

What makes you feel sick after inflammation? Predictors of acute and persisting physical sickness symptoms induced by experimental endotoxemia

Running title: Predictors of sickness symptoms in endotoxemia

Sven Benson, PhD^a, Harald Engler, PhD^a, Alexander Wegner, MD^b,
Laura Rebernik, M Sc^a, Ingo Spreitzer, PhD^c, Manfred Schedlowski, PhD^a, Sigrid
Elsenbruch, PhD^a

^a*Institute of Medical Psychology & Behavioral Immunobiology, University Hospital Essen, University of Duisburg-Essen, Germany*

^b*Clinic for Trauma and Orthopedic Surgery, University Hospital Essen, University of Duisburg-Essen, Germany*

^c*Paul Ehrlich Institute, Federal Agency for Sera and Vaccines, Langen, Germany*

WORD COUNT: 3924

NUMBER OF REFERENCES: 48

NUMBER OF TABLES: 4

NUMBER OF FIGURES: 3

KEY WORDS: Sickness symptoms, Endotoxin, LPS, Interleukin-6, Cortisol, Depression

Corresponding author:

Sven Benson, Ph.D.
Inst. of Medical Psychology & Behavioral Immunobiology
University Hospital Essen
Hufelandstr. 55
45147 Essen
Germany
Phone: +49 201 723 4516
Fax: +49 201 723 5948
Email: sven.benson@uk-essen.de
Homepage: www.uk-essen.de/medizinische-psychologie

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1002/cpt.618

ABSTRACT

We aimed to identify statistical predictor variables of LPS-induced physical sickness symptoms during the acute and late inflammatory phases using multivariate regression analyses. Data from N=128 healthy volunteers who received i.v. LPS injection (0.4 or 0.8 ng/kg) or placebo were pooled for analyses. Physical sickness symptoms experienced during the acute (0-6h post-injection) and late (6-24h post-injection) phases were assessed with the validated General-Assessment-of-Side-Effects (GASE) questionnaire. LPS-treated subjects reported significantly more physical sickness symptoms. Physical symptoms during the acute phase were associated with LPS-induced mood impairments and IL-6 increases, explaining 28.5% of variance in GASE scores. During late phase, LPS-induced increases in cortisol and IL-6 plasma concentrations and baseline depression were significant predictor variables, explaining 38.5% of variance. In patients with recurrent or chronic inflammatory states these factors may act as risk factors ultimately contributing to an exacerbation of sickness symptoms, and should be considered as potential targets for therapeutic strategies.

INTRODUCTION

Physical sickness symptoms, comprising malaise, fatigue, or pain, are commonly experienced by medically-ill patients. These symptoms are non-specific and occur in a number of highly prevalent medical conditions including coronary heart disease, obesity, cancer, and depression as well as in patients treated with immunotherapies (1-5). Peripheral inflammation constitutes a common mechanism in the pathophysiology of these conditions, and conceivably explains the occurrence of similar sickness symptoms across a variety of diagnoses (1). Support for this notion comes from evidence that the severity of physical sickness symptoms correlates with peripheral pro-inflammatory cytokine concentrations (2, 6). Since a close association among physical sickness symptoms, negative mood and inflammation has been established (4, 7), it is conceivable that inflammation contributes both directly, e.g., via afferent immune-to-brain pathways and indirectly, via immune-related mood disturbances to physical sickness symptoms. Despite the high prevalence and broad clinical impact, individual risk factors predicting the presence, severity and persistence of physical sickness symptoms remain incompletely understood, hindering the development of tailored treatment approaches.

The administration of endotoxin (e.g., lipopolysaccharide, LPS) has proved an established translational tool to analyze the mechanisms of immune-mediated sickness symptoms in animal models and in humans (8, 9). LPS, the major component of the outer membrane of Gram-negative bacteria, is a prototypic pathogen-associated molecular pattern. Via Toll-like receptor (TLR) 4-dependent pathways, it triggers a well-characterized cascade of peripheral inflammatory mediators along with neuroendocrine feedback loops, signaling the central nervous system via molecular, cellular, and neural routes (8, 10). At the behavioral level,

centrally-mediated LPS effects include a range of physical sickness symptoms and mood changes [reviewed in (11), (12)], which closely resemble the symptoms experienced by patients with chronic health conditions. Hence, experimental endotoxemia allows to analyze cause-effect relationships between peripheral immune activation, inflammation-induced mood impairments, and physical sickness symptoms in healthy volunteers without potentially confounding effects of medical comorbidities.

The majority of studies in human endotoxin research focused on mechanisms underlying specific psychological or physical sickness symptoms during the acute inflammatory phase. However, a broader and clinically-relevant range of different physical sickness symptoms in response to low-dose endotoxin has rarely been characterized in detail, especially for the late phase. Further, risk factors that could contribute to persisting physical symptom beyond the acute phase of experimental endotoxemia remain largely unknown. Therefore, ~~the first aim of this study was to we~~ characterized clinically-relevant physical sickness symptoms in response to low and moderate LPS doses. A validated questionnaire was implemented to assess symptoms not only during the acute but also the late inflammatory phases (i.e., 6 and 24h post-injection). In addition, we explored if (1) both acute and persisting physical sickness symptoms are triggered by inflammatory mediators in a dose-dependent manner, and if (2) the severity of physical sickness symptoms is associated not only with inflammatory mediators but also with symptoms of negative mood. Finally, we implemented different sets of multivariate regression analyses including relevant psychological state and trait variables in addition to pro-inflammatory cytokines [Tumor necrosis factor (TNF)- α , Interleukin (IL)-6] and cortisol in order to identify predictor variables for number and intensity of sickness symptoms.

RESULTS

Sample characteristics

Data from 128 healthy volunteers (age: mean 26.8 ± 0.4 , median 26.0, range 18-43 years; BMI: mean 23.5 ± 0.3 , median 22.6, range 18.6 – 29.4 kg/m²) were analyzed.

Subjects randomized to the LPS or control (placebo) groups did not differ in sociodemographic or psychological variables. Only a small, but statistically significant difference in coping style (i.e., catastrophizing) between groups was found (Table 1).

LPS effects on immune, neuroendocrine, and mood parameters

To characterize the effects of LPS, we compared immunological parameters, cortisol, body temperature and mood parameters between LPS (i.e. 0.4 ng/kg LPS; 0.8 ng/kg LPS) and placebo treated subjects. LPS induced significant increases in plasma TNF- α ($F=4.3$, $p<0.001$, $\eta_p^2=0.16$) and IL-6 ($F=3.8$, $p<0.001$, $\eta_p^2=0.13$), plasma cortisol ($F=11.1$, $p<0.001$, $\eta_p^2=0.32$), heart rate ($F=9.5$, $p<0.001$, $\eta_p^2=0.30$) as well as body temperature ($F=9.6$, $p < 0.001$, $\eta_p^2=0.29$) in a transient and dose-dependent manner (all ANOVA interaction effects, see Figure 1 for posthoc tests and comparisons of AUC values). Plasma cytokine and cortisol concentrations returned to baseline 6 hours post-injection. Twenty-four hours post-injection of LPS, CRP concentrations were significantly increased compared to placebo [0.5 ± 0.1 mg/dl (placebo), 2.0 ± 0.7 mg/dl (0.4 ng/kg LPS), 2.5 ± 0.9 mg/dl (0.8 ng/kg LPS); $F=106.0$, $p<0.001$, $\eta_p^2=0.64$, ANOVA interaction)]. LPS application induced mood disturbances, indicated by a significant and transient increase in state anxiety ($F=7.5$, $p<0.001$, $\eta_p^2=0.12$) as well as decreases in positive mood ($F=14.5$, $p<0.001$, $\eta_p^2=0.21$), alertness ($F=10.1$, $p<0.001$, $\eta_p^2=0.16$) and calmness ($F=9.6$, $p<0.001$, $\eta_p^2=0.15$), with more pronounced effects in response to the higher LPS dose (ANOVA interaction effects; for post-hoc test and AUC values, see Figure 2).

LPS-induced physical sickness symptoms (GASE scores)

During LPS-induced systemic inflammation, a significantly greater number and intensity of physical sickness symptoms (i.e., higher GASE scores) was reported for

both the acute ($F=20.9$, $p<0.001$, $\eta_p^2=0.25$) and the late inflammatory phases ($F=4.7$, $p=0.010$, $\eta_p^2=0.07$) (Figure 3). Bonferroni-corrected posthoc test revealed that during the acute phase, both LPS doses led to significantly increased symptoms compared to placebo (0.4 ng/kg LPS vs. placebo: $t=-6.0$, $p<0.001$; 0.8 ng/kg LPS vs. placebo: $t=7.6$, $p<0.001$). Interestingly, for the late phase (i.e., 24h post-injection), LPS-treated subjects still reported increased physical sickness symptoms (0.4 ng/kg LPS vs. placebo: $t=-3.0$, $p=0.006$; 0.8 ng LPS vs. placebo: $t=-2.9$, $p=0.015$). The higher LPS dose induced more symptoms 6h after administration compared to the lower LPS dose, however, this difference was not significant after controlling for multiple testing ($t=-1.8$, $p=0.072$). No difference between LPS groups was observed 24h after LPS administration ($t=0.1$, $p=0.89$). To address putative sex differences in physical sickness symptoms, we additionally computed two-factorial ANOVAs with the factors LPS (0.4ng LPS vs. placebo) and sex (males vs. females) for GASE scores. While sex did not affect sickness symptom scores for the early phase, females displayed increased sickness symptom scores for the late phase ($F=5.1$, $p=0.026$, $\eta_p^2=0.05$; LPS x sex interaction; see Supplementary Information for detailed results). To exclude that these sex differences affected the above reported results for GASE scores, we repeated the analysis in male subjects only. Herein, our previous results were confirmed (see Supplementary Information for detailed results).

LPS-induced physical sickness symptoms comprised a broad range of pain-related, vegetative, fever-related, and gastrointestinal symptoms. With prevalence rates of up to 64% in response to 0.4 ng/kg LPS, and 75% in response to 0.8 ng/kg LPS, the

most common symptoms were headache, fatigue, and dizziness (for details, see Table 2).

Statistical predictors of LPS-induced physical sickness symptoms

Correlation analyses within LPS-treated subjects revealed that physical sickness symptoms during the acute inflammatory phase (6h post-injection) were significantly associated with changes (i.e., AUC increase) in plasma TNF- α , IL-6, and cortisol concentrations, state anxiety, and mood (all $p<.05$, see Table 3). In contrast, no significant correlations were observed for changes in heart rate and body temperature, age, BMI, or psychological trait variables (Suppl. Table 1). Physical sickness symptoms during the late inflammatory phase (24h post-injection) were significantly correlated with LPS-induced TNF- α , IL-6, cortisol, and mood changes, as well as with baseline depression scores (assessed with the Beck depression Inventory, BDI) (all $p<.05$, see Table 3). Of note, BDI scores were not significantly correlated with baseline TNF- α , IL-6, or cortisol concentrations (all $p>.13$).

Next, different sets of stepwise multiple regression analyses (accounting for sex, age, and cytokine assay method) were conducted within LPS-treated subjects to predict the variance of physical sickness symptoms (i.e., GASE scores). In a first model, TNF- α and IL-6 were simultaneously entered as predictor variables. Herein, greater IL-6 concentrations were identified as significant predictor variable for LPS-induced physical sickness symptoms both during the acute and the late phases (Model I, Table 4). In Model II, all variables which showed 1) changes in response to LPS-treatment and 2) were significantly associated with GASE scores in bivariate correlation analyses (see Table 3) were entered. Herein, a greater number of LPS-induced physical sickness symptoms during the acute phase was statistically predicted by greater LPS-induced mood impairments and IL-6 increases, which explained a total of 28.5% of the variance in GASE scores (adj. $R^2=0.285$). Higher

GASE scores during the late phase were predicted by greater LPS-induced IL-6 and cortisol responses, accounting for 26.8% of GASE score variance (adj. $R^2=0.268$). Finally, in a third model, baseline depression (BDI) scores were additionally entered given the significant bivariate association with GASE scores 24h post-injection (Table 4, Model III). This model revealed that baseline depression (BDI) scores as well as LPS-induced cortisol and IL-6 increases were significant predictor variables of physical sickness symptoms during the late phase, explaining a total of 38.5% of variance in GASE scores (adj. $R^2=0.385$). Of note, all predictor variables remained significant after additionally controlling for baseline IL-6 and TNF-alpha concentrations (data not shown). Model III was not computed for the acute inflammatory phase given that no significant correlations with trait variables including BDI scores were observed.

DISCUSSION

Research in experimental endotoxemia has often been narrowly focused on very specific sickness symptoms during the acute inflammatory phase. In addition, the persistence of physical sickness symptoms in later phases of the inflammatory response has rarely been addressed, and predictor variables remain virtually unknown. In this randomized, placebo-controlled analysis including a large cohort of participants, administration of LPS induced increases in the pro-inflammatory cytokines TNF- α and IL-6 as well as in cortisol, along with increased body temperature, heart rate and mood changes. These effects were dose-dependent and transient, with normalized values 6 hours after LPS administration (acute phase), with the exception of elevated CRP concentrations at the 24 hour time point (late phase).

Using a validated questionnaire to assess a range of clinically-relevant physical sickness symptoms, we here document that high proportions of participants randomized to LPS reported spontaneous pain in several body regions, various fever-related and vegetative symptoms as well as gastrointestinal disturbances during the acute inflammatory phase. These findings complement and extend data showing acute increases in self-reported physical sickness symptoms, including headaches, muscle pain, shivering, nausea, breathing difficulties and fatigue in response to 0.8 ng/kg LPS (13, 14). Interestingly, herein overall physical sickness symptom scores remained significantly elevated in the late phase (i.e., 6-24 hours after LPS), indicating persisting symptoms despite normalized cytokines responses 6 hours post LPS. This is a new finding and underscores that systemic inflammation triggers a broad range of persisting clinically-relevant symptoms reported by numerous patients with medical conditions.

During the acute phase, the overall sickness symptom response showed a dose-dependent increase, which extends earlier findings on specific sickness symptoms, such as hyperalgesia (15), state anxiety (16), and cognitive impairments (17). However, there was no evidence of a dose-dependent effect on symptoms experienced in the late inflammatory phase. We expected a contribution of both inflammatory mediators and psychological variables as responsible predictor variables of physical sickness symptoms. To test this assumption, we entered multiple variables simultaneously in regression models to identify predictor variables of LPS-induced sickness symptoms while accounting for various confounding variables. For the acute inflammatory phase, more pronounced sickness symptoms were associated with greater LPS-induced increases in depressive mood and IL-6 concentrations. For the late phase, IL-6 also emerged as a significant and independent predictor variable of sickness symptoms, along with plasma cortisol

concentrations and baseline depression (BDI) scores. Both statistical models explained a substantial amount of variance in sickness symptom scores. Together, these results support our hypothesis that both immunological and psychological parameters contribute to more exaggerated physical sickness symptoms during systemic inflammation.

The pro-inflammatory cytokine IL-6 was a significant predictor variable in both regression models, which fits well with previous experimental and clinical evidence. For example, we and others have reported significant correlations between IL-6 and specific physical and psychological sickness symptoms, such as hyperalgesia (15), anorexia (18), cognitive impairments (19) or negative mood and feelings of social disconnection (16, 17, 20). Recently, an inverse relationship between IL-6 and self-rated health has been established in a large cohort of older adults (21). In medical conditions, IL-6 has been proposed as a potential driver of sickness symptoms in cancer and cancer treatment-related side effects (6, 22). Our finding that IL-6 also predicted late sickness symptoms even though plasma concentrations had normalized during this time period can be explained by neural immune-to-brain pathways that seemed to trigger immediate but also delayed effects on central processes. While cytokines cannot directly cross the blood-brain barrier, humoral pathways include volume diffusion via structures outside the blood-brain barrier, active transport via saturable cytokine transporters, and prostaglandin E₂ (PGE₂)-mediated transmission of inflammatory signals produced by brain endothelial cells and perivascular macrophages (4, 8, 11). Activation of microglia by cytokines and PGE₂ may contribute to central inflammation (23), a process which has been implicated in chronic pain and depression (4, 24, 25). It has recently been demonstrated that COX1-mediated cerebral PGE₂ synthesis targets dopaminergic cells in the striatum and modulates motivational brain circuitry, leading to

inflammation-induced sickness symptoms (26). A second and independent immune-to-brain pathway encompasses cytokine-mediated activation of vagal afferents, which notably project to areas involved in the processing of sickness symptoms including the thalamus, hypothalamus, and amygdala (7, 8, 27). Indeed, altered neural activation during acute experimental endotoxemia has recently been documented for these and other key structures such as the insula and cingulate cortex using task-related (e.g., (13, 28-30) and resting-state brain imaging techniques (31), which was related to sickness symptoms (28, 30, 32). The clinical relevance of delayed cytokine-induced sickness symptoms is corroborated by a recent report that the initiation of interferon (IFN)- α treatment in patients with hepatitis C acutely evoked an inflammatory cytokine response along with acute changes in striatal microstructure, while fatigue and depressed mood persisted as long as 24 weeks after IFN- α application (33). Our correlative results, along with the notion that IL-6 is a “keystone cytokine in infection, cancer and inflammation, in which it drives disease progression or supports the maintenance of immunological reaction” (34), suggest that IL-6 might be an interesting target for immune-related therapies aimed at sickness symptoms. Interestingly, we did not observe an association between TNF- α and sickness symptoms in any regression model, which is in line with compensatory mechanisms of TNF- α and IL-1 in signaling sickness symptoms (26, 35).

It is not only a new but also intriguing finding that pre-existing depression scores measured prior to LPS-administration emerged as significant predictor variable of late physical sickness symptoms. This may indicate that even depressive symptoms in a low and non-pathological range (all subjects herein presented with BDI scores below the cutoff for mild depression; i.e., <11) may constitute a risk factor for an increased perception (or reporting) of physical sickness symptoms, which is independent of at baseline and LPS-induced cytokine concentrations. Indeed, an

association between depressive symptoms and physical symptom reporting has previously been shown (36). While our findings are based on a model of acute and transient immune activation, one would expect even greater effects in patients with chronic inflammation, where persistent negative affectivity and altered neuroendocrine-immune axes (e.g. chronic activation and blunted feedback loops of the hypothalamus-pituitary-adrenal axis) may contribute to symptom exacerbation (27); (4).

Our data show that increases in plasma cortisol concentrations were associated with significantly more pronounced sickness symptoms during the late phase of inflammation. This is in line with previous observations documenting that cortisol may also exert pro-inflammatory effects within the CNS (37). It may well be that this effect of cortisol on sickness symptoms, in conjunction with IL-6 levels, might be mediated by an activation of immune-cell derived indoleamine-2,3-dioxygenase (IDO) and hepatic tryptophan-2,3-dioxygenase (TDO), leading to enhanced degradation of tryptophan via the kynurene pathway. These processes have been implicated as a link between inflammation and depression (27, 38), and may ultimately also contribute to more pronounced sickness symptoms during the late inflammatory phase.

The present study has strengths and limitations. Strengths include the large study population providing sufficient statistical power to conduct predictor analyses. Further, all primary studies implemented randomized, placebo-controlled study designs, and all subjects were blinded to the study condition and naïve to LPS. Finally, sickness symptoms were assessed with a validated questionnaire. Limitations include retrospective assessment of sickness symptoms, which bears the risk of a recall bias. Moreover, experimental pain testing was conducted during the

acute phase as part of the primary study aims, which could have influenced questionnaire responses in the pain domain for the acute phase. However, pain stimuli were identical in the LPS and control groups, and questionnaire items clearly addressed symptoms of spontaneous rather than evoked pain. In additional analyses comparing physical sickness symptom scores in male and female subjects, we found evidence of increased symptoms in females during the late phase, but not during the early phase. This complements and extends earlier findings of no sex differences during the acute phase, both in a small subgroup of this study cohort (39), as well as in a study by Moieni et al. (20). To exclude that the results for sickness symptom scores were confounded by sex differences, we repeated analysis in men only, confirming previous results. To account for sex differences in regression analyses, sex was included as control variable. Lastly, we did not assess cytokine and cortisol concentrations 24h after injection. However, we previously found that these parameters had completely returned to baseline 24 hours post injection (40). Finally, given that our findings are based on correlational approaches, one should keep in mind that the predictor models reported herein reflect statistical associations and do not provide causality.

In summary, our results demonstrate that a range of clinically-relevant physical sickness symptoms commonly experienced by patients with chronic medical conditions involving inflammation can be experimentally induced using experimental endotoxemia, supporting the notion that the LPS-model is an valuable tool for research in the field of neuropsychopharmacology and drug development (9, 41, 42).

We could identify statistical predictors of exaggerated sickness symptoms in response to an acute immune challenge, which included increased IL-6 and cortisol concentrations, as well as greater state and trait depressive symptoms. In patients with recurrent or chronic inflammatory states, these factors may act as risk factors

ultimately contributing to a prolongation and / or exacerbation of sickness symptoms, and should be considered as potential targets for therapeutic strategies.

MATERIALS AND METHODS

Participants

Data from a total of N=128 subjects randomized to a low dose of LPS (0.4ng/kg: n=61), a medium dose of LPS (0.8ng/kg: n=20) or placebo (n=47, control group) were used in the present analyses. The sample included data from 20 women (15.6%; all using hormonal contraceptives) who received either 0.4 ng/kg LPS (n=10) or placebo (N=10). Data were pooled from four previously published randomized, placebo-controlled studies (15, 31, 43, 44). To exclude that the primary outcome (i.e., symptom reporting) was affected by a previous experience with LPS treatment, all subjects included herein were naïve to LPS. In addition, data from crossover studies were only included from the first of two consecutive study days. Of note, sickness symptom scores from a small subgroup of participants (n=19 subjects) of the current analysis were also included in a previous report that focused on sex differences (39).

The study was approved by the local ethics committee for human investigations of the University Hospital Essen (permit no. 09-4271) and follows the rules stated in the Declaration of Helsinki. All subjects provided written informed consent and were paid for their participation.

Recruitment and safety routine

Screening, safety routines and inclusion criteria were standardized across all primary studies (for detail, see supplementary material and methods). Briefly, healthy volunteers aged 18-45 years were subjected to an in-depth screening procedure consisting of a physical examination and a personal interview conducted by a

physician, as well as repeated assessments of blood and clinical chemistry parameters. Safety measures included monitoring up to at least 6h post LPS-injections, and follow-up examinations 24h and 7 days after the study day.

Study protocol

In all studies, subjects were randomized to receive an intravenous injection of either LPS (0.4 ng or 0.8 ng per kilogram of body weight; reference standard endotoxin from *Escherichia coli*, serotype O113:H10:K-negative, United States Pharmacopeia, Rockville, MD, dissolved in sterile water; LPS conditions or the same volume of saline (placebo condition) via an intravenous catheter placed in an antecubital forearm vein. Blood for cytokine analyses was collected in EDTA-coated tubes 15 min prior to the injection (baseline) as well as 1h, 2h, 3h, 4h, and 6h after injection.

Plasma was separated by centrifugation and stored at -80°C until analysis. Following blood draws, body temperature (with an intra-aural thermometer), blood pressure (with a manual blood pressure cuff), and heart rate (palpated at radial artery) were assessed. Physical sickness symptoms were assessed six hours post-injection for the acute (i.e., 0-6h), and 24h post-injection for the late (i.e., 6-24h) inflammatory phases (see below).

Questionnaires

Physical sickness symptoms: Physical sickness symptoms were assessed with the validated General-Assessment-of-Side-Effects (GASE) questionnaire (45). Briefly, subjects rated the severity of 17 different physical symptoms (see Table 1) from 0 (“not present”) to 3 (“severe”), and sum scores were calculated. Physical symptoms were retrospectively rated at two separate time points: 1) Six hours after injection, subjects were asked to rate sickness symptoms experienced from the time point of

injection until 6 hours post-injection (acute inflammatory phase). 2) 24h post injection, subjects returned to the lab for a medical check-up. During this visit, subjects were asked to rate sickness symptoms experienced during the time between 6h post-injection and 24h post-injection (late inflammatory phase). Subjects received written instructions to ensure that symptoms were specifically rated for the acute or late inflammatory phase, respectively. Subjects and the investigators involved in assessment of physical sickness symptoms were blinded to the study condition (i.e., LPS or saline).

State anxiety and mood questionnaires: State anxiety was measured with the state-trait-anxiety-inventory (STAI) (46), with higher scores indicating higher state anxiety.

Mood was assessed with the standardized and validated German multidimensional mood questionnaire (MDBF) (47), with lower scores indicating impaired mood (see supplement).

Psychological trait variables: A questionnaire battery assessing various psychological trait variables (to be included in regression analyses) was completed after study inclusion (i.e., up to seven days before application of LPS or placebo) (see supplement).

Cytokines, cortisol, and C-reactive protein

Plasma concentrations of TNF- α and IL-6 were analyzed using enzyme-linked immunosorbent assays (ELISA) or with multiplexed bead-based assays, and plasma cortisol was analyzed with commercial ELISA according to the manufacturer's instructions. Cytokine data from individual studies were normalized by z-transformation to allow comparison across experiments and assay systems. C-reactive protein (CRP) was assessed before and 24h post-injection with a

polyethylene glycol (PEG)-enhanced immunoturbidimetric assay. See supplement for detailed information.

Statistical analyses

All data were tested for normal distribution with Kolmogorov-Smirnov-test, and non-normally distributed data were log-transformed to achieve normal distribution prior to analysis. Sociodemographic and psychological data from LPS- and placebo-treated subjects were compared using univariate ANOVA or Chi²-tests. To characterize the dose-dependent effects of LPS, repeated measures analyses of variance (rmANOVA) with the between-subject factor group (i.e., medium-dose LPS, low-dose LPS, placebo) and time (i.e., measurement points) were computed. For all subsequent correlation and regression analyses, the area under curve with respect to increase (AUC) was calculated according Pruessner, Kirschbaum (48) for the time period from baseline to 6h post-injection to provide an integrative measure of absolute changes in repeated measures. Group differences in severity of sickness symptoms (i.e., GASE sum scores) and AUC values were compared with univariate ANOVA. All post-hoc tests were Bonferroni-corrected for multiple testing. Additionally, the prevalence of physical sickness symptoms (i.e., single GASE items) was compared between placebo and LPS-treated subjects with Chi²-tests.

To analyze the associations between trait and state parameters and physical sickness symptoms (i.e., GASE sum scores) within LPS-treated subjects, correlation and regression analyses were conducted. In a first exploratory step, correlation analyses were computed as Pearson's r. Next, separate sets of step-wise multiple regression analyses (accounting for sex, age, and cytokine assay method) were performed to identify predictors variables of LPS-induced sickness symptoms (i.e., GASE scores) 6h and 24h post-injection. In model I, AUC values for TNF- α and IL-6

were entered to simultaneously assess the association between these two pro-inflammatory cytokines and GASE scores. In model II, AUC values for variables which showed 1) LPS-induced changes and 2) a significant bivariate correlation with GASE scores were additionally entered. Finally, in model III, psychological trait variables which showed a significant bivariate correlation with GASE scores (i.e., Beck Depression Inventory score) were additionally included as predictor variables. All regression models were also run while additionally controlling for baseline IL-6 and cytokine concentrations. The alpha level was set at 0.05 in all analyses. All data are presented as mean and standard error of the mean (SEM) unless indicated otherwise. Data analysis was performed using PASW statistics version 18 (SPSS, Chicago, IL, USA).

STUDY HIGHLIGHTS

What is the current knowledge on the topic?

The administration of endotoxin (e.g., LPS) is an established translational tool to analyze the mechanisms of immune-mediated sickness symptoms in humans. However, possible persistence of symptoms beyond the acute inflammatory phase has rarely been addressed, and predictor variables of physical sickness symptoms remain largely unknown.

What question did this study address?

Do physical sickness symptoms persist after the acute inflammatory response to LPS has?

Which parameters including inflammatory mediators and psychological state and trait variables statistically predict more pronounced physical sickness symptoms?

Accepted Article

What does this study add to our knowledge?

Physical sickness symptoms were reported up to 24 hours after LPS despite normalized cytokines responses. Multiple regression models showed that persisting sickness symptoms were predicted by LPS-induced increases in cortisol and IL-6 plasma concentrations and baseline depression.

How this might change clinical pharmacology and translational science?

In patients with recurrent or chronic inflammatory states, these factors may act as risk factors ultimately contributing to an exacerbation of sickness symptoms, and should be considered as potential targets for therapeutic strategies.

ACKNOWLEDGEMENT: The authors would like to express their gratitude to Jan Sebastian Grigoleit, Alexandra Kornowski and Magdalene Vogelsang for excellent technical support, to Elisa Engelbrecht, Joswin Kattoor, Julian Kleine-Borgmann, Janina Maluck, Daniel Reidick, and Till Roderigo for data collection, and to Bettina Löschner and the staff of Section 1/3 "Microbial safety" of the Paul-Ehrlich-Institute (Langen, Germany) for endotoxin and sterility testing.

CONFLICT OF INTEREST / DISCLOSURE: The authors declare no conflict of interest. The study was funded by the German Research Foundation (Deutsche Forschungsgemeinschaft; DFG) (BE 5173/2-1; EL 236/11-1). Alexander Wegner received an IFORES stipend for clinicians of the Medical Faculty, University Duisburg Essen. The funding organizations were not involved in study design; in collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

AUTHOR CONTRIBUTIONS: S.B., H.E., M.S., and S.E. wrote the manuscript; S.B., M.S., and S.E. designed the research; S.B., H.E., A.W., L.R., and I.S. performed the research; S.B., H.E., and I.S. analyzed the data; I.S. contributed new reagents/analytical tools.

REFERENCES

1. Dantzer R, Capuron L, Irwin MR, Miller AH, Ollat H, Perry VH, et al. Identification and treatment of symptoms associated with inflammation in medically ill patients. *Psychoneuroendocrinology*. 2008;33(1):18-29.
2. Kiecolt-Glaser JK, Derry HM, Fagundes CP. Inflammation: Depression Fans the Flames and Feasts on the Heat. *The American journal of psychiatry*. 2015;172(11):1075-91.
3. Reyes-Gibby CC, Wu X, Spitz M, Kurzrock R, Fisch M, Bruera E, et al. Molecular epidemiology, cancer-related symptoms, and cytokines pathway. *The Lancet Oncology*. 2008;9(8):777-85.
4. Walker AK, Kavelaars A, Heijnen CJ, Dantzer R. Neuroinflammation and comorbidity of pain and depression. *Pharmacological reviews*. 2014;66(1):80-101.
5. Capuron L, Miller AH. Immune system to brain signaling: neuropsychopharmacological implications. *Pharmacology & therapeutics*. 2011;130(2):226-38.
6. Seruga B, Zhang H, Bernstein LJ, Tannock IF. Cytokines and their relationship to the symptoms and outcome of cancer. *Nature reviews Cancer*. 2008;8(11):887-99.
7. Miller AH, Raison CL. The role of inflammation in depression: from evolutionary imperative to modern treatment target. *Nature reviews Immunology*. 2016;16(1):22-34.
8. DellaGioia N, Hannestad J. A critical review of human endotoxin administration as an experimental paradigm of depression. *Neuroscience and biobehavioral reviews*. 2010;34(1):130-43.
9. Suffredini AF, Noveck RJ. Human endotoxin administration as an experimental model in drug development. *Clinical pharmacology and therapeutics*. 2014;96(4):418-22.
10. Grace PM, Hutchinson MR, Maier SF, Watkins LR. Pathological pain and the neuroimmune interface. *Nature reviews Immunology*. 2014;14(4):217-31.
11. Schedlowski M, Engler H, Grigoleit JS. Endotoxin-induced experimental systemic inflammation in humans: A model to disentangle immune-to-brain communication. *Brain Behavior and Immunity*. 2014;35:1-8.
12. Shattuck EC, Muehlenbein MP. Human sickness behavior: Ultimate and proximate explanations. *American journal of physical anthropology*. 2015;157(1):1-18.
13. Eisenberger NI, Inagaki TK, Rameson LT, Mashal NM, Irwin MR. An fMRI study of cytokine-induced depressed mood and social pain: The role of sex differences. *NeuroImage*. 2009;47(3):881-90.
14. Moieni M, Irwin MR, Jevtic I, Breen EC, Eisenberger NI. Inflammation impairs social cognitive processing: A randomized controlled trial of endotoxin. *Brain, behavior, and immunity*. 2015;48:132-8.
15. Wegner A, Elsenbruch S, Maluck J, Grigoleit JS, Engler H, Jager M, et al. Inflammation-induced hyperalgesia: effects of timing, dosage, and negative affect on somatic pain sensitivity in human experimental endotoxemia. *Brain, behavior, and immunity*. 2014;41:46-54.
16. Lasselin J, Elsenbruch S, Lekander M, Axelsson J, Karshikoff B, Grigoleit JS, et al. Mood disturbance during experimental endotoxemia: Predictors of state anxiety as a psychological component of sickness behavior. *Brain, behavior, and immunity*. 2016.
17. Grigoleit J-S, Kullmann, J.S., Wolf, O.T., Hammes, F., Wegner, A., Jablonowski, S., Engler, H., Gizewski, E.R., Oberbeck, R., Schedlowski, M. Dose-

- Dependent Effects of Endotoxin on Neurobehavioral Functions in Humans. *PLoS one*. 2011;6(12):e28330.
18. Reichenberg A, Kraus T, Haack M, Schuld A, Pollmacher T, Yirmiya R. Endotoxin-induced changes in food consumption in healthy volunteers are associated with TNF-alpha and IL-6 secretion. *Psychoneuroendocrinology*. 2002;27(8):945-56.
 19. Reichenberg A, Yirmiya R, Schuld A, Kraus T, Haack M, Morag A, et al. Cytokine-associated emotional and cognitive disturbances in humans. *Archives of General Psychiatry*. 2001;58(5):445-52.
 20. Moieni M, Irwin MR, Jevtic I, Olmstead R, Breen EC, Eisenberger NI. Sex differences in depressive and socioemotional responses to an inflammatory challenge: implications for sex differences in depression. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*. 2015;40(7):1709-16.
 21. Arnberg FK, Lekander M, Morey JN, Segerstrom SC. Self-rated health and interleukin-6: Longitudinal relationships in older adults. *Brain, behavior, and immunity*. 2016;54:226-32.
 22. Wood LJ, Weymann K. Inflammation and neural signaling: etiologic mechanisms of the cancer treatment-related symptom cluster. *Current opinion in supportive and palliative care*. 2013;7(1):54-9.
 23. Sandiego CM, Gallezot JD, Pittman B, Nabulsi N, Lim K, Lin SF, et al. Imaging robust microglial activation after lipopolysaccharide administration in humans with PET. *Proceedings of the National Academy of Sciences of the United States of America*. 2015;112(40):12468-73.
 24. Ren K, Dubner R. Interactions between the immune and nervous systems in pain. *Nature medicine*. 2010;16(11):1267-76.
 25. Yirmiya R, Rimmerman N, Reshef R. Depression as a microglial disease. *Trends in neurosciences*. 2015;38(10):637-58.
 26. Fritz M, Klawonn AM, Nilsson A, Singh AK, Zajdel J, Wilhelms DB, et al. Prostaglandin-dependent modulation of dopaminergic neurotransmission elicits inflammation-induced aversion in mice. *J Clin Invest*. 2016;126(2):695-705.
 27. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nature Reviews Neuroscience*. 2008;9(1):46-57.
 28. Benson S, Rebernik L, Wegner A, Kleine-Borgmann J, Engler H, Schlamann M, et al. Neural circuitry mediating inflammation-induced central pain amplification in human experimental endotoxemia. *Brain, behavior, and immunity*. 2015;48:222-31.
 29. Harrison NA, Cercignani M, Voon V, Critchley HD. Effects of inflammation on hippocampus and substantia nigra responses to novelty in healthy human participants. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*. 2015;40(4):831-8.
 30. Karshikoff B, Jensen KB, Kosek E, Kalpouzos G, Soop A, Ingvar M, et al. Why sickness hurts: A central mechanism for pain induced by peripheral inflammation. *Brain, behavior, and immunity*. 2016.
 31. Labrenz F, Wrede K, Forsting M, Engler H, Schedlowski M, Elsenbruch S, et al. Alterations in functional connectivity of resting state networks during experimental endotoxemia - An exploratory study in healthy men. *Brain, behavior, and immunity*. 2016;54:17-26.
 32. Lekander M, Karshikoff B, Johansson E, Soop A, Fransson P, Lundstrom JN, et al. Intrinsic functional connectivity of insular cortex and symptoms of sickness

- during acute experimental inflammation. *Brain, behavior, and immunity*. 2016;56:34-41.
33. Dowell NG, Cooper EA, Tibble J, Voon V, Critchley HD, Cercignani M, et al. Acute Changes in Striatal Microstructure Predict the Development of Interferon-Alpha Induced Fatigue. *Biological psychiatry*. 2016;79(4):320-8.
 34. Hunter CA, Jones SA. IL-6 as a keystone cytokine in health and disease. *Nature immunology*. 2015;16(5):448-57.
 35. Bluthe RM, Laye S, Michaud B, Combe C, Dantzer R, Parnet P. Role of interleukin-1beta and tumour necrosis factor-alpha in lipopolysaccharide-induced sickness behaviour: a study with interleukin-1 type I receptor-deficient mice. *The European journal of neuroscience*. 2000;12(12):4447-56.
 36. Howren MB, Suls J, Martin R. Depressive symptomatology, rather than neuroticism, predicts inflated physical symptom reports in community-residing women. *Psychosomatic medicine*. 2009;71(9):951-7.
 37. Bellavance MA, Rivest S. The HPA - Immune Axis and the Immunomodulatory Actions of Glucocorticoids in the Brain. *Frontiers in immunology*. 2014;5:136.
 38. Haroon E, Raison CL, Miller AH. Psychoneuroimmunology meets neuropsychopharmacology: translational implications of the impact of inflammation on behavior. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*. 2012;37(1):137-62.
 39. Engler H, Benson S, Wegner A, Spreitzer I, Schedlowski M, Elsenbruch S. Men and women differ in inflammatory and neuroendocrine responses to endotoxin but not in the severity of sickness symptoms. *Brain, behavior, and immunity*. 2016;52:18-26.
 40. Grigoleit J-S, Kullmann JS, Wolf OT, Hammes F, Wegner A, Jablonowski S, et al. Dose-Dependent Effects of Endotoxin on Neurobehavioral Functions in Humans. *PloS one*. 2011;6(12).
 41. Hutchinson MR. Want more pain? Just add a dash of endotoxin to enhance your clinical pain model. *Brain, behavior, and immunity*. 2014;41:44-5.
 42. Kett DH, Breitmeyer JB, Ang R, Royal MA. A randomized study of the efficacy and safety of intravenous acetaminophen vs. intravenous placebo for the treatment of fever. *Clinical pharmacology and therapeutics*. 2011;90(1):32-9.
 43. Benson S, Kattoor J, Wegner A, Hammes F, Reidick D, Grigoleit J-S, et al. Acute experimental endotoxemia induces visceral hypersensitivity and altered pain evaluation in healthy humans. *Pain*. 2012;153(4):794-9.
 44. Wegner A, Elsenbruch S, Rebernik L, Roderigo T, Engelbrecht E, Jager M, et al. Inflammation-induced pain sensitization in men and women: does sex matter in experimental endotoxemia? *Pain*. 2015;156(10):1954-64.
 45. Rief W, Barsky AJ, Glombiewski JA, Nestoriuc Y, Glaesmer H, Braehler E. Assessing general side effects in clinical trials: reference data from the general population. *Pharmacoepidemiology and drug safety*. 2011;20(4):405-15.
 46. Spielberger CD, Gorsuch RL, Lushene RE. Manual for the state-trait anxiety inventory. Palo Alto, CA: Consulting Psychology Press; 1970.
 47. Steyer R, Schwenkmezger, P., Notz P., Eid, M. Der Mehrdimensionale Befindlichkeitsfragebogen (MDBF). Goettingen: Hogrefe; 1997.
 48. Pruessner JC, Kirschbaum C, Meinlschmid G, Hellhammer DH. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology*. 2003;28(7):916-31.

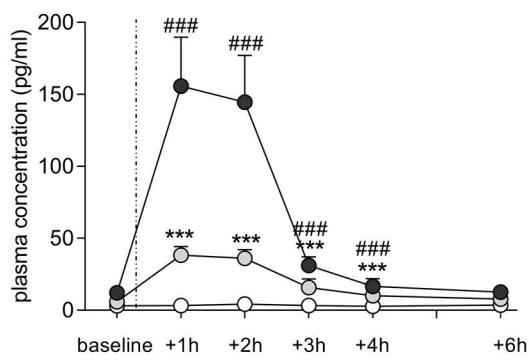
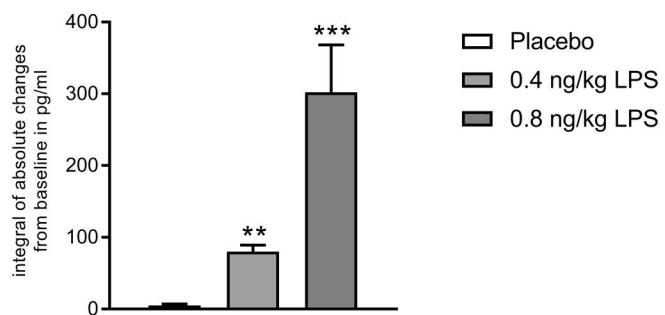
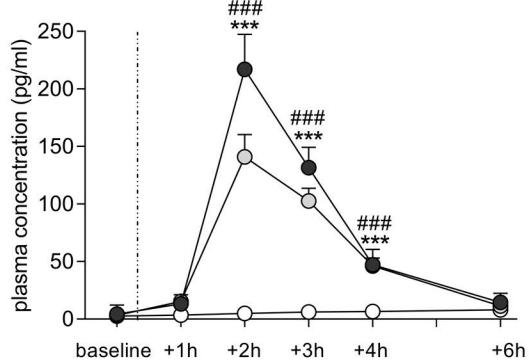
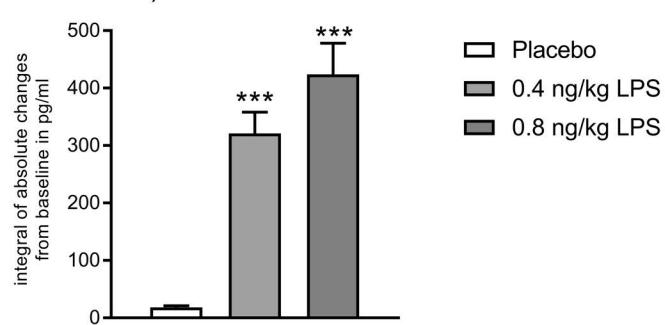
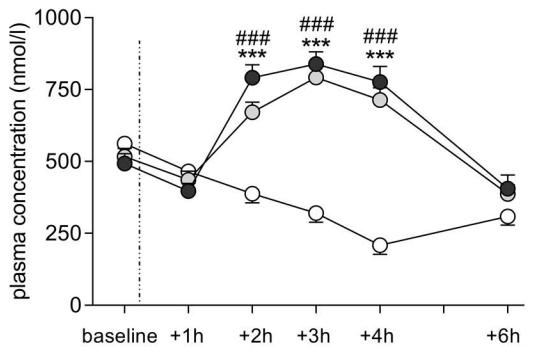
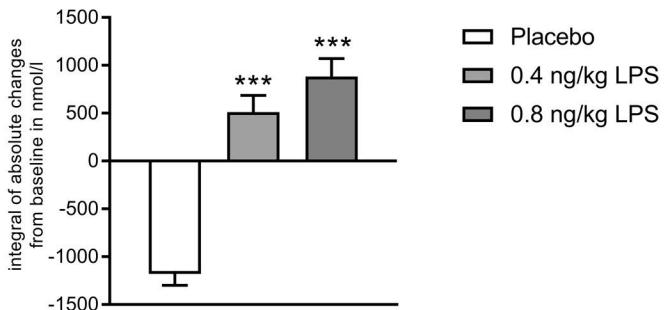
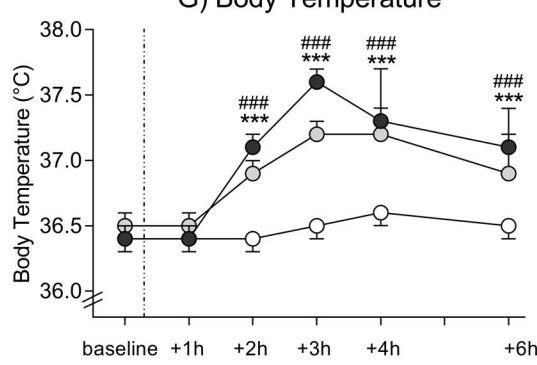
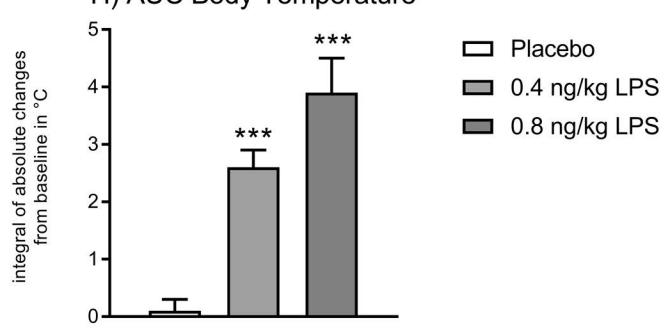
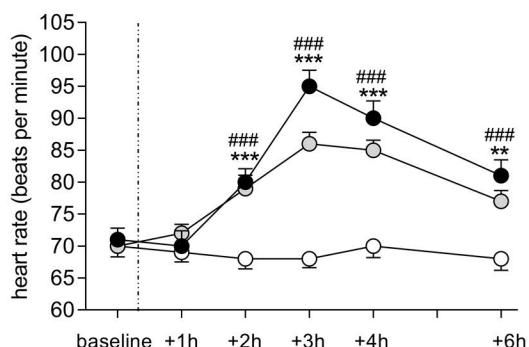
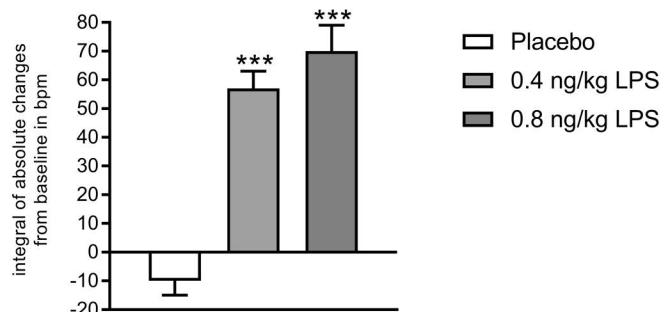
FIGURE LEGENDS:

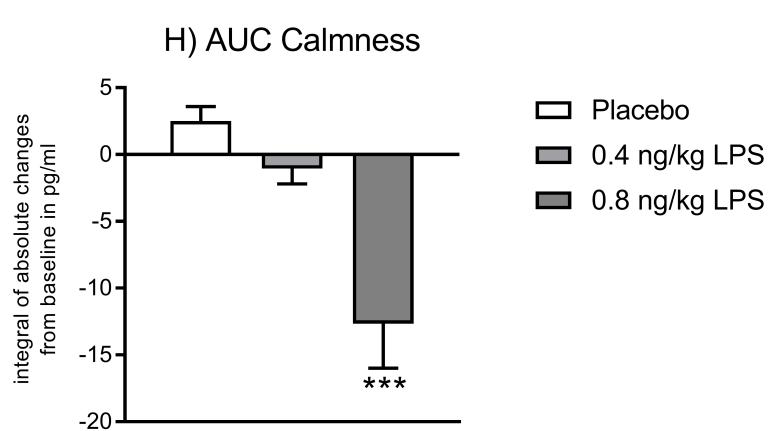
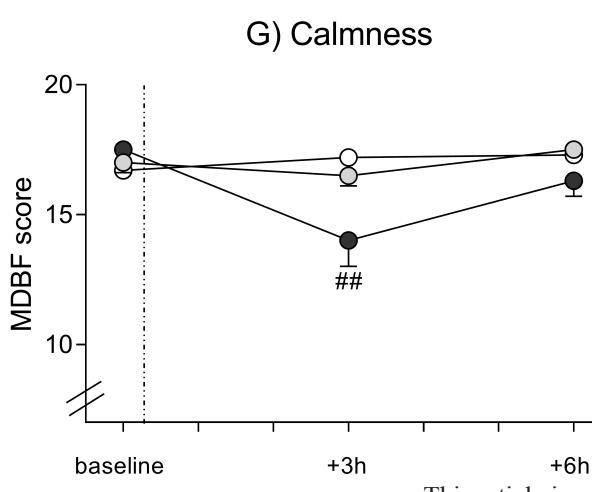
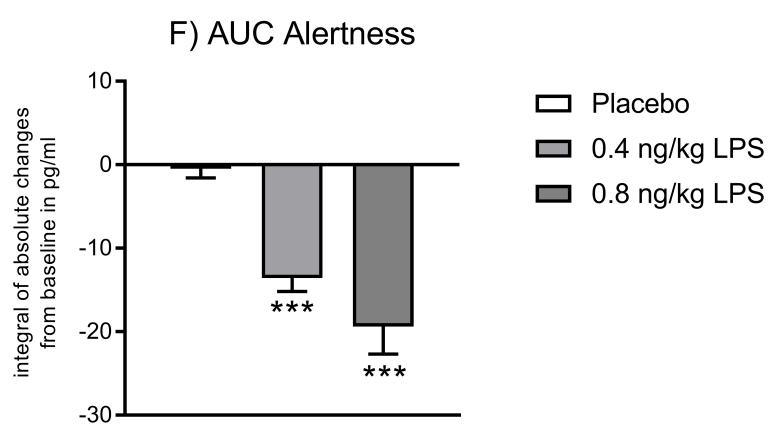
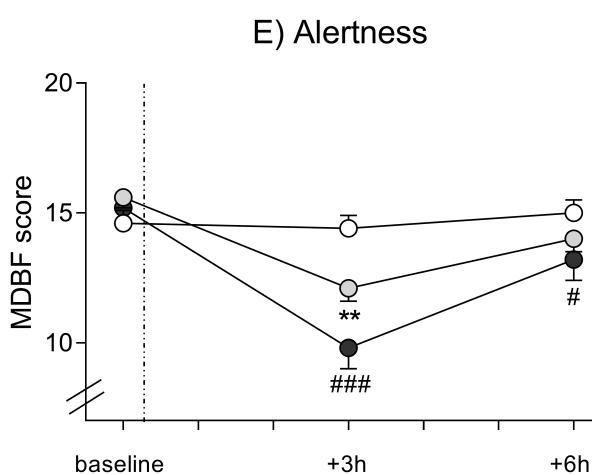
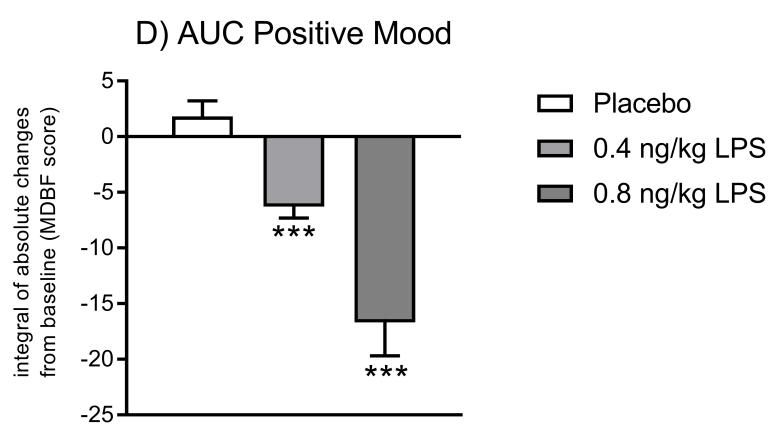
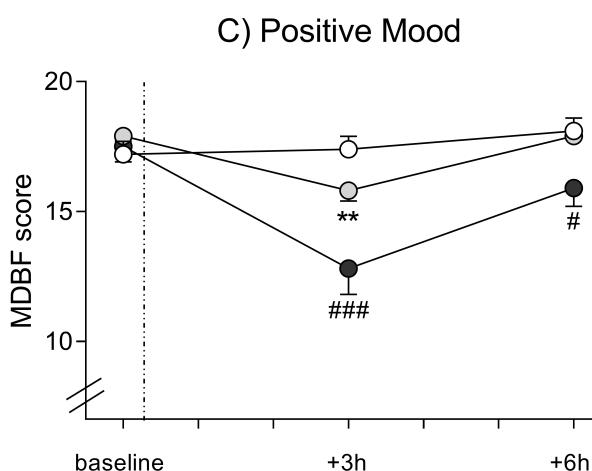
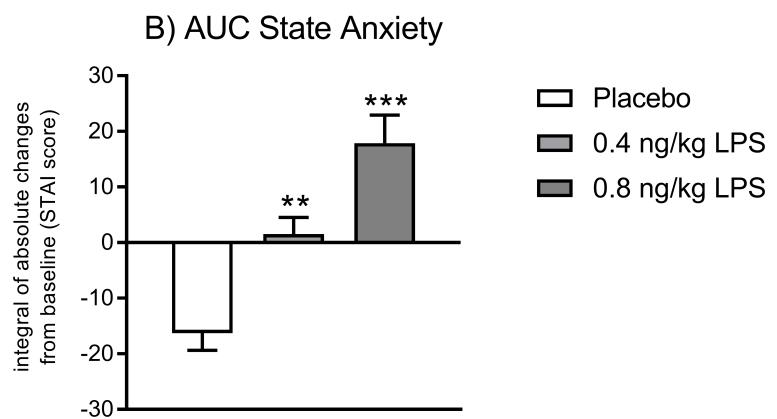
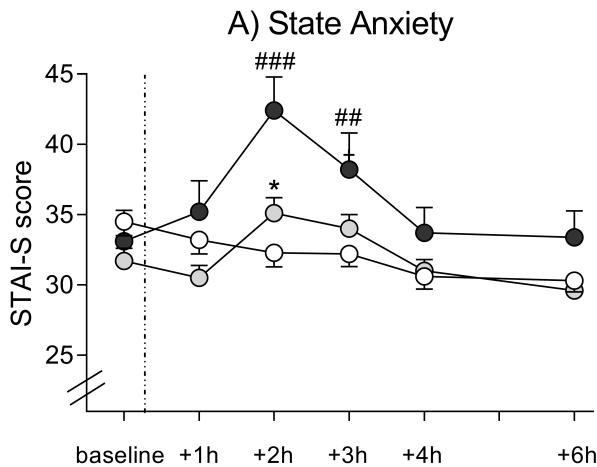
Figure 1, left panel: Plasma levels of TNF- α , IL-6, plasma cortisol, as well as body temperature and heart rate were repeatedly measured at a baseline and up to 6h post injection of either lipopolysaccharide (LPS; 0.8 ng or 0.4 ng / kg body weight) or saline (placebo) (left column). Of note, while z-standardized cytokine data were used in analyses (to allow comparisons across experiments and assay systems), raw data are presented herein for a better visualization of LPS effects. LPS administration led to a significant increase in plasma cytokines cortisol, body temperature, and heart rate, indicating a systemic immune activation, with more pronounced effects after application of 0.8 ng/kg LPS. **p<0.01, ***p<0.001, 0.4 ng/kg LPS vs. placebo; ##p<0.01, ###p< 0.001; 0.8 ng / kg LPS vs. placebo; results of posthoc Bonferroni tests. For ANOVA results, see text. **Figure 1, right panel:** The area under curve with respect to increase (AUC) was calculated according Pruessner, Kirschbaum (48) for the time period from baseline to 6h post-injection to provide an integrative measure of absolute changes in repeated measures, i.e., TNF- α , Interleukin (IL)-6, plasma cortisol, body temperature, and heart rate. Univariate ANOVA revealed that LPS application led to significant increases in all parameters (all F>30.2, all p<0.001). **p<0.01, ***p<0.001, results of posthoc Bonferroni tests comparing LPS groups vs. placebo group.

Figure 2, left panel: State anxiety (State-Trait-Anxiety-Inventory, STAI, state version), mood, alertness, and calmness (Multi-Dimensional-Mood-Questionnaire, MDBF), repeatedly measured at a baseline and up to 6h post injection of either lipopolysaccharide (LPS; 0.8 ng or 0.4 ng / kg body weight) or saline (placebo) (left column). LPS administration led to a significant increase in state anxiety, and significantly decreases in positive mood, alertness, and calmness, with more pronounced effects after application of 0.8 ng/kg LPS. *p<0.05, **p<0.01, 0.4 ng/kg

LPS vs. placebo; ${}^{\#} p<0.05$, ${}^{\#\#} p<0.01$, ${}^{\#\#\#} p<0.001$; 0.8 ng / kg LPS vs. placebo; results of posthoc Bonferroni tests. For ANOVA results, see text. **Figure 2, right panel:** The area under curve with respect to increase (AUC) was calculated according Pruessner, Kirschbaum (48) for the time period from baseline to 6h post-injection to provide an integrative measure of absolute changes in repeated measures, i.e., STAI state and MDBF scores. Univariate ANOVA revealed that LPS application led to significant changes in all parameters (all $F>17.7$, all $p<0.001$). ${}^{**}p<0.01$, ${}^{***}p<0.001$, results of posthoc Bonferroni tests comparing LPS groups vs. placebo group.

Figure 3: LPS-induced sickness symptoms were assessed using a standardized symptoms checklist 6h (acute inflammatory phase) and 24h (late inflammatory phase) after injection of either lipopolysaccharide (LPS; 0.8 ng or 0.4 ng / kg body weight) or saline (placebo). ${}^{*}p<0.05$, ${}^{**}p<0.01$, ${}^{***}p<0.001$, results of posthoc Bonferroni tests comparing LPS groups vs. placebo group. For ANOVA results, see text.

A) TNF- α **B) AUC TNF- α** **C) Interleukin-6****D) AUC Interleukin-6****E) Plasma Cortisol****F) AUC Plasma Cortisol****G) Body Temperature****H) AUC Body Temperature****I) Heart Rate****J) AUC Heart Rate**



Sickness symptoms

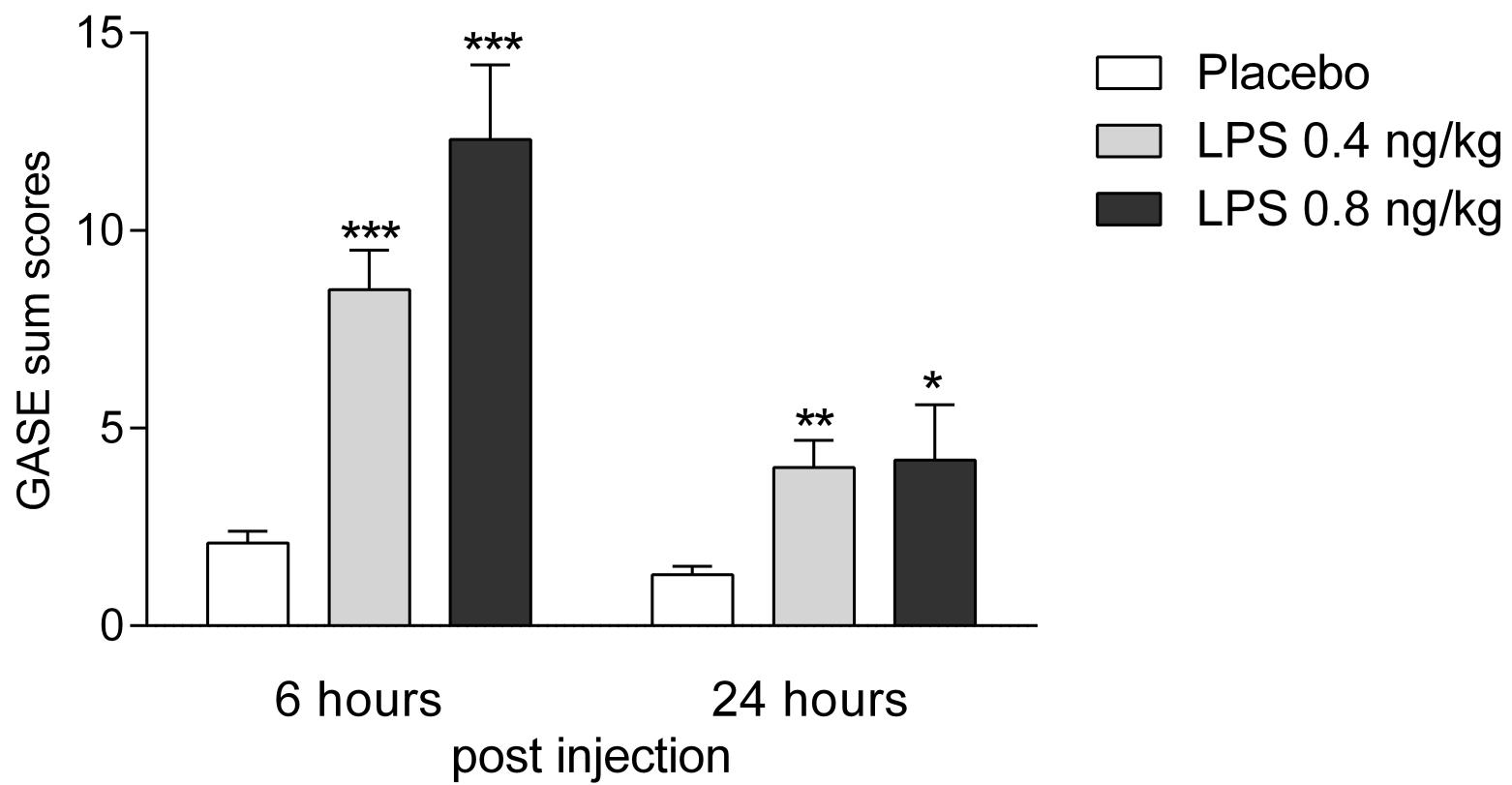


Table 1: Sociodemographic and psychological characteristics

	Placebo (N=47)	0.4 ng/kg LPS (N=61)	0.8 ng/kg LPS (N=20)	F / X ²	P
Age (years)	27.4 ± 0.7	26.5 ± 0.4	26.5 ± 0.4	F = 1.1	0.28
Body Mass Index (kg/m ²)	23.6 ± 0.4	26.3 ± 0.5	27.4 ± 0.8	F = 1.2	0.31
Education > 12 years % (N)	93.6 (44)	95.1 (58)	95.0 (19)	X ² = 0.12	0.94
HADS anxiety score	2.6 ± 0.3	3.2 ± 0.3	3.3 ± 0.6	F = 1.2	0.31
HADS depression score	1.3 ± 0.2	1.1 ± 0.2	1.7 ± 0.4	F = 1.4	0.25
STAI trait	32.9 ± 1.1	31.2 ± 0.8	33.3 ± 2.1	F = 1.0	0.37
BDI	3.6 ± 0.4	2.5 ± 0.4	3.7 ± 1.0	F = 1.9	0.15
NEO-FFI neuroticism	2.5 ± 0.1	2.3 ± 0.1	2.3 ± 0.2	F = 1.1	0.33
NEO-FFI extraversion	3.9 ± 0.1	4.2 ± 0.1	4.1 ± 0.2	F = 0.7	0.49
PRSS catastrophizing	1.1 ± 0.2	0.9 ± 0.1*	1.4 ± 0.1	F = 9.6	<0.001
PRSS active coping	3.3 ± 0.2	3.4 ± 0.1	3.4 ± 0.2	F = 0.2	0.85

HADS = Hospital Anxiety and Depression Scale. STAI = State-Trait-Anxiety-Inventory. BDI = Beck depression Inventory. NEO-FFI = Neo Five Factor Inventory.

PRSS = Pain-Related Self Statements Scale. All data are shown as mean ± SEM unless otherwise indicated. P = P-value for results of univariate ANOVA or chi²-tests for dichotomous variables. *p<.05 vs. placebo group and 0.8 ng/kg LPS group, results from post-hoc Bonferroni-tests

Table 2: Prevalence of physical sickness symptoms in placebo and LPS groups

	Acute inflammatory phase (0-6h post-injection)			Late inflammatory phase (6-24h post-injection)		
	Placebo (N=46)	0.4 ng/kg LPS (N=61)	0.8 ng/kg LPS (N=20)	Placebo (N=46)	0.4 ng/kg LPS (N=61)	0.8 ng/kg LPS (N=17)
Pain-related symptoms						
Headache % (N)	15.2 (7)	63.9 (39)***	75.0 (15)***	13.0 (6)	42.6 (26)**	52.9 (9)***
Back pain % (N)	6.5 (3)	27.9 (17)*	25.0 (5)*	4.3 (2)	11.5 (7)	17.6 (3)
Muscle pain % (N)	2.2 (1)	32.8 (20)**	40.0 (8)***	2.2 (1)	14.8 (9)*	23.5 (4)**
Joint pain % (N)	2.2 (1)	29.5 (18)**	30.0 (6)**	2.2 (1)	8.2 (5)	11.8 (2)
Fever-related symptoms						
Fever / increased body temperature % (N)	8.7 (4)	42.6 (26)***	55.0 (1)***	4.3 (2)	16.4 (10)	29.4 (5)
Abnormal sweating % (N)	4.3 (2)	19.7 (12)	30.0 (6)**	2.2 (1)	11.5 (7)	5.9 (1)
Hot flashes % (N)	4.3 (2)	22.3 (13)	25.0 (5)*	0.0 (0)	11.5 (7)	11.8 (2)
Shivering, chills % (N)	6.5 (3)	41.0 (25)**	75.0 (15)***	2.2 (1)	13.1 (8)	11.8 (2)
Vegetative symptoms						

	Acute	Placebo	LPS 10 ng/kg	LPS 50 ng/kg	LPS 100 ng/kg	LPS 200 ng/kg
Fatigue, loss of energy	39.1 (18)	54.1 (33)*	75.0 (15)***	21.7 (10)	31.1 (19)	29.4 (5)
Low blood pressure, circulation problems % (N)	8.7 (4)	36.1 (22)*	45.0 (9)**	6.5 (3)	13.1 (8)	23.6 (4)*
Dizziness % (N)	15.2 (7)	50.8 (31)***	55.0 (11)***	4.3 (2)	26.2 (16)*	35.3 (6)**
Palpation % (N)	4.3 (2)	18.0 (11)	20.0 (4)*	0.0 (0)	9.8 (6)*	0.0 (0)
Dry mouth % (N)	19.6 (9)	27.9 (17)	45.0 (9)	13.0 (6)	24.6 (15)	29.4 (5)
Gastro-intestinal symptoms						
Nausea % (N)	2.2 (1)	31.1 (19)**	35.0 (7)***	2.2 (1)	14.8 (9)	5.9 (1)
Abdominal pain % (N)	10.9 (5)	9.8 (6)	5.0 (1)	4.3 (2)	3.2 (2)	0.0 (0)
Vomiting % (N)	0.0 (0)	0.0 (0)	5.0 (1)	0.0 (0)	0.0 (0)	0.0 (0)
Loss of appetite % (N)	0.0 (0)	13.1 (8)*	25.0 (5)***	2.2 (1)	8.2 (5)	5.9 (1)

Prevalence of LPS-induced physical sickness symptoms reported for the acute (i.e., 0-6h post-injection) and late (i.e., 6-24h post-injection). *p<.05, **p<.01,

***p<.001, results of Chi²-tests between placebo and respective LPS-group.

Table 3: Correlations with LPS-induced physical sickness symptom

	Acute inflammatory phase (0-6h post-injection of LPS)		Late inflammatory phase (6-24h post-injection of LPS)	
	r	p	r	p
AUC TNF- α (n=80)	0.27	0.014	0.26	0.022
AUC IL-6 (n=80)	0.29	0.010	0.40	<0.001
AUC cortisol (n=79)	0.28	0.012	0.37	0.001
AUC STAI state anxiety (n=75)	0.40	<0.001	0.22	0.061
AUC MDBF mood (n=69)	-0.47	<0.001	-0.28	0.019
AUC MDBF alertness (n=69)	-0.43	<0.001	-0.27	0.026
BDI score (n=78)	0.15	0.19	0.27	0.017
CRP 24h post-injection (n=75)	---	---	0.35	0.002

Correlations between LPS-induced sickness symptoms (i.e., GASE = General Assessment of Side Effects Scale scores) assessed 6h (acute inflammatory phase) and 24h (late inflammatory phase) post-injection of LPS. Correlations were computed as Pearson's r within LPS-treated subjects (see table for exact N). Significant correlations are printed in bold font. No significant correlations were found for age, body mass index, AUC body temperature, AUC heart rate, AUC MDBF subscale "calmness", coping strategies (PRSS subscales catastrophizing; active coping), personality traits (NEO-FFI neuroticism, extraversion), trait anxiety (STAI-trait). Please note that complete results from correlation analysis including non-significant results are provided as supplementary Table 1.

AUC = area under the curve with respect to increase. STAI = State-Trait-Anxiety-Inventory. MDBF = German Multidimensional Mood Questionnaire. BDI = Beck Depression Inventory.

Table 4: Results of stepwise multiple regression analyses with LPS-induced physical sickness symptoms (GASE sum scores) as criterion

Model	Physical sickness symptoms during the acute inflammatory phase (0-6h post-injection of LPS) were predicted by						Physical sickness symptoms during the late inflammatory phase (6-24h post-injection of LPS) were predicted by					
	Predictor variables	B	β	t	P	adj. R ²	Predictor variables	B	β	t	P	adj. R ²
Model I	AUC IL-6	0.008	0.29	2.6	0.010	0.069	AUC IL-6	0.009	0.46	2.1	0.035	0.202
	Constant	6.565		4.6	<0.001		Constant	1.181		5.5	<0.001	
Model II	AUC MDBF mood	-0.290	-0.44	-4.1	<0.001	0.238	AUC IL-6	0.005	0.33	2.9	0.005	0.241
	AUC IL-6	0.006	-0.25	2.3	0.024	0.285	AUC cortisol	0.001	0.25	2.2	0.033	0.268
	constant	4.112		3.2	0.002		constant	1.655		2.9	0.005	
Model III*	(not computed)	---	---	---	---	---	AUC cortisol	0.001	0.27	2.2	0.033	0.216
							BDI score	0.530	0.37	3.6	0.001	0.304
							AUC IL-6	0.007	0.36	2.9	0.005	0.385
							constant	-1.099		-1.1		

As predictor variables of LPS-induced physical sickness symptoms (i.e., GASE = General Assessment of Side Effects scores) assessed 6h (acute inflammatory phase) and 24h (late inflammatory phase) post-injection of lipopolysaccharide (LPS), the area under the curve (AUC) for IL-6 and TNF-alpha (Model I), as well as AUCs for IL-6, TNF-alpha, cortisol, state anxiety (STAI-state score), mood (MDBF subscale positive-negative mood), and C-reactive protein (CRP; included only at 24h post-injection) (model II) were entered in stepwise multiple regression models.

Model III for GASE scores 24h post-injection included as predictor variables: AUCs for IL-6, TNF-alpha, cortisol, state anxiety, MDBF subscale positive-negative mood, C-reactive protein, BDI scores.

*Since no significant bivariate correlation between GASE score and any psychological trait variable was observed 6h post LPS-injection, no model was computed.

All models were adjusted for age, sex, and cytokine assay method (i.e., ELISA vs. bead-based assay). Of note, all predictor variables remained significant after additionally controlling for baseline IL-6 and TNF-alpha concentrations (data not shown).

Accepted Article

B = regression coefficient. β = standardized regression coefficient. adj. R² = adjusted R². STAI = State-Trait-Anxiety-Inventory. MDBF = Multi-dimensional mood questionnaire.