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Wound-healing ability is conserved during periods of chronic stress and costly life history events in a wild-caught bird

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ABSTRACT

Chronic stress, potentially through the actions of corticosterone, is thought to directly impair the function of immune cells. However, chronic stress may also have an indirect effect by influencing allocation of energy, ultimately shifting resources away from the immune system. If so, the effects of chronic stress on immune responses may be greater during energetically-costly life history events. To test whether the effects of chronic stress on immune responses differ during expensive life history events we measured wound healing rate in molting and non-molting European starlings (*Sturnus vulgaris*) exposed to control or chronic stress conditions. To determine whether corticosterone correlated with wound healing rates before starting chronic stress, we measured baseline and stress-induced corticosterone and two estimates of corticosterone release and regulation, negative feedback (using dexamethasone injection), and maximal capacity of the adrenals to secrete corticosterone (using adrenocorticotropin hormone [ACTH] injection). After 8 days of exposure to chronic stress, we wounded both control and chronically stressed birds and monitored healing daily. We monitored nighttime heart rate, which strongly correlates with energy expenditure, and body mass throughout the study. Measures of corticosterone did not differ with molt status. Contrary to work on lizards and small mammals, all birds, regardless of stress or molt status, fully-healed wounds at similar rates. Although chronic stress did not influence healing rates, individuals with low baseline corticosterone or strong negative feedback had faster healing rates than individuals with high baseline corticosterone or weak negative feedback. In addition, wound healing does appear to be linked to energy expenditure and body mass. Non-molting, chronically stressed birds decreased nighttime heart rate during healing, but this pattern did not exist in molting birds. Additionally, birds of heavier body mass at the start of the experiment healed wounds more rapidly than lighter birds. Finally, chronically stressed birds lost body mass at the start of chronic stress, but after wounding all birds regardless of stress or molt status started gaining weight, which continued for the remainder of the study. Increased body mass could suggest compensatory feeding to offset energetic or resource demands (e.g., proteins) of wound healing. Although chronic stress did not inhibit healing, our data suggest that corticosterone may play an important role in mediating healing processes and that molt could influence energy saving tactics during periods of chronic stress. Although the experiment was designed to test allostasis, interpretation of data through reactive scope appears to be a better fit.

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1. Introduction

Chronic stress can cause behavioral and physiological effects in organisms that have implications for survival and reproduction (McEwen and Wingfield, 2003; Romero et al., 2009; Sapolsky et al., 2000). Several frameworks have been proposed to understand when stress will have negative consequences for animals, and the literature provides examples that support and refute these frameworks (Cyr et al., 2008; DuRant et al., 2008; Romero et al., 2000; Romero and Wikelski, 2001, 2010). One framework, the allostatic overload model, is rooted in energy expenditure and availability, and predicts that stress will cause pathology in an organism when the energetic cost of the stressor and the animal's basic maintenance costs (termed allostatic load) become too great an energetic burden (McEwen and Wingfield, 2003). The allostasis model thus predicts that the ability of an individual to withstand stress should vary with their life history stage, such that life history stages associated with increased energy expenditure

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(e.g., reproduction, or molt in birds) should also correspond with a reduction in an animal's ability to cope with stress. An alternative framework, reactive scope, posits that an organism has a range in which various physiological mediators of stress can increase or decrease (e.g., heart rate, corticosterone, etc.) and only when an organism fluctuates outside this range, termed the reactive homeostasis range, does an animal experience pathology from stress (Romero et al., 2009). Thus reactive scope allows a suite of variables, including energy-associated variables, to cause or protect an individual from stress-induced pathology. For instance, marine iguanas with robust negative feedback of corticosterone (the primary glucocorticoid in reptiles and birds) release were more likely to survive famine that individuals with poor negative feedback ability (Romero and Wikelski, 2010). Shutting down corticosterone presumably prevented these iguanas from moving outside the reactive homeostatic range.

One of the pathological effects often associated with chronic stress, is reduced immune function (e.g., Dhabar, 2006; Dhabhar, 2014; French et al., 2006; Segerstrom and Miller, 2004; Walburn et al., 2009). In humans, periods of intense stress (e.g., nursing a parent or spouse through chronic illness) are often followed by the development of disease (Fonareva and Oken, 2014; Tsukamoto and Machida, 2014). Although relationships between chronic stress and immune function are less well studied in wild populations of animals (Martin, 2009), existing studies suggest that chronic stress can negatively impact immune function of wild animals (Archie, 2013; Archie et al., 2012; French et al., 2006; Kinsey et al., 2003; Morales et al., 2004). Stress could directly impact the immune system through the actions of glucocorticoid hormones (Sternberg, 2006), which are known to alter immune function (Apanius, 1998; Dhabhar, 2002; Sternberg, 2006). Tissues and cells associated with immune processes contain glucocorticoid receptors (Rabin, 1999; Planey and Litswack, 2000), and glucocorticoids have well-documented anti-inflammatory effects (Sternberg, 2006). Glucocorticoids can inhibit production of some T-helper cells which are important in adaptive immune responses, and suppress maturation and proliferation of most immune cells associated with innate immunity by inhibiting gene expression (Sternberg, 2006). Negative feedback of glucocorticoid release influences how long an individual is exposed to elevated glucocorticoids, and has been associated with higher rates of survival during chronic stress events (Romero and Wikelski, 2010). Thus, high negative feedback efficacy could reduce the negative effects of chronic stress on physiological processes like immune responses.

Chronic stress may also indirectly influence immune processes by altering energy expenditure and allocation, an idea that fits nicely within the allostatic overload model. Corticosterone is important in liberating energy stores (Sapolsky et al., 2000), and during times of stress, energy may be allocated to immediate survival needs and away from other energetically costly behavioral or physiological processes (Wingfield et al., 1998; McEwen and Wingfield, 2003). Thus, physiological processes like immune responses may be reduced in times of chronic stress as an energy-saving mechanism, or because of energetic constraints induced by chronic stress (Apanius, 1998). Research suggests that immune responses can upregulate energy expenditure, increase protein and lipid breakdown, and are greater in individuals of better condition (Hasselgren and Fischer, 1998; Lochmiller and Deerenberg, 2000; Martin et al., 2003, 2008; Navarro et al., 2003). If immune processes are down-regulated during periods of chronic stress to help conserve energy that can be allocated towards immediate survival needs (Segerstrom and Miller, 2004), then costly life-history events with high maintenance costs (i.e., high allostatic load) could exacerbate immune-stress interactions.

In birds, molt is a particularly costly life history event and is one of the most physiologically-costly processes birds undergo (Klaassen, 1995; Lindström et al., 1993). Molt has been shown to alter physiological responses to chronic stress, and increase nighttime energy expenditure by 60% and protein synthesis by 3.5-fold (Murphy, 1996; Cyr et al., 2008; Kostelanetz et al., 2009). During molt there is increased deposition of keratins and increased protein synthesis in the liver, muscle, and whole body (Murphy and Taruscio, 1995). Thus, based on the allostatic overload type I model whereby the sum of energetic requirements exceeds available energy, a molting bird, which has increased basic maintenance costs, may attempt to cope with chronic stress by mounting a weaker immune response compared to a non-molting bird (McEwen and Wingfield, 2003).

To test whether chronic stress differentially affects immune processes in relation to life-history events, we measured wound healing, an integrative measure of immune function (French et al., 2006), in molting and non-molting European Starlings either exposed to chronic stress or control conditions (Table 1). We predicted that chronic stress and molt would have negative and additive effects on wound healing. We also examined the role energy expenditure plays in mediating immune-stress interactions by measuring nighttime heart rate, which strongly correlates with energy expenditure (Cyr et al., 2008), throughout the study. Finally, we measured various components of corticosterone release and regulation prior to wounding to determine whether they predicted wound healing ability. Specifically, we hypothesized that individuals with lower baseline corticosterone and better negative feedback ability would heal wounds more quickly than birds with higher corticosterone and less robust negative feedback ability (Romero and Wikelski, 2001, 2010).

2. Materials and methods

2.1. Study species and animal husbandry and collection

We used adult European starlings (*Sturnus vulgaris*, Linnaeus) in this study, because they were well-suited for studies of chronic stress. Physiological changes associated with stress in both wild-caught and free-living starlings are well understood, including heart rate and hormonal responses to acute and chronic stress. In addition, the chronic stress protocols described below successfully induce chronic stress in starlings (e.g., Cyr et al., 2007; Kostelanetz et al., 2009; Rich and Romero, 2005). Starlings have also been the focus of several laboratory studies regarding molt (Cyr et al., 2008; De Bruijn and Romero, 2013; Dawson, 2003; Kostelanetz et al., 2009).

We trapped wild starlings on the Tufts University, Medford campus and in surrounding neighborhoods. Birds used in this study were captured at different times throughout the year. Due to logistical constraints of the heart rate software we could only conduct the experiment on 8 birds at a time. The first round of birds were captured in January–February 2012, the second round of birds were captured in May–June 2012, the third round of birds were captured in August 2012, and the last round was captured in September–October 2012. All birds had at least 2 weeks to acclimate to captive conditions, and time to acclimate varied across birds. For each

Table 1
Experimental design and predicted results based upon allostasis.

		Chronic Stress	
		No	Yes
Molt	No	Low allostatic load = fastest wound healing *N* = 9	Medium allostatic load = slower wound healing *N* = 7
	Yes	Medium allostatic load = slower wound healing *N* = 7	High allostatic load = slowest wound healing *N* = 8