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Revisiting the stress recovery hypothesis: Differential associations of cortisol stress reactivity and recovery after acute psychosocial stress with markers of long-term stress and health

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ABSTRACT

Exposure to excessive and long-term stress may result in dysregulation of the stress system, including the acute stress response. In particular, failure to downregulate stress-related reactivity may lead to prolonged stress responses and the accumulation of allostatic load. However, the contribution of altered acute cortisol recovery to chronic stress and associated health impairments has often been neglected. Addressing this lack of research, we explored whether recovery from – more so than reactivity to – acute stress captures the basal stress load of an individual. Using Piecewise Growth Curve Models with Landmark Registration, we analyzed cortisol reactivity and recovery slopes of 130 healthy participants exposed to a standardized psychosocial laboratory stressor. Reactivity and recovery were predicted by measures indicative of long-term stress and its downstream effects, including self-report questionnaires, diurnal cortisol indices [cortisol awakening response (CAR); diurnal cortisol slope], markers of pro-inflammatory activity (interleukin-6; high-sensitive C-reactive protein), and hippocampal volume (HCV). Among these measures, only an increased CAR was specifically and consistently associated with relatively impaired recovery. Since the CAR represents the physiological enhancement needed to meet the anticipated demands of the forthcoming day, this finding may highlight the contribution of cognitive processes in determining both CAR and acute stress recovery. Furthermore, greater cortisol reactivity covaried with smaller HCV, showing that increased acute reactivity translates to health-relevant downstream effects. The lack of further associations between long-term and acute stress measures may arise from biases in self-reported chronic stress and the rigorously health-screened study sample. Overall, our findings suggest that while cortisol stress recovery might not supersede reactivity as an indicator of the long-term stress load or associated health effects, recovery and reactivity have differential utility in describing individuals' allostatic states.

1. Introduction

Psychosocial stress is an inherent part of modern lifestyle. Accordingly, the incidence of stress-associated medical conditions, including cardiovascular, metabolic, and autoimmune diseases, is steadily on the rise (e.g., Agorastos and Chrousos, 2022; Chrousos, 2009). Individual and societal costs of excess stress are substantial (Hassard et al., 2018; Patel et al., 2018), and the young report higher levels of stress than any generation prior (American Psychological Association, 2018).

The subjective experience of psychosocial stress is accompanied by the activation of sympathetic nervous system and hypothalamic-pituitary-adrenal (HPA) axis, which cause the release of catecholamines and the steroid hormone cortisol. In the acutely threatening event, this stress cascade provides an organism with the necessary motivation and energy to survive. However, if activated over an extended time period, the principal stress mediators and their downstream effects may lead to a wear and tear on the body termed "allostatic load" (Guidi et al., 2021; McEwen, 1998).

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There are several ways to capture the long-term stress load. For instance, self-reports of stress experience within a retrospective time frame can be assessed using standardized questionnaires. On the level of HPA axis activity, repeated cortisol samples over the course of the day reflect the dynamics of diurnal cortisol secretion. However, the diurnal cortisol profile only provides a narrow window into an individual's long-term cortisol exposure. Hair cortisol levels, by contrast, capture systemic cortisol exposure over up to three months, and are less prone to state-related variance (Stalder et al., 2017). Typically, studies infer individuals' habitual psychosocial stress responding from a single assessment of stress reactivity in the laboratory. In other words, if a participant shows high cortisol release in response to an acute stressor, it is assumed that stress responses in daily life are equally high, thus accumulating to long-term elevated cortisol release.

With the repeated activation of the stress system in states of chronic stress, the organism is exposed to high levels of catecholamines and cortisol over an extended period of time. As has been shown in the animal model over many years, this process may wear out the various interconnected mechanisms in charge of keeping the stress response flexible (Herman et al., 2016), thus preventing proper shut down and recovery after acute stress exposure (McEwen, 2006, 2019). Based on this rationale, we hypothesized that rather than the absolute amplitude of acute cortisol release, the ability for cortisol recovery after stressor termination may be the best indicator of an individual's long-term stress load. This hypothesis is akin to the concept of resilience, which is broadly described as the capacity to "bounce back" in the face of adversity (Windle et al., 2011). Thus, a resilient individual would still be expected to show a stress response, yet recuperate successfully once the stressor has ended. Accordingly, we tested whether cortisol recovery following acute psychosocial stress induction, as compared to cortisol reactivity, is a more valid proxy of long-term stress and its physiological sequelae.

Considering the complexity of the stress network (Engert et al., 2018), dysregulation in one part of the system entails compensational alterations within other parts. Catecholamines and glucocorticoids, for example, influence immune function by regulating the production of pro- and anti-inflammatory cytokines (McEwen, 2006). The inflammatory processes prompted by prolonged psychosocial stress are chronic, not limited to focal sites of tissue damage, and occur at low level (see Slavich, 2020 for a review). Such systemic low-grade inflammation is assumed to increase vulnerability to deleterious health outcomes, including mental health conditions, cardiovascular and Alzheimer's disease (Rohleder, 2019; Slavich, 2020). Although empirical evidence suggests an association between chronic stress and systemic inflammation (see Rohleder, 2019 for a review), it is unclear whether poor stress recovery contributes to these immunological effects.

The hippocampal formation with its abundance of mineral- and glucocorticoid receptors is another critical target for neurotoxic glucocorticoid effects (Lupien et al., 2018; McEwen et al., 2016; Sheline et al., 2019). Accordingly, studies have linked sustained exposure to high glucocorticoid levels with hippocampal pyramidal neuronal damage (McEwen et al., 1995; McKittrick et al., 2000), dendrite atrophy, loss of spines (McEwen, 2017), and disruption of hippocampal neurogenesis (Lupien et al., 2018). Importantly, through glucocorticoid binding, the hippocampus participates in the inhibitory feedback of the HPA axis (Herman et al., 2020; Jacobson and Sapolsky, 1991). In case of hippocampal damage and consequent failure to inhibit HPA axis activation, the hippocampus may be further exposed to excessive cortisol secretion, creating a cascade of hippocampal damage (Oitzl et al., 2010; Sapolsky et al., 1986). In acute stress settings, HPA recovery after stressor termination may be decelerated or less effective if the hippocampal feedback mechanism is disrupted due to prior long-term stress exposure.

In order to capture a comprehensive picture of long-term stress and its physiological sequelae, we collected a variety of measures associated with prolonged stress exposure. Psychological effects of general life stress were assessed with two of the most widely used stress questionnaires, the Trier Inventory of Chronic Stress (TICS; Schulz and Schlotz, 1999) and the Perceived Stress Scale (PSS; Cohen et al., 1983). On a physiological level, altered diurnal cortisol profiles were collected to reflect medium-term HPA axis activity. Precisely, we considered the cortisol awakening response (CAR), suggested to capture the anticipated demands of the upcoming day (Law et al., 2013), and the diurnal cortisol slope (DCS), a proxy of cortisol recovery over the course of the day (Ross et al., 2014). Glucocorticoid concentrations in hair were assessed as reliable long-term stress biomarkers. Drawing on their incorporation in the growing hair, hair glucocorticoids reflect the cumulative cortisol secretion over up to three months (Stalder and Kirschbaum, 2012). As mentioned above, downstream effects of long-term stress may be detected within immune system and brain. First, systemic low-grade inflammation, frequently measured via plasma concentrations of the pro-inflammatory cytokine interleukin-6 (IL-6) and acute-phase protein high-sensitive C-reactive protein (hsCRP), may mirror relevant stress-related alterations within the immune system (see Rohleder, 2019 for a review). Second, repeated or prolonged glucocorticoid exposure has been associated with deleterious effects on hippocampal structure, including smaller hippocampal volume (for reviews see Lupien et al., 2018; Sheline et al., 2019). Due to its critical role for HPA axis negative feedback control, the examination of hippocampal volume in the context acute cortisol stress recovery is particularly relevant.

We are not the first to emphasize the importance of acute stress recovery for long-term stress system integrity and health. Already in 1997, Linden and colleagues made a call for the more systematic investigation of acute stress recovery, seeing that between 1993 and 1994 only 23% of the studies targeting stress responding reported data from the recovery period (Linden et al., 1997). From those studies, many focused on cardiovascular and catecholaminergic parameters, disregarding the health consequences of HPA axis dysregulation, which are considered particularly disease-relevant (Linden et al., 1997). Since then, the relative scarcity of recovery findings continued. Among the studies focusing on cortisol recovery after acute stress exposure, one found that greater self-reported chronic stress was associated with slower recovery (Matthews et al., 2001). A meta-analysis found no association between HPA axis recovery and general life stress (Chida and Hamer, 2008). Challenging the reliability of this null finding, however, only five out of the 729 included studies reported effect sizes for cortisol recovery, and only two out of these related cortisol recovery to chronic stress.

In order to investigate acute cortisol recovery in the current study, we used Piecewise Growth Curve Models with Landmark Registration (Lopez-Duran et al., 2014). This procedure simultaneously models individual peak-adjusted stress reactivity and recovery with respect to a variety of predictors, allowing for the observation of distinct reactivity and recovery effects. To further investigate potentially confounding effects of stress reactivity on recovery, we additionally considered two relatively unadulterated one-index measures of reactivity and recovery, stemming from a data-driven study by Miller et al. (2018): the change score between maximal and minimal cortisol concentration (*MaxMin*) and the minimal cortisol level throughout the testing period (*Cmin*).

Based on the summarized literature, we hypothesized that a poorer (i.e., less steep) cortisol recovery would be associated with greater long-term stress load, reflected in (1) greater self-reported chronic stress, (2) a steeper CAR and flattened DCS, as well as (3) greater hair glucocorticoid concentration. Additionally, we expected an association between less efficient recovery and downstream stress effects, in particular (4) increased levels of the inflammatory markers IL-6 and hsCRP, and (5) decreased HCV.

2. Materials and methods

2.1. Participants

The current data was collected at the baseline measurement timepoint of the *ReSource Project* (Singer et al., 2016), a longitudinal

mental training intervention. Study volunteers were screened in multiple steps, starting with an online application form assessing demographics, time constraints, and mental and physical health. Potential participants also completed a series of mental health questionnaires. Afterwards they underwent an extensive diagnostic interview with a trained clinical psychologist, which included the SCID-I DIA-X (Wittchen and Pfister, 1997) and the SCID-II (First et al., 1997; Wittchen et al., 1997) for the assessment of DSM-IV Axis-I and Axis-II disorders. Volunteers were excluded if they fulfilled criteria for an Axis-I disorder, including psychotic disorder, bipolar disorder, and substance dependency, within the past two years, or an Axis-II disorder at any time in their life. Also, a variety of chronic physical pathologies and intake of medication affecting the central nervous system or HPA axis served as exclusion criteria. A detailed description of the recruitment procedure and information about the final sample of the *ReSource Project* can be found in Singer et al. (2016). Registration of the *ReSource Project* was implemented via the Protocol Registration System of ClinicalTrial.gov (Identifier NCT01833104). The study was approved by the research ethics boards of Leipzig University (ethic number: 376/12-ff) and Humboldt University Berlin (ethic numbers: 2013–20, 2013–29, 2014–10). Participants gave written informed consent, received financial compensation, and could withdraw from the study at any time without providing reasons.

The *ReSource Project* is a longitudinal intervention study aimed towards the evaluation of three distinct mental training modules (for more detail on the study see Singer et al., 2016). Training effects were repeatedly examined in 332 healthy participants (197 women; mean age ± SD: 40.74 ± 9.24 years; age range: 20–55 years). For the current analyses, we included a subset of 134 participants who were exposed to a psychosocial laboratory stressor, the Trier Social Stress Test (TSST; Kirschbaum et al., 1993), either as part of a training-free retest control cohort (n = 88), or at the baseline measurement time-point, before mental training had taken place (n = 46). Of those 134 participants, four dropped out because they were repeatedly unavailable for stress testing. Thus, 130 participants (74 women; mean age ± SD: 40.1 ± 9.1 years; age range: 22–55) were available for statistical analysis. Further 24 subjects were excluded due to missing data in one or more of the predictor variables (for details see 3.1), resulting in a final sample of 106 participants. Although the data reported here were previously published in the context of other research questions (Engert et al., 2016, 2017, 2018; Puhlmann et al., 2019, 2021a, 2021b), none of these studies linked measures of acute stress responsivity to measures of long-term stress and health. The current study is an a-posteriori exploratory study not planned during the designing of the *ReSource Project*.

2.2. Procedure

Participants filled out self-report questionnaires (see 2.4.2 and Supplementary Material A for details) via an online Internet platform. On the day of stress testing, they attended the TSST in a 130 min session. To circumvent possible confounds due to the cortisol circadian rhythm (Dallman et al., 2000), testing took place between 12pm and 5pm. Participants were asked to eat a standardized snack upon arrival (−70 min relative to stressor onset) for the adjustment of blood sugar levels. Afterwards, they did not consume any food or beverages, except for water, until the end of the testing session. Prior to their experience sampling days, participants received experience sampling kits including 14 saliva collection devices for home-sampling, and a preprogrammed mobile device utilized to standardize and streamline the home-sampling procedure. To ensure proficiency in self-administering saliva samples and handling the mobile device, they attended a short training session. For brain imaging via Magnetic Resonance Imaging (MRI), as well as for blood and hair sampling, participants returned to the institute for two additional visits (see Fig. S1 for the time in days between TSST and the other assessments).

2.3. Stress induction

Psychosocial stress was induced with the Trier Social Stress Test (TSST; Kirschbaum et al., 1993), a standardized laboratory paradigm that reliably provokes psychological and physical stress responses by exposing the subject to a socially evaluative challenge. The test starts with an anticipation phase of variable duration (15 min in the current study), followed by the stress phase in which the participant gives a 5-min free speech, and performs a 5-min mental arithmetic task in front of an evaluation committee. The committee communicates with the participant in a neutral manner without giving verbal or non-verbal feedback. The combination of free speech and a mental arithmetic conducted in front of alleged behavioral analysts creates novelty, unpredictability, and social-evaluative threat. In comparison to other laboratory stress paradigms, the TSST is associated with the longest HPA axis recovery period, and therefore ideally suited for research on cortisol stress recovery (see Dickerson and Kemeny, 2004 for a review).

2.4. Measures

See Supplementary Material A for additional details on the collection and analysis of the described measures.

2.4.1. Acute salivary cortisol stress response

Salivary cortisol served as biomarker for acute stress and was assessed at −55, +10, +20, +30, +40 and + 55 min relative to stressor onset (at 0 min). For saliva sampling, Salivette collection devices (Sarstedt, Nümbrecht, Germany) were used. Participants placed a collection swab in their mouth and refrained from chewing for 2 min. Samples were stored at −30 °C until assay (at the Department of Biological and Clinical Psychology, University of Trier, Germany). A time-resolved fluorescence immunoassay (Dressendörfer et al., 1992) determined cortisol levels (in nmol/l) with 10% intra-assay and 12% inter-assay variability.

2.4.2. Indicators of long-term stress load

To evaluate subjective experience of chronic stress, participants completed the ten-item Perceived Stress Scale (PSS; Cohen et al., 1983) and the 39-item Trier Inventory of Chronic Stress (TICS; Schulz and Schlotz, 1999).

Diurnal cortisol secretion was assessed via self-collected saliva (altogether 14 samples) on two consecutive workdays (Mondays/Tuesdays, Wednesdays/Thursdays, or Thursdays/Fridays, depending on participant availability). Per day, saliva was collected immediately upon awakening (0 min), 30 min and 60 min thereafter, and at +240, +360, +480, +600 min throughout the day. Sampling time of the non-morning process was jittered (±15 min) to avoid complete predictability. Diurnal cortisol data was used to calculate distinct measures of cortisol secretion dynamics, the cortisol awakening response (CAR), quantified as a change score from the awakening to the 30-min post awakening sample, and, the diurnal cortisol slope (DCS), calculated as a change score from the awakening to the last sample (+600 min). While the CAR is assumed to capture dynamic aspects of the diurnal cortisol secretion pattern associated with anticipated stress experiences (e.g. Fries et al., 2009), the DCS serves as a marker of more persistent alterations within the diurnal cortisol pattern (Adam et al., 2006). Increased CAR and flattened DCS are linked to chronic stress experience (Adam and Kumari, 2009; Chida and Steptoe, 2009).

Concentrations of cortisol and cortisone in hair (HC, HE) were assessed as markers of systemic glucocorticoid exposure and long-term stress load (Stalder et al., 2017). Three cm segments of hair were analyzed. Given the average hair growth rate of 1 cm per month, the resulting HC and HE levels reflect the cumulative hormone secretion over the past three months (Stalder and Kirschbaum, 2012). The hair sampling procedure resulted in selective dropout and considerable sample size reduction. Therefore, HC and HE levels were not included in

our main analysis, but separate analysis as described in 2.5.4 were conducted.

Serum levels of the pro-inflammatory cytokine interleukin-6 (IL-6; measured in pg/ml) and high-sensitive C-reactive protein (hsCRP; measured in mg/L) were used as indicators of low-grade systemic inflammation.

To acquire brain images for the analysis of hippocampal volume, a 3T Siemens Verio scanner (Siemens Medical Systems, Erlangen) with a 32-channel head coil was used. For T1-weighted magnetic resonance imaging (MRI), a 3-dimensional magnetization-prepared rapid gradient-echo sequence was used (176 sagittal slices; repetition time (TR) 2300 ms; echo time (TE) 2.98 ms; inversion time (TI) 900 ms; flip angle = 7°; matrix size 240 × 256; field of view (FOV) 240 × 256 mm^2; voxel size 1 × 1 × 1 mm^3). Imaging hardware and console software (Syngo B17; Siemens Healthineers) remained the same throughout data collection. T1-weighted MRI data was linearly registered to MNI152. In order to segment the hippocampal subfields CA1-3, CA4/DG, and subiculum (SUB), an automated patch-based algorithm was performed for every subject (for details see Caldairou et al., 2016). The algorithm employs a population-based patch normalization relative to a template library, providing good space and time complexity. Two independent raters assessed the quality of hippocampus segmentation and data. Cases of insufficient segmentation quality, based on a predefined standard, were excluded from analysis (for details see Puhlmann et al., 2021b). In the current data analysis, we included only total bilateral hippocampal volume (HCV).

2.5. Statistical analyses

2.5.1. Missing data and data replacement

Missing values in level 2 predictor data (i.e., questionnaire, diurnal cortisol, HC, HE, immune, and HCV) were not imputed or otherwise replaced. Only subjects with the entire set of level 2 predictor values were included in the main analysis. Four participants were missing a subset of TSST saliva samples (two samples at +10 min, two samples at +20 min). In these cases, all available cortisol data were included into the model.

2.5.2. Data processing

Data cleaning and pre-processing was implemented via IBM SPSS Statistics version 24 (IBM Corp, 2016) and R version 4.0.1 (R Core Team, 2020). All data were checked for normal distribution. Saliva cortisol, HC, HE, IL-6 and hsCRP data were ln-transformed to normalize skewness. Subsequently, data were checked for outliers, defined as values deviating more than 3 SDs from the respective sample mean, and detected outliers were winsorized to ±3 SDs from the mean. All dependent variables were z-transformed to simplify interpretation of estimates. Statistical significance was assessed at an alpha level of 0.05, and all tests were two-sided.

2.5.3. Main analysis: piecewise growth curve modeling with landmark registration

In order to differentiate acute cortisol stress reactivity and recovery phases, we modeled cortisol reactivity and recovery slopes relative to the individual cortisol peak via Piecewise Growth Curve Modeling with Landmark Registration (pGCM-LR; Lopez-Duran et al., 2014). Piecewise GCM-LR was performed in R version 4.0.1 (R Core Team, 2020) using the *lmer* function of the *lme4* package (Bates et al., 2015). Models were summarized and compared using the *summary* and *anova* functions of the *lmerTest* package (Kuznetsova et al., 2017). Effect sizes ω^2 were determined using the *F_to_omega2* function of the *effectsize* package (Ben-Shachar et al., 2020). According to the rule of thumb proposed by Field et al. (2012) ω^2 values of 0.01, 0.06, and 0.14 were considered small, medium, and large effects.

2.5.3.1. Landmark Registration. Individual peak time (peak_time) was defined as the sample time (in min) of the highest cortisol level within the post-TSST period. If single values were missing, the highest cortisol value within the remaining data was used. In case of a post-peak plateau or another sample with the same peak concentration, the earliest post-TSST peak time was defined as peak_time. After longitudinal data transformation, adjusted time variables were created. First, the *time to peak* variable (time_to_peak) was calculated (see Supplementary Material B, Equation S1), then, two coded time variables representing the individual *time before peak* (time_before_peak) and *time after peak* (time_after_peak) were created for every subject (Lopez-Duran et al., 2014; see Supplementary Material B, Equation S2).

2.5.3.2. Model building procedure. We followed a forward stepwise model building procedure, starting with an unconditional basic model. To test the assumed two-piece structure of the cortisol secretion pattern, the initial model was specified as an unconditional fixed effects model with random intercept and fixed slopes (for details see Supplementary Material B). In a second step, we added random reactivity and recovery slopes to the model, before in a third step, sex and age were included as covariates (Allen et al., 2017; Kajantie and Phillips, 2006).

In order to examine the interactions of long-term stress and health measures with individual cortisol trajectories, we clustered the level 2 predictors into three groups of stress correlates with shared variance, that is, diurnal cortisol measures, self-report questionnaires, and measures of downstream sequelae including immune markers and hippocampal volume, before adding them to the model. In the next and fourth step of model building, CAR and DCS were included in the model, due to their conceptual proximity to the acute cortisol stress response. TICS (Schulz and Schlotz, 1999) and PSS (Cohen et al., 1983) were added next. Levels of the proinflammatory markers IL-6 and hsCRP, and HCV completed the model building procedure in a last step. A detailed description, R codes, and fit statistics of the model building procedure can be found in the Supplementary Material B. As stated above, when adding HC and HE to the model, we were confronted with considerable sample size reduction ($N_{HC} = 64$, $N_{HE} = 73$). Therefore, HC and HE were excluded from our main model. However, due to their relevance as measures of long-term stress load, we built an additional model with only HC and HE as level 2 predictors (see 2.5.4).

The main model was specified as presented in Equation S7, Supplementary Material B. The model reflects a two-level linear mixed effects model with random intercept and random reactivity and recovery slopes. Level 1 represents the within-subject estimation of cortisol levels by time_before_peak, time_after_peak, sex, age and sampling time. On between-subject level 2, cortisol peak levels, reactivity slope, and recovery slope were predicted by seven measures of long-term stress and health.

If level 2 variables of interest interacted with both reactivity and recovery slope in our main analyses, we conducted exploratory analyses with one-index measures of stress reactivity and recovery to examine whether an effect on stress recovery was primarily driven by a parallel effect on stress reactivity – or not. Details on the statistical analysis and results are presented in Supplementary Material C.

2.5.4. Hair glucocorticoids analysis

Due to the optional nature of the hair glucocorticoid assessment, the number of dropouts was considerable. In order to prevent massive sample size reduction in our main model, interactions of hair glucocorticoids with cortisol reactivity and recovery were explored in a separate model with only HC and HE as level 2 predictors ($N_{HC} = 64$, $N_{HE} = 73$). The model was specified as presented in the Supplementary Material B, Equation S8.

3. Results

3.1. Descriptive statistics and preliminary analyses

Out of initially available 130 participants, 24 were excluded due to missing data in one or more level 2 predictor (CAR n = 1; DCS n = 2; TICS/PSS n = 1; IL-6/hsCRP n = 7; HCV n = 15). Reasons for missingness included dropouts, missed appointments, and assay failure [more detail on missingness in level 2 predictors is provided in Engert et al., 2018 (TICS, PSS, CAR, DCS, HC, HE); Puhlmann et al., 2021b (TICS, PSS, HC, HE), 2021a (HCV), 2019 (TICS, PSS, IL-6, hsCRP)]. The final sample comprised of 106 participants (57 women; see Table 1 for descriptive statistics), 78.3% of whom responded to the TSST with a baseline-to-peak cortisol release of >1.5 nmol/l, demonstrating successful stress induction (Miller et al., 2013). Importantly, all available participants (responders and non-responders) were included into the statistical analysis.

3.2. Main analysis: piecewise growth curve modeling with landmark registration

The basic random intercept model with fixed reactivity and recovery slopes indicated significantly increasing cortisol levels towards the peak (reactivity slope: $\beta = 0.41$, $t(526.23) = 22.42$, $p < .001$), and significantly declining cortisol levels after the peak (recovery slope: $\beta = -0.42$, $t(578.22) = -16.33$, $p < .001$), confirming the expected two-piece structure of cortisol secretion before and after the TSST. This pattern of significance remained, and the model fit significantly improved, after adding random reactivity and recovery slopes to the model (for details see Supplementary Material B, Tables S1 and S2).

Level 2 predictors were added to the basic model in three steps, resulting in Model 1 (with CAR, DCS), Model 2 (with CAR, DCS; TICS, PSS), and the main Model 3 (with CAR, DCS; TICS, PSS; hsCRP, IL-6, HCV). The respective results are presented in Table 2. The main model revealed that female subjects had significantly lower cortisol peak levels than male subjects ($\beta = -0.37$, $t(94.29) = -2.38$, $p = .019$, $\omega^2 = 0.05$), which can be considered a small effect. Age did not contribute to cortisol peak levels. There was a significant interaction of HCV with reactivity slopes ($\beta = -0.08$, $t(91.48) = -2.70$, $p = .008$, $\omega^2 = 0.06$), and a marginal interaction of HCV with recovery slopes ($\beta = 0.06$, $t(87.63) = 1.78$, $p = .078$, $\omega^2 = 0.02$), such that steeper cortisol reactivity and recovery slopes were linked to a smaller HCV (Fig. 1). Exploring this pattern further by employing alternative proxies for reactivity and recovery confirmed a robust association with reactivity, and no association with recovery (see Supplementary Material B). Also, the CAR showed a marginal interaction specifically with the recovery slope ($\beta = 0.07$, $t(115.9) = 1.83$, $p = .070$, $\omega^2 = 0.02$), whereas no significant interaction with the reactivity slope was identified. This interaction was significant throughout the model building procedure, until HCV was added to the model (Table 2). In detail, a higher CAR was linked to a decreased (i.e., more shallow) stress recovery (Fig. 2), which means that participants with habitually higher CARs took longer to recover from stress. The recovery effect linked to the CAR can be considered small; the association of reactivity and HCV corresponds to a medium effect. Besides HCV and CAR, no other Level-2 predictors were significantly associated with either reactivity or recovery slopes (Table 2).

Table 1
Descriptive statistics of the final sample (N = 106).

	Mean	SD	Min	Max
Age (years)	40.42	8.96	22	55
CAR (log nmol/l)	0.34	0.49	−0.90	1.54
DCS (log nmol/l)	−0.14	0.06	−0.29	−0.01
TICS	14.85	7.40	0	35
PSS	13.97	5.78	1	28
hsCRP (mg/L)	1.09	1.20	0.14	10.04
IL-6 (pg/ml)	1.53	0.43	1.24	5.65
HCV (mm^3)	12435.57	1031.81	10648	17283

CAR = Cortisol awakening response. DCS = Diurnal cortisol slope. TICS = Trier Inventory of Chronic Stress (Schulz and Schlotz, 1999). PSS = Perceived Stress Scale (Cohen et al., 1983). hsCRP = High-sensitive C-reactive protein. IL-6 = Interleukin-6. HCV = Hippocampal volume.

3.3. Hair glucocorticoids analysis

The analysis of hair glucocorticoid levels yielded no main or interaction effects of HC or HE with recovery or reactivity slopes, as presented in Table 3.

4. Discussion

The aim of our work was to investigate how cortisol recovery after an acute psychosocial stressor – in contrast to cortisol reactivity – is linked to the basal stress load of healthy adults. We hypothesized that difficulty to recover from acute stress exposure, as measured in the context of a psychosocial laboratory stress task, the TSST (Kirschbaum et al., 1993), would be associated with less favorable outcomes in a range of longer-term stress and health markers. As such, self-reports of chronic stress (TICS, PSS), diurnal cortisol activity (CAR, DCS), proinflammatory immune activity (IL-6, hsCRP), hippocampal volume (HCV), and, in a separate analysis, hair cortisol and cortisone levels (HC, HE) were assessed. We used Piecewise Growth Curve Models with Landmark Registration (Lopez-Duran et al., 2014) to simultaneously model cortisol reactivity and recovery relative to the individual cortisol peak.

Of the various markers examined, only the CAR was specifically and consistently associated with the progression of cortisol stress recovery. In detail, slower cortisol recovery from acute stress was linked to a relatively increased CAR. Furthermore, when adding HCV as a marker of neuronal integrity to our final model, smaller HCV was associated with higher cortisol stress reactivity.

A possible explanation for the specificity of the association between cortisol stress recovery and the CAR may be that, compared to the other markers, the CAR has the highest functional overlap with the acute cortisol stress response. Both measures reflect dynamic aspects of HPA axis activity, albeit in relation to very different stimuli. In the acute stress response, an individual is confronted with a real challenge (in our study, the demands of psychosocial evaluation in the TSST). The CAR, on the other hand, is considered a response to the anticipated demands of the upcoming day (Fries et al., 2009; Law et al., 2013). Accordingly, higher CAR has been reported on working days relative to weekends (Kunz-Ebrecht et al., 2004; Schlotz et al., 2004; Thorn et al., 2006), and on days of upcoming competitions relative to non-competition days (Rohleder et al., 2007). Among the few studies directly testing a link of acute cortisol response dynamics with the CAR, one found no relationship in a large sample of older adults (Kidd et al., 2014), while another suggested a negative relation between acute and morning cortisol peaks (Quirin et al., 2008). A pattern of blunted CAR and impaired acute *recovery* has been shown in depressed patients (Dienes et al., 2019) and in samples including depressed patients (Burke et al., 2005). Our study provides first evidence for an inverse pattern in rigorously health-screened adults, indicating an association between increased CAR and slowed acute recovery.

The observed association of CAR and acute stress recovery may be due to both measures being strongly dependent on cognitive processes. In their perseverative cognition hypothesis, Brosschot and colleagues (2010; 2006) propose that cognitive representations of stressors can extend stress-related affective and physiological activation, both in advance and in the aftermath of stressful experience. Such thinking patterns likely also play a substantial role in how demanding we anticipate the upcoming day to be, and would consequently influence the daily rise in CAR. Along those lines, rumination of events that occurred throughout the day and worries about events to come, were shown to

Table 2
Model summaries of linear mixed models with sex/age as covariates and level 2 predictors CAR/DCS (Model 1), TICS/PSS (Model 2), and hsCRP/IL-6/HCV (Model 3).

Predictors	Model 1					Model 2					Model 3 (Main Model)				
	β	*std. CI*	*t*	*p*	*df*	*β*	*std. CI*	*t*	*p*	*df*	*β*	*std. CI*	*t*	*p*	*df*
(Intercept)	0.16	−0.05 – 0.38	9.46	**<0.001**	103.43	0.15	−0.07 – 0.36	9.42	**<0.001**	101.25	0.14	−0.08 – 0.36	8.88	**<0.001**	97.23
sex [women]	−0.41	−0.71–−0.12	−2.77	**0.007**	99.84	−0.39	−0.68–−0.09	−2.56	**0.012**	97.78	−0.37	−0.68–−0.07	−2.38	**0.019**	94.29
Age	−0.02	−0.16 – 0.13	−0.21	0.834	99.46	−0.02	−0.16 – 0.13	−0.24	0.814	97.36	−0.01	−0.16 – 0.14	−0.17	0.868	94.32
time_before_peak	0.43	0.37–0.49	14.04	**<0.001**	92.88	0.43	0.37–0.49	13.90	**<0.001**	91.90	0.43	0.37–0.49	14.38	**<0.001**	90.15
time_after_peak	−0.54	−0.60–−0.48	−17.34	**<0.001**	109.07	−0.54	−0.61–−0.48	−17.39	**<0.001**	100.77	−0.55	−0.61–−0.48	−17.53	**<0.001**	94.81
CAR	0.07	−0.09 – 0.23	0.85	0.903	100.48	0.06	−0.10 – 0.23	0.76	0.931	98.54	0.08	−0.09 – 0.25	0.95	0.725	95.47
DCS	−0.00	−0.16 – 0.16	−0.05	0.989	100.14	0.00	−0.16 – 0.16	0.03	0.963	98.27	−0.01	−0.17 – 0.15	−0.12	0.841	95.43
time_before_peak *CAR	−0.02	−0.08 – 0.05	−0.48	0.636	89.66	−0.01	−0.08 – 0.05	−0.40	0.690	88.43	−0.01	−0.07 – 0.06	−0.17	0.866	86.42
time_after_peak *CAR	0.08	0.01–0.14	2.16	**0.032**	130.38	0.07	0.00–0.14	2.05	**0.042**	122.40	0.07	−0.00 – 0.14	1.83	**0.070**	115.90
time_before_peak *DCS	−0.01	−0.07 – 0.06	−0.25	0.805	90.79	−0.01	−0.08 – 0.05	−0.32	0.749	89.70	−0.02	−0.09 – 0.04	−0.70	0.484	87.97
time_after_peak *DCS	−0.01	−0.08 – 0.05	−0.43	0.669	122.20	−0.01	−0.08 – 0.06	−0.33	0.743	112.52	−0.00	−0.07 – 0.07	−0.10	0.921	104.75
TICS						−0.02	−0.23 – 0.20	−0.16	0.636	98.22	−0.07	−0.29 – 0.15	−0.59	0.335	95.68
PSS						−0.10	−0.32 – 0.11	−0.94	0.463	97.72	−0.08	−0.30 – 0.14	−0.72	0.570	95.03
time_before_peak *TICS						−0.04	−0.13 – 0.05	−0.88	0.381	93.57	−0.05	−0.14 – 0.04	−1.09	0.278	91.79
time_after_peak *TICS						0.03	−0.06 – 0.12	0.76	0.451	94.52	0.05	−0.04 – 0.14	1.09	0.279	86.82
time_before_peak *PSS						0.01	−0.08 – 0.10	0.17	0.868	90.50	0.00	−0.08 – 0.09	0.10	0.919	89.51
time_after_peak *PSS						−0.01	−0.10 – 0.09	−0.13	0.893	109.25	−0.01	−0.10 – 0.08	−0.17	0.866	99.78
hsCRP											0.04	−0.12 – 0.20	0.48	0.681	95.21
IL-6											−0.14	−0.29 – 0.02	−1.70	0.084	100.90
HCV											−0.09	−0.24 – 0.07	−1.10	0.067	95.34
time_before_peak *hsCRP											−0.01	−0.07 – 0.05	−0.18	0.854	87.48
time_after_peak *hsCRP											−0.00	−0.07 – 0.06	−0.10	0.920	110.97
time_before_peak *IL-6											−0.01	−0.08 – 0.07	−0.13	0.896	103.58
time_after_peak *IL-6											0.03	−0.02 – 0.09	1.11	0.271	48.37
time_before_peak *HCV											−0.08	−0.15–−0.02	−2.70	**0.008**	91.48
time_after_peak *HCV											0.06	−0.01 – 0.12	1.78	0.078	87.63
Random Effects															
σ^2	0.04					0.04					0.04				
τ_{00}	0.37 $_{id}$					0.37 $_{id}$					0.35 $_{id}$				
τ_{11}	0.01 $_{id.time\_before\_peak\_ind}$					0.01 $_{id.time\_before\_peak\_ind}$					0.00 $_{id.time\_before\_peak\_ind}$				
	0.00 $_{id.time\_after\_peak\_ind}$					0.00 $_{id.time\_after\_peak\_ind}$					0.00 $_{id.time\_after\_peak\_ind}$				
ρ_{01}	0.55					0.55					0.51				
	−0.47					−0.47					−0.43				
ICC	0.89					0.89					0.89				
N	106 $_{id}$					106 $_{id}$					106 $_{id}$				
Observations	632					632					632				
Marginal R^2/Conditional R^2	0.330/0.927					0.340/0.928					0.362/0.930				

CAR = Cortisol awakening response. DCS = Diurnal cortisol slope. TICS = Trier Inventory of Chronic Stress (Schulz and Schlotz, 1999). PSS = Perceived Stress Scale (Cohen et al., 1983). hsCRP = high-sensitive C-reactive protein. IL-6 = Interleukin-6. HCV = Hippocampal volume.

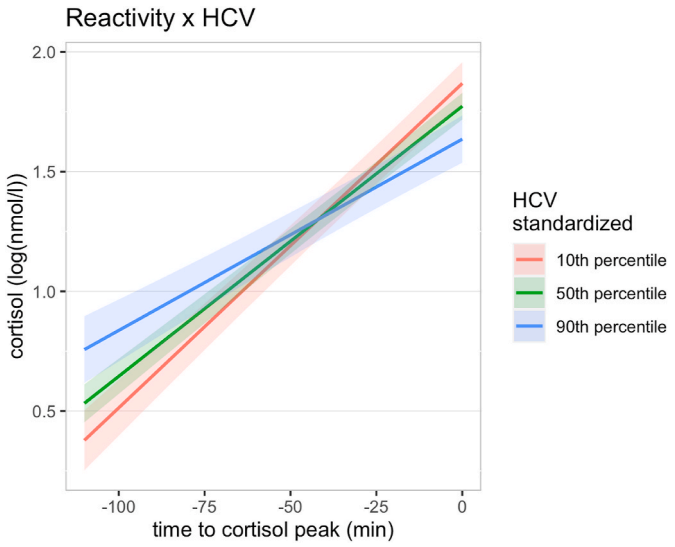

Fig. 1. Associations of cortisol stress reactivity with hippocampal volume. A Piecewise Growth Curve Model with Landmark-Registration (pGCM-LR) shows associations of individual peak-adjusted cortisol stress reactivity with HCV. Predicted values of cortisol before the individual peak (reactivity slope) dependening on HCV (p = .008) are depicted. Estimated slopes are presented for 10th (red line), 50th (green line) and 90th percentiles (blue line) of standardized HCV. Shaded areas represent the standard error (SE). HCV = Hippocampal volume. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

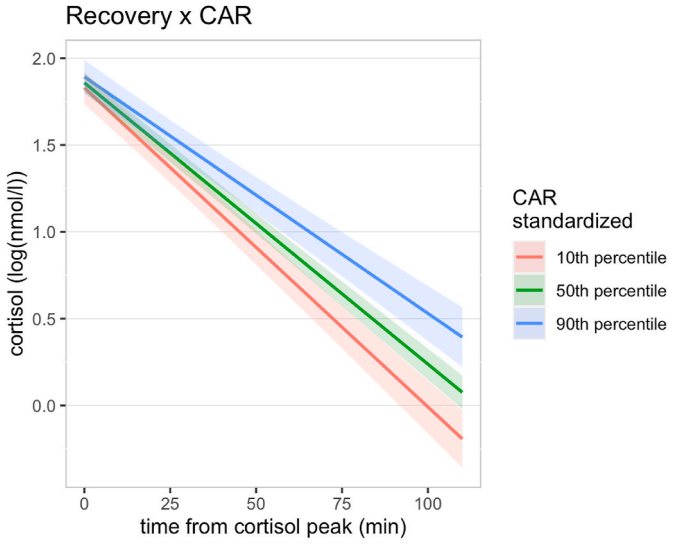

Fig. 2. Associations of cortisol stress recovery with the cortisol awakening response. A Piecewise Growth Curve Model with Landmark-Registration (pGCM-LR) shows marginal associations of individual peak-adjusted cortisol stress recovery with CAR. Predicted values of cortisol after the individual peak (recovery slope) depending on CAR (p = .070) are depicted. Estimated slopes are presented for 10th (red line), 50th (green line) and 90th percentiles (blue line) of standardized CAR. Shaded areas represent the standard error (SE). CAR = Cortisol awakening response. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

predict the next-day CAR (Zoccola et al., 2010). Further, increased rumination after an acute stressor was found to predict both higher and prolonged cortisol responses (Zoccola et al., 2008) as well as diminished stressor habituation (Gianferante et al., 2014).

Other than expected, cortisol reactivity rather than recovery was linked to HCV. The inverse relationship, meaning increased cortisol reactivity going along with smaller HCV, supports the often-made assumption that increased cortisol reactivity in the acute setting does indeed translate to health-relevant downstream effects. Most evidence suggesting a negative relation of HCV and HPA-axis activity stems from studies investigating basal glucocorticoid levels, samples at greater risk for neuronal challenge (e.g., due to increasing age, chronic stress, or psychopathology; see Frodl and O'Keane, 2013; Sheline et al., 2019 for reviews), or small sample sizes (Pruessner et al., 2007; Tessner et al., 2007). Therefore, our findings provide first robust evidence for this link in healthy adults. It should be noted that, while our study sample had a fairly broad age range (20–55 years, mean age = 40.7 years), substantial age-related HCV decline is unlikely with a maximal age of 55 years (Fjell et al., 2013; Nobis et al., 2019). The moderating role of age on both HCV and the cortisol stress response (Kudielka et al., 2009; Nobis et al., 2019; Otte et al., 2005) may best explain the inconsistent reports on their relationship, and remains a promising target for future research.

We did not find associations of either cortisol recovery or reactivity with the remaining indices of long-term stress and associated downstream effects, particularly TICS and PSS, DCS, HC and HE, as well as IL-6 and hsCRP. Different factors may account for these null results. First, regarding self-report measures, a dissociation between the psychological and endocrine aspects of stress is a well-described phenomenon in the literature (Campbell and Ehlert, 2012; Dalile et al., 2022; Engert et al., 2018; Schlotz et al., 2008). While different factors may contribute to this "lack of psychoendocrine covariance", biases in retrospective self-report methods, such as social desirability or recall bias, likely play a prominent role (Althubaiti, 2016).

Second, an association between acute reactivity or recovery and DCS would have been conceivable. In prior research, less steep acute cortisol reactivity and recovery, as well as a flattened DCS have been related to long-term stress (Adam and Kumari, 2009; McEwen, 2006). From a methodological perspective, both measures are assessed in saliva, strongly rely on awakening cortisol levels (see Stalder et al., 2016 for a review), and tend to show methodological clustering (Engert et al., 2018). However, in simultaneously testing a link of both CAR and DCS with acute reactivity and recovery, our results suggest that, above and beyond the CAR, the DCS does not account for a significant share of

Table 3
Model summary of the additional model (Model 4) with hair cortisol concentration (HC) and hair cortisone concentration (HE) as predictors of cortisol trajectories.

Predictors	Model 4				
	β	*std. CI*	*t*	*p*	*df*
(Intercept)	0.28	−0.01 – 0.57	8.51	**<0.001**	57.79
sex [women]	−0.50	−0.86–−0.13	−2.67	**0.010**	55.74
Age	0.01	−0.15 – 0.18	0.16	0.870	55.62
time_before_peak	0.50	0.41–0.58	11.54	**<0.001**	53.47
time_after_peak	−0.59	−0.67–−0.50	−13.58	**<0.001**	53.44
HC	0.03	−0.20 – 0.25	0.22	0.737	59.13
HE	0.02	−0.21 – 0.26	0.20	0.825	59.65
time_before_peak *HC	0.04	−0.07 – 0.15	0.71	0.482	52.39
time_after_peak *HC	0.00	−0.11 – 0.11	0.00	0.998	59.96
time_before_peak *HE	0.00	−0.11 – 0.11	0.03	0.977	52.94
time_after_peak *HE	−0.01	−0.12 – 0.10	−0.13	0.893	57.42
Random Effects					
σ^2	0.04				
$\tau_{00\ id}$	0.27				
$\tau_{11\ id.time\_before\_peak}$	0.01				
$\tau_{11\ id.time\_after\_peak}$	0.01				
ρ_{01}	0.58				
	−0.26				
ICC	0.87				
N_{id}	64				
Observations	382				
Marginal R^2/ Conditional R^2	0.407/0.924				

HC = Hair cortisol. HE = Hair cortisone.

incremental variance in recovery.

Third, the lack of an association of reactivity or recovery with systemic long-term stress markers (HC and HE) may be attributable to the comparably lower N, and accordingly reduced analysis power. In contrast, a recent study reported a link of lower cortisol reactivity with higher HC, although the authors acknowledge the limited generalizability of their findings due to low sample size (Sandner et al., 2020). While future studies are needed to clarify this link, in the current sample, neither increased cortisol reactivity to, nor prolonged recovery from, acute psychosocial stress seem to accumulate to a measurable effect in systemic glucocorticoid levels in healthy adults.

Fourth, acute cortisol reactivity or recovery did not covary with indicators of low-grade systemic inflammation, particularly IL-6 and hsCRP. Both inflammatory markers capture the downstream effects of long-term stress and are indicative of long-term health (Elenkov et al., 2005; Straub and Schradin, 2016), even though these associations might not be as straightforward as previously assumed (Del Giudice and Gangestad, 2018). Since our participants underwent a strict inclusion procedure ruling out the presence or history of physical and mental health issues, the resulting sample was extremely healthy. Consequently, there may have been insufficient variance in proinflammatory activity to detect an association (see also Puhlmann et al., 2019).

There are several limitations to the current study. First, the present data do not fully conform to the consensus guidelines on the assessment of the CAR (Stalder et al., 2022), which were published after the conception of our study. Most importantly, we did not employ objective measures for the verification of participants' sampling times. Hence, respective CAR data must be treated with some caution since the possibility of non-adherence-related confounding cannot be excluded (Kudielka et al., 2003). Likewise, confounding effects in our DCS data cannot be ruled out. We nevertheless addressed the issue of non-adherence through an experience sampling approach based on mobile phones handed out to our participants. As shown by the relatively low proportion of missingness, these devices may have boosted adherence by reminding participants of a forthcoming sampling time-point. Second, due to our rigorously health-screened sample of middle-aged adults, meaningful associations may have been missed due to relatively little variance in long-term stress and downstream health effects. Third, we did not track whether participants suffered from minor diseases or infections, which may have influenced the assayed inflammatory biomarkers. Lastly, several participants did not reach their pre-TSST cortisol baseline levels within the window of data assessment. This may have led to biased estimation of recovery slopes.

Building on these limitations, we encourage future research into the topic of stress recovery to widen the window of cortisol assessment beyond the 60–70 min typically seen in TSST studies, and to increase the amount of post-stress cortisol samples to enable a more detailed analysis of cortisol recovery. Also, it would be interesting to investigate the common role of perseverative cognition for cortisol recovery and long-term stress, and to explore associations of acute stress reactivity and recovery with measures of long-term stress, health and resilience in samples with a broader age range and more variance in health-related variables.

Overall, we found that cortisol stress recovery and reactivity differentially relate to distinct indicators of long-term stress and downstream health sequelae, possibly reflecting different aspects of an individual's stress burden. There was no evidence to suggest that recovery is the better indicator of the two. The only one measure associated with cortisol recovery after an acute psychosocial stressor was the cortisol response to awakening. We suggest that this association is driven by the role of perseverative thought patterns in both, recovering from acute stress exposure, and awakening with the challenges of the upcoming day on one's mind. HCV as a more permanent downstream consequence of stress was linked to cortisol stress reactivity. These recovery- and reactivity-specific findings point to the complex nature of stress and should encourage future research into stress recovery, including the design of novel recovery protocols and the development of improved statistical methods for recovery data analysis.

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Author contributions

M.D. analyzed the data and drafted the manuscript. R.L. and L.M.C.P. supported data analysis. V.E. designed the study, supported data curation and supported the drafting of the manuscript. T.S. initiated and developed the *ReSource Project* and secured all funding. She also co-developed all stress-related measures and tasks as PI of the *ReSource Project* and was leader of all *ReSource* related meetings with her entire staff including meetings with the teachers, the researchers, the *ReSource* support and testing staff etc. All authors critically revised the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbih.2023.100598.

References

Adam, E.K., Hawkley, L.C., Kudielka, B.M., Cacioppo, J.T., 2006. Day-to-day dynamics of experience-cortisol associations in a population-based sample of older adults. Proc. Natl. Acad. Sci. USA 103, 17058–17063. https://doi.org/10.1073/pnas.0605053103.

Adam, E.K., Kumari, M., 2009. Assessing salivary cortisol in large-scale, epidemiological research. Psychoneuroendocrinology 34, 1423–1436. https://doi.org/10.1016/j.psyneuen.2009.06.011.

Agorastos, A., Chrousos, G.P., 2022. The neuroendocrinology of stress: the stress-related continuum of chronic disease development. Mol. Psychiatr. 27, 502–513. https://doi.org/10.1038/s41380-021-01224-9.

Allen, A.P., Kennedy, P.J., Dockray, S., Cryan, J.F., Dinan, T.G., Clarke, G., 2017. The trier social stress test: principles and practice. Neurobiol. Stress 6, 113–126. https://doi.org/10.1016/j.ynstr.2016.11.001.

Althubaiti, A., 2016. Information bias in health research: definition, pitfalls, and adjustment methods. J. Multidiscip. Healthc. 211 https://doi.org/10.2147/JMDH.S104807.

American Psychological Association, 2018. Stress in America: stress and generation Z. Stress Am. Surv. 11.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Software 67. https://doi.org/10.18637/jss.v067.i01.

Ben-Shachar, M., Lüdecke, D., Makowski, D., 2020. Effectsize: estimation of effect size indices and standardized parameters. J. Open Source Softw. 5, 2815. https://doi.org/10.21105/joss.02815.

Brosschot, J.F., 2010. Markers of chronic stress: prolonged physiological activation and (un)conscious perseverative cognition. Neurosci. Biobehav. Rev. 35, 46–50. https://doi.org/10.1016/j.neubiorev.2010.01.004.

Brosschot, J.F., Gerin, W., Thayer, J.F., 2006. The perseverative cognition hypothesis: a review of worry, prolonged stress-related physiological activation, and health. J. Psychosom. Res. 60, 113–124. https://doi.org/10.1016/j.jpsychores.2005.06.074.

Burke, H.M., Davis, M.C., Otte, C., Mohr, D.C., 2005. Depression and cortisol responses to psychological stress: a meta-analysis. Psychoneuroendocrinology 30, 846–856. https://doi.org/10.1016/j.psyneuen.2005.02.010.

Caldairou, B., Bernhardt, B.C., Kulaga-Yoskovitz, J., Kim, H., Bernasconi, N., Bernasconi, A., 2016. A surface patch-based segmentation method for hippocampal subfields. In: Ourselin, S., Joskowicz, L., Sabuncu, M.R., Unal, G., Wells, W. (Eds.), Medical Image Computing and Computer-Assisted Intervention – MICCAI 2016, Lecture Notes in Computer Science. Springer International Publishing, Cham, pp. 379–387. https://doi.org/10.1007/978-3-319-46723-8_44.

Campbell, J., Ehlert, U., 2012. Acute psychosocial stress: does the emotional stress response correspond with physiological responses? Psychoneuroendocrinology 37, 1111–1134. https://doi.org/10.1016/j.psyneuen.2011.12.010.

Chida, Y., Hamer, M., 2008. Chronic psychosocial factors and acute physiological responses to laboratory-induced stress in healthy populations: a quantitative review of 30 years of investigations. Psychol. Bull. 134, 829–885. https://doi.org/10.1037/a0013342.

Chida, Y., Steptoe, A., 2009. Cortisol awakening response and psychosocial factors: a systematic review and meta-analysis. Biol. Psychol. 80, 265–278. https://doi.org/10.1016/j.biopsycho.2008.10.004.

Chrousos, G.P., 2009. Stress and disorders of the stress system. Nat. Rev. Endocrinol. 5, 374–381. https://doi.org/10.1038/nrendo.2009.106.

Cohen, S., Kamarck, T., Mermelstein, R., 1983. A global measure of perceived stress. J. Health Soc. Behav. 24, 385–396.

Dalile, B., La Torre, D., Verbeke, K., Van Oudenhove, L., Vervliet, B., 2022. When the mind says one thing, but the HPA axis says another: lack of coherence between subjective and neuroendocrine stress response trajectories in healthy men. Psychoneuroendocrinology 139, 105692. https://doi.org/10.1016/j.psyneuen.2022.105692.

Dallman, M.F., Bhatnagar, S., Viau, V., 2000. Hypothalamo-pituitary-adrenal axis. In: Fink, G. (Ed.), Encyclopedia of Stress. Academic Press, New York, pp. 468–477.

Del Giudice, M., Gangestad, S.W., 2018. Rethinking IL-6 and CRP: why they are more than inflammatory biomarkers, and why it matters. Brain Behav. Immun. 70, 61–75. https://doi.org/10.1016/j.bbi.2018.02.013.

Dickerson, S.S., Kemeny, M.E., 2004. Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. Psychol. Bull. 130, 355–391. https://doi.org/10.1037/0033-2909.130.3.355.

Dienes, K., Gartland, N., Ferguson, E., 2019. The relationship between the cortisol awakening response and cortisol reactivity to a laboratory stressor. Br. J. Health Psychol. 24, 265–281. https://doi.org/10.1111/bjhp.12352.

Dressendörfer, R.A., Kirschbaum, C., Rohde, W., Stahl, F., Strasburger, C.J., 1992. Synthesis of a cortisol-biotin conjugate and evaluation as a tracer in an immunoassay for salivary cortisol measurement. J. Steroid Biochem. Mol. Biol. 43, 683–692. https://doi.org/10.1016/0960-0760(92)90294-S.

Elenkov, I.J., Iezzoni, D.G., Daly, A., Harris, A.G., Chrousos, G.P., 2005. Cytokine dysregulation, inflammation and well-being. Neuroimmunomodulation 12, 255–269. https://doi.org/10.1159/000087104.

Engert, V., Koester, A.M., Riepenhausen, A., Singer, T., 2016. Boosting recovery rather than buffering reactivity: higher stress-induced oxytocin secretion is associated with increased cortisol reactivity and faster vagal recovery after acute psychosocial stress. Psychoneuroendocrinology 74, 111–120. https://doi.org/10.1016/j.psyneuen.2016.08.029.

Engert, V., Kok, B.E., Papassotiriou, I., Chrousos, G.P., Singer, T., 2017. Specific reduction in cortisol stress reactivity after social but not attention-based mental training. Sci. Adv. 3, e1700495 https://doi.org/10.1126/sciadv.1700495.

Engert, V., Kok, B.E., Puhlmann, L.M.C., Stalder, T., Kirschbaum, C., Apostolakou, F., Papanastasopoulou, C., Papassotiriou, I., Pervanidou, P., Chrousos, G.P., Singer, T., 2018. Exploring the multidimensional complex systems structure of the stress response and its relation to health and sleep outcomes. Brain Behav. Immun. 73, 390–402. https://doi.org/10.1016/j.bbi.2018.05.023.

Field, A.P., Miles, J., Field, Z., 2012. Discovering Statistics Using R. Sage, London ; Thousand Oaks, Calif.

First, M.B., Gibbon, M., Spitzer, R.L., Williams, J.B.W., Benjamin, L.S., 1997. Structured Clinical Interview for DSM-IV® Axis II Personality Disorders (SCID-II). American Psychiatric Press Inc., Washington D.C.

Fjell, A.M., Westlye, L.T., Grydeland, H., Amlien, I., Espeseth, T., Reinvang, I., Raz, N., Holland, D., Dale, A.M., Walhovd, K.B., 2013. Critical ages in the life course of the adult brain: nonlinear subcortical aging. Neurobiol. Aging 34, 2239–2247. https://doi.org/10.1016/j.neurobiolaging.2013.04.006.

Fries, E., Dettenborn, L., Kirschbaum, C., 2009. The cortisol awakening response (CAR): facts and future directions. Int. J. Psychophysiol. 72, 67–73. https://doi.org/10.1016/j.ijpsycho.2008.03.014.

Gianferante, D., Thoma, M.V., Hanlin, L., Chen, X., Breines, J.G., Zoccola, P.M., Rohleder, N., 2014. Post-stress rumination predicts HPA axis responses to repeated acute stress. Psychoneuroendocrinology 49, 244–252. https://doi.org/10.1016/j.psyneuen.2014.07.021.

Guidi, J., Lucente, M., Sonino, N., Fava, G.A., 2021. Allostatic load and its impact on health: a systematic review. Psychother. Psychosom. 90, 11–27. https://doi.org/10.1159/000510696.

Hassard, J., Teoh, K.R.H., Visockaite, G., Dewe, P., Cox, T., 2018. The cost of work-related stress to society: a systematic review. J. Occup. Health Psychol. 23, 1–17. https://doi.org/10.1037/ocp0000069.

Herman, J.P., McKlveen, J.M., Ghosal, S., Kopp, B., Wulsin, A., Makinson, R., Scheimann, J., Myers, B., 2016. Regulation of the hypothalamic-pituitary-adrenocortical stress response. In: Terjung, R. (Ed.), Comprehensive Physiology. Wiley, pp. 603–621. https://doi.org/10.1002/cphy.c150015.

Herman, J.P., Nawreen, N., Smail, M.A., Cotella, E.M., 2020. Brain mechanisms of HPA axis regulation: neurocircuitry and feedback in context Richard Kvetnansky lecture. Stress 23, 617–632. https://doi.org/10.1080/10253890.2020.1859475.

IBM Corp, 2016. IBM SPSS Statistics for Windows.

Jacobson, L., Sapolsky, R.M., 1991. The role of the Hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical Axis. Endocr. Rev. 12, 118–134. https://doi.org/10.1210/edrv-12-2-118.

Kajantie, E., Phillips, D., 2006. The effects of sex and hormonal status on the physiological response to acute psychosocial stress. Psychoneuroendocrinology 31, 151–178. https://doi.org/10.1016/j.psyneuen.2005.07.002.

Kidd, T., Carvalho, L.A., Steptoe, A., 2014. The relationship between cortisol responses to laboratory stress and cortisol profiles in daily life. Biol. Psychol. 99, 34–40. https://doi.org/10.1016/j.biopsycho.2014.02.010.

Kirschbaum, C., Pirke, K.-M., Hellhammer, D.H., 1993. The 'trier social stress test' – a tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiology 28, 76–81. https://doi.org/10.1159/000119004.

Kudielka, B.M., Broderick, J.E., Kirschbaum, C., 2003. Compliance with saliva sampling protocols: electronic monitoring reveals invalid cortisol daytime profiles in noncompliant subjects: psychosom. Med 65, 313–319. https://doi.org/10.1097/01.PSY.0000058374.50240.BF.

Kudielka, B.M., Hellhammer, D.H., Wüst, S., 2009. Why do we respond so differently? Reviewing determinants of human salivary cortisol responses to challenge. Psychoneuroendocrinology 34, 2–18. https://doi.org/10.1016/j.psyneuen.2008.10.004.

Kunz-Ebrecht, S.R., Kirschbaum, C., Marmot, M., Steptoe, A., 2004. Differences in cortisol awakening response on work days and weekends in women and men from the Whitehall II cohort. Psychoneuroendocrinology 29, 516–528. https://doi.org/10.1016/S0306-4530(03)00072-6.

Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2017. lmerTest package: tests in linear mixed effects models. J. Stat. Software 82. https://doi.org/10.18637/jss.v082.i13.

Law, R., Hucklebridge, F., Thorn, L., Evans, P., Clow, A., 2013. State variation in the cortisol awakening response. Stress 16, 483–492. https://doi.org/10.3109/10253890.2013.817552.

Linden, W., Earle, T.L., Gerin, W., Christenfeld, N., 1997. Physiological stress reactivity and recovery: conceptual siblings separated at birth? J. Psychosom. Res. 42, 117–135. https://doi.org/10.1016/S0022-3999(96)00240-1.

Lopez-Duran, N.L., Mayer, S.E., Abelson, J.L., 2014. Modeling neuroendocrine stress reactivity in salivary cortisol: adjusting for peak latency variability. Stress 17, 285–295. https://doi.org/10.3109/10253890.2014.915517.

Lupien, S.J., Juster, R.-P., Raymond, C., Marin, M.-F., 2018. The effects of chronic stress on the human brain: from neurotoxicity, to vulnerability, to opportunity. Front. Neuroendocrinol. 49, 91–105. https://doi.org/10.1016/j.yfrne.2018.02.001.

Matthews, K.A., Gump, B.B., Owens, J.F., 2001. Chronic stress influences cardiovascular and neuroendocrine responses during acute stress and recovery, especially in men. Health Psychol. 20, 403–410. https://doi.org/10.1037/0278-6133.20.6.403.

McEwen, B.S., 2019. What is the confusion with cortisol? Chronic Stress 3, 247054701983364. https://doi.org/10.1177/2470547019833647.

McEwen, B.S., 2017. Neurobiological and systemic effects of chronic stress. Chronic Stress 1, 247054701769232. https://doi.org/10.1177/2470547017692328.

McEwen, B.S., 2006. Protective and damaging effects of stress mediators: central role of the brain. In: Progress in Brain Research. Elsevier, pp. 25–34. https://doi.org/10.1016/S0079-6123(08)62128-7.

McEwen, B.S., 1998. Stress, adaptation, and disease: allostasis and allostatic load. Ann. N. Y. Acad. Sci. 840, 33–44. https://doi.org/10.1111/j.1749-6632.1998.tb09546.x.

McEwen, B.S., Albeck, D., Cameron, H., Chao, H.M., Gould, E., Hastings, N., Kuroda, Y., Luine, V., Magarinos, A.M., Mckittrick, C.R., Orchinik, M., Pavlides, C., Vaher, P., Watanabe, Y., Weiland, N., 1995. Stress and the brain: a paradoxical role for adrenal steroids. In: Litwack, G. (Ed.), Vitamins & Hormones. Academic Press, pp. 371–402. https://doi.org/10.1016/S0083-6729(08)61045-6.

McEwen, B.S., Nasca, C., Gray, J.D., 2016. Stress effects on neuronal structure: Hippocampus, amygdala, and prefrontal cortex. Neuropsychopharmacology 41, 3–23. https://doi.org/10.1038/npp.2015.171.

McKittrick, C.R., Magariños, A.M., Blanchard, D.C., Blanchard, R.J., McEwen, B.S., Sakai, R.R., 2000. Chronic social stress reduces dendritic arbors in CA3 of

hippocampus and decreases binding to serotonin transporter sites. Synapse 36, 85–94. https://doi.org/10.1002/(SICI)1098-2396(200005)36:2<85::AID-SYN1>3.0.CO;2-Y.
Miller, R., Plessow, F., Kirschbaum, C., Stalder, T., 2013. Classification criteria for distinguishing cortisol responders from nonresponders to psychosocial stress: evaluation of salivary cortisol pulse detection in panel designs. Psychosom. Med. 75, 832–840. https://doi.org/10.1097/PSY.0000000000000002.
Miller, R., Wojtyniak, J.-G., Weckesser, L.J., Alexander, N.C., Engert, V., Lehr, T., 2018. How to disentangle psychobiological stress reactivity and recovery: a comparison of model-based and non-compartmental analyses of cortisol concentrations. Psychoneuroendocrinology 90, 194–210. https://doi.org/10.1016/j.psyneuen.2017.12.019.
Nobis, L., Manohar, S.G., Smith, S.M., Alfaro-Almagro, F., Jenkinson, M., Mackay, C.E., Husain, M., 2019. Hippocampal volume across age: nomograms derived from over 19,700 people in UK Biobank. NeuroImage Clin 23, 101904. https://doi.org/10.1016/j.nicl.2019.101904.
Oitzl, M.S., Champagne, D.L., van der Veen, R., de Kloet, E.R., 2010. Brain development under stress: hypotheses of glucocorticoid actions revisited. Neurosci. Biobehav. Rev. 34, 853–866. https://doi.org/10.1016/j.neubiorev.2009.07.006.
Otte, C., Hart, S., Neylan, T.C., Marmar, C.R., Yaffe, K., Mohr, D.C., 2005. A meta-analysis of cortisol response to challenge in human aging: importance of gender. Psychoneuroendocrinology 30, 80–91. https://doi.org/10.1016/j.psyneuen.2004.06.002.
Patel, V., Saxena, S., Lund, C., Thornicroft, G., Baingana, F., Bolton, P., Chisholm, D., Collins, P.Y., Cooper, J.L., Eaton, J., Herrman, H., Herzallah, M.M., Huang, Y., Jordans, M.J.D., Kleinman, A., Medina-Mora, M.E., Morgan, E., Niaz, U., Omigbodun, O., Prince, M., Rahman, A., Saraceno, B., Sarkar, B.K., De Silva, M., Singh, I., Stein, D.J., Sunkel, C., UnÜtzer, Jü, 2018. The Lancet Commission on global mental health and sustainable development. Lancet 392, 1553–1598. https://doi.org/10.1016/S0140-6736(18)31612-X.
Pruessner, M., Pruessner, J.C., Hellhammer, D.H., Bruce Pike, G., Lupien, S.J., 2007. The associations among hippocampal volume, cortisol reactivity, and memory performance in healthy young men. Psychiatry Res. Neuroimaging. 155, 1–10. https://doi.org/10.1016/j.pscychresns.2006.12.007.
Puhlmann, L.M.C., Engert, V., Apostolakou, F., Papassotiriou, I., Chrousos, G.P., Vrtička, P., Singer, T., 2019. Only vulnerable adults show change in chronic low-grade inflammation after contemplative mental training: evidence from a randomized clinical trial. Sci. Rep. 9, 19323 https://doi.org/10.1038/s41598-019-55250-3.
Puhlmann, L.M.C., Linz, R., Valk, S.L., Vrticka, P., Vos de Wael, R., Bernasconi, A., Bernasconi, N., Caldairou, B., Papassotiriou, I., Chrousos, G.P., Bernhardt, B.C., Singer, T., Engert, V., 2021a. Association between hippocampal structure and serum Brain-Derived Neurotrophic Factor (BDNF) in healthy adults: a registered report. Neuroimage 236, 118011. https://doi.org/10.1016/j.neuroimage.2021.118011.
Puhlmann, L.M.C., Vrtička, P., Linz, R., Stalder, T., Kirschbaum, C., Engert, V., Singer, T., 2021b. Contemplative mental training reduces hair glucocorticoid levels in a randomized clinical trial. Psychosom. Med. 83, 894–905. https://doi.org/10.1097/PSY.0000000000000970.
Quirin, M., Pruessner, J.C., Kuhl, J., 2008. HPA system regulation and adult attachment anxiety: individual differences in reactive and awakening cortisol. Psychoneuroendocrinology 33, 581–590. https://doi.org/10.1016/j.psyneuen.2008.01.013.
R Core Team, 2020. R: A Language and Environment for Statistical Computing.
Rohleder, N., 2019. Stress and inflammation – the need to address the gap in the transition between acute and chronic stress effects. Psychoneuroendocrinology 105, 164–171. https://doi.org/10.1016/j.psyneuen.2019.02.021.
Rohleder, N., Beulen, S.E., Chen, E., Wolf, J.M., Kirschbaum, C., 2007. Stress on the dance floor: the cortisol stress response to social-evaluative threat in competitive ballroom dancers. Pers. Soc. Psychol. Bull. 33, 69–84. https://doi.org/10.1177/0146167206293986.
Ross, K.M., Murphy, M.L.M., Adam, E.K., Chen, E., Miller, G.E., 2014. How stable are diurnal cortisol activity indices in healthy individuals? Evidence from three multi-wave studies. Psychoneuroendocrinology 39, 184–193. https://doi.org/10.1016/j.psyneuen.2013.09.016.
Sandner, M., Lois, G., Streit, F., Zeier, P., Kirsch, P., Wüst, S., Wessa, M., 2020. Investigating individual stress reactivity: high hair cortisol predicts lower acute stress responses. Psychoneuroendocrinology 118, 104660. https://doi.org/10.1016/j.psyneuen.2020.104660.
Sapolsky, R.M., Krey, L.C., McEwen, B.S., 1986. The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis. Endocr. Rev. 7, 284–301. https://doi.org/10.1210/edrv-7-3-284.
Schlotz, W., Hellhammer, J., Schulz, P., Stone, A.A., 2004. Perceived work overload and chronic worrying predict weekend–weekday differences in the cortisol awakening response. Psychosom. Med. 66, 207–214. https://doi.org/10.1097/01.psy.0000116715.78238.56.
Schlotz, W., Kumsta, R., Layes, I., Entringer, S., Jones, A., Wüst, S., 2008. Covariance between psychological and endocrine responses to pharmacological challenge and psychosocial stress: a question of timing. Psychosom. Med. 70, 787–796. https://doi.org/10.1097/PSY.0b013e3181810658.
Schulz, P., Schlotz, W., 1999. Trierer Inventar zur Erfassung von chronischem Streß (TICS): skalenkonstruktion, teststatistische Überprüfung und Validierung der Skala Arbeitsüberlastung. Diagnostica 45, 8–19. https://doi.org/10.1026//0012-1924.45.1.8.
Sheline, Y.I., Liston, C., McEwen, B.S., 2019. Parsing the Hippocampus in depression: chronic stress, hippocampal volume, and major depressive disorder. Biol. Psychiatr. 85, 436–438. https://doi.org/10.1016/j.biopsych.2019.01.011.
Singer, T., Kok, B.E., Bornemann, B., Bolz, M., Bochow, C.A., 2016. The ReSource Project. Background, Design, Samples, and Measurements, 2nd. Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig.
Slavich, G.M., 2020. Psychoneuroimmunology of stress and mental health. In: Harkness, K.L., Hayden, E.P. (Eds.), The Oxford Handbook of Stress and Mental Health. Oxford University Press, pp. 518–546. https://doi.org/10.1093/oxfordhb/9780190681777.013.24.
Stalder, T., Kirschbaum, C., 2012. Analysis of cortisol in hair – state of the art and future directions. Brain Behav. Immun. 26, 1019–1029. https://doi.org/10.1016/j.bbi.2012.02.002.
Stalder, T., Kirschbaum, C., Kudielka, B.M., Adam, E.K., Pruessner, J.C., Wüst, S., Dockray, S., Smyth, N., Evans, P., Hellhammer, D.H., Miller, R., Wetherell, M.A., Lupien, S.J., Clow, A., 2016. Assessment of the cortisol awakening response: expert consensus guidelines. Psychoneuroendocrinology 63, 414–432. https://doi.org/10.1016/j.psyneuen.2015.10.010.
Stalder, T., Lupien, S.J., Kudielka, B.M., Adam, E.K., Pruessner, J.C., Wüst, S., Dockray, S., Smyth, N., Evans, P., Kirschbaum, C., Miller, R., Wetherell, M.A., Finke, J.B., Klucken, T., Clow, A., 2022. Evaluation and update of the expert consensus guidelines for the assessment of the cortisol awakening response (CAR). Psychoneuroendocrinology 146, 105946. https://doi.org/10.1016/j.psyneuen.2022.105946.
Stalder, T., Steudte-Schmiedgen, S., Alexander, N., Klucken, T., Vater, A., Wichmann, S., Kirschbaum, C., Miller, R., 2017. Stress-related and basic determinants of hair cortisol in humans: a meta-analysis. Psychoneuroendocrinology 77, 261–274. https://doi.org/10.1016/j.psyneuen.2016.12.017.
Straub, R.H., Schradin, C., 2016. Chronic inflammatory systemic diseases – an evolutionary trade-off between acutely beneficial but chronically harmful programs. Evol. Med. Public Health eow001. https://doi.org/10.1093/emph/eow001.
Tessner, K.D., Walker, E.F., Dhruv, S.H., Hochman, K., Hamann, S., 2007. The relation of cortisol levels with hippocampus volumes under baseline and challenge conditions. Brain Res. 1179, 70–78. https://doi.org/10.1016/j.brainres.2007.05.027.
Thorn, L., Hucklebridge, F., Evans, P., Clow, A., 2006. Suspected non-adherence and weekend versus week day differences in the awakening cortisol response. Psychoneuroendocrinology 31, 1009–1018. https://doi.org/10.1016/j.psyneuen.2006.05.012.
Windle, G., Bennett, K.M., Noyes, J., 2011. A methodological review of resilience measurement scales. Health Qual. Life Outcome 9, 8. https://doi.org/10.1186/1477-7525-9-8.
Wittchen, H.-U., Gruschwitz, S., Fydrich, T., 1997. SKID-Strukturiertes Klinisches Interview für DSM-IV. Achse I und II. Hogrefe, Göttingen.
Wittchen, H.-U., Pfister, H., 1997. Diagnostisches Expertensystem für Psychische Störungen (DIA-X). Swets & Zeitlinger, Frankfurt.
Zoccola, P.M., Dickerson, S.S., Zaldivar, F.P., 2008. Rumination and cortisol responses to laboratory stressors. Psychosom. Med. 70, 661–667. https://doi.org/10.1097/PSY.0b013e31817bbc77.
Zoccola, P.M., Quas, J.A., Yim, I.S., 2010. Salivary cortisol responses to a psychosocial laboratory stressor and later verbal recall of the stressor: the role of trait and state rumination. Stress 13, 435–443. https://doi.org/10.3109/10253891003713765.