

"I'm Ready This Time": Investigating physiological and emotional habituation to repetitive social stress

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Abstract

Background: Repetitive stress is at the nexus of acute and chronic stress, and there is limited knowledge about how physiological and emotional responses change with repeated exposure.

Purpose: We examined stress-related biomarkers and emotional responses to repeated social stressors, and we tested behavioral moderators.

Methods: In Study 1, 42 adults completed the Trier Social Stress Test (TSST) twice, 4 months apart. Serum inflammatory cytokines (interleukin-6 [IL-6], tumor necrosis factor- α [TNF- α]), blood pressure, pulse, salivary cortisol, and state-level anxiety were measured surrounding the stressor. In Study 2, 84 married individuals completed two 20-minute discussions of contentious topics in the marriage, 1 month apart. Serum IL-6, TNF- α , blood pressure, pulse, salivary cortisol, and state affect were collected surrounding the conflict. Trained experimenters rated positive and negative behavior during the conflict.

Results: In the repetitive Trier paradigm, participants reported less anxiety (P s = .048) and had higher anticipatory IL-6 responses (P = .014) at Visit 2, compared to Visit 1. In the repetitive marital conflict paradigm, participants had lower positive affect (P = .0004), as well as systolic blood pressure (SBP) (P = .009), diastolic blood pressure (P = .0003), and pulse (P = .027) habituation at the second visit. Objectively rated negative conflict behavior interacted with visit to predict TNF- α (P = .025) and SBP (P = .037) responses. Positive conflict behavior did not moderate outcomes (P s > .06).

Conclusions: Stress-sensitive systems can habituate or sensitize to even nontraumatic, repetitive social stressors. Patterns of habituation or sensitization may vary by time between repetition, type of social stressor, stress-sensitive system, and participant behavior.

Key words: stress; habituation; sensitization; Trier; conflict; inflammation; IL-6; TNF- α ; cytokines; cardiovascular; sympathetic; HPA axis; cortisol.

Introduction

Aberrant physiological responses to acute laboratory social stressors signal risk for poorer mental and physical health outcomes.^{1,2} For example, heightened and persistent inflammatory responses to acute social stress may pave the way for depressive symptom increases.² Slavich and Irwin emphasized the importance of social stressors, compared to other stressors in their Social Signal Transduction Theory of Depression, in

which they outline how the immune system may be uniquely primed to respond to social threats, especially conflict or exclusion, and over time these responses can set the stage for depression.³ Especially if exposed to adversity during vulnerable developmental periods, stress-responsive systems can respond in a heightened manner to future stressors.⁴ However, it is less obvious how stress-responsive systems behave in the context of repetitive stress in adulthood, especially nontraumatic,

relatively routine stressors. As reviewed below, existing evidence suggests that the immune system, hypothalamic-pituitary-adrenal (HPA) axis, and cardiovascular system may respond differently with subsequent stressors. Repetitive stress is the nexus of acute and chronic stress, and therefore it is an important bridge between these constructs.⁵

A healthy, adaptive response to repeatedly encountering the same stressor is to habituate, that is, by responding less and less over time.⁶ By contrast, when repeated stress does not lead to habituation, or even causes an escalating response (ie, sensitization), this reflects a failure to adapt that, over time, will increase risks for psychopathology and chronic disease. Prior empirical studies that have primarily focused on the HPA axis largely align with theoretical expectations: In a series of studies conducted in small samples of healthy adults, on average, cortisol responses diminished from the first to the second administration of a well-established laboratory psychosocial stress paradigm, the Trier Social Stress Test (TSST)⁷ on 2 consecutive days,^{8–11} and even on a longer timescale (ie, 1 month).¹² When the stressor is repeated more than 2 times, there is no further reduction in the cortisol response.^{12,13}

Evidence concerning sympathetic-adrenal medullary (SAM) axis habituation is mixed. For example, there is some evidence that epinephrine and norepinephrine may not habituate to repeated exposures^{12,14}; yet the cardiovascular response consistently habituates in both men and women and across stressors.¹⁵ Catecholamines change rapidly and have a very short half-life (ie, clearance from the blood within a few minutes), so it can be difficult to ascertain their stress response trajectory, which may help to explain this otherwise nonsensical divergence. The SAM axis drives the inflammatory response, and cortisol from the HPA axis mutes it, begging the question of whether the inflammatory response habituates or sensitizes.

Despite the fact that cardiovascular and cortisol responses habituate to repetitive stress, multiple small studies have shown that interleukin-6 (IL-6) responses do not dissipate over repeated TSST sessions with 1-week intervals¹⁶ as well as on consecutive days¹⁰; even so, both pro- and anti-inflammatory gene expression may habituate to repeated stressors.¹⁰ One study found that IL-6 responses sensitized to the second TSST session, 1 day after the first, and those who showed stronger cortisol habituation had weaker IL-6 sensitization, suggesting that SAM axis responses that initiate inflammatory cascades may have habituated as well.¹¹ More work is needed in larger samples with differing time intervals to understand the sympathetic, cortisol, and inflammatory dynamics following repeated stressors.

As first conceptualized, habituation referred to a lower response magnitude with repeated exposure to the stressor/stimulus.¹⁷ Therefore, most prior empirical studies featuring repeated stress paradigms index habituation via response magnitude (ie, change from baseline to a specified post-stress timepoint). However, anticipation can provoke changes in stress-sensitive biomarkers even before the stressor manifests^{18,19}—a critical factor to account for in repetitive stress paradigms. When a stressor repeats, individuals' pre-stress data may, in fact, be a part of the sensitized response rather than a true baseline (eg, Brosschot¹⁸); in this context, examining change scores would obscure the sensitized response and perhaps misleadingly suggest habituation. Therefore, stress-responsive biomarker trajectories, rather than simply baseline to post-stress change scores, can help

to provide a more complete picture of stress habituation and sensitization. Also, response duration may be just as important as response magnitude, as failing to return to baseline (ie, recover) in a timely manner may change the homeostatic set point.²⁰

Most repetitive psychosocial stress studies used the TSST, a 20-minute social-evaluative threat paradigm in which a participant completes a complex mental arithmetic paradigm aloud and then gives a speech in front of a panel of stern-faced judges. Habituation and sensitization are largely unexplored in other ecologically relevant contexts, such as repetitive conflict with a close other. Yet, examining stress responses in these ecologically relevant contexts may help to explain the robust connection between strained marriages and poor health.²¹ Here, we examined the full stress response trajectory (to include anticipatory responses and recovery) first in the TSST repeated over a longer period of time—4 months (Study 1) and then, we extended the literature by using another stress paradigm—two 20-minute marital conflicts separated by 1 month (Study 2). We examined inflammatory, HPA (ie, cortisol) axis, cardiovascular (ie, blood pressure, heart rate), and emotional responses. Based on prior literature, we expected cortisol and cardiovascular responses to habituate^{8–13}; however, other outcomes were exploratory in nature given prior mixed evidence or lack of evidence to date, particularly in the context of Study 2's repeated marital conflict.

As an additional aim, in Study 2, we investigated whether conflict behavior moderated habituation/sensitization. While prior research provides evidence that healthy adults' cortisol and cardiovascular responses habituate in the face of repeated stress on average, physiological responses to repeated stressors can vary widely between individuals.^{8–11,15,22–25} For many years, it has been established that intensity of the stressor itself inversely relates to extent of habituation^{26,27}; yet, even with low-intensity, common stressors, there may be interindividual differences that help to determine whether people habituate. Beyond physiological contributors, such as central adiposity²⁸ and a flatter cortisol awakening response,⁸ individuals' behavior during the stressor may play a role. Prior research from our lab has shown that objectively rated behavior during a conflict between romantic partners relates to cortisol slopes throughout the day; that is, when couples used more negative and fewer positive behaviors during a conflict, those with more stressed partners had elevated cortisol levels than those with stressed partners.²⁹ More negative and fewer positive conflict behaviors may also relate to slower wound healing, lower levels of positive emotion, and heightened and persistent inflammatory responses.^{30,31} Here, we explored whether negative and positive conflict behavior moderated physiological and emotional responses to repetitive stressors. Study method and results are reported below following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement for cross-sectional studies.³²

Study 1: methods

Participants

Participants were recruited through campus and community print and web-based advertisements for a parent study assessing omega-3 supplementation's effects on inflammation, mood, anxiety, and accelerated aging^{33,34}. In total, 138 sedentary, primarily overweight ($22.5 \text{ kg/m}^2 \leq \text{body mass index}$

[BMI] ≤ 30 kg/m²), middle-aged and older adults participated in this double-blind, randomized, placebo-controlled trial (RCT). Using a permuted block randomization sequence created by a data manager who had no contact with participants, participants were randomized to receive 2.5 or 1.25 g/d of omega-3, or a placebo approximating the fatty acid ratio in a typical US diet (saturated:monounsaturated:polyunsaturated ratio of 37:42:21; USDA Continuing Survey of Food Intake by Individuals). OmegaBrite (Waltham, MA) supplied the supplement and placebo. Elsewhere, we have reported the effect of omega-3 supplementation on physiological stress reactivity³⁵, and in the current secondary analyses, we only used data from the placebo group ($n = 46$) to answer the question of interest about habituation or sensitization to acute stress. The parent study's strict exclusionary criteria yielded a sample that did not take cardiovascular medications, antidepressants, or immunomodulators and did not have metabolic, autoimmune, or inflammatory diseases. This study was approved by The Ohio State University Institutional Review Board, and all participants provided written informed consent before participating.

Procedure

Data were collected between July 2006 and February 2011. At a screening visit, a nurse measured participants' height and weight to calculate BMI. Throughout the 4-month RCT, participants had a baseline visit, as well as a visit every month. Only data from the baseline pre-supplementation visit and the final post-supplementation visit were relevant to these secondary analyses. On both visit days at 07:45, participants arrived at The Ohio State University's Clinical Research Center (CRC), a hospital research unit. Then, they completed questionnaires and ate a standardized breakfast. After breakfast, they had a 20-minute seated resting period, during which time the Dinamap/Critikon 1846SX/P monitor (GE Healthcare, Milwaukee, WI) took their blood pressure and pulse every 3 minutes; these instances were averaged together as a baseline measurement. At 08:50, they had a baseline blood draw to assess pre-stressor inflammation and provided saliva to assess pre-stressor cortisol. At 10:10, participants completed the TSST (described below), and they reported their state anxiety immediately before and after the stress protocol. Immediately after the stressor ended, they had 6 blood pressure and pulse measurements (one every 2 minutes), which were averaged together to produce a single post-stress value. Participants had their blood drawn 0.75 and 2 hours after the stressor to assess inflammation, and they provided saliva immediately post-stressor and 0.75 hours after the stressor to assess cortisol. These measurement timepoints capture the timing of known post-stress increases for these biomarkers.^{36,37}

Trier Social Stress Test

Participants completed the TSST (Kirschbaum et al.⁷), a well-validated laboratory stressor that provokes reliable changes in biological functioning.³⁸ After spending 10 minutes preparing a speech about why they were the best candidate for a job, a research assistant escorted them to a room where they saw a microphone, video camera, and an "audience" panel of 2 researchers wearing white laboratory coats. While seated, the participants gave their 5-minute speech and then performed mental arithmetic serial subtraction tasks for 5 minutes in front of this panel.

State anxiety

Participants rated their state anxiety on the 20-item State Anxiety Inventory, on which they rated to what extent they are presently experiencing a variety of anxiety-related feelings (eg, "I feel tense") on a scale from 0 "Not at All" to 3 "Very much" ($.89 < \text{Cronbach's } \alpha < .93$).³⁹

Salivary cortisol

Participants placed a salivette (Sarstedt, Newton, NC), an untreated sterile cotton roll, into their mouth for approximately 2 minutes or until saturated. From this sample, salivary cortisol was assayed with the Cortisol Coat-A-Count Radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA), which was modified to measure free cortisol in saliva, rather than plasma, per the manufacturer's instructions. The assay was counted and calculated on the Packard Cobra II Gamma Counter (Packard Instrument Company, Downers Grove, IL). The sensitivity was 0.025 $\mu\text{L/dL}$ and, inter- and intra-assay coefficients of variation were 5.2% and 4.3%, respectively.

Proinflammatory cytokines

Using an electrochemiluminescence method with Meso Scale Discovery kits, serum levels of IL-6, TNF- α , IL-10, and IL-12 were multiplexed and measured, and they were read with the Meso Scale Discovery Sector Imager 2400. To use the same controls for all timepoints for each participant, each participant's stored samples were assayed in 1 run. For the current study, we chose to examine IL-6 and TNF- α only, given that they are most commonly measured following acute stress, and we wanted to minimize the number of statistical tests. Sensitivity for these cytokines was 0.3 pg/mL. Intra-assay coefficients of variation were 2.8% for IL-6 and 4.3% for TNF- α . Inter-assay coefficients of variation were 12.5% for IL-6 and 12.1% for TNF- α .

Analytic method

For the primary models, linear mixed-effects models were used with subject-specific random visit effects that were allowed to be correlated to account for repeated measurements both within visit and between visits. Cytokine models also included a random assay plate effect. Because the anticipatory stress response is part of the habituation or sensitization phenomenon, the pre-stress baseline timepoint was modeled as an outcome, rather than used as a covariate. Participants were included in models if they had the pre-stressor baseline measurement and at least one post-stressor measurement at both visits; therefore, 4 individuals were excluded, yielding a total sample of 42 people. Outcomes of interest, modeled separately, were IL-6, TNF- α , salivary cortisol, heart rate, diastolic blood pressure (DBP), systolic blood pressure (SBP), and state anxiety. Key parameters in the models included the interaction between visit and time, which captures whether within-visit outcome trajectories differed between the first and second visits, and the main effect of visit, which captures how overall outcome levels differed between the visits. All models controlled for age, sex, and BMI. See [Supplementary Methods](#) for model equations.

Study 1: results

Preliminary results

Participants ($n = 42$) were middle-aged ($M = 51.0$, $SD = 8.4$), primarily female (76%), and mostly White (76%) ([Table 1](#)).

Across visits, all biomarkers, except for TNF- α ($P = .79$) and DBP ($P = .09$), responded to the speech stressor with significant post-stress increases (P s < .001). Likewise, participants reported higher anxiety after the stressor, compared to before the stressor (P s < .008).

Primary results

At all timepoints, anxiety levels at the second visit were lower than the first visit ($B = -2.38$, $SE = 1.17$, $t(38) = -2.04$,

Table 1. Sample information for both studies.

	Study 1 (N = 42)	Study 2 (N = 84)
Age	51.02 (8.37)	38.18 (8.26)
Sex		
Male	10 (23.8%)	42 (50%)
Female	32 (76.2%)	42 (50%)
BMI	29.78 (4.82)	32.20 (5.83)
Race		
White	32 (76.2%)	68 (81.0%)
Black	6 (14.3%)	16 (19.0%)
Asian	2 (4.8%)	0(0.0%)
Native American	1 (2.4%)	0(0.0%)
Mixed	1 (2.4%)	0(0.0%)
Education		
High school or some college	9 (21.4%)	27 (32.1%)
College graduate	22 (52.4%)	21 (25.0%)
Graduate or professional	11 (26.2%)	36 (42.9%)
Income		
<50K	16 (38.1%)	20 (23.8%)
50K-100K	17 (40.4%)	45 (53.6%)
> 100K	8 (19.1%)	14 (16.7%)
Negative emotion words, %	1.90 (1.29)	
Negative conflict behavior		22.27 (36.98)
Positive conflict behavior		173.34 (58.54)

Abbreviation: BMI, body mass index.

$P = .048$), but there were no other visit main effects (P s > .10). There was a significant visit by timepoint interaction for IL-6 ($F(2, 141) = 4.4$, $P = .014$), indicating that response patterns were different at each visit (Figure 1). That is, at the second visit, participants had a higher anticipatory IL-6 response than they did at the first visit ($B = 0.27$, $SE = 0.12$, $t(110) = 2.4$, $P = .020$), but there were no between-visit differences at the other timepoints (P s > .42). None of the other biomarkers or anxiety trajectories varied by visit (P s > .09). In terms of covariates, those who were older had higher TNF- α ($P = .022$), males had higher DBP ($P = .010$) and salivary cortisol levels ($P = .034$) across timepoints, and those with higher BMIs had higher SBP across timepoints ($P < .001$). No other covariates were significant (P s > .06) (see [Supplementary Tables 1 and 2](#)).

Study 2: methods

Participants

Participants were recruited for a parent study examining immune and metabolic responses to a high-fat meal⁴⁰. Because the study protocol featured a marital conflict as a lab stressor paradigm, there was an extensive 2-phase screening process (initially online and then in-person) to ensure adequate representation of distressed couples, as happier couples are generally overrepresented in marital research. In line with the parent study’s aims, we had strict exclusionary criteria to yield a sample that was disease-free yet overweight or obese. Specifically, couples were excluded if they were married fewer than 3 years, or if either partner had chronic health conditions (eg, anemia, diabetes), smoked, abused substances, used prescription medications other than birth control or levothyroxine, or had sensory impairments that would interfere with study completion. In total, 86 participants (43 couples) were included, and 350 were excluded because they did not meet the strict exclusionary criteria. This study was approved by The Ohio State University’s Institutional Review Board, and all participants provided written informed consent prior to study participation.

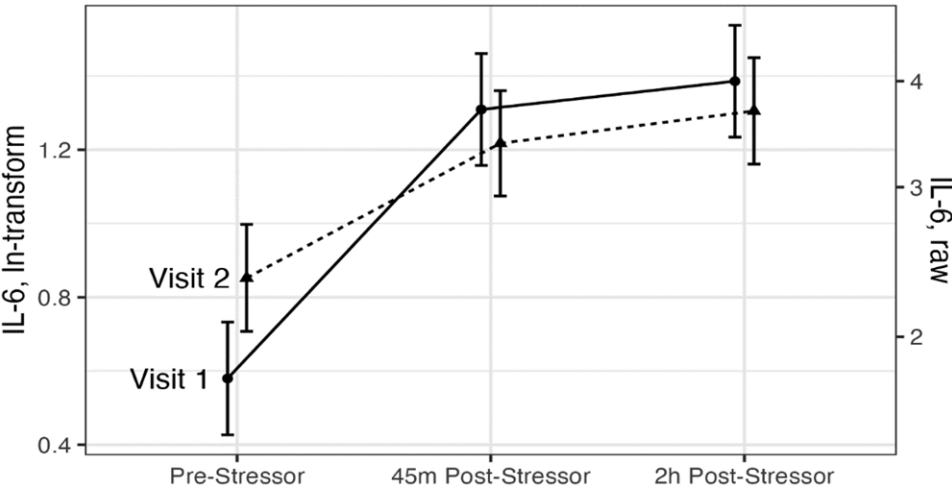


Figure 1. Inflammatory anticipatory response sensitization to repeated speech stressor. IL-6 trajectories were different at each visit ($P = .019$), such that participants had a higher anticipatory IL-6 response to the Trier Social Stress Test at the second visit, compared to the first.

Procedure

Data were collected between May 2011 and September 2013. Although it is not a focus of these secondary analyses, the parent study involved a double-blind, crossover high-fat meal paradigm, in which participants ate a high saturated fat meal at one visit and a high oleic sunflower oil meal at the other, in a random sequence 1–25 weeks apart ($M = 4.45$, $SD = 4.76$); most visits occurred within 3 weeks of one another, but some were more widely spaced due to participants' work schedules. Participants were told to avoid alcohol and caffeine use for one day prior, vigorous physical activity for 2 days prior, and aspirin, vitamins (except multivitamins), antioxidants, and other dietary supplements for 1 week before the 2 full-day study visits. The day before each visit, participants received 3 standardized meals from The Ohio State University's CRC metabolic kitchen, and they began a 12-hour fast at 19:30 on the evening before each study visit.

On the morning of each visit, couples arrived at the CRC at 07:30, and a catheter was inserted into each partner's arm. For the parent study, a baseline metabolic measurement was then obtained, and 3 blood pressure measurements were taken in 2-minute intervals.

Then each participant ate the study meal. The husband and wife received the same meal at each visit and were required to eat the full meal. Approximately 2 hours post-meal, participants provided another blood and saliva sample and then couples engaged in a marital problems discussion (ie, conflict) described below. Approximately 30 minutes and 1 hour after the conflict, participants provided saliva samples to assess cortisol, and approximately 1 and 5 hours post-conflict, participants provided blood samples to assess inflammatory responses. Although inflammatory reactivity is not often measured beyond 2 hours, we have previously shown sustained post-stress IL-6 elevations even 5 hours post-conflict in this sample—beyond what would be expected with IL-6's normal diurnal rhythm.² Participants reported their positive and negative affect prior to the conflict, as well as immediately post-conflict, and approximately 1 hour later. Blood pressure and pulse measurements were obtained using the Dinamap/Critikon 1846SX/P monitor (GE Healthcare, Milwaukee, WI) before the conflict, as well as immediately after the conflict. Before the conflict, 2 blood pressure measurements were taken 2 minutes apart, and averaged together, and after the conflict, blood pressure was taken every 2 minutes for 10 minutes, and these readings were averaged together.

Marital conflict

Using an inventory of potential areas of disagreement that both partners had completed, a trained experimenter initiated a 10- to 20-minute interview to identify a mutually contentious topic in the marriage. Couples were then told that they had 20 minutes to discuss and try to resolve one or more of the issues that the experimenter had determined to be the most conflictual. The conflict was videotaped, and the experimenters remained out of sight.

Trained research assistants used the Rapid Marital Interaction Coding System (RMICS), which discriminates between distressed and non-distressed couples.⁴¹ In line with prior research, we used a couple-level composite score of the 4 negative RMICS codes: psychological abuse (eg, disgust, beligerence, and nonverbal behaviors like glowering), distress-maintaining attributions (eg, "You were being mean on

purpose"), hostility (eg, criticism, nonverbal behavior like eye rolling), and withdrawal (nonverbal behaviors that suggest pulling back from the interaction). Couples' positive behavior was a composite of 5 RMICS codes: acceptance (eg, verbal and nonverbal behaviors that communicate active listening and care); relationship-enhancing attributions (eg, using situational or unintentional factors to explain negative behavior); self-disclosure (eg, verbal expression of feelings, wishes, or beliefs that are not hostile toward the partner); humor (eg, playful joking); and constructive problem discussion (eg, collaborative approaches to solving problems or nonverbal communication of agreement). The interrater agreement was high for both positive and negative conflict behaviors (Holley and Guilford's G index for negative behaviors = 0.97, for positive behaviors = 0.87)⁴².

Proinflammatory cytokines and salivary cortisol

Cytokine assays were performed with the same method as in Study 1. For the cytokines, sensitivity was 0.3 pg/mL, the intra-assay coefficient of variation was 3.42% for IL-6 and 259 for TNF- α , and the inter-assay coefficient of variation was 8.43% for IL-6 and 8.14% for TNF- α .

Salivary cortisol was collected and analyzed using the same method specified in Study 1.

Positive and negative affect

Participants completed the Positive and Negative Affect Schedule (PANAS), short form,⁴³ which features 5 positive items (inspired, alert, excited, enthusiastic, and determined) and 5 negative items (afraid, upset, nervous, scared, and distressed). Participants were asked to what extent they feel this way in the present moment, and they answered on a 5-point scale ranging from 0 "very slightly or not at all" to 4 "extremely." Cronbach α s ranged from .61 to .83 for each of the PANAS negative scale administrations and .85 to .89 for each for each of the PANAS positive scale administrations.

Analytic method

Similar to Study 1, linear mixed-effects models were used to capture the multiple sources of correlation induced by the study design. These models included random subject-specific meal effects that were allowed to be correlated to account for repeated measurements between and within visits, random couple-level intercepts to capture within-couple correlation, and, for cytokine models only, random intercepts for assay plate. We additionally included fixed effects for meal type, as well as the interaction between meal type and categorical timepoint. The outcomes of interest were IL-6, TNF- α , salivary cortisol, DBP, SBP, pulse, positive affect, and negative affect. Key parameters in the models included the interaction between visit and timepoint, which captures whether within-visit changes in outcomes differed between visits, and the main effect of visit, which captures changes in overall outcome levels between visits. As in Study 1, we included people who had the pre-stressor baseline measurement and at least one post-stressor measurement; thus, 84 people were included in primary models.

In a second set of models, we assessed potential moderators of habituation and sensitization. Because objective ratings of marital interaction are more sensitive predictors of health outcomes and physiological responses than self-report measures,⁴⁴ we examined couples' objectively rated positive and negative behavior during the conflict as potential moderators,

in separate models. In these models, the 3-way interaction between conflict behavior, visit, and timepoint tested whether conflict behavior moderated changes in the within-visit outcome trajectories across visits, and the 2-way interaction between conflict behavior and visit tested whether conflict behavior moderated changes in overall outcome levels between visits. Conflict behavior was time-varying (ie, an individual's conflict behavior may differ across visits), based on the recognition that couples' conflict could have started earlier that day and affected baseline measurements. Model equations are included in the [Supplementary Methods](#).

Study 2: results

Preliminary results

The sample consisted of younger to middle-aged adults ($M = 38$ years old, $SD = 8.2$, range: 24–61), and they were primarily White (81%) and employed full-time (70%). Couples had been married for an average of 11.5 years ($SD = 6.7$, range = 3–27) ([Table 1](#)). Across visits, all biomarkers, except for $TNF-\alpha$ ($P = .76$), SBP ($P = .06$), and DBP ($P = .29$), responded to the marital conflict with significant post-stress increases ($P_s < .001$). Participants also reported post-stress increases in negative and positive affect immediately after the stressor ($P_s < .002$).

Primary results

Participants reported lower levels of positive affect ($B = -1.09$, $SE = 0.30$, $t(84) = -3.69$, $P = .0004$), had lower SBP ($B = -2.84$, $SE = 1.06$, $t(82) = -2.67$, $P = .009$), DBP ($B = -2.30$, $SE = 0.61$, $t(82) = -3.75$, $P = .0003$), and pulse rate ($B = -1.52$, $SE = 0.67$, $t(82) = -2.25$, $P = .027$) across timepoints at the second visit, compared to the first; there were no other visit main effects ($P_s > .05$). No visit by time interactions were significant ($P_s > .05$). In terms of covariates, males had lower IL-6 ($P = .001$) and negative affect ($P = .001$), but higher SBP ($P < .0001$) and DBP ($P < .0001$) across timepoints compared to females. Those with higher BMIs had higher levels of $TNF-\alpha$ ($P = .002$), IL-6 ($P = .008$), negative affect ($P = .032$), SBP ($P < .0001$), DBP ($P = .008$), and pulse rate ($P = .026$), but lower salivary cortisol ($P = .004$) across timepoints compared to those with lower BMIs. No other covariates were significant ($P_s > .16$). See [Supplementary Tables 3 and 4](#).

Behavioral moderators of habituation

When testing moderators of habituation, the couple's negative behavior during the conflict moderated visit-related differences in $TNF-\alpha$ levels ($F(1,93) = 5.2$, $P = .025$), such that higher levels of negative behavior were associated with higher $TNF-\alpha$ levels during the second visit ($B = .002$, $SE = 0.0003$, $t(113) = 2.34$, $P = .021$) compared to the first visit ($P = .37$) ([Figure 2](#)); the between-visit difference was significant only for individuals with negative behavior of 37 and higher. Also, couple's negative behavior moderated visit-related differences in SBP trajectories ($F(1,161) = 4.4$, $P = .037$). At both high and low levels of negative behavior, spouses' pre-conflict SBP was higher at Visit 1 than Visit 2 (25th percentile of behavior: $B = 4.06$, $SE = 1.79$, $t(199) = -2.27$, $P = .024$); 75th percentile of behavior: $B = 3.92$, $SE = 1.48$, $t(194) = -2.65$, $P = .009$), but there were no visit differences in post-conflict SBP ($P_s > .18$). As a result, spouses' SBP significantly decreased from pre- to post-conflict at Visit 1 (25th percentile of behavior:

$B = -4.23$, $SE = 1.57$, $t(161) = -2.69$, $P = .008$; 75th percentile of behavior: $B = -2.83$, $SE = 1.41$, $t(161) = -2.01$, $P = .045$) but not at Visit 2 ($P_s > .52$), and this decline was steeper for couples with low levels of hostile behavior ([Figure 3](#)). Negative behavior during the conflict did not moderate habituation or sensitization for any other biomarker or for affect (2-way interaction effect $P_s > .07$; 3-way interaction effect $P_s > .32$). On average across visits, negative conflict behavior predicted trajectories of cortisol ($P = .037$), positive affect ($P = .019$), and negative affect ($P = .001$).

Positive conflict behavior did not modulate habituation of any outcome of interest (2-way interaction $P_s > .14$; 3-way interaction $P_s > .06$). However, on average across visits, positive conflict behavior predicted IL-6 ($P = .025$) and pulse ($P = .030$) trajectories, but not trajectories of any other outcome of interest ($P_s > .06$).

Discussion

Here, we have provided evidence that even in adulthood, stress-responsive systems can habituate or sensitize to nontraumatic, repetitive social stressors, even when a month or more passes before repetition. More specifically, the proinflammatory (ie, IL-6) anticipatory response sensitized, but cortisol and cardiovascular reactivity remained unchanged when the TSST was repeated 4 months after the first TSST. Also, in our study, people reported lower levels of state anxiety surrounding the second TSST compared to the first. A different pattern was observed in our repetitive marital conflict paradigm. There was evidence of cardiovascular response habituation; even so, people reported lower positive affect surrounding the second marital conflict, compared to the first. In short, our findings demonstrate that repetitive social-evaluative stress yields a different profile of habituation than repetitive marital conflict, underscoring the value of using an array of paradigms to understand the dynamics of reactivity and recovery following diverse, repeated stressors.

Our results are somewhat distinct from prior findings, perhaps because the stress exposures were separated by a longer timeframe. The inflammatory response sensitization generally accords with some prior studies with repetitive TSST^{10,11}; yet we observed anticipatory sensitization rather than post-stressor sensitization. We also did not observe cortisol response habituation in either repetitive stress paradigm, even though it is a well-established phenomenon.^{8–12} This difference may result from our analytical framework, in that we predicted all cortisol timepoints (even the pre-stressor timepoint) because anticipation of the repeated stressor is an important part of the response. In other studies, response habituation was indexed as the difference between the post-stress and pre-stress timepoints; thus, even slight cortisol anticipatory sensitization, documented elsewhere,⁴⁵ may be construed as response habituation instead. In these repeated stress paradigms, null results are also interesting because they indicate that the stress-responsive system maintained a similar response regardless of whether the stressor was familiar; indeed, cardiovascular reactivity did not diverge between the first and second repetition of the TSST, which is at odds with prior work that found cardiovascular response habituation when stressors were repeated on a shorter timescale.¹⁵

We extended the existing repetitive stress literature to a repeated marital conflict paradigm and found that cardiovascular responses habituated even though participants reported

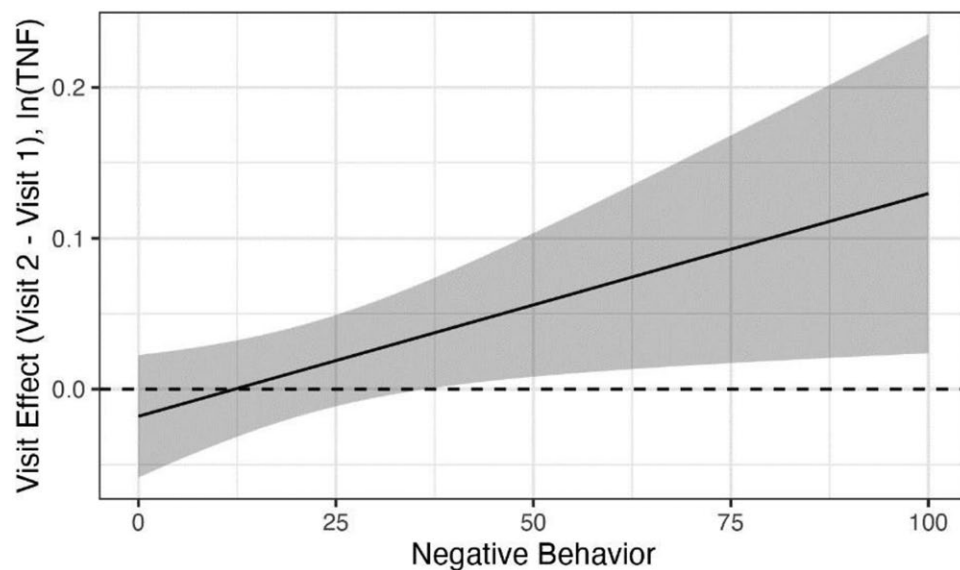


Figure 2. Negative conflict behavior moderates visit-related differences in inflammation. This Johnson-Neyman plots depicts the region of significance (ie, values of the conflict negative behavior) in which there is a between-visit difference in TNF- α responses to conflict. In the shaded regions that do not include a 0 effect (the dotted, horizontal line), greater negative behavior during the conflict predicted higher TNF- α responses at the second visit, compared to the first visit ($P = .025$). Abbreviation: TNF- α , tumor necrosis factor- α .

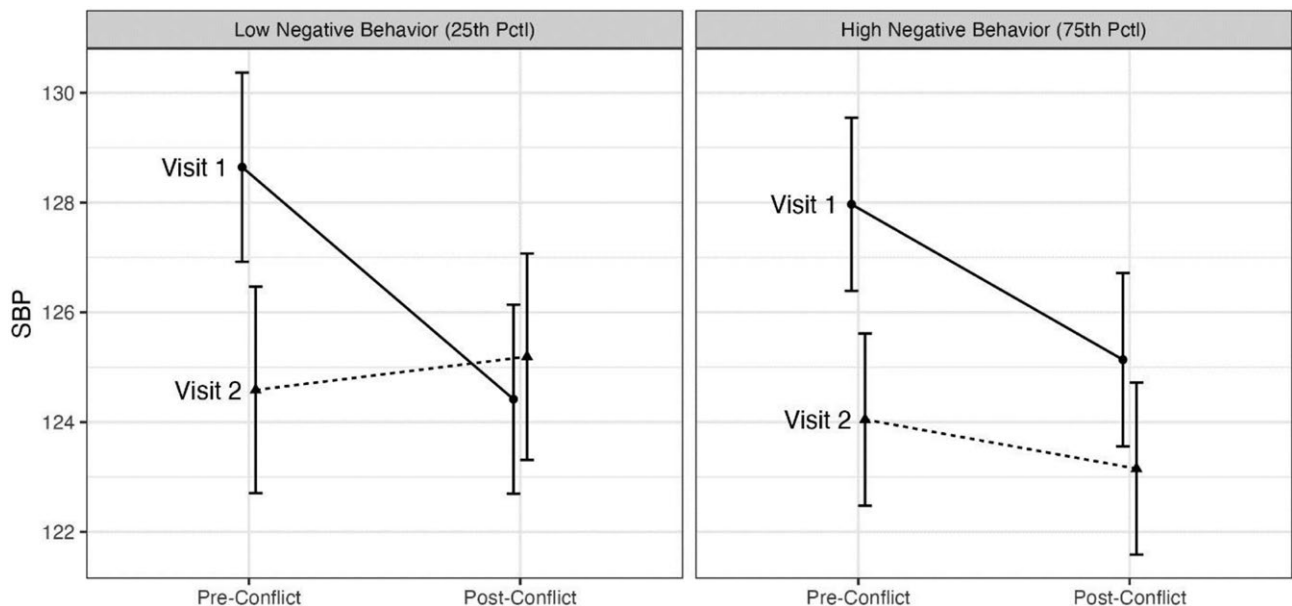


Figure 3. Negative conflict behavior moderates visit-related differences in blood pressure. Negative behavior during the marital conflict interacted with visit to predict systolic blood pressure (SBP) ($P = .037$). At both high and low levels of negative behavior, spouses' pre-conflict SBP was higher at Visit 1 than Visit 2, suggesting anticipatory habituation, but there were no visit differences in post-conflict SBP. As a result, spouses' SBP significantly decreased from pre- to post-conflict at Visit 1 but not at Visit 2, and this decline was steeper for couples with low levels of hostile behavior.

lower positive affect at the second conflict. This differing pattern of results for our repetitive marital conflict paradigm and the repetitive social-evaluative stress paradigm deserves further comment. The cardiovascular response may habituate to marital conflict because the conflict partner is the same and perhaps more predictable, whereas it did not habituate in the repetitive social-evaluative stress paradigm because the audience members differ and are again unfamiliar. Another possibility is that the longer between-stress timeframe (4 months in Study 1 vs. 1 month in Study 2) precluded cardiovascular response habituation.

Prior research suggests that people differ in their extent of stress response habituation or sensitization, but this work primarily used repeated administrations of the TSST. Here, we investigated moderators of stress response trajectories in the repetitive marital conflict paradigm and showed that objectively rated negative conflict behavior moderated visit-related differences in the inflammatory (ie, TNF- α) and SBP responses. That is, those who had more negative conflict behaviors had higher TNF- α responses, compared to their peers with less negative conflict behaviors, but this difference did not exist at Visit 1. Stress-induced TNF- α

responses, in particular, may diverge between individuals, as we observed that, on average, it did not respond to the stress paradigm nor did it habituate/sensitize, in contrast to IL-6. In addition, spouses' pre-conflict SBP was higher at Visit 1 than Visit 2, and couples who had less negative conflict behavior recovered faster at Visit 1. These results position negative conflict behavior, but not positive conflict behavior, as an important factor to consider when using marital conflict as a repetitive psychosocial stress paradigm. Moreover, these results may help to explain why poor marital communication and marital strain predicts adverse health outcomes,⁴⁶ as those with the most harmful conflict styles may not experience inflammatory response habituation nor quick blood pressure recovery, compared to their more satisfied peers.

These repetitive stress paradigms are an important, ecologically relevant design to fill in the gap between acute stress and chronic stress. Chronic stress fuels low-grade, systemic inflammation, dampens antiviral activity, and sets the stage for disease and depression. Acute stress transiently increases inflammation and antiviral activity, but if there is no time for adequate recovery, these stress-induced transient changes can become the new, elevated baseline.^{6,20} Therefore, repetitive stress paradigms can help to identify who is most at risk for delayed recovery, lack of habituation, and even sensitization of stress-responsive systems. Moreover, although stress-responsive systems respond to both social and nonsocial stress, prior research has demonstrated that social stress may uniquely set the stage for inflammation-associated mood and behavioral symptoms.^{2,47} Therefore, lack of habituation—and even sensitization—to repetitive social stress, may be relevant to depression etiology.¹⁷

Strengths and limitations

A strength of these findings is that they are from 2 different social stressors repeated across distinct time periods. That said, these study differences may help to explain inconsistent findings. Future studies should evaluate habituation and sensitization across other stress paradigms and timescales. As another strength, both studies featured repetitive measurements of the outcomes of interest before and after the stressor. Including the pre-stressor (anticipatory) timepoint as an outcome in our primary models was a critical and deliberate choice that likely impacted the pattern of our results. The anticipatory response is indeed part of the habituation/sensitization process, in that people who have already completed the study protocol can anticipate the upcoming lab stressor, and therefore they may already be responding before the stress paradigm is introduced at the second visit. If the anticipatory response were not measured or were controlled for rather than modeled as an outcome, the opposite pattern of results may emerge. For example, if IL-6's anticipatory sensitization is adjusted for, it may appear as though the IL-6 response habituated post-stressor because if this biomarker is elevated to start, it may not rise as much as it did at the first visit. Thus, future work in this domain must carefully consider the role of anticipatory responses. As another strength, we investigated objectively rated conflict behaviors, rather than self-report measures that may have been confounded with the outcomes, as potential moderators of habituation/sensitization in Study 2. Moreover, we measured multiple physiological systems' response habituation (or lack thereof), as well as subjective response habituation, within the same study—a unique contribution to the literature.

In terms of limitations, it is important to note that although we only used the placebo group in Study 1, the placebo was not neutral; it contained the fatty acid profile consistent with a standard US diet. Therefore, if participants did not previously consume a standard US diet, this placebo may have contributed to between-visit differences in physiological stress responses.

Similarly, in Study 2, the parent study featured a high-fat breakfast in which participants were randomized to sequence to either a high oleic acid meal or a high saturated fat meal (and their partner ate the same meal) 2 hours prior to stress; although we controlled for the meal in our primary models, it may not completely negate meal-related effects. There is mixed evidence concerning whether a high-fat meal impacts physiological stress responses,^{48,49} and a dearth of evidence concerning whether a single high-fat meal affects stress habituation. Of note, participant's stress responses after a high-fat meal were compared to their own stress responses after another high-fat meal. The meals at both visits had the same percentage of calories from fat, thus allowing for a fair comparison of stress response trajectories. As another limitation, our measurement timepoints were not the same across studies and did not always align with peak biomarkers responses (eg, 30 minutes post-stress for cortisol, 90 minutes post-stress for IL-6), but our timepoints aligned with known periods of post-stress elevations for these biomarkers.^{36,37} Moreover, participants were primarily White and well-educated. Although our sample sizes were at least as large, if not larger, than many relevant studies to date, our sample sizes were still small; relatedly, we did not conduct a priori power analyses given that these are secondary analyses of parent studies. Post hoc power analyses are contraindicated because they are conceptually flawed and misleading.⁵⁰ Even so, our results should be interpreted with caution given that we conducted secondary analyses. Lastly, although we examined average stress response habituation (Studies 1 and 2) as well as conflict behavior as a potential moderator of habituation (Study 2), interindividual variability in stress response habituation—as found in prior studies of HPA axis^{22,25} and cardiovascular response habituation^{15,23,24}—deserves continued attention and exploration.

Conclusion

Repetitive social stress, even when separated by at least 1 month, may provoke habituation or sensitization. Repeat social-evaluative stress may lead to anticipatory inflammatory response sensitization, as well as lower levels of state anxiety. In contrast, repetitive marital conflict may predict cardiovascular response habituation, as well as positive affect declines. Moreover, habituation may depend on how much couples employ negative behavior (eg, stonewalling) during the conflict. Therefore, among hostile couples, who likely have the most frequent and the most negative conflicts, stress-responsive biological systems may have difficulty adapting. In this context, risk for stress-related disorders may steadily increase over time.

Supplementary material

Supplementary material is available at *Annals of Behavioral Medicine* online.

Author contributions

Annelise Madison (Conceptualization [lead], Investigation [lead], Methodology [lead], Project administration [lead], Supervision [lead], Writing—original draft [lead], Writing—review & editing [lead]), Rosie Shrout (Conceptualization [supporting], Writing—original draft [supporting], Writing—review & editing [supporting]), Stephanie J. Wilson (Writing—original draft [supporting], Writing—review & editing [supporting]), Megan E. Renna (Writing—original draft [supporting], Writing—review & editing [supporting]), Juang Peng (Data curation [equal], Formal analysis [equal], Visualization [supporting], Writing—review & editing [supporting]), Rebecca Andridge (Data curation [equal], Formal analysis [equal], Visualization [lead], Writing—review & editing [supporting]), Lisa Jaremk (Writing—review & editing [supporting]), Christopher Fagundes (Writing—review & editing [supporting]), Martha Belury (Writing—review & editing [supporting]), William B. Malarkey (Resources [supporting], Writing—review & editing [supporting]), and Janice K. Kiecolt-Glaser (Resources [lead], Writing—review & editing [supporting])

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Conflicts of interest

None declared.

Transparency statements

The parent study was registered at NCT00385723. The analysis plan was not formally pre-registered. De-identified data from this study are not available in a public archive. De-identified data from this study will be made available (as allowable according to institutional IRB standards) by emailing the corresponding author. Analytic codes used to conduct the analyses presented in this study are not available in a public archive. They may be available by emailing the corresponding author. Materials used to conduct the study are not publically available.

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