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Full-length Article

# Stronger hypothalamus-pituitary-adrenal axis habituation predicts lesser sensitization of inflammatory response to repeated acute stress exposures in healthy young adults

Myriam V. Thoma [a,b,*], Danielle Gianferante [a], Luke Hanlin [a], Alexander Fiksdal [a], Xuejie Chen [a], Nicolas Rohleder [a,c]

[a] Department of Psychology, Brandeis University, Waltham, MA, USA
[b] University of Zurich, Psychopathology and Clinical Intervention & Dynamics of Healthy Aging, Binzmuehlestr. 14/17, 8050 Zurich, Switzerland
[c] Chair of Health Psychology, Department of Psychology, Friedrich-Alexander-University Erlangen-Nuernberg, Erlangen, Germany

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ABSTRACT

Effective adjustment of the stress systems to repeated stress is regarded as an adaptive response of the organism facing environmental threats. Given the intertwined relationship between the stress systems and the inflammatory system, it could be expected that inflammatory processes should adapt to repeated stress as well. However, only little is known about adaptational processes of the different components of the immune system in response to repeated stress, and how these might be related to adaptational processes of the hypothalamus-pituitary-adrenal (HPA) axis.

We here examined $N = 22$ healthy participants (mean age 23 years, 50% female) and exposed them to a standardized laboratory stressor twice, 24 h apart. Plasma interleukin 6 (IL-6), salivary cortisol and psychometric parameters were assessed repeatedly up to 120 min post stress.

Results revealed a significant day by time interaction for cortisol ($F = 5.06$; $p = 0.013$) and IL-6 ($F = 4.42$; $p = 0.041$), indicating habituation of HPA axis and sensitization of inflammatory stress responses. Cortisol habituation and inflammatory sensitization were inversely related when controlling for sex ($r = -0.44$; $p = 0.044$). Explorative analyses revealed significant associations between the IL-6 response on the second exposure with perceived stress ($r = 0.58$; $p = 0.004$), vital exhaustion ($r = 0.57$; $p = 0.009$), depression ($r = 0.47$; $p = 0.026$) and purpose in life ($r = -0.50$; $p = 0.04$).

These findings may help to increase understanding of the still only rudimentary understood interplay of adaptational processes of endocrine and immune responses to repeated stress and might indicate a link between inflammatory disinhibition and psychological indicators of well-being.



## 1. Introduction

Human beings can be considered well-adapted organisms, and this is likely based on the availability of highly efficient and finely orchestrated systems responding to environmental threats, such as the sympathetic nervous system (SNS), the hypothalamus-pituitary-adrenal (HPA) axis, and the immune system. In a healthy, adaptive response to environmental threats, these systems are constantly tuning their optimal level of functioning to match the demands from an incessantly changing environment (McEwen, 1998; Sterling and Eyer, 1988). An adaptive short-term response to acute threat starts with the activation of the SNS, which instantly releases the catecholamines norepinephrine (NE) and epinephrine (E) to provide the organism with fast energy to either 'fight or flight'. Within minutes, the HPA axis is activated and releases its main effector, the glucocorticoid hormone cortisol, to further increase available energy (del Rey et al., 2008; Jacobson, 2005; Sapolsky et al., 2000; Tsigos and Chrousos, 2002). Further downstream, a temporary increase in plasma inflammatory cytokines, such as interleukin (IL-) 6 can be observed (Steptoe et al., 2007; van Gool et al., 1990), which functions as a stimulator of the acute-phase reaction enhancing the body's defense against invading pathogens (Heinrich et al., 1990; Sapolsky et al., 2000; Segerstrom and Miller, 2004; Steptoe et al., 2007). This activation is later down regulated by the anti-inflammatory actions of cortisol (Kunz-Ebrecht et al., 2003).

* Corresponding author at: University of Zurich, Psychopathology and Clinical Intervention, Binzmuehlestr. 14/17, CH-8050 Zurich, Switzerland.
*E-mail address:* m.thoma@psychologie.uzh.ch (M.V. Thoma).

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While previous research has concentrated either on short-term activation of these adaptive mechanisms, i.e. responses to single situations representing environmental threat, or on long-term adaptation such as under chronic stress, less is known about the medium-term adaptation of these systems. In other words, while we know more about the effect of singular acute events, and about long-term conditions on these systems, we know much less about systemic responses to repeated acute environmental threats. As for the SNS, existing literature does not suggest the existence of a medium-term adaptational mechanism to repeated stress exposures, as response to repeated stressors tend to be of unchanged magnitude, i.e. not showing habituation (Gerra et al., 2001; Schommer et al., 2003; Strahler et al., 2015). In contrast, in the HPA axis, repeated stress-exposure has frequently been found to lead to habituation, i.e. a decrease in the released cortisol in animals and healthy individuals across repeated exposure to similar stressors (e.g. Dhabhar et al. (1997), Figueiredo et al. (2003), Gerra et al. (2001), Gunnar et al. (1989), Johnson et al. (2002), Kirschbaum et al. (1995), Mason (1968), Mason et al. (1968), Schommer et al. (2003), Strahler et al. (2015), Wust et al. (2005)). Whether or not an individual habituates to repeated stress seems to depend on various interindividual differences (e.g. Deinzer et al. (1997), Gerra et al. (2001), Kirschbaum et al. (1995), Schommer et al. (2003)), such as personality factors, i.e. social dominance or locus of control (Pruessner et al., 1997), body-mass index (BMI) and body fat (McInnis et al., 2014), rumination after stress (Gianferante et al., 2014), exhaustion (Kudielka et al., 2006), or cortisol release in response to the first stress exposure (Wust et al., 2005). Much less is known about the medium-term adaptive response of the inflammatory response system. A valuable exception is the study by von Kanel and colleagues, in which twenty-one middle-aged male participants were subjected to a psychosocial stress task on three occasions, each one week apart (von Kanel et al., 2006). In this study, the authors did not find habituation in the IL-6 response to repeated stress. In sum, existing data indicate that only the HPA axis appears to adapt to repeated stress in the form of habituation; however, the SNS and the inflammatory response system do not adapt.

This is remarkable as with the initial, acute stress response it was found that the SNS does stimulate the inflammatory response (Bierhaus et al., 2003) and glucocorticoids then down-regulate the inflammatory response (Wolf et al., 2009). A repeated exposure to stress would thus, hypothetically, lead to the activation of the inflammatory response (by the SNS), but not to its containment (by the HPA axis) due to the lack of the inhibitory effect by the glucocorticoids (Sapolsky et al., 2000). While technically, a non-habituated SNS response together with a habituated HPA response, is compatible with non-habituating plasma IL-6, it would potentially permit an increased or prolonged inflammatory stress response, in form of a low-grade peripheral inflammation of the system when confronted with repeated (or chronic) stress (Rohleder, 2014), which can be considered a maladaptive response of the system. It is thus, on a theoretical basis, expected that there should be variability of the IL-6 response to repeated stress, which again should be related to variability of the HPA axis response (habituation). One study has found this expected inverse relationship between cortisol and IL-6 in response to a single acute stress paradigm (Kunz-Ebrecht et al., 2003). The only (published) indication from a repeated-stress paradigm comes from von Kanel et al. (2006), who did find an inverse link between HPA axis activation and peripheral immune system activation in middle aged men, yet, only on the third out of three stress exposures.

Given the potentially harmful effect of increased levels of inflammatory markers over time (e.g. Danesh et al. (2008), Ershler and Keller (2000)), it would be essential to know whether the inflammatory response adapts to repeated stress, and how this adaptation of the peripheral immune system activation is related to the HPA axis habituation. We therefore set out to examine whether IL-6 adapts to repeated stress, and whether habituation or sensitization of the inflammatory response is related to hypothesized cortisol habituation in healthy young adults.

## 2. Methods

### 2.1. Study participants

Participants were recruited using fliers and print advertisements. Eligibility requirements were checked during a standardized telephone screening. Participants were included in the study if they were native speakers of English, 18 to 35 years old, had a BMI between 18 and 30 $kg/m^2$, and were non-smokers. Non-smoking was defined as smoking less than ten cigarettes per week. Additional inclusion criteria for female participants were a regular menstrual cycle and non-usage of oral contraceptives, as the use of oral contraceptives has been linked to changes in the stress-reactivity of the HPA axis (Rohleder et al., 2003).

Furthermore, participants had to be free of chronic diseases (e.g. allergies, atopic, autoimmune or infectious diseases), psychiatric diseases (e.g. depression, anxiety), and were free of regular prescribed medications. Those who were currently undergoing severe and unusual stress (e.g. exams, death of a loved one, or separation) at the time of screening were also excluded. If all inclusion criteria were met, participants were scheduled for two appointments on consecutive weekdays in the laboratory. Female participants were tested in the luteal phase of their menstrual cycle (day 22–27), because previous studies have shown that during the luteal phase, women and men have comparable cortisol reactivity in response to a psychosocial stress situation (Kirschbaum et al., 1999). Informed consent was received from all participants. Monetary reimbursement for study participation was US $100. The study was conducted in accordance with the declaration of Helsinki, and the study protocol was approved by the local Institutional Review Board (IRB).

### 2.2. Procedures

Both appointments, from now on referred to as Day 1 and Day 2, were scheduled in the afternoon, between 1300 h and 1830 h. Afternoon hours were chosen to minimize potential confounding effects by the circadian rhythmicity of the biochemical parameters of interest. We chose to repeat the stress test only once (for a total of two exposures) because previous studies have shown that the majority of participants display HPA axis habituation upon secondary exposure (Gerra et al., 2001; Kirschbaum et al., 1995). Participants were instructed to come to the laboratory well-hydrated, but to refrain from eating or brushing their teeth one hour before their appointments. Furthermore, participants were not allowed to drink alcoholic or caffeinated beverages 24 h prior the experiments. The same procedure and stress protocol was applied for both days. Upon arrival on Day 1, participants were informed about the nature and procedures of Day 1 and Day 2, and in case of agreement, signed the written informed consent. Upon signing the consent form, the main experimenter assessed basic medical data of the participants, including body fat, waist-to-hip ratio (WHR) and body mass index (BMI). A first saliva sample was collected using a salivette (Sarstedt, Newton, NC) to allow assessment of baseline cortisol before catheter placement. Following this, a registered nurse placed a venous catheter in an antecubital vein of the non-dominant arm (BD Nexiva, BD, Franklin Lakes, NJ USA), followed by a resting period of 30 min. A second saliva sample, and the first blood sample (Vacutainer, 9 ml, EDTA, BD, Franklin Lakes, NJ) were

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