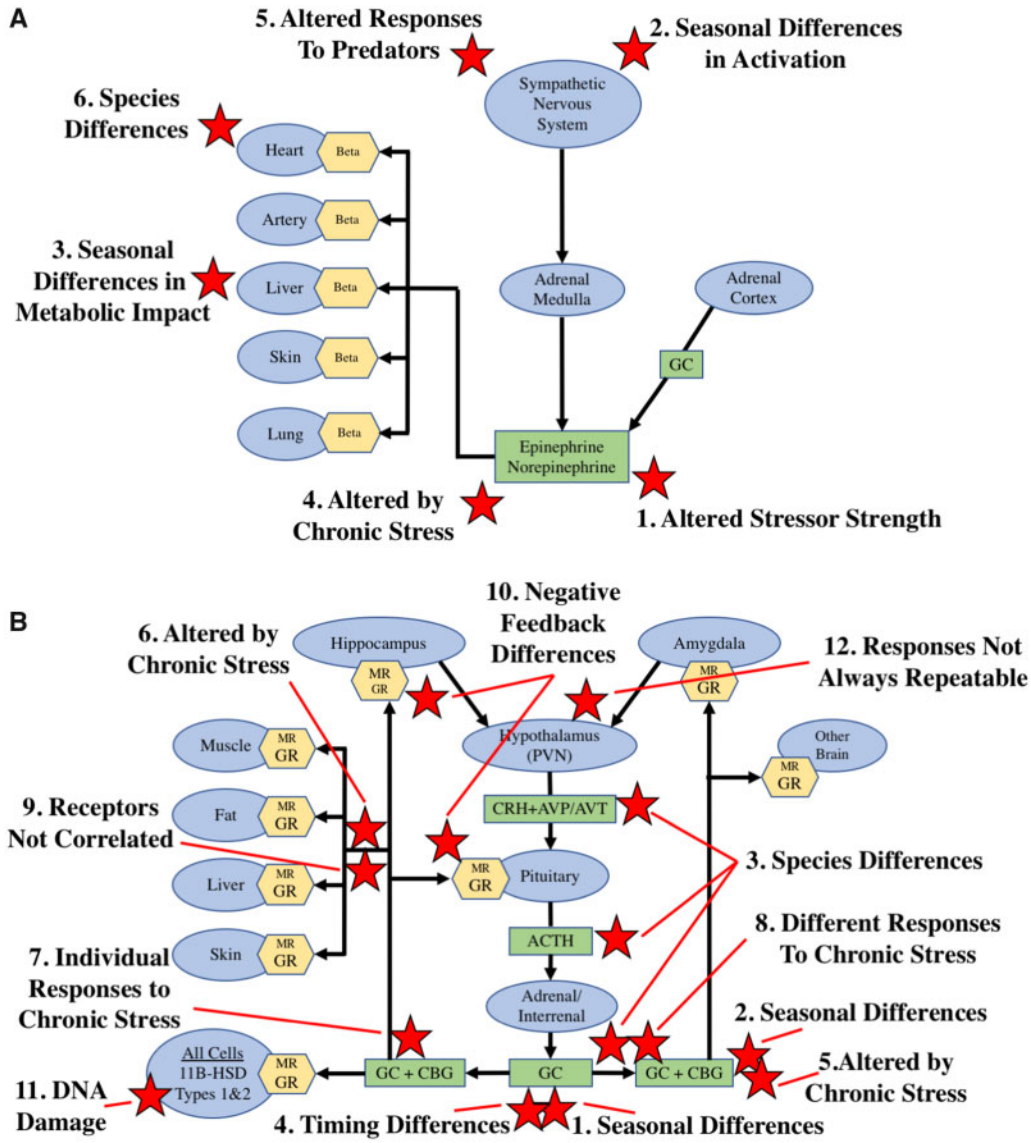

Fig. 2 The catecholamine (**A**) and glucocorticoid (**B**) pathways that comprise the stress response. The major tissues leading to hormone release and the major target tissues of those hormones are represented by blue ovals. Hormones are represented by green squares and their receptors are represented by yellow hexagons. Arrows show the sequence of the pathways. Red stars indicate where there is substantial variation in the pathway, with the discussion in the text referring to the numbers pointing to those stars. GC, glucocorticoids; Beta, beta-adrenergic receptors; CBG, corticosteroid binding globulin; MR, mineralocorticoid receptor; GR, glucocorticoid receptor; 11B-HSD, 11B hydroxysteroid dehydrogenase; PVN, paraventricular nucleus; CRH, corticotropin releasing hormone; AVP, arginine vasopressin; AVT, arginine vasotocin; ACTH, adrenocorticotropin hormone.

Similarly, there can be great differences in heart rate over the course of a year (2 in Fig. 2A). Early work showed that white-tailed deer (*Odocoileus virginianus*) held in semi-natural outdoor pens had seasonal differences in heart rates during the same types of activity (Moen 1978). For example, deer that were running averaged 150 beats per minute (bpm) in winter but 200 bpm in late summer. Other evidence suggests that this seasonal variation reflects differences in the strength of the fight-or-flight response. Captive starlings held on short day photoperiod (mimicking winter) had an attenuated heart rate response to the same stressor relative to starlings held on long days (mimicking summer) (Dickens et al. 2006). In addition, free-ranging breeding king penguins (*Aptenodytes patagonicus*) exposed to three different types of stressors showed a dramatic attenuation of the heart rate response to each stressor over the course of the breeding season (Viblanc et al. 2015). However, this is not true for all species. Roe deer (*Capreolus capreolus*) showed seasonal variation in heart rate for many behaviors,

but not for fleeing (Theil et al. 2004). Clearly, for some species but not others, how an individual responds to a stressor will depend upon the time of year the stressor occurs.

Time of year can also greatly impact the downstream effects of catecholamines. Because of the close relationship between heart rate and oxygen consumption (Butler et al. 2004), heart rate is often used to estimate the catecholamine effects on metabolism that are primarily mediated by the liver. Seasonal physiological changes can have a dramatic impact on these estimates (3 in Fig. 2A). For example, the increase in heart rate in captive European starlings subjected to a 30 min stressor results in an estimate that it requires an extra 64% of the daily energy budget to cope with the stressor (Cyr et al. 2008). During molt (the annual replacement of feathers), however, the heart rate response is so low that the estimate is that zero additional energy is required to cope with an identical 30 min stressor (Cyr et al. 2008). If stressors do not elicit a fight-or-flight response at some times of the year, and those small responses do not require much additional energy, the impact of the fight-or-flight response on fitness will be difficult to estimate at these times of year.

Prior chronic stress can also dramatically alter fight-or-flight responses (4 in Fig. 2A). Introduction to captivity greatly attenuated the heart rate response to startle in both house sparrows (*Passer domesticus*) and European starlings (Dickens and Romero 2009; Fischer et al. 2018), and exposure of starlings to 2 weeks of chronic stress greatly attenuated the heart rate response to restraint (Cyr et al. 2009). That chronic stress can be bad is a substantial feature of the hypothesis that stress responses are correlated with fitness, and yet chronic stress itself can fundamentally alter the fight-or-flight response, thereby altering the balance between positive and negative impacts of acute and chronic stress.

Perhaps the clearest illustration that fight-or-flight responses can vary comes from work on the heart rate responses to stressors in wild animals (5 in Fig. 2A). The literature indicates that there is no consistent response. When humans approach incubating Humboldt penguins (*Spheniscus humboldti*; Ellenberg et al. 2006), there is a marked tachycardia (increase in heart rate). In contrast, when black-capped (*Vireo atricapilla*) or white-eyed (*Vireo griseus*) vireos were experimentally chased, there was no change in heart rate—they didn't seem to be disturbed. Finally, when humans approach incubating ptarmigan (*Lagopus lagopus* and *L. mutus*) there is a distinct bradycardia (decrease in heart rate) (Gabrielsen et al. 1985). Intriguingly, the bradycardia in the ptarmigan continued until the humans moved off in a different direction, at which point there was a strong tachycardia. Bradycardia is not restricted to incubating birds. "Feigned death" is a phenomenon in many species, with strong bradycardia being documented in beavers (*Castor canadensis*; Swain et al. 1988), American opposums (*Didelphis marsupialis*; Gabrielsen and Smith 1985), and American alligators (*Alligator mississippiensis*; Smith et al. 1974). The bradycardia makes sense. In order to successfully feign death, or avoid being seen by an approaching predator, a strong damping of physiological systems would be of great advantage to avoid inadvertent movement that would draw attention to the animal's position. In fact, the freeze response is often an underappreciated feature of the fight-or-flight response. However, the contrast in these studies is striking—the same stressor (human approach) can elicit increased, no change, and decreased heart rate. Any connection to fitness would require substantial knowledge of the typical species response and detailed knowledge of the circumstances surrounding the stressor. Clearly, the implicit assumption of a "conserved" increase in heart rate elicited by catecholamines is not true.

Finally, the fight-or-flight response might not even be regulated the same way in every species (6 in Fig. 2A). Changes in heart rate, the classic index of a fight-or-flight response, are regulated both by increases in sympathetic activation and decreases in parasympathetic activation. In general, moderate exercise increases heart rate via decreased parasympathetic, whereas stressors increase heart rate via increased sympathetic. However, this dogma has recently been questioned. In European starlings, stress-induced increases in heart rate were clearly being driven by sympathetic activation (Cyr et al. 2009). The key evidence was that propranolol, a beta-adrenergic antagonist, blocked the increase in heart rate during a stressor. In contrast, recent work in streaked shearwaters (*Calonectris leucomelas*) indicated that stress-induced heart rate in this species was primarily a result of lowered parasympathetic input (Muller et al. 2017), although the critical propranolol experiment has not yet been done. Stress resulted in heart rate increases in both species, yet the underlying mechanism appears to be quite different. As far as we know, the shearwaters are the only species where this has been documented, and far more work needs to be done, but there is the possibility that even the "highly conserved" backbone of the fight-or-flight response might not be as conserved as once thought.

Glucocorticoid release

Figure 2B shows the backbone of the glucocorticoid response (Romero and Wingfield 2016). Glucocorticoid release is the culmination of the HPA axis, with the paraventricular nucleus of the hypothalamus secreting corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP—or arginine vasotocin, AVT, depending upon the species), which stimulate adrenocorticotropin (ACTH) hormone release from the pituitary, which in turn stimulates the release of glucocorticoids from the adrenal cortex (or interrenal tissue, depending upon the species). Glucocorticoids, either free or bound to corticosteroid binding globulins (CBG), then circulate through the plasma until they reach their target tissues and interact with two major intracellular receptors, mineralocorticoid receptors (MR) and glucocorticoid receptors (GR) that must form homo- or heterodimers to function. MR and GR are present in different ratios in different tissues. Once inside the cell, glucocorticoids can be deactivated by 11beta-hydroxysteroiddehydrogenase (11B-HSD) type 2, and metabolites can be reactivated by 11B-HSD type 1. Glucocorticoids affect many tissues in the body. In the brain, glucocorticoids exert negative feedback effects both in the pituitary and the hippocampus, and can stimulate the amygdala to increase glucocorticoid release. Included in Fig. 2B are starred steps in the pathway where current data indicate that the function of the pathway is not consistent either across time within an individual, between individuals of the same species, or across different species.

Perhaps the best-known variation in the glucocorticoid release pathway is the substantial seasonal variation (1 in Fig. 2B). Approximately 75% of species studied to date either alter baseline concentrations or alter their glucocorticoid response to stressors depending upon the life-history stage or physiological state (Romero 2002). Consequently, like with the fight or flight response, finding fitness correlates with glucocorticoid responses requires species-specific knowledge of how glucocorticoid function is regulated at each life-history stage. To illustrate the problem, consider the response of white-crowned sparrows (*Zonotrichia leucophrys*) undergoing a molt. At this time of year, their response is attenuated to the point that they essentially do not increase glucocorticoid secretion in response to a stressor (Romero 2002). The seasonal challenges are exacerbated when CBG is considered (2 in Fig. 2B). Many taxa also alter CBG levels seasonally (Breuner and Orchinik 2001; Romero et al. 2006; Malisch and Breuner 2010). To make the situation even more confusing, it is still not clear what physiological role CBG plays on the impact of glucocorticoid effects, with CBG potentially increasing or decreasing glucocorticoid's access to its receptors (Breuner et al. 2013; Schoech et al. 2013). Consequently, even though the pathway to glucocorticoid release may be conserved, how much glucocorticoid reaches receptors can vary enormously over the course of the year, with an ultimate impact that is still not completely understood. Notice, however, that simply controlling for season does not solve the problem. When fitness is ultimately about grandchildren (Fig. 1), an assessment throughout the life cycle is important.

The HPA axis also varies taxonomically (3 in Fig. 2B). There can be enormous species variation in the total amount of glucocorticoids in the blood, ranging from 1 ng/mL or less in some fish and reptile species, up to >1000 ng/mL in some rodents and primates (Romero 2004). In other words, some species have more than enough naturally circulating glucocorticoids to kill other species. The source of this taxonomic variation is essentially unknown. A part of the explanation may relate to differences in CRH and/or AVP release from the hypothalamus, differences in ACTH release from the pituitary, or sensitivity of adrenal cortical tissue to ACTH. Whether these taxonomic differences have any functional significance is also unknown, but the assumption has always been that there isn't. For example, whereas rats have 10-fold higher glucocorticoid levels than many reptiles and birds, few argue that rats are 10-fold more stressed. On the other hand, several Arctic bird species show different glucocorticoid responses to similar storms, with the differences regulated at different levels of the HPA axis (Romero et al. 2000), so species differences in total glucocorticoid levels may, in fact, have undiscovered significance.

Glucocorticoid release is the end result of the HPA cascade, but how quickly that cascade functions also is a source of interspecies variation (4 in Fig. 2B). Although researchers generally assume that glucocorticoids begin to increase in the blood in approximately 3 min, i.e., the HPA cascade takes about 3 min to complete (Romero and Reed 2005), the reality is far more nuanced. Many species begin to have measurable glucocorticoid release much earlier (e.g., Dawson and Howe 1983) or later (e.g., Tyrrell and Cree 1998) than 3 min. Even so, such variation was not thought to have much functional relevance, but a recent paper has begun to question that assumption. Individual Florida scrub-jays (*Aphelocoma coerulescens*) varied in how fast the initial rise in glucocorticoids were detected, and furthermore, the individual speed of the initial increase predicted that individual's magnitude of glucocorticoid release after a 30 min stressor (Small

et al. 2017). Although it is not clear how widespread is this phenomenon (in many species, baseline glucocorticoid concentrations do not predict an individual's magnitude of response to a stressor; Romero and Wingfield 2016), this experiment makes clear that how quickly the signal traverses the HPA cascade can potentially have functional significance.

Many studies that attempt to correlate glucocorticoid responses with fitness do so with the assumption that chronic stress will decrease fitness. However, it is clear that chronic stress can alter many features of the stress response. We discussed earlier that glucocorticoid responses vary seasonally, but the impacts of chronic stress also vary seasonally (5 in Fig. 2B). For example, house sparrows subjected to the chronic stressor of introduction to captivity alter both baseline and stress-induced glucocorticoid release when captured in some seasons, but not in others (Lattin et al. 2012). In addition, chronic stress in house sparrows results in increased GR and MR, but only in some tissues and not in others (Lattin and Romero 2014), indicating that the impact of chronic stress will be different in different tissues (6 in Fig. 2B). The changes in anatomy and physiology that occur with chronic stress would make it very difficult to compare chronically stressed and non-chronically stressed individuals to evaluate impacts on fitness. For example, it is not clear how to assess the overall impact on fitness if chronic stress increased GR in the liver (e.g., increased impact on metabolism) yet decreased GR in the spleen (e.g., decreased impact on immune function).

In fact, it would be difficult even to compare chronically stressed individuals because individuals can react quite differently to chronic stress (7 in Fig. 2B). A classic example is the Visible Burrow System (Blanchard and Blanchard 1989). In these experiments, five male and two female rats are placed in an apparatus with a single open area containing the food and water surrounded by tunnels and chambers. One male quickly becomes dominant and controls access to food and water for the other four males. These four subordinates become highly stressed and two-thirds show highly elevated baseline glucocorticoids that continue to respond to further stressors (Blanchard et al. 1995). These "responders" contrast with the other one-third of subordinates that also have elevated baseline glucocorticoids but no longer respond to further stressors ("non-responders"). However, both responders and non-responders show equivalent pathology resulting from the chronic subordination. Not knowing whether an individual was a responder or non-responder to chronic stress would greatly complicate any attempt to correlate responses with fitness.

Perhaps even more problematic (8 in Fig. 2B), a paper reviewing the literature on chronic stress from many different species determined that there is no consistent "endocrine profile" of a chronically stressed animal (Dickens and Romero 2013). After experimental exposure to chronic stress, for both baseline and stress-induced glucocorticoid levels about a third of studies report that glucocorticoids increased, about a third report that glucocorticoids decreased, and about a third report that glucocorticoids did not change. It is currently impossible to predict which response will result from a new experiment, which again would greatly complicate any attempt to connect the chronic response to fitness.

Although hormone titers are important, hormones are only chemical messengers and their power comes from what they induce other cells to do. Unfortunately, evidence is building that downstream effects of glucocorticoids are also highly variable. For example, a common assumption of studies focusing on hormone titers is that a change in glucocorticoids will have a consistent impact across the body. In other words, a five-fold glucocorticoid increase will result in a five-fold increase in glucose mobilization, a five-fold impact on immune function, a five-fold increase on impacts in the brain, etc. Recent data, however, suggest that a consistent response is unlikely (9 in Fig. 2B). One study measured GR and MR in 13 tissues from 72 house sparrows and asked whether birds had consistent ranks of receptor numbers across those tissues (Lattin et al. 2015). In other words, did some individuals have high GR and/or MR across all tissues, or were receptor numbers independent across tissues? Although individuals did tend to show higher or lower receptor numbers across tissues, the statistical correlations were very weak. This suggests that tissues do not generally respond in a consistent manner to the same hormonal signal.

It is not even clear that most studies have been trying to connect fitness to the relevant aspects of the glucocorticoid system. When assessing fitness, it is necessary to consider the *impact* of glucocorticoids (and the HPA axis more broadly) on physiological and behavioral systems; simply measuring the release of hormones fails to capture these downstream effects. It has become increasingly obvious that there is truly too much variability in both the raw concentrations as well as receptor expressions to connect interindividual variation in fitness solely with interindividual variation in glucocorticoid levels.

It is potentially more productive and biologically meaningful to examine downstream consequences of the signaling hormones. Some studies have drawn connections between HPA axis regulation and fitness by focusing on the strength of negative feedback (10

in Fig. 2B). Efficient control over the release of glucocorticoids is important and it is maladaptive for organisms to continue to release them past the acute stressor (Romero 2004). For example, acute corticosterone elevation in tree swallows (*Tachycineta bicolor*) leads to weakened negative feedback strength (Taff et al. 2018) and in marine iguanas (*Amblyrhynchus cristatus*), stronger HPA axis negative feedback predicted survival following an El Niño (Romero and Wikelski 2010). These studies reflect the potential usefulness of measuring other elements of the HPA axis beyond simply baseline glucocorticoids.

Studies also have begun to explore how glucocorticoids could induce DNA damage—an impact that may have more biologically relevant connections to fitness because it is a downstream effect of glucocorticoid function rather than just a measure of release (11 in Fig. 2B). Evidence has been accumulating that glucocorticoids can shorten telomeres, the DNA caps on the ends of chromosomes that are necessary for normal chromosome replication, presumably by mediating oxidative damage (Haussmann and Heidinger 2015; Angelier et al. 2018). Glucocorticoids can also damage DNA more directly (Flint et al. 2007). For example introduction to captivity elicited increases in both DNA damage and baseline corticosterone, although these two metrics were independent of each other (Gormally et al. 2019).

Finally, it is not currently clear whether individual studies that attempt to connect glucocorticoids to fitness are even repeatable. Most studies of this kind only measure glucocorticoids once, or at most a few times, over the life-span of the animal. This is primarily a result of the difficulty of repeated captures of most free-living animals. However, one fundamental assumption of this approach is that the glucocorticoid concentration that is measured reflects the glucocorticoid concentration at other time points—in other words, is repeatable (Romero and Wingfield 2016). The idea is that a single measurement of glucocorticoids can provide a good estimate of the "endocrine phenotype" of the individual. This is important, because selection works on the phenotype. If a single (or even multiple) measurement of glucocorticoids does not accurately estimate the individual's endocrine phenotype, i.e., is not repeatable, then connecting glucocorticoid concentrations to fitness becomes nearly impossible.

Although early research suggested that glucocorticoid concentrations were repeatable (12 in Fig. 2B), at least within a life-history stage, a recent comprehensive review indicated that the data are more complicated (Taff et al. 2018). Baseline glucocorticoid concentrations were very weakly repeatable when averaged from 68 estimates, whereas stress-induced concentrations had a bit better repeatability when averaged from 51 estimates (Taff et al. 2018). However, absolute concentrations might not be the best metric to assess for repeatability. The relative rank concentration across individuals has a better theoretical connection with fitness (Romero and Reed 2008). As an example, if we assume that higher glucocorticoids provide greater fitness, then the fitter individual is the one with more glucocorticoids relative to another individual (i.e., has a higher rank), not the one with simply high concentrations. In the end, however, even measuring repeatability of ranks may not be the best approach. Glucocorticoid release takes place over time and thus produces a curve of concentrations in the blood. Until recently, estimates of repeatability were restricted to comparing multiple measures of a single time point. Thus repeatability estimates were calculated for baseline concentrations, then stress-induced concentrations, etc. (Taff et al. 2018). A new mathematical tool, profile repeatability that estimates the repeatability of the entire glucocorticoid response to a stressor over time (Reed et al. 2019), should provide a more biologically relevant estimate of repeatability of the glucocorticoid response to a stressor.

Conclusion

This review identified numerous points in both the catecholamine and glucocorticoid pathways that show substantial variation across time and across taxa. Other parts of these pathways are also known to vary but are not presented. In addition, many parts of these pathways, especially the catecholamine pathway, have been understudied, so that more studies may indicate other points in the pathways that vary. The overall conclusion is that the "well-conserved" vertebrate stress response is, in fact, not so highly conserved. The way in which an individual responds to a stimulus (elicits a stress response) and the resulting success of that individual could very well connect to fitness; however, as of yet, the variation in the metrics of this response has not supported a stress–fitness hypothesis.

It is important to point out, however, that the backbone of these pathways remain highly conserved. It is the function that is so variable. An analogy can illustrate the difference. In terrestrial vertebrates (and species like whales that are their descendants), the bones at the end of the forelimbs (the hand) are highly conserved. However, those same bones support limbs that walk, climb, grasp,

swim, and fly, depending upon the taxa. Similarly, the structures involved in the catecholamine and glucocorticoid pathways are highly conserved, but the functions differ depending upon the taxa. Consequently, it is not contradictory to claim that structures are both conserved and vary.

However, this substantial interindividual variation makes it extremely difficult to connect catecholamine and/or glucocorticoid responses to fitness. Exploring alternative (e.g., DNA damage) and multiple measures of stress may shed additional light on the complexities that are so prevalent in stress biology (Romero et al. 2015). If anything has become clear since the time of Cannon and Selye, it is that there is no magic bullet when it comes to measuring and understanding the vertebrate stress response. The fight-or-flight and glucocorticoid arms contribute to an organism's survival in unique and variable ways. They both integrate a number of key physiological, behavioral, and genetic components. This integration suggests that taking a multimodal approach to questions in stress biology is a potential solution to understanding this variability, and ultimately connecting stress physiology to fitness.

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