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

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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).

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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

taken immediately before exposure to the Trier Social Stress Test (TSST, Kirschbaum et al., 1993). Briefly, participants were informed that they were expected to hold a speech highlighting positive personal characteristics in front of an evaluative panel of trained confederates. After the speech, participants underwent a mental arithmetic task, which requires serial subtraction for another 5 min. To limit learning effects, this math task was modified used different numbers during the second TSST (see also Schommer et al. (2003), von Kanel et al. (2006)). The TSST has been shown to be a highly reliable tool for inducing strong psychophysiological stress responses (see Dickerson and Kemeny (2004)). After the TSST, participants were escorted back to their room, where further saliva samples were taken 1, 10, 30, 60, and 120 min, and further blood samples were taken 30 and 120 min after the end of the TSST. While resting, participants filled out a battery of questionnaires (see in psychometric measures section below) and were allowed to read in provided magazines. Procedures were repeated in the same way, but with a different version of the math task of the TSST on Day 2 of the study.

2.3. Measurement of cortisol and Interleukin-6

For measuring cortisol, saliva was collected using the salivette collection system (Sarstedt, Newton, NC), and was stored at room temperature until completion of the session. Samples were then stored at −30 °C until analysis. Prior to analysis, salivettes were centrifuged for 10 min at 2000 g and 4 °C. Salivary free cortisol concentrations were then measured using commercial chemiluminescence immunoassay (CLIA; IBL-International, Toronto, Canada). Intra- and inter-assay CVs were below 10%.

To assess inflammatory responses to acute stress, blood was taken at three time points on each of the study days using EDTA-coated Vacutainer tubes with 9 ml volume (BD, Franklin Lakes, NJ). Vacutainers were centrifuged immediately for 10 min at 2000 g and 4 °C, followed by transfer of plasma into microtubes, and immediate storage at -80C until batch analysis. Prior to measurement of IL-6, plasma was thawed and centrifuged at 15,000*g* for 2 min. Interleukin-6 concentrations were measured using commercial high sensitivity immunoassay (Quantikine HS human IL-6 kit, R&D Systems, Minneapolis, MN, USA) with minimum detection limit of 0.039 pg/ml. Intra- and inter-assay CVs were below 10%.

2.4. Self-Report measures of psychosocial health and Well-being

The 10-item version of the *Perceived Stress Scale* (PSS, Cohen et al., 1983) was used to assess an individual's subjective appraisal of particular life events/situations as being unpredictable, uncontrollable, and/or overloaded. On a 5-point Likert scale ranging from 0 'never' to 4 'very often', participants rated how often in the previous month they felt or thought as described in 10 examples. Higher scores are associated with higher levels of chronic stress.

For the assessment of vital exhaustion, which is a concept used to group feelings of demoralization or disproportionate fatigue, including low levels of energy and increased irritability, we applied the 23-item *Maastricht Vital Exhaustion Scale* (MVES, Appels and Mulder, 1988). Questions with regard to how the individual felt lately, such as "do you feel tired", or "do you feel weak all over", have to be answered on a yes, no, or question-mark scale. Higher scores indicate that the individual recently experienced more vital exhaustion.

The 20-item *Center for Epidemiological Studies Depression Scale* (CES-D, Andresen et al., 1994) has been used to quantify depressive symptoms in participants. On a 4-point Likert scale ranging from 0 'rarely or none of the time (less than 1 day)' to 3 'most or all of the time (5–7 days)', individuals rate the frequency of depressive symptoms during the previous week. Higher scores are associated with higher chance of clinical depression; scores of 16 or more have been related to clinical depression.

To assess purpose in life, we applied the *Purpose in Life* scale (Boyle et al., 2009). Purpose in life describes a psychological predisposition to see meaning in one's life, to have a sense of intentionality as well as a sense of behavior guiding goal directedness. On a 5-point Likert scale ranging from 1 "strongly disagree" to 5 "strongly agree", participants rated questions with regard to their purpose in life, such as for example: "I have a sense of direction and purpose in life". Higher scores are associated with a higher sense of purpose in life.

2.5. Statistical analyses

Prior to hypothesis testing, normality of distribution and homogeneity of variance were tested using Kolmogorov-Smirnov and Levene's test. Cortisol and IL-6 data fulfilled assumptions of a normal distribution and all respective analyses were performed using raw data. In the case of a violation of the sphericity assumption, corrections by the Greenhouse-Geisser procedure were applied. To estimate the extent of stress reactivity of the biochemical stress measures cortisol and IL-6, we calculated *response indices*, i.e. the maximum increase (delta max, i.e. peak values after stressor minus baseline values before stressor) separately for Day 1 and Day 2. In the following, we refer to these indices of stress reactivity of cortisol and IL-6 as 'cortisol response' and 'IL-6 response' either of Day 1 or Day 2. To estimate the extent of habituation of cortisol and sensitization of IL-6 stress responses upon repeated exposure, we computed double delta indices, differently for cortisol and IL-6. Because habituation was expected for cortisol, we computed an adaptation index termed 'cortisol habituation' by subtracting Day 2 response from Day 1 response. Therefore, higher values indicate stronger habituation. For IL-6, we were interested in the extent of sensitization, as no habituation was expected. We therefore calculated an index termed 'IL-6 sensitization' by subtracting Day 1 response from Day 2 response. Therefore, higher values indicate higher sensitization. To test for sex differences in dependent variables of interest we computed One-Way ANOVAs. To compare Day 1 and Day 2 measures, we used paired-sample t-tests. To test for stress-induced activation, as well as habituation of cortisol and sensitization of IL-6, we further computed analysis of variance (ANOVAs) for repeated measures with the within-subjects factors day (Day 1 vs. Day 2) and time (six time points for cortisol, three time points for IL-6). Pearson and partial correlations (controlling for sex) were computed to analyze the relationship of cortisol and IL-6 adaptation. All reported results were considered to be significant at the $p \leqslant 0.05$ level, and were considered a trend at the $p \leqslant 0.1$ level. All tests were two-tailed. Unless otherwise indicated, all reported results shown are means ± standard deviations (SD). All statistical analyses were performed using SPSS Statistics 19 for Mac OSX (IBM, Chicago, IL, USA).

3. Results

3.1. Sample characteristics

Statistical analyses were based on data provided by $N = 22$ healthy participants, 11 women and 11 men. Women and men did not differ in terms of age (women: 21.76 years ± 3.01 SD; men: 24.15 years ± 6.22 SD; $F(1/20) = 1.3$; $p = 0.27$) and BMI (women: 23.42 kg/m^2 ± 3.24 SD; men: 23.84 kg/m^2 ± 3.21 SD; $F(1/21) = 0.09$; $p = 0.76$). Women and men significantly differed in terms of waist-to-hip ratio (WHR, women: 0.82 ± 0.06 SD; men: 0.88 ± 0.03 SD; $F(1/21) = 7.72$; $p = 0.012$). Mean PSS scores (women: 19.0 ± 6.34 SD; men 15.0 ± 8.11 SD; $F(1/21) = 1.66$;

$p = 0.212$) were comparable to a large, age-matched population (21.1 ± 7.2 SD) (Cohen and Williamson, 1988), indicating no elevated perceived stress levels in our sample. Mean MVES scores (women: 14.00 ± 6.15 SD; men 8.30 ± 10.33 SD; $F(1/18) = 2.25$; $p = 0.151$) suggesting low vital exhaustion in our sample (Appels and Mulder, 1988). Mean CES-D scores (women: 12.55 ± 7.39 SD; men 14.18 ± 13.73 SD; $F(1/21) = 0.12$; $p = 0.731$) were below the cut-off value of 16 for clinical depression suggested by Andresen et al. (1994). Mean Purpose in Life scores (women: 4.11 ± 0.62 SD; men 3.95 ± 0.37 SD; $F(1/15) = 0.41$; $p = 0.53$), indicating no meaningful sex differences in purpose in life.

3.2. Acute HPA axis stress responses and habituation

To test whether the acute stress induction succeeded in activating the HPA axis, we first computed separate repeated measures ANOVAs for both study days. Results for Day 1 revealed a significant change in the cortisol concentrations over time (time effect: $F[1.74, 36.62] = 17.07$; $p < 0.001$), indicating successful HPA axis activation after primary stress exposure in the whole sample. Although cortisol responses were lower on average in women compared to men, there were no significant main effects of sex ($F[1,20] = 2.99$; $p = 0.10$), or sex by time interaction ($F[1.83, 36.59] = 2.11$; $p = 0.14$). Both, men and women responded with a significant cortisol stress response on Day 1 (men: $F[1,10] = 11.87$; $p = 0.002$; women $F[1,10] = 6.17$; $p = 0.011$). HPA axis responses were also found on Day 2 (time effect: $F[2.53, 53.19] = 10.09$; $p < 0.001$), and similarly, no sex differences (main effect: $F[1,20] = 0.03$; $p = 0.86$; sex by time interaction: $F[2.54, 50.81] = 0.47$; $p = 0.67$) (see Fig. 1a and b). While women showed a significant cortisol stress response on Day 2 ($F[1,10] = 7.60$; $p = 0.006$), men's cortisol response was only marginally significant ($F[1,10] = 3.26$; $p = 0.054$).

We next computed repeated-measures ANOVA to test for HPA axis habituation. We found a significant day by time interaction ($F[1.82/38.29] = 5.06$; $p = 0.013$), indicating differential HPA axis activation on Day 1 vs. Day 2. Comparison of cortisol delta increase ($t(21) = 3.75$; $p = 0.001$) revealed lower HPA axis reactivity on Day 2, consistent with HPA axis habituation. No sex differences were found (cortisol delta increase: $F(1/21) = 1.7$; $p = 0.21$).

3.3. Interleukin-6

To test whether the acute stress induction succeeded in activating the inflammatory response, we first computed separate repeated measures ANOVAs for both study days. Results for Day 1 revealed a significant change in the IL-6 concentrations over time (time effect: $F[1.11, 23.45] = 18.22$; $p < 0.001$), indicating a successful activation of the inflammatory response after the first stress exposure. There was no significant main effect of sex ($F[1,20] = 0.01$; $p = 0.908$). The inflammatory response was also found on Day 2 (time effect: $F[1.12, 23.49] = 34.05$; $p < 0.001$). No sex differences were found on Day 2 either (main effect: $F[1,20] = 0.17$; $p = 0.686$) (see Fig. 2a and b).

We then computed repeated measures ANOVA to test for IL-6 habituation, and found a significant day by time interaction ($F(1.15/24.08) = 4.42$; $p = 0.041$), indicating differential IL-6 activation on Day 1 vs. Day 2. Comparison of IL-6 delta increase ($t(21) = -2.26$; $p = 0.035$) revealed higher IL-6 reactivity on Day 2, which is consistent with IL-6 sensitization. No sex differences were found (IL-6 delta increase: $F(1/21) = 0.07$; $p = 0.796$).

3.4. Relationship of HPA axis habituation with inflammatory responses to repeated stress

We next tested our main hypothesis, i.e. whether HPA axis habituation is related to IL-6 sensitization. Pearson correlation of the index for cortisol habituation and the index for IL-6 sensitization revealed a statistical trend ($r = -0.409$; $p = 0.058$). Controlling for sex, partial correlation revealed a significant negative correlation between these indices ($r = -0.444$; $p = 0.044$) (see Fig. 3).

3.5. Explorative statistical analyses

Furthermore, explorative statistical analyses were conducted in order to identify psychological determinants of the cortisol and IL-6 stress responses on Day 1 and Day 2, as well as the cortisol habituation and IL-6 sensitization (see Table 1). Pearson correlation revealed no significant correlations between cortisol response indices and psychological determinants neither on Day 1 nor on Day 2. With regard to the IL-6 response on Day 1, we found a significant correlation only with MVES ($r = 0.48$; $p = 0.032$). However, significant correlations were found between the IL-6 response on Day 2 with PSS ($r = 0.58$; $p = 0.004$), MVES ($r = 0.57$; $p = 0.009$), CES-D ($r = 0.47$; $p = 0.026$), and purpose in life ($r = -0.50$; $p = 0.04$) (see also Fig. 4a–d). In addition, we also calculated Pearson correlations between the indices of cortisol habituation and IL-6 sensitization with the psychological determinants. We found no meaningful correlations (all n.s., see Table 1).

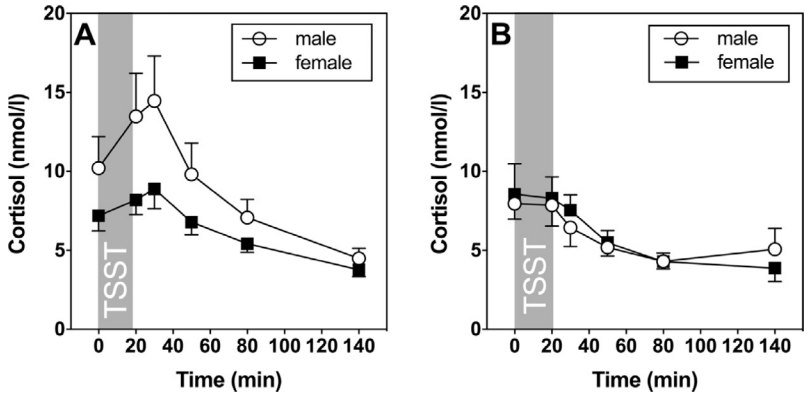

Fig. 1. (a) Salivary cortisol responses to the Trier Social Stress Test (TSST) on Day 1, separately for female and male participants. (b) Salivary cortisol responses to the Trier Social Stress Test (TSST) on Day 2, separately for female and male participants. Graphs show mean cortisol concentrations and standard error of mean at each measurement point.

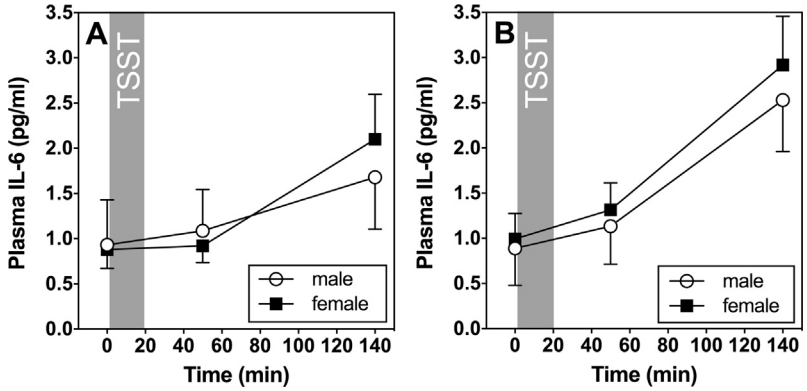

Fig. 2. (a) Inflammatory responses to the Trier Social Stress Test (TSST) on Day 1, separately for female and male participants. (b) Inflammatory responses to the Trier Social Stress Test (TSST) on Day 2, separately for female and male participants. Graphs show means of plasma interleukin 6 and standard error of mean at each measurement point.

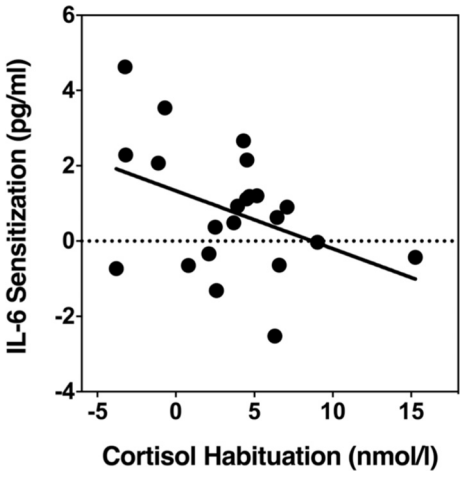

Fig. 3. Scatterplot showing the association of salivary cortisol habituation and interleukin 6 sensitization.

4. Discussion

In the current study we were interested in medium-term adaptation of inflammatory reactivity, as indexed by plasma IL-6 concentration, and its interaction with HPA axis reactivity, as indexed by salivary cortisol, when challenged with two repeated psychosocial stress exposures on two consecutive days. We were able to show that on average, IL-6 stress responses sensitized, i.e. were higher in response to a second stress exposure, while cortisol responses habituated as previously shown. Moreover, we found that stronger habituation of the HPA axis was associated with lesser IL-6 sensitization, meaning that those participants with stronger HPA axis habituation, also showed lesser sensitization of their IL-6 response to stress. Finally, explorative analyses revealed that higher IL-6 responses on Day 2, equivalent to an increased disinhibition of the inflammation response, were linked to self-reports of higher levels of perceived stress, depression and vital exhaustion, as well as lower perception of having purpose in life.

Our finding of a decreased cortisol response to repeated stress, which is consistent with HPA axis habituation, was expected and is in line with previous research (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)). It also corresponds to classic stress theories (e.g. Mason (1968)) stating that significant endocrine responses are expected in those situations that are novel, unpredictable, and uncontrollable, and a re-exposure minimizes these factors and thus also stress induced cortisol output. This habituation with regard to the HPA axis seems to be independent of sex, as we were not able to detect meaningful sex differences in the habituation of cortisol to repeated stress.

Our finding of a slow increase of IL-6 after acute stress induction up to two hours after termination of the stress exposure is in line with previous reports (Brydon et al., 2004; Steptoe et al., 2001; Takaki et al., 1994). Our result with regard to higher IL-6 reactivity to repeated stress, which we interpret as IL-6

Table 1
Correlation matrix of response indices of cortisol and interleukin (IL-) 6 on Day 1 and Day 2, the indices for cortisol habituation and IL-6 sensitization, with PSS, MVES, CES-D and Purpose in Life.

		1	2	3	4	5	6	7	8	9	10
1	Cortisol response D1	–									
2	Cortisol response D2	**0.61**∗∗	–								
3	IL-6 response D1	0.05	−0.19	–							
4	IL-6 response D2	−0.21	0.06	0.09	–						
5	Cortisol habituation	**0.63**∗∗	−0.23	0.24	−0.31	–					
6	IL-6 sensitization	−0.20	0.17	−**0.55**∗∗	**0.79**∗∗	−0.41†	–				
7	PSS	0.14	0.23	0.41†	**0.58**∗∗	−0.05	0.24	–			
8	MVES	−0.11	−0.06	**0.48**∗	**0.57**∗∗	−0.07	0.17	**0.83**∗∗	–		
9	CES-D	0.16	0.21	0.30	**0.47**∗	−0.01	0.21	**0.68**∗∗	**0.60**∗∗	–	
10	Purpose in Life	−0.40	−0.45†	0.00	−**0.50**∗	−0.09	−0.45†	−**0.49**∗	−**0.53**∗	−0.46†	–

Note. ∗ = correlation is significant at the 0.05 level; ∗∗ = correlation is significant at the 0.01 level; † = marginally significant.
CES-D = Center for Epidemiological Studies Depression Scale; D1 = day 1; D2 = day 2; IL-6 = interleukin 6; MVES = Maastricht Vital Exhaustion Scale; PSS = Perceived Stress Scale.
The bold values indicate a statistically significant correlation with a *p*-value less than 0.05.

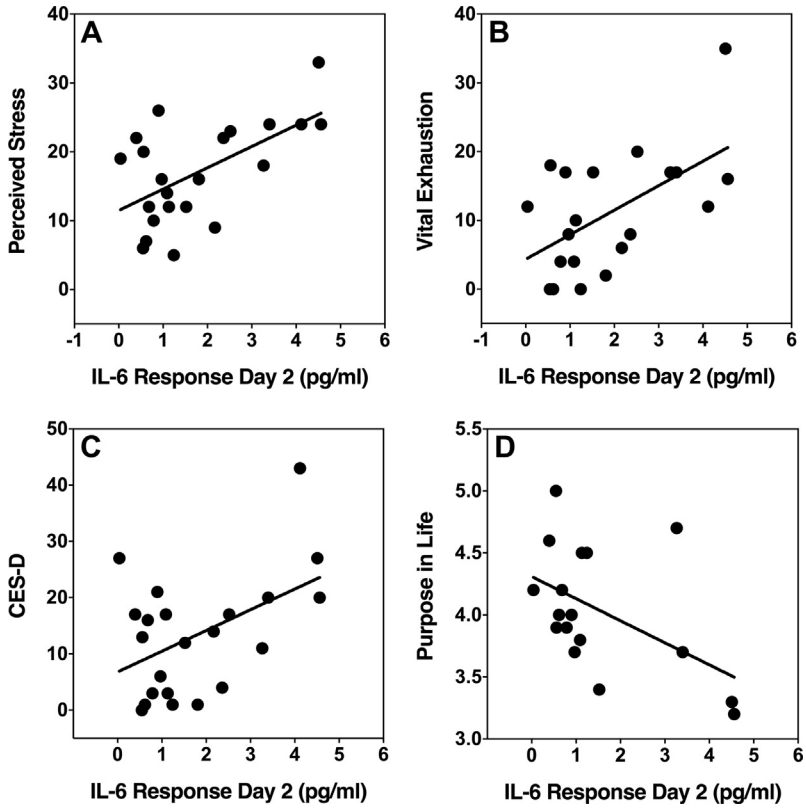

Fig. 4. Scatterplots showing associations of interleukin 6 response to the Trier Social Stress Test (TSST) on Day 2 with (a) perceived stress (PSS), (b) vital exhaustion (MVES), (c) depressive symptoms (CES-D), and (d) purpose in life.

sensitization, is in line with a previous report of our own research group (McInnis et al., 2014). However, other recent work by von Kanel et al. (2006) did not report such sensitization. One reason for this discrepancy might be that in the study by von Kanel et al., the last measurement of IL-6 occurred earlier (105 min post stress), and thus might only have captured a portion of the inflammatory stress response (von Kanel et al., 2006). Furthermore, the longer interval between TSSTs in von Kanel's study (7 days) compared to ours (1 day) might have accounted for the differences in our findings. In the face of almost no existing findings with regard to the medium-term adaptation of inflammatory reactivity, as indexed here with IL-6, this interpretation is of course speculative. In fact, it may be that the findings by von Kanel et al. and ours may not contradict each other as they may equally describe various forms of non-habituation, analogous to the maladaptive stress-response patterns of the HPA axis formulated by McEwen (e.g. missing or exaggerated response, or non-recovery, 1998).

Our finding of an association between HPA axis habituation and IL-6 sensitization partly replicates results by von Kanel et al., who observed that cortisol and IL-6 were inversely related on the last out of three repeated stress exposures, each one week apart, in middle-aged male participants (von Kanel et al., 2006). We were able to extend these findings by showing that HPA axis habituation and IL-6 sensitization are meaningfully linked also in women, and also already in the response to a second stress exposure. The fact that we assessed IL-6 longer after the stress induction than von Kanel et al. may have been responsible for the fact that we did find this meaningful link between HPA axis habituation and IL-6 sensitization already in response to the second stress induction.

In the absence of longitudinal data, it is too early to draw any conclusions about the consequences of a sensitizing inflammatory response to repeated stress, but based on cross-sectional data, it might be early enough to assume that it is likely not beneficial for health (Rohleder, 2014). Only very recently, our research group found cross-sectional evidence that higher body fat (McInnis et al., 2014), and lower subjective social status (Rohleder, 2014), are inversely linked to sensitization of the inflammatory response as indexed with IL-6, indicating that disinhibition of the inflammatory response might be related with biomedical and psychological health issues. In addition to that, exploratory analyses in the current study revealed that IL-6 responses on Day 2 were associated with self-reported well-being. Individuals with higher levels of perceived stress, more vital exhaustion, higher depressive symptom scores, and lower perceived purpose in life displayed stronger disinhibition of peripheral inflammation. It is important to note here that we did not find relationships of these psychological factors with IL-6 sensitization. While this difference in relationships cannot be explained at present, the associations of self-reports of well-being with responses to repeated stress still indicate that individuals with less psychological well-being are subject to higher IL-6 levels in the context of repeated stress, which underscores that inflammatory disinhibition might be a maladaptive stress response pattern.

Of particular significance, in our opinion, is the finding that IL-6 responses to the second TSST seem to be a better index for psychological health assessed in our study, than IL-6 responses to initial stress, or cortisol responses to any of the two TSSTs. This might not come as a surprise as single stress responses allow room for misinterpretations: individuals display extensive intra- and inter-individual variability in their acute stress responses due to confounding factors such as age, sex, sex steroid levels, behavioral and lifestyle choices (e.g. smoking, coffee, alcohol consumption, diet), time of the assessment, etc. (see reviews in Foley and Kirschbaum (2010), Kudielka et al. (2009)). Although investigators

strive for controlling these factors, stress patterns derived from the assessment of only one stress response may be misleading and several measurements would be advised. This would also allow the elimination of other, underestimated biasing factors, such as the impact of novelty. Although novelty leads to a more efficient stress induction in the laboratory (in the sense of a larger response), it is not a required pre-condition to induce a strong stress response in the laboratory (Dickerson and Kemeny, 2004). In fact, novelty has the potential to "mask" the impact of individual differences, such as for instance personality variables (see Pruessner et al. (1997)). These reasons may also be accountable for the apparent (unexpected) dissociation between psychological and physiological stress responses (for a review see Campbell and Ehlert (2012)). The fact that data aggregation of repeated-stress exposures (Pruessner et al., 1997), or data derived from the second stress exposure or computed stress adaptation indices seem to be a better index for general health indicators, implies that determinants derived from a repeated-stress paradigm may also be better indicators to explain inter-individual differences in adaptational processes of allostatic systems. Therefore, future studies interested in the relation between biological stress parameters and psychological indicators should favor repeated stress paradigms. However, it should be critically added here that our effects on Day 2 may not (only) be due to a potential habituation or non-habituation, respectively, but may (also) be due to the fact that the second stressor lacks the feature "novelty". But then again, it is the definition of the repeated stress paradigm to experience the same stressor at least twice and thus, the "novelty issue" must be critically acknowledged here but may not be regarded as a limitation of the paradigm per se.

Our results have to be considered in the light of some limitations: as Steptoe et al. (2007) concluded in their important review about the effects of stress on inflammation, we still do not know when IL-6 peaks, and when it recovers after acute stress induction (Rohleder, 2014). It is likely that our last blood sample taken two hours after stress cessation may still have been in the middle of the IL-6 stress response, so that we were unable to capture peak and recovery. We were therefore left comparing a complete HPA axis response with an incomplete IL-6 stress response. Future studies are required to answer the question with regard to the time point of when IL-6 peaks after stress. What is more, our study sample consisted mainly of healthy young students of a private university, which may restrict generalization of our results to older or less privileged adults. Furthermore, our study design would have benefited from including additional measurements of cortisol in blood besides the measurement of cortisol in saliva only. This would have allowed us to draw more specific conclusions with regard to the interplay of the HPA axis and the inflammatory system. Finally, we still do not know whether a short-term sensitization in the peripheral immune system activity has, or reflects positive or negative adaptational processes. While we have reasons to believe that a sensitization in the IL-6 response may be related to less advantageous health outcomes, it is unclear why a response pattern like this might have developed. It could be speculated that individuals living in more threatening environments might, from an evolutionary perspective, have been better able to survive with a more responsive innate immune system, albeit at the expense of longevity. Future studies are required to investigate the medium-term effects of a sensitizing IL-6 response.

In conclusion, our findings support the notion of an adaptation of the peripheral inflammatory system activation, as indexed with IL-6, to repeated stress exposures. Furthermore, we were able to show that habituation in HPA axis activity was related to sensitization in the inflammatory response in healthy young adults. Finally, we found meaningful inverse links between psychological indicators of well-being and inflammatory disinhibition. These findings may help to better understand the still only rudimentary understood interplay of adaptational processes of endocrine and immune system responses.

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