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How do duration, frequency, and intensity of exogenous CORT elevation affect immune outcomes of stress?

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ABSTRACT

Stress is typically characterized as "acute" (lasting from minutes to hours) or "chronic" (lasting from days to months). These terms are of limited use as they are inconsistently used and only encompass one aspect of the stressor (duration). Short and long duration stress are generally thought to produce specific outcomes (e.g. acute stress enhances while chronic stress suppresses immune function). We propose that aspects of stress other than duration, such as frequency and intensity, are important in determining its outcome. We experimentally manipulated duration, frequency, and intensity of application of exogenous corticosterone, CORT, in *Sceloporus undulatus* (Eastern fence lizards) and measured the immune outcomes. Our findings reveal that immune outcomes of stress are not easily predicted from the average amount or duration of CORT elevation, but that intensity plays an important role. Although three of our treatments received the same average amount of CORT, they produced different effects on immune outcomes (hemagglutination). As predicted by the literature, short-duration exposure to *low*-dose CORT enhanced hemagglutination; however, short-duration exposure to *high*-dose CORT suppressed hemagglutination, suggesting that stressor intensity affects immune outcomes of stress. While both are traditionally termed "acute" based on duration, these treatments produced different immune outcomes. Long-duration ("chronic") exposure to CORT did not produce the expected suppression of hemagglutination. Frequency of CORT application did not alter immune outcomes at low intensities. These results highlight the need to quantify more than just the duration of a stressor if we are to understand and manage the ecological consequences of stress. Specifically, we should consider stressor frequency and intensity, as well as duration, for a more complete characterization and understanding of stress.

1. Introduction

The influence of stressor duration on physiological outcomes is well recognized; however, less is known about the role of other stress aspects. Stress is typically characterized by its duration, as "acute" or "chronic". Acute stress is characterized as "short" in duration (Burchfield, 1979; Romero, 2004), lasting from minutes to hours (Boonstra, 2012; Harbuz and Lightman, 1992; Martin, 2009), and the stressor is usually not repeated. The stress response is generally considered to be adaptive in the short term as it can facilitate the response to and recovery from a threat (Munck et al., 1984; Sapolsky et al., 2000). For example, the production of glucocorticoids (such as cortisol or corticosterone, CORT) in response to a stressor can mobilize energy to allow a fight or flight response (Sapolsky et al., 2000), enhance immune function to deal with increased risk of injury and infection (Dhabhar, 2009; Martin, 2009), and alter metabolism to help an individual maintain homeostasis (Stratakis and Chrousosa, 1995). Chronic stress is characterized as "long" in duration (Burchfield, 1979; Romero, 2004; Sapolsky et al., 2000), lasting from days to months (Boonstra, 2012; Dhabhar, 2009; Martin, 2009), and includes persistent or frequent repeated stress (Harbuz and Lightman, 1992; Romero, 2004), such as frequent tourist visits or sub-lethal predator attacks ("repeated acute;" Burchfield, 1979; Busch et al., 2008a,b). Persistent or long term activation of the physiological stress response can push animals into allostatic overload, whereby more energy is required for allostasis than can be obtained from the environment (McEwen and Wingfield, 2003). This can lead to energy being diverted away from other energy-sensitive functions, which in turn may lead to suppressed immune function (Dhabhar, 2009; French et al., 2007; Guillette et al., 1994; Martin, 2009), growth rates (Chrousos and Gold, 1992; Laugero and Moberg,

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2000), and reproductive output (Greenberg and Wingfield, 1987; McGrady, 1984). However, long-duration exposure to stress does not always have these suppressive effects (Chester et al., 2010; McCormick and Langkilde, 2014; Miles et al., 2007). It is also unclear at what point in time acute stress become chronic, and where repeated acute stressors fall on the acute – chronic spectrum (Busch et al., 2008a,b). The terms "acute" and "chronic" may not be entirely useful, as they are inconsistently used and only encompass one aspect of the stressor (duration).

While it is clear that stressor duration influences the physiological outcome of stress (citations above), other characteristics of a stressor may also play a role. Intensity and frequency may have important influences on the outcomes of stress. These characteristics are largely ignored in the literature (but see intensity: Ottenweller et al., 1989; McEwen et al., 1997; frequency: McCormick et al., 1998; Busch et al., 2008a,b), and are not considered simultaneously or in combination with duration. We suspect that considering only duration of stress is hampering progress towards understanding how stress can have different outcomes (Romero et al., 2009).

In this study, we systematically investigated the effects of duration, frequency, and intensity of CORT elevation on immune outcomes using *Sceloporus undulatus* (Eastern fence lizards). We experimentally elevated CORT, the primary glucocorticoid stress-relevant hormone in reptiles, using various application regimes that differed in duration (length of time exposed), intensity (concentration of CORT dosage), and frequency (how often exposed), and determined how these stress aspects affected immune outcomes (Fig. 1). We then determined whether the traditional classification of these treatments as "acute" or "chronic" matched the expected immune outcomes (immune enhancement vs suppression, respectively). We chose to manipulate CORT. Many other components of the HPA axis, including hypothalamic and pituitary hormones, as well as the sympathetic nervous system are also important in the physiological stress response and may respond differently to various stress aspects. CORT plays an important role in the stress response, its elevation is frequently linked to negative consequences (Dhabhar, 2009; French et al., 2007; Guillette et al., 1994; Martin, 2009), and our manipulation of CORT will provide insight into how organisms respond to stressors with different characteristics.

2. Methods

2.1. Study system

Between May and July 2013, we collected 32 adult male *S. undulatus* (Eastern fence lizards) using a hand-held noose. Lizards were collected from five sites across the Southern United States: (1) Standing Stone State Park, Overton County, Tennessee; (2) Fall Creek Falls State Park, Van Buren County, Tennessee; (3) Holly Springs National Forest, Marshall County, Mississippi; (4) Conecuh National Forest, Covington County, Alabama; and (5) Blakeley State Park, Baldwin County, Alabama. We measured lizards for snout-vent length (SVL) at capture and placed lizards in individual cloth bags for transport.

(A)

Treatment	Frequency	Intensity	Duration	Total CORT (over 3 days)
High Acute (HA)	Infrequent (every 3 days)	***High (18μg)***	***Short (hours)***	18μg
Low Acute (LA)	***Infrequent (every 3 days)***	***Low (6μg)***	Short (hours)	6μg
Repeated Acute (RA)	***Frequent (daily)***	Low (6μg)	Short (hours)	18μg
Chronic (Ch)	Infrequent (every 3 days)	High (18μg)	***Long (days)***	18μg

(B)

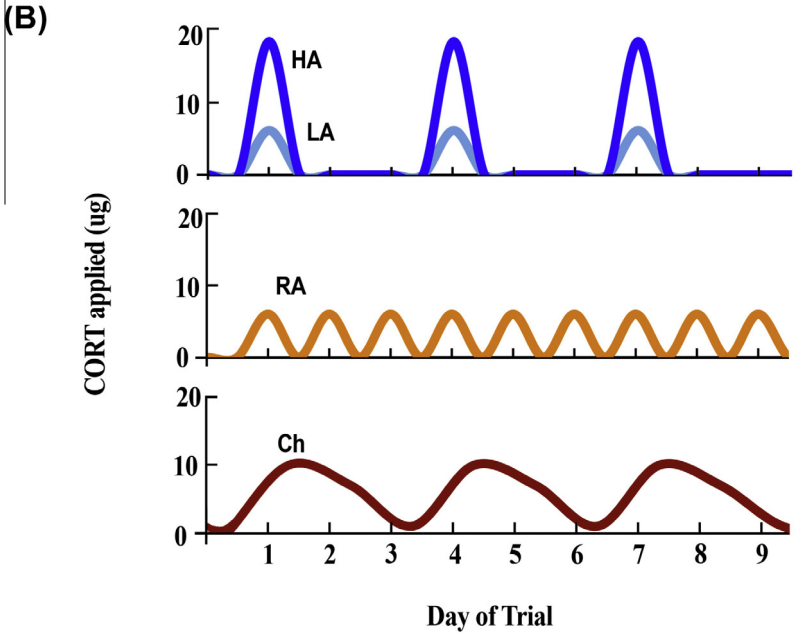

Fig. 1. (A) The frequency, intensity, and duration of CORT application in each of the treatments used in this study, and the total amount of CORT received in each 3-day period. Text in parentheses indicates: for frequency, how frequently a CORT-oil solution was applied (oil-vehicle only was applied on remaining days); for intensity, the amount of CORT applied during each application; and for duration, whether the period of CORT elevation was short or long. Italicized pairs in each column represents treatments that differ in only the parameter shown in that column. (B) A graphical representation of the amount of CORT applied for each of the treatments used in this study (Control (Ctl) had no CORT applied). This is provided for illustrative purposes to convey the expected duration of CORT release following application.

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