Association Between Interleukin-6 and Striatal Prediction-Error Signals Following Acute Stress in Healthy Females

Supplemental Information

SUPPLEMENTAL METHODS

Participants and Study Description: A total of 88, right-handed, medically, psychiatrically, and neurologically healthy female participants were included in this study. All participants were recruited in response to community advertisements. Exclusion criteria included any current or past psychiatric disorder as assessed by a Structured Clinical Interview (1). Additionally, individuals were excluded for five or more lifetime exposures to any illegal substance. Individuals were also excluded on the basis of recent substance use of illegal drugs, psychotropic medications or nicotine (established using a urine drug test at leach session). Of the 88 participants, 75 completed a second neuroimaging session. Of these, 60 had IL-6 levels from at least two blood draws (inflammatory markers were added after study onset) and 65 had useable neuroimaging data after exclusion for motion (>3mm) and artifacts. Additionally, 70 of the 88 participants (79.5%) from session 1 completed one or more self-report sessions during the 4-month follow-up period. There were no differences in baseline PSS scores, STAI scores or baseline IL-6 levels in individuals who completed a majority of follow-up assessments vs those who did not (all p-values > 0.75). To minimize the effects of diurnal variation on all biological measures, all sessions occurred between 11am and 4pm.

Prior to the first visit, all participants completed an online screening questionnaire and/or phone screen to assess initial eligibility. Participants meeting criteria were then invited for the first session, which included a Structured Clinical Interview (SCID; (1)) conducted by a masters-level clinician to confirm all psychiatric eligibility criteria were met. After the interview, an IV catheter was placed in the participants' arm and a baseline plasma sample was drawn. Participants then completed a series of mood ratings and computer tasks before and after the MAST acute stress paradigm (described below).

The computer tasks and resulting data will be described as part of a separate manuscript. Following this first laboratory session, participants were then asked to return to the lab within approximately one month to complete an fMRI scanning session (mean days = 25, SD = 21). The scanning session included a second stress paradigm (described below) interleaved with blocks of a reinforcement-learning task. Upon the conclusion of all study procedures, participants were fully debriefed regarding the nature of the stressors and the objectives of the study. Menstrual cycle data were collected at each visit. Cycle phase was determined by asking female participants to report the approximate date of their prior two menses and estimate the onset of their next. Menstrual cycle data were unavailable for 4 participants for session 1, and 9 participants in session 2.

Session 1 – MAST Laboratory Stressor: To induce stress during the first session, participants completed the Maastricht Acute Stress Test (MAST; (2)). The MAST is a laboratory stress paradigm that combines alternating periods of well-validated stress-

inducing procedures including a cold pressor and performance of serial subtraction in front of evaluators. During the cold pressor, participants were instructed to immerse their hand up to and including the wrist in ice water (1–3°C). Water immersion occurred 5 times for varying time intervals (60-90s) that were controlled by the computer and presented in the same fixed order for all participants. In-between water immersion periods, participants were asked to perform serial subtraction starting from 2043 and counting down by 17. There were 4 serial subtraction blocks, varying in duration between 45-90s. Throughout the task, participants were monitored by a taciturn and stone-faced experimenter. Finally, in order to further prolong the effect of the stressor, participants were told that their performance was not good enough and that the task would need to be repeated following administration of remaining tasks and questionnaires. Later in the session, however, they were informed that this was not necessary as their performance was deemed “good enough”.

To monitor affective responses to the MAST, all participants completed a mood rating scale using the visual analogue mood scale (VAMS) (3). The VAMS consists of five 100mm horizontal lines each representing a bipolar dimensional mood state: Happy-Sad, Relaxed-Tense, Friendly-Hostile, Sociable-Withdrawn, Quick Witted-Mentally Slow. Participants indicated their response by moving a computer cursor on each line to the point that best describes their current mood state. This VAMS scale was administered at 8 time points: -90 minutes (before stressor), -25 minutes, -3 minutes, +3 minutes following onset of stressor, +25 minutes, +35 minutes, +60 minutes, +80 minutes. All VAMS ratings were transformed so that higher scores indicated greater negative emotional experience.

Session 1: Plasma Collection and IL-6 Analysis: To assess IL-6 responses, plasma samples were drawn intravenously at -10 minutes (before stressor), +45 minutes following stressor and +90 minutes following stressor. Prior to the MAST, each subject had an 18-gauge intravenous catheter placed in a major vein in the antecubital fossa of their non-dominant arm. Using sterile procedure, the catheter was connected to a 3-way stopcock valve, which in turn was connected to a Vacutainer holder assembly on one port and a normal saline drip on the other port. The saline drip rate was set to approximately 20cc/hr in order to maintain patency of the intravenous line between serial blood draws. For each blood draw, the 3-way stopcock valve was operated to turn off the saline drip and allow for blood draws using Vacutainer blood collection tubes inserted into the Vacutainer holder assembly. Each blood sample collection was preceded by a "waste" tube collection of approximately 6mL to 8mL to clear saline from the proximal line and IV catheter prior to blood sample collection. Following blood sample collection, the 20cc/hr saline drip was resumed. Approximately 6mL of whole blood was collected from each participant. Samples were collected in EDTA tubes, and were centrifuged at 1300 ×g for 10 min at room temperature in a Vanguard V6500 centrifuge model (Hamilton Bell) within 20 minutes of collection, yielding about 3 mL of one-step centrifugation plasma sample. Plasma was then apportioned, collected, and transferred in aliquots of 1mL into two 2mL clean cryovials with a pipette, and immediately stored in a –80°C freezer.

IL-6 was measured by an ultra-sensitive enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN) employing the quantitative sandwich enzyme

immunoassay technique. All assays were performed at the Clinical and Epidemiologic Laboratory (CERLab) within the Department of Laboratory Medicine at Children's Hospital, which specializes in plasma analysis. The assays were run in duplicate, and all assays were required to have an inter-assay covariance of less than 10%. No IL-6 measurements were excluded due to samples falling outside the assay range. The assay had a sensitivity of 0.094 pg/mL, and the day-to-day variabilities of the assay at concentrations of 0.49, 2.78 and 5.65 pg/mL were 9.6, 7.2 and 6.5%, respectively.

Sessions 1 & 2: Salivary Cortisol Analysis: Salivary cortisol levels collected at both time points was assessed using a chemiluminescence immunoassay (CLIA) from IBL-International, Hamburg, Germany (Cortisol Luminescence Immunoassay). Salivettes were first centrifuged at 2,000g for 10 minutes at 20°C to extract saliva. All inter- and intra-assay coefficients were below 10%. Cortisol assays were performed within the Laboratory for Biological Health Psychology at Brandeis University (Directors: Drs. Nicolas Rohleder and Jutta Wolf).

Session 2: Laboratory Stressor: For the session 2 laboratory stressor (**Figure 1**), which was performed during an fMRI scan, we used a modified version of the Montreal Imaging Stress Task (MIST; (4)), a widely used and well-validated stress-paradigm. Briefly, this task requires participants to solve arithmetic problems while their performance is publicly evaluated. The problems vary in terms of time allotted and difficulty level such that "Easy" runs of the MIST involved only very simple arithmetic problems (e.g., 4 – 0 +2) while "Hard" runs of the MIST involved more difficult problems

and shorter response times (e.g., 65/15 + 27/3). Our combination of easy and hard MIST runs was done to ensure that the flow events for runs of the RL task (interleaved between runs of the MIST) was the same for stress and non-stress runs. The comparison of easy vs. hard blocks of the MIST follows the standard protocol for this task as originally developed (4). Participants were instructed that they had to maintain an 80% accuracy level. In reality, maintaining 80% was very easy for Easy blocks and made impossible for Hard blocks by increasing difficulty and reducing response times. After Hard blocks, participants were exposed to pre-recorded videos that they were told were live video-conference calls from an unfriendly and impatient experimenter who complained that their performance was not adequately maintained at the 80% level (videos have been included separately as supplemental materials).

As with the MAST during session 1, affective responses to the MIST were measured using the VAMS. For the scanning session, the VAMS scale was administered at 5 time points: 20 minutes prior to onset of stress blocks, 3 minutes prior to onset of stress blocks, immediately following first negative feedback video, immediately following second negative feedback video, and 5 minutes following last negative feedback video. Based on debriefing, 94% of participants reported finding this MIST stressful.

Session 2: RL Task: To assess reward prediction error (RPE) signals participants were asked to complete a well-validated instrumental conditioning paradigm (5). For each trial, participants were instructed to choose between two visual stimuli displayed on a screen. Each of the stimuli pairs was associated with a given outcome (gain: win $1 or $0; loss:

lose $1 or $0; neutral: look at gray square or nothing). For gain and loss pairs, the probabilities of winning $1/$0 varied between 80/20% and 20/80% for each stimulus in the pair. In the neutral pair, there was no monetary outcome. For each trial, one pair was randomly presented, with one stimulus above and one below a fixation cross (counterbalanced). The subject was instructed to choose the upper or lower stimulus by pressing one of two keys. After a jittered delay interval, participants received feedback (either “Nothing”, “Gain”, “Loss” or a gray square with no monetary value for neutral trials). Each run lasted approximately 4 minutes, and consisted of 36 trials (12 per condition).

Behavioral Data Analysis: Change in self-report ratings and plasma IL-6 following the acute stressor was analyzed using repeated measures ANOVAs. For cases that violated the sphericity assumption, a Greenhouse-Geisser correction was used. For skewed distributions (e.g., IL-6 levels), a standard log-transform was used and spearman correlations were performed. In order to examine the relationships between variables of interest (IL-6 levels, self-report measures, ROI data and nuisance covariates), standard linear regression or correlation analyses were performed. Analyses were conducted using MATLAB 2013B (Mathworks, Natick, MA) and SPSS v22 (IBM, Armok, NY). To estimate prediction errors for the RL task, a standard Q-learning model was fit to participants’ choice data.

fMRI Acquisition: All data were acquired using a 3-Tesla Siemens Tim Trio scanner with a 32-channel head coil at the McLean Imaging Center. Trial presentation was

synchronized to initial volume acquisition. Scanning protocol included low- and high-resolution structural images using standard parameters. Functional (T2* weighted) images were acquired using a GRAPPA EPI protocol with the following parameters: TR 3000ms, TE =30, flip angle 75°, FOV 224 X 224 x 170 mm with 57 interleaved axial slices.

Neuroimaging Analysis: All neuroimaging data were preprocessed and analyzed using SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/). Preprocessing in SPM8 included slice timing correction, realignment estimation and implementation, template-based normalization to the SPM8 EPI image template and spatial smoothing using a 6mm Gaussian kernel. For single-subject fixed-effects models, a single GLM was used to estimate BOLD signal across the 6 runs that separately modeled the cue and feedback phases for win, loss, and neutral trials. To examine neural RPE signals, model-derived estimates of trial-wise prediction errors were entered as a parametric modulator (pmod) during trial feedback for win and loss trials. This pmod contrast representing RPE signals across all runs was then entered into a random effects analysis to examine the main effect of PE signals across all stress conditions. For the positive RPE pmod contrast, an SVC correction was included using the NAcc mask drawn from the Harvard-Oxford Probabilistic atlas and threshold of pFWE < 0.005. Whole-brain analysis focused on the prediction error signaling contrast, which was examined using a cluster-corrected threshold of $p_{cluster}$ <0.05. To examine the effects of stress on RPE signaling, additional random-effects models were tested examining the

interactions between RPE and pre-stress vs. during-stress runs, pre-stress vs. post-stress runs, and during-stress vs. post-stress runs.

ROI Analysis: To further probe the effects of stress on striatal RPE signals, as well as the relationships between RPE signals and other variables of interest (e.g., IL-6), beta weights from the RPE contrast were extracted separately for Pre-, During- and Post-stress runs. To ensure statistical independence of this ROI analysis, the striatal ROI were anatomically defined using the NAcc mask for right and left hemispheres drawn from the Harvard-Oxford probabilistic atlas.

Computational RL Model: To estimate prediction errors, a standard Q-learning model was fit to participants' choice data. For this model, individual choices and outcomes for each pair of stimuli, A and B, were entered into a Q-learning algorithm to estimate the expected values of choosing stimulus A (Q_a) or stimulus B (Q_b) (6). For each condition (Pre-Stress, During-Stress, Post-Stress), Q values were initialized at 0. For every subsequent trial *t,* the value of the chosen stimulus (A or B) was updated according to the rule $Q_a(t+1)=Q_a(t)+\alpha*\delta(t)$, where $\delta(t)$ represented a prediction error [$\delta(t)=R(t)-Q_a(t)$] representing the difference between the expected outcome [Q(t)] and the actual outcome [R(t)]. The reinforcement magnitude R was set to be +1, -1 and 0 for winning, losing and neutral outcomes, respectively. Based on the Q value for each option at each trial, the probability of selecting an option was then estimated using the softmax selection rule:

Pa(t) = exp(Qa(t)/beta) / (exp(Qa(t)/beta))+exp(Qb(t)/beta) (7)

For generation of prediction error-signals for neuroimaging analyses, pre-determined alpha and beta parameters were drawn from a prior study using this paradigm (5), in keeping with the recommendation to use population-level free-parameters for the purpose of fMRI modeling (8).

Model Fitting: Consistent with best-fitting learning rate (alpha) parameters identified by Pessiglione and colleagues (5), we observed a best-fitting alpha of 0.28 (vs. 0.29 as reported in (5)) for gain pairs and 0.46 for loss pairs (identical to that reported in (5)). For temperature (beta) parameters, we observed an optimal beta of 2.24 for gain pairs and 5.23 for loss pairs.

Although prior studies suggest that our Q-learning model would fit the data well, the Akaike information Criterion (AIC) was used to evaluate model fits against two comparison (null) models. The first null model was just an equal probability model where each stimulus within a pair was assigned a 50% probability of being chosen for every trial. No parameters are fit for this model. The second comparison model used a single bias parameter that favors one of the two options for each pair. This model is fit such that the bias is fixed across all trials (i.e., the model cannot learn from feedback).

Consistent with our expectations, these model fit comparisons suggested the Q-learning model provided a superior fit to the data as compared to the null models. Below is a summary of the AIC values for each model for gain and loss pairs respectively:

	Gain Pairs		Loss Pairs	
	Model Fit*	AIC	Model Fit	AIC
Equal Probability Model	48.4	(NA)**	48.4	(NA)
Bias Model	34.4	70.8	40.3	82.5
Q-Learning Model	30.9	65.9	37.5	79.0

**Negative log likelihood*
***Not applicable*

We note that model fits for the Q-learning model were only moderately superior to those for the bias model, which is not unexpected given the simple task design. Our goal was to demonstrate that the Q-learning model fit the data well enough to support our application of this model for the purpose of estimating prediction error signals.

SUPPLEMENTAL RESULTS

Baseline Associations with Trait Variables: We examined baseline associations between trait personality factors believed to be related to stress reactivity and mood, cortisol and IL-6 responses to the MAST stressor during session 1. We found that the trait-anxiety subscale of the State-Trait Anxiety Inventory (STAI; (9)) was positively correlated with increased negative affect during the VAMS (average AUC for all VAMS questions: Spearman r = 0.68, $p = 5.09 \times 10^{-12}$), increased IL-6 blunted (Spearman r = 0.28, p = 0.031) with no relationship to change in cortisol (Spearman r = -0.16, p = 0.17).

Associations between NAcc RPE Signals and Performance Accuracy: Consistent with prior studies (5, 10), the strength of positive RPE signals in the NAcc was positively associated with performance accuracy across win and loss trials accuracy during the Pre-Stress (Right: r = 0.28, p = 0.026; Left: r = 0.27, p = 0.033) and During-Stress (Right: r = 0.41, p = 0.001; Left: r = 0.22, p = 0.075) runs, but not for the Post-Stress runs (Right: r = 0.16, p = 0.217; Left: r = 0.07, p = 0.588). None of these correlations significantly differed from each other as a function of stress condition (all Fisher Z-tests p > 0.127). Independent-sample t-tests revealed no effect of menstrual cycle phase on the magnitude of positive RPE signals for either the right or left NAcc for any stress condition (all p's > 0.11).

SUPPLEMENTAL TABLES

Supplemental Table S1: Sample demographic information

Gender		
Female	88	100%
Male	0	0%
Race		
Caucasian	61	69%
Black	15	17%
Asian	10	12%
Unknown	2	2%
Ethnicity		
Hispanic	5	6%
Non-Hispanic	81	92%
Unknown	2	2%
Years of Education (Median +/- SD)	16	± 1.68
BMI (Median +/- SD)	22.6	± 3.4
with BMI > 25 (overweight)	15	17%
with BMI > 30 (obese)	4	5%
Income		
<$10,000	11	13%
$10,000–$25,000	10	11%
$25,000–$50,000	21	24%
$50,000–$75,000	18	20%
$75,000–$100,000	17	19%
>$100,000	11	13%
Marital Status		
Married	17	19%
Unmarried	71	81%
Smoking Status		
Smoker	0	0%
Non-Smoker	88	100%

Supplemental Table S2: Associations between IL-6 and Body Mass Index (BMI)

	Spearman r	p-value
Il-6 Time 1	0.52***	0.0001
Il-6 Time 2	0.58***	0.00002
Il-6 Time 3	0.55**	0.001
Change in IL-6 Time 1-Time 2	0.22	0.14
Change in IL-6 Time 1-Time 3	-0.068	0.697

Supplemental Table S3. Whole-brain fMRI BOLD Amplitude Results: Positive and Negative Prediction Error Contrasts

Region	Peak Coordinates (MNI) X	Y	Z	t-statistic (peak)	Cluster Size	p-value (cluster)
Positive Prediction Error						
R Posterior Cingulate	10	-52	14	4.38	326	0.022
L Calcarine*	-4	-66	22	3.91		
L Posterior Cingulate*	-6	-56	14	3.83		
L Mid Occipital Gyrus (BA 39)	-44	-74	36	4.73	303	0.032
L Angular Gyrus*	-50	-72	26	3.73		
L Angular Gyrus (BA 39)*	-42	-60	24	3.70		
Negative Prediction Error						
R Inferior Frontal Gyrus (BA 45)	50	20	8	4.91	636	<0.001
R Anterior Insula*	38	14	-10	4.67		
R Temporal Pole (BA 38)*	40	16	-26	3.96		
Mid Cingulate	8	20	36	4.96	1172	<0.001
R Supplementary Motor Area (BA 6)*	8	18	64	4.64		
Mid Cingulate*	-4	24	32	4.50		
R Middle Frontal Gyrus	30	54	16	4.06	569	0.001
R Superior Frontal*	22	54	32	4.00		
R Superior Frontal*	18	58	26	3.73		
L Occipital Pole (BA 19)	-28	-100	12	5.04	344	0.017
L Mid Occipital*	-30	-90	12	4.54		
L Inferior Occipital Gyrus*	-42	-86	0	3.36		
R Mid Occipital (BA 19)	28	-92	20	5.36	1124	<0.001
R Mid Occipital*	26	-90	6	5.22		
R Lingual*	24	-80	-10	3.77		
L Anterior Insula	-42	14	-4	4.16	437	0.004
L Temporal Pole (BA 38)*	-38	14	-14	4.01		
L Inferior Frontal Gyrus (BA 45)*	-42	20	6	3.87		
Mid Frontal (BA 10)	-26	50	28	4.79	290	0.040
L Cerebellum	-46	-60	-34	3.90	298	0.035
L Fusiform (BA 18)*	-26	-72	-12	3.89		
L Cerebellum*	-40	-70	-28	3.66		

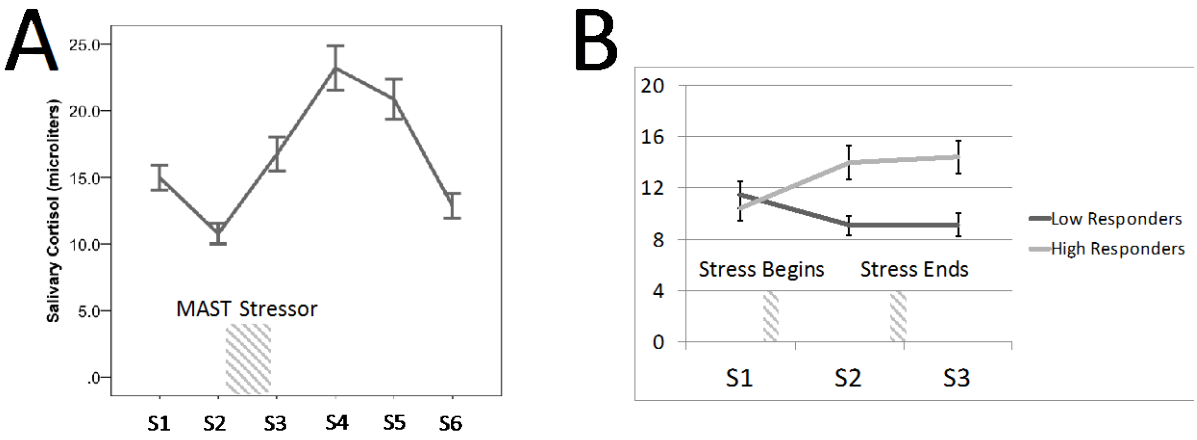
** Indicates subregion in larger cluster*

Supplemental Table S4. Spearman correlations between IL-6 and NAcc RPE for each individual condition.

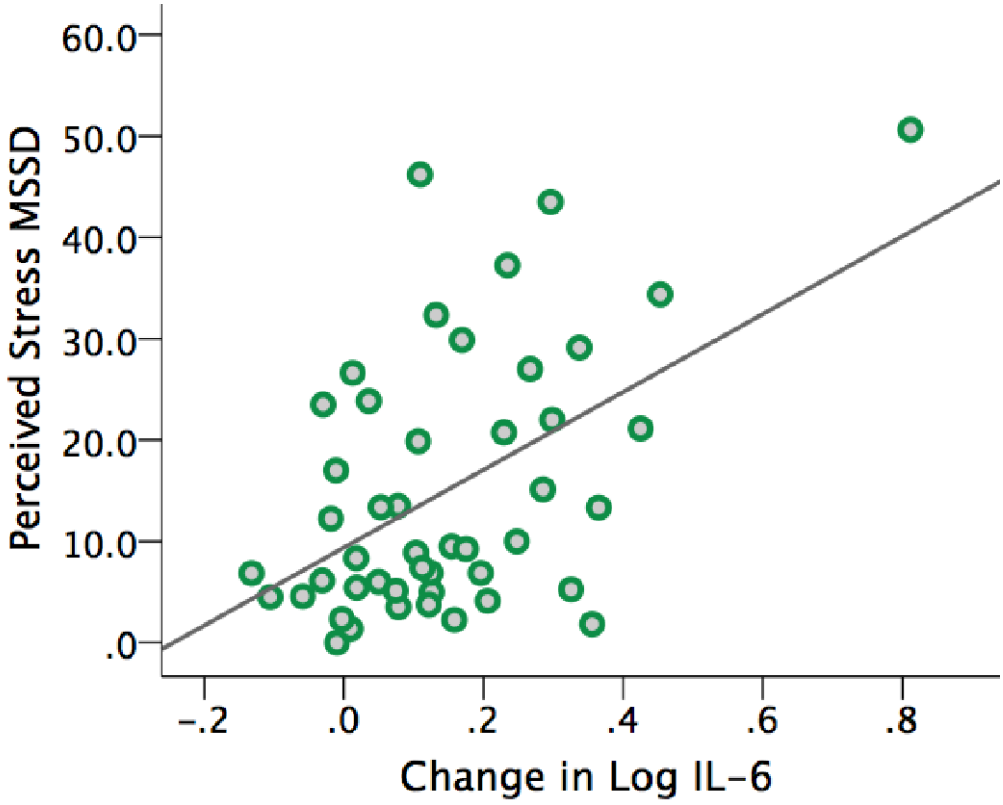
	IL-6 Time 1	IL-6 Time 2	IL-6 Time 3
LNacc Pre	-0.28	-0.17	-0.07
RNacc Pre	-0.20	-0.16	-0.13
LNacc Dur	-0.09	-0.05	<0.01
RNacc Dur	0.05	0.05	-0.02
LNacc Post	0.13	0.09	-0.03
RNacc Post	0.05	0.08	0.02

All associations p > 0.12

SUPPLEMENTAL FIGURES



Supplemental Figure S1: Stress-induced change cortisol for session 1 (A) and Session 2 (B). **A.** For session 1, the MAST induced a significant overall increase in salivary cortisol ($F_{(2.34,182.38.)} = 27.87$, $p = 1.5 \times 10^{-12}$), with a strong quadratic effect ($F_{(1,78)} = 33.14$, $p = 1.62 \times 10^{-7}$). **B**. For session 2, the MIST did not produce a significant main effect on cortisol ($F_{(1.71,116.06)} = 21.31$, $p = 0.437$), though a subset of participants did show a positive change in cortisol (High Responders).



Supplemental Figure S2: Stress-induced change in IL-6 predicts variability (mean sum of squared differences, MSSD) in reported perceived stress during a 4-month follow-up assessment period (n = 47; Spearman r = 0.39, p = 0.007). Scatter plot depicted shows data from all participants with data available from two or more follow-up time points. Note that results remain significant without influential data point (Spearman r = 0.35, p = 0.019).

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