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Research paper

A test of reactive scope: Reducing reactive scope causes delayed wound healing

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ABSTRACT

Reactive scope predicts that all animals have an adaptive ability to respond to stressors in their environment, termed reactive homeostasis, and that only when an animal's response to stressful stimuli exceeds a certain threshold (homeostatic overload) will stress have pathological effects. While this framework has successfully helped interpret effects of stressors on wildlife, no study has designed an experiment to directly test this framework. This study was designed to expose house sparrows (*Passer domesticus*) to treatments that would result in varying ranges of reactive homeostasis during chronic stress, which based on the reactive scope model should cause birds with the lowest reactive homeostasis range to exhibit signs of pathology during a subsequent challenge. To modulate the reactive homeostasis range, we altered allostatic load of birds by exposing them to chronic stress while either elevating, blocking, or not manipulating corticosterone. After concluding chronic stress treatments, birds were exposed to the subsequent challenge of a superficial wound. Individuals treated with corticosterone during chronic stress (high allostatic load) experienced the most pathology, including both weight loss and slower wound healing. Unmanipulated birds (medium allostatic load) also experienced weight loss but had normal healing rates, while birds with blocked corticosterone (low allostatic load) had minimal weight loss and normal healing rates. Our results indicate that increased allostatic load reduces the reactive homeostasis range, thereby causing individuals to cross the homeostatic overload threshold sooner, and thus support the reactive scope framework.

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1. Introduction

Reactive scope provides a framework for understanding how and when stress may have pathological effects on an individual (Romero et al., 2009). Animals all have the capacity to respond to stress, termed reactive homeostasis in the reactive scope model, an adaptive process whereby an organism modulates physiological variables to respond to unpredictable and/or threatening changes in their environment. The reactive scope model proposes that only when an organism's responses are pushed outside their reactive homeostasis range and into homeostatic overload does stress begin to have pathological effects (Romero et al., 2009). Thus, a reduction in an animal's reactive scope, or reactive homeostasis range, should increase the likelihood that a subsequent stressor will push the animal into homeostatic overload and induce pathology. To put this in terms of a physiological variable associated with responses to unpredictable stimuli, like glucocorticoids, an organism needs varying circulating concentrations of glucocorticoids throughout the day or season to maintain basic physiological processes, termed predictive homeostasis in the reactive scope model. Upon exposure to a stressful stimulus an organism can increase glucocorticoid concentrations (i.e., reactive homeostasis). Although these transient increases in glucocorticoids are adaptive, repeated or chronic exposure to stress can cause pathology by reducing the reactive homeostasis range, causing normal glucocorticoid responses to stress to push an animal into homeostatic overload.

Many factors can constrain an individual's reactive scope. For instance, as physiological parameters of an animal vary over the season or throughout the day, referred to as predictive homeostasis, so does the animal's ability to cope with stress in their environment (Romero et al., 2009). An animal would have a reduced reactive scope when a physiological variable was high (e.g., increased metabolic rate; Cyr et al., 2008). Another possibility for altering an individual's reactive scope is a decrease in the threshold at which an animal is pushed into homeostatic overload. A reduction in the threshold for homeostatic overload can occur when a

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prior event, such as an early developmental experience, has long term consequences. For instance, reduced grooming early in life increases stress-induced corticosterone in rat pups resulting in individuals that are more fearful later in life (Meaney, 2001; Weaver et al., 2004). A reduction in the homeostatic overload threshold (i.e. decreased reactive scope) can also be temporary, decreasing as an organism is faced with a persistent stressor in their environment (e.g., low food availability; Romero, 2012) and then recovering once the stressor is removed. If an animal is exposed to a subsequent stressor while reactive scope is decreased, they are more likely to be pushed into overload, but less likely if they have sufficient time to recover from the initial stressor.

Although numerous studies have discussed their findings in the context of reactive scope (198 studies to date based on a web of science search), no study has empirically tested this framework. In this study, we designed an experiment to test the reactive scope model. We pre-treated three groups of house sparrows (*Passer domesticus*) with different protocols that the reactive scope model predicts would result in different magnitudes of a decreased threshold to enter homeostatic overload. In other words, at the end of the pre-treatment period, each group should have a different range of reactive scope. Then, each group was challenged with the identical stressor of a subcutaneous wound. If the reactive scope model is correct, then only the treatment with the smallest reactive scope (i.e. lowest threshold) will enter homeostatic overload during the period of wound healing (Fig. 1). To produce treatments with differing reactive scopes we manipulated allostatic load of birds by exposing them to chronic stress while also manipulating corticosterone concentrations. Allostatic load is the summation of an individual's cost to live, including physiological and behavioral factors (e.g., circulating hormones, metabolic rate, social interactions, foraging, etc.), and life history events, like reproduction or molt (McEwen and Wingfield, 2003). During

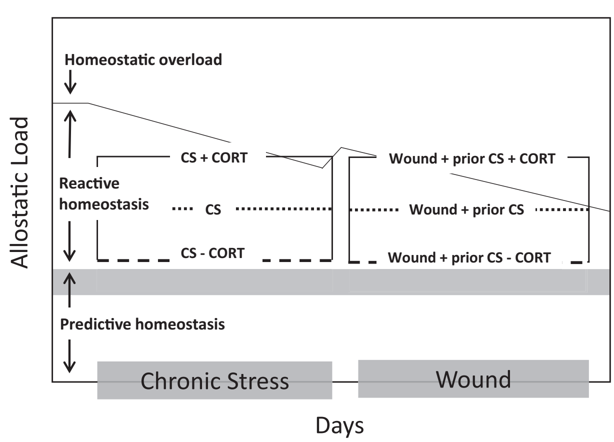

Fig. 1. A conceptual figure depicting the primary components of the reactive scope model in relation to our experimental design. Predictive homeostasis represents the range (due to diel or seasonal rhythms) at which various mediators (e.g. hormones, metabolic rate, etc.) vary, the gray bar represents the theoretical allostatic load of birds in this study in the absence of stress. Reactive homeostasis is the range in which an organism can alter a physiological mediator to respond to a stressful stimulus without experiencing any deleterious effects of stress. Homeostatic overload represents the threshold at which a response to stress becomes deleterious. In this study, we manipulated reactive scope of our treatments by altering allostatic load of birds *via* exposure to chronic stress while simultaneously either increasing circulating corticosterone (high allostatic load), blocking corticosterone release (low allostatic load), or leaving corticosterone unmanipulated (moderate allostatic load; controls). The reactive scope model proposes that the threshold to entering homeostatic overload would steadily decrease over the 10 day course of chronic stress, rebound once the stressors are removed, then decrease again once the subsequent challenge, healing a wound, was applied. Groups with higher allostatic load should experience deleterious effects during the subsequent challenge, whereas those with lower allostatic load should not.

chronic stress we artificially elevated corticosterone in one treatment group (high allostatic load), blocked corticosterone production in another treatment group (low allostatic load), and did not manipulate corticosterone in the control treatment group (moderate allostatic load). After a two day recovery from our chronic stress protocol (described below), we exposed all treatments to a subsequent increase in allostatic load, specifically a small superficial wound. Stress is known to impact immune processes; acute stressors often have stimulatory effects, while chronic stressors or more severe stressors have inhibitory effects (Dhabhar, 2003, 2009; Dhabhar and McEwen, 1999; Carter et al., 2013). We predicted that individuals with the highest allostatic load would cross the homeostatic threshold and experience reduced healing and exhibit other indicators of chronic stress (e.g., weight loss), whereas individuals with the lowest allostatic load would not cross this threshold, exhibiting normal healing and no deleterious effects of chronic stress (Fig. 1).

2. Materials and methods

2.1. Study species, trapping, and animal husbandry

We used house sparrows in this study because they are invasive to North America and also have been a popular model organism for stress physiology studies (e.g., Hegner and Wingfield, 1987; Kuhlman and Martin, 2010; Martin et al., 2011; Lattin et al., 2012a,b, 2015). Twenty-four wild house sparrows were captured with baited Potter traps in Medford, MA in May, 2013. Birds were taken to indoor facilities and housed in mixed sex or female only pairs based on treatment groups in 45 cm × 37 cm × 33 cm cages. All cages were housed in the same room. Sparrows were held on a 14L:12D light cycle at 22 °C with food and water available ad libitum. Birds were allowed at least 2 weeks to acclimate to captivity before starting the experiment. Birds were fed on a mixed seed diet (Supreme Wild Bird Mix, Lebanon Seaboard Corporation) and were also provided grit. All experiments were approved by the Institutional Animal Care and Use Committee at Tufts University and were carried out according to the guidelines of the Association for Assessment of Laboratory Animal Care.

2.2. Experimental design and timeline

To understand how an individual's reactive scope affects their likelihood of entering homeostatic overload, we manipulated corticosterone concentrations of birds during exposure to a 10 day chronic stress protocol and monitored changes in body mass, corticosterone, and their ability to handle a subsequent increase in allostatic load—wound healing (Fig. 2). Based on assumptions of the reactive scope model, we hypothesized that the 10 days of chronic stress would gradually reduce the threshold at which animals enter homeostatic overload (Fig. 1). The degree of underlying allostatic load will then alter how likely an individual animal is to enter homeostatic overload. To augment allostatic load (thereby reducing reactive scope) we increased circulating corticosterone in the corticosterone-elevated treatment group (n = 8; 5F and 3M) (described below). We ameliorated allostatic load (thereby increasing reactive scope) by blocking corticosterone release in the corticosterone-blocked treatment group (n = 7; 4F and 3M). Finally, we did not manipulate allostatic load (resulting in moderate reactive scope from the chronic stress protocol) in control birds (n = 8; 6F and 2M). Using previously-established methods, corticosterone-elevated birds received dermal application on the neck between the feather tracts of 20 μg corticosterone dissolved in 20 μL DMSO twice daily throughout the duration of chronic stress (Shallin-Busch et al., 2008). To block secretion of corticosterone we