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Treatment with corticosterone delays cutaneous wound healing in male and female salamanders

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ABSTRACT

In vertebrates, exposure to stressors and stress hormones has a number of physiological effects including modulation of immune function. These effects on immune function have been well studied in mammals, but less is known in other groups, in particular amphibians. To analyze the effects of exposure to stressors and the stress hormone corticosterone, we monitored cutaneous wound healing as a measure of integrated immunity in male and female semi-terrestrial salamanders (*Desmognathus ochrophaeus*) that were chased to induce endogenous release of corticosterone or were treated with physiologically relevant doses of corticosterone. As predicted, subjects treated daily with corticosterone healed more slowly than did controls. In contrast, subjects that had been chased daily healed at the same rate as controls. Surprisingly, repeated chasing did not elevate plasma corticosterone despite causing drops in body mass and survival. Additionally, females healed more slowly than males, possibly due to energetic constraints.

1. Introduction

Stress is broadly defined as any real or perceived event which disrupts homeostasis (Sapolsky, 2002). Upon perceiving a stressor, the hypothalamus activates the sympathetic nervous system to cause a release of epinephrine from the adrenal medulla triggering the "fight-or-flight" response. The stressor also activates the hypothalamic–pituitary–adrenal (HPA) axis to release glucocorticoids (GCs) from the adrenal cortex. At stress-induced levels, GCs are implicated in various physiological responses including increased glucose production, decreased reproduction, and altered immune responses (Blotta et al., 1997; Carr, 2010; Ramirez et al., 1996; Rollins-Smith, 2001b; Sapolsky, 2002; Webster Marketon and Glaser, 2008).

Studies of humans and rodents find that stressors and GCs have widespread impacts on immune function by altering natural killer cell activity, lymphocyte production, T-cell ratios, and antibody and cytokine production (Webster Marketon and Glaser, 2008). The relationship between stress and immune function has also been widely studies in birds, where again the impacts on immune function are broad (Martin Ii et al., 2005; Matson et al., 2006). Responses vary with the context of the stress, such as duration, as well as the type of immune response examined. For example, acute physiological increases in GCs have immune-enhancing effects while chronic stress conditions become immunosuppressive (Dhabhar, 2000; Webster Marketon and Glaser, 2008). Additionally, mounting immune responses is energetically costly (Demas, 2004), and the effects of GCs on immune function may depend on energy availability (French et al., 2007a; Nelson et al., 2002; Rollins-Smith, 2001a). Finally, the effects of chronic stress on at least one aspect of immune function, wound healing, may be modulated by sex and gonadal status (Romana-Souza et al., 2014).

The effects of stressors and stress hormones on immune function have been well studied in mammals and birds, but less is known in other groups, in particular amphibians. It is important to study stress-immune interactions in amphibians because it has been proposed that environmental stressors increase susceptibility to infection and disease, which has been the cause of many amphibian declines and extinctions (Berger et al., 1998; Carey et al., 1999; Gray et al., 2009). In amphibians undergoing metamorphosis, plasma corticosterone (CORT, the primary amphibian GC) levels surge, and induce apoptosis of lymphocytes, presumably to prevent autoimmune responses to new tissues formed during metamorphosis (Rollins-Smith, 1998). In salamanders, injections of hydrocortisone decreased lymphocytes in the thymus, spleen, and blood (Bennett et al., 1972; Tournefier, 1982). However, CORT had no effect on susceptibility of larval or post-metamorphic amphibians to a chytrid fungus (Searle et al., 2014), and chronic exposure to natural stressors did not increase susceptibility of larval wood frogs to a ranavirus (Reeve et al., 2013).

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To better understand how stressors and GCs modulate amphibian immunity, we monitored cutaneous wound healing in response to repeated exposure to a chasing stressor and CORT elevation. Wound healing is a biologically relevant and integrative measure of immunity (Demas et al., 2011). Animals frequently receive cuts from predator attacks or social interactions, and cuts are points of access for infections. Cutaneous wound healing consists of three sequential yet overlapping phases: inflammation, re-epithelialization, and tissue remodeling. In order for successful healing to occur, the phases must take place in the appropriate order and for sufficient time. Any interruptions can result in delayed or abnormal healing (Christian et al., 2006; Li et al., 2007). Stressors and GCs delay wound healing in humans, rodents, and lizards (Ebrecht et al., 2004; French et al., 2006; Kiecolt-Glaser et al., 1995; Padgett et al., 1998). Interesting sex differences have also been seen in humans and rodents, with females healing faster than males due to the differing effects of androgens and estrogens on the inflammatory stage of healing (Gilliver and Ashcroft, 2007; Gilliver et al., 2007).

Here, we asked whether daily exposure to a handling stressor (specifically, chasing) or daily elevations of CORT delayed cutaneous wound healing in female and male Allegheny Mountain dusky salamander, *Desmognathus ochrophaeus*. Capture and handling elicits a CORT response in many species (Wingfield and Romero, 2001) including dusky salamanders (Ricciardella et al., 2010; Woodley et al., 2014; Woodley and Lacy, 2010). To exogenously elevate plasma CORT to physiological levels, we used a dermal patch containing CORT previously validated for use in Allegheny Mountain dusky salamanders (Wack et al., 2010). Controls consisted of unmanipulated subjects and subjects that received a vehicle on patches. To determine if sex affects wound healing, we included both males and females. We predicted that (1) subjects that were chased or treated with CORT patches would heal more slowly than unmanipulated or vehicle controls, and (2) female subjects would heal more quickly than males.

2. Methods

2.1. Animals

All procedures were approved by Duquesne University's Institutional Animal Care and Use Committee. Appropriate collecting permits were obtained prior to any animal collection. Male and female Allegheny Mountain dusky salamanders were collected from Brooks Run (41N°24′ 31.69″ 78W°4′ 3.991″) in Elk State Forest, Austin, Pennsylvania on May 20, 2013. Animals were sexually mature, with males possessing enlarged premaxillary teeth and females possessing enlarged, yolky follicles. Animals were individually housed in 16 × 16 × 5 cm Gladware containers with moist paper towels. Home boxes were placed in incubators and kept at 16 °C on a long-day (14L:10D) photoperiod. Animals were fed a single wax worm once a week for the duration of the study.

2.2. Dermal biopsies

To induce wounding, dermal biopsies were given on June 11, 2013 (Fig. 1). Animals were anesthetized using Orajel (10% benzocaine) applied to the dorsal surface of the head. All animals were given a cutaneous biopsy, dorsally between the hind limbs, using a sterile 1 mm diameter punch (Miltex Instrument Company).

2.3. Treatments

Animals were randomly assigned to one of the following four treatment groups: unmanipulated, sesame oil patch, sesame oil patch + chasing, and CORT patch. Each group consisted of 6–7 males and 6–7 females. Treatments were begun on the day after biopsies and were given once a day in the morning (between 9:00 AM and 12:00 PM) for the next 29 days (Fig. 1). At the time of each treatment, animals were removed from their incubators. For those animals receiving dermal patches, the paper towels were removed from their home boxes. With the animal in its home box, a 1.5 × 3 mm patch (Whatman Glass microfiber filter paper, cat. #1820 070) was placed dorsally between the forelimbs using clean forceps. The patch was then moistened with 2.5 μL of either 0.25 mg/mL CORT (final dose of 0.625 μg) or sesame oil (vehicle used to dissolve CORT). After 30 min, patches were removed, paper towels were returned to the home boxes, and animals were placed back in their incubators. This method of transdermal delivery of exogenous CORT has been validated in *D. ochrophaeus* and, at the concentration used, significantly elevates plasma CORT to levels similar to those induced by a handling stressor (Wack et al., 2010). For the sesame oil patch + chasing group, animals were exposed to a 2-min chasing stressor before patches were applied. To do so, a sterile cotton-tipped applicator was touched to the animal's tail, head, and forelimbs to induce an escape response for two minutes, taking care not to physically disturb the area of the

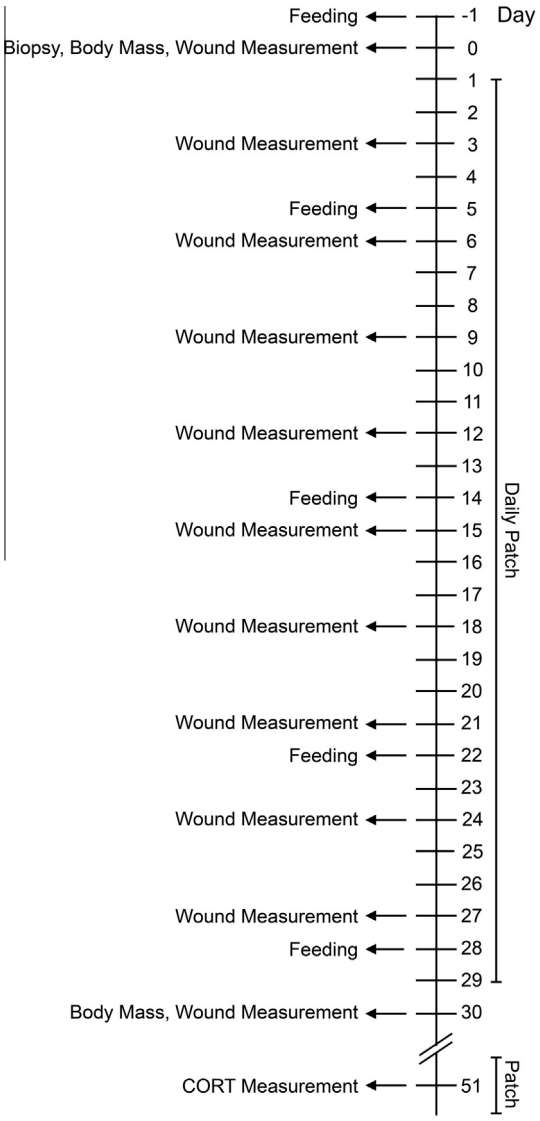

Fig. 1. Timeline showing sequence of patch treatments (no patch, oil patch, oil patch + chasing, CORT patch) and measurements collected on each day of the study.