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SYMPOSIUM INTRODUCTION

Introduction to the Symposium: Stress Phenotype: Linking Molecular, Cellular, and Physiological Stress Responses to Fitness

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From the symposium "Stress phenotype: linking molecular, cellular, and physiological stress responses to fitness" presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2019 at Tampa, Florida.

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Synopsis Although most organisms respond to environmental and social stressors by initiating a stress response that is expected to increase fitness, we currently lack information about how the stress response is integrated across levels of biological organization. Organismal biologists and physiological ecologists have tended to focus on questions related to how the glucocorticoid stress response varies across ecological contexts and is related to fitness, whereas, molecular and cellular biologists have typically investigated the fundamental underlying mechanisms. However, it is becoming increasingly clear that a comprehensive understanding of the evolution of the stress response will require integrative studies that span levels of analyses. This information will be critical for predicting how selection will influence the expression of this complex phenotype at the organismal level, as well as how the integration of the underlying mechanisms will influence the evolutionary response to selection. As diverse organisms are expected to experience rising stress exposure in the face of anthropogenic disturbance and climate change, this information is becoming increasingly urgent. The overarching goals of this symposium were to bring together researchers that study the stress response across levels of organization in diverse organisms to identify important gaps in knowledge and novel research approaches that could be used to advance the field.

Introduction

Most organisms respond to noxious environmental and social stimuli such as reduced food availability, inclement weather, and predation by initiating a stress response that is expected to increase coping ability and enhance survival (Wingfield et al. 1995; Wingfield et al. 1998; Sapolsky et al. 2000; Romero and Wikelski 2001; McEwen and Wingfield 2003; Romero et al. 2009). Knowledge about how organisms respond to stressors and how variation in resilience impacts fitness is critical for evolutionary ecologists interested in predicting the consequences of rapidly changing environments (Wingfield 2008) and biomedical researchers seeking to mitigate the impacts of life's adversities (McEwen and Wingfield 2003; Epel et al. 2004; Shalev et al. 2013; Scheffer et al. 2018).

Over the past 30 years, a wealth of studies in diverse captive and free-living systems has established that the way in which individuals respond to stressors varies considerably depending on developmental circumstances (Seckl and Meaney 2004; Monaghan 2008; Wada 2008; Crino and Breuner 2015), season (Romero 2002), and age (Wingfield and Sapolsky 2003; Heidinger et al. 2006; Angelier et al. 2007; Heidinger et al. 2010). This context-dependent variation in the stress response is often assumed to be adaptive, however studies relating variation in the stress response to fitness are equivocal and a multitude of factors could contribute to discrepancies among studies (Breuner et al. 2008; Bonier et al. 2009a, 2009b; Crino and Breuner 2015; Henderson et al. 2017).

One important issue is that measures of the stress response are often collected at one time point, lifestage, or in a single environment and then related to fitness proxies such as annual reproductive success

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and/or survival (Bonier and Martin 2016; Dantzer et al. 2016; Taff and Vitousek 2016). However, the stress response is a flexible phenotype that allows organisms to dynamically respond to changes in the environment and their own internal state across the lifespan (Bonier and Martin 2016; Dantzer et al. 2016; Taff and Vitousek 2016). This is problematic because the same environmental and/or internal conditions that influence the stress response can also affect fitness parameters. For example, in vertebrates, individuals less able to acquire resources during breeding might have higher glucocorticoids and lower reproductive output than individuals better able to acquire resources (Bonier and Martin 2016; Dantzer et al. 2016). In this example, the negative relationship between glucocorticoids and fitness is driven by variation in resource levels among individuals, which can obscure the true relationship between the trait of interest (in this case glucocorticoids) and fitness (Bonier and Martin 2016; Dantzer et al. 2016). Instead, individuals better able to flexibly modulate glucocorticoids and reproductive output with respect to resource availability likely experience greater lifetime fitness. In addition to resources, several other factors including the developmental environment and age could also shape the relationship between the stress response and fitness. Thus, there is a growing appreciation that because the stress response is a flexible phenotype, it will be essential to use a reaction norm approach across a range of environments and time scales to understand how the stress response is related to fitness (Bonier and Martin 2016; Dantzer et al. 2016; Taff and Vitousek 2016).

Another critical issue is that although the stress response is a whole organismal response that requires the integration of diverse cellular, physiological, and behavioral processes, it is often characterized by a single or very few physiological and/or cellular parameters (i.e., glucocorticoids and/or oxidative stress measures) and we currently lack a comprehensive understanding of how these levels of biological organization are connected to one another (Cohen et al. 2012; Romero et al. 2015; Del Giudice et al. 2018). Evolutionary and physiological ecologists tend to focus on how organismal stress responses influence fitness parameters (Breuner et al. 2008; Bonier et al. 2009a), whereas cellular and molecular biologists typically concentrate on identifying the key underlying mechanisms and cellular pathways (Grad and Picard 2007; Manoli et al. 2007; Picard et al. 2014). Traditionally, these fields have been somewhat isolated from one another. However, it is becoming increasingly clear that integrative studies that span levels of analyses from genomes to phenomes will be critical for predicting how selection will influence the integration of this complex phenotype at the organismal level, as well as how the relationships among the underlying mechanisms will influence the evolutionary response to selection (Cohen et al. 2012).

Goals of the symposium

The primary objective of this symposium was to bring together researchers studying the relationship between the stress response at different biological levels (i.e., molecular, cellular, physiological, and behavioral) and fitness in diverse systems including vertebrates and invertebrates. We had three overarching goals for the symposium: (1) to provide opportunities for researchers who do not typically cross paths (i.e., because they work at different levels of organization or with different organisms) to interact and discuss the underlying mechanisms and functional consequences of variation in the stress response, (2) to stimulate new collaborations and promote the development of integrative approaches for studying the stress response, and (3) to identify important future research directions.

Overview of the symposium contributions

Here, we provide a brief overview of the eight symposium contributions. We begin by summarizing studies examining the relationship between glucocorticoids and traits related to fitness. Breuner and Berk (2019) first review studies investigating links between glucocorticoids and fitness relevant traits and then propose a new framework, deeply rooted in life-history theory, to experimentally test for relationships between glucocorticoids and investment in reproduction and survival. The basic premise is that although glucocorticoids are expected to play a role in mediating the trade-off between investment in reproduction and survival, allocation trade-offs can be masked by variation in resource availability among individuals. This is because individuals that are better able to acquire resources will have more to invest in both reproduction and survival, which will result in a positive rather than negative correlation between these two traits (van Noordwijk and de Jong 1986; Reznick et al. 2000). Breuner and Berk (2019) then describe a captive study in mountain bluebirds (*Sialia currucoides*) supporting this idea. Glucocorticoids were negatively related to blue chroma, an aspect of feather coloration involved in mate choice, and positively related to barbule density, a feather trait expected to increase survival (i.e., the expected negative relationship between investment in reproduction and survival), when food was restricted, but not when it was freely available.

Studies investigating the relationships between glucocorticoids and fitness have typically focused on baseline or peak stress-induced glucocorticoid levels, however sustained glucocorticoid exposure can have pathological effects and much less is known about how an individual's ability to terminate the stress response impacts fitness (Romero and Wikelski 2010). Vitousek et al. (2019) present novel data examining relationships between several aspects of glucocorticoid regulation and reproductive success and survival in free-living tree swallows (*Tachycineta bicolor*). Interestingly, birds that terminate the glucocorticoid stress response more quickly via negative feedback, and under some circumstances also mount a more robust response have greater fitness. Importantly, these results suggest that the ability to respond dynamically to stressors is critical, particularly for individuals living in environments characterized by frequent stressor exposure.

Regulation of the glucocorticoid stress response can be influenced by environmental conditions during development via epigenetic modifications of glucocorticoid receptors in the brain (Weaver et al. 2004). However, because much of this research comes from laboratory studies, we currently have little information about how these epigenetic modifications impact fitness (Siller and Rubenstein 2019). One challenge in linking variation in epigenetic modifications of glucocorticoid receptors in the brain to fitness is that it requires terminal sampling (Siller and Rubenstein 2019). As an important first step in addressing this issue, Siller and Rubenstein (2019) investigated whether DNA methylation of the glucocorticoid receptor gene (*Nr3c1*) promoter was correlated between regions of the brain (hippocampus and hypothalamus) and blood, a tissue that can be non-destructively sampled and related to longitudinal fitness measures in European starlings (*Sturnus vulgaris*). DNA methylation of the Nr3c1 promoter was not correlated across tissues, but a similar cluster of correlated Nr3c1 putative promoter 5'—C—phosphate—G—3' (CpG) sites were identified within each tissue, which may yield promise in future studies. However, as methylation of the Nr3c1 promoter in the blood was not predictive of that in the brain, these results also demonstrate the difficulty of linking methylation of tissues that need to be terminally sampled to longitudinal fitness measures.

Romero and Gormally (2019) then provide a rich review of the vertebrate stress response including the two major pathways of catecholamine and glucocorticoid release. They conclude that although the anatomical structure is highly conserved, there is also tremendous variation in its regulation and functional consequences. They then make several suggestions for future studies interested in relating variation in the stress response to fitness. Importantly, most research examining links between the stress response and fitness in vertebrates have focused on glucocorticoids and much less is known about how variation in catecholamine release is related to fitness. In addition, future studies should also assess additional multifactorial downstream measures of catecholamine and glucocorticoid release, rather than relying on hormone levels alone. Echoing this sentiment, Wada and Heidinger (2019) discuss the recently proposed Damage-Fitness Model (H. Wada, manuscript under review), which suggests that because downstream damage markers (e.g., lipid peroxidation, protein oxidation, and telomere loss) better reflect how organisms have coped with past stress experiences, they may be more predictive of fitness outcomes than the magnitude of stress responses. Wada and Heidinger (2019) then review correlational and experimental studies demonstrating explicit links between damage markers and measures of reproductive success and survival.

Although, glucocorticoids are expected to play an important role in coordinating the stress response in vertebrates, the degree to which downstream effects vary depending on the type and timing of stressor exposure remains poorly understood (Telemeco et al. 2019). Telemeco et al. (2019) experimentally investigated this idea by exposing Eastern fence lizards (*Sceloporus undulatus*) to two different stressors (fire ants and high temperature) at two different life stages (juvenile and adults) and examining the effects on the stress response across levels of organization (i.e., behavior, glucocorticoids, innate immune function, and the expression of heat shock proteins in the blood and liver). Interestingly, although the behavioral and endocrine responses were largely overlapping across stressors and life-stages, the cellular responses were not. This finding is critical because it suggests that the same generalized endocrine response can translate into very different, context-specific responses at the cellular level, which likely has important functional consequences and contributes to why studies relating glucocorticoids to fitness are often incongruent.

Jones et al. (2019) then present data in Eastern oysters (*Crassostrea virginica*) lending additional support to the idea that exposure to different stressors elicits divergent downstream responses. Eastern oysters (*C. virginica*) are expected to experience decreasing salinity and rising temperatures in response to climate change with negative consequences for fitness (Jones et al. 2019). Using a comparative transcriptomic approach, Jones et al. (2019) experimentally examined the effects of these two stressors (salinity and temperatures) independently and in combination on gene expression profiles. Importantly, they found that there was low overlap in gene expression between these two stressors and that a greater number of genes were differentially expressed in response to a combination of low salinity and warm temperature than to either stressor alone. These intriguing results suggest these two stressors have divergent, synergistic effects at the transcription level. Future studies like these will be essential for determining the mechanistic underpinnings and functional consequences of interactive and non-additive effects of exposures to multiple stressors.

The above studies emphasize that a significant challenge in relating the stress responses to fitness is that the mechanisms and functional consequences are often context specific. Another important issue is that the effects of stress exposure are also often non-linear and the mechanisms that underlie these non-linear effects are not well understood (McEwen and Sapolsky 1995; Melicher et al. 2019). In the last contribution, Melicher et al. experimentally examined the effects of constant and fluctuating temperature on gene expression profiles and mortality in pupal alfalfa leafcutting bees (*Megachile rotundata*). Pupae that experienced a brief, 1-hour warming pulse were characterized by an increase in the expression of a suite of genes associated with membrane homeostasis, metabolism, oxidative stress responses, ion homeostasis, and anti-freeze proteins, relative to pupae that were maintained at a constant cold temperature. Importantly, these protective effects extended beyond the warming period, highlighting that even a brief respite from the cold stressor was sufficient to induce persistent positive effects.

Conclusions and future directions

Several important, reoccurring themes emerged throughout the symposium, roundtable discussion, and post-symposium survey. First, although most organisms respond to environmental and social stressors by initiating a stress response, the underlying mechanisms and functional consequences are highly context and time dependent, making it difficult to correlate variation in the stress response collected at a single time point or environment to fitness. There is a growing appreciation that moving forward will require a better understanding of how this dynamic phenotype is related to fitness under diverse environmental conditions and across the lifespan. Second, there was large agreement that we need to take a more integrated approach to characterizing the stress response across levels of biological organization rather than relying on single measurements. Specifically, existing research is biased toward vertebrate taxa and certain parameters (e.g., glucocorticoids). Integrative approaches that incorporate behavioral, hormonal, and multifactorial downstream measurements including damage markers are expected to be particularly fruitful. Transcriptomic approaches are also expected to offer great promise for identifying common pathways and novel mechanisms involved in mediating the stress response, but will also present distinct challenges including decisions about what time points and tissues to sample, how to test for casual relationships, and if samples need to be terminally collected, then how to relate these measurements to longitudinal fitness measures. Despite these challenges, taking a more integrative approach to studying the stress response across levels of biological organization and in diverse contexts is likely to yield important insight into how organisms respond to and cope with stressors and the consequences for fitness.

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