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Mood disturbance during experimental endotoxemia: Predictors of state anxiety as a psychological component of sickness behavior

Julie Lasselin [a,b,c], Sigrid Elsenbruch [c], Mats Lekander [a,b,d], John Axelsson [a,b,d], Bianka Karshikoff [a,b], Jan-Sebastian Grigoleit [c,e], Harald Engler [c], Manfred Schedlowski [c], Sven Benson [c,*]

[a] Stockholm University, Stress Research Institute, Stockholm, Sweden
[b] Karolinska Institutet, Department of Clinical Neuroscience, Division of Psychology, Solna, Stockholm, Sweden
[c] Institute of Medical Psychology and Behavioral Immunobiology, University Hospital Essen, University of Duisburg-Essen, Germany
[d] Osher Center for Integrative Medicine, Karolinska Institutet, Solna, Stockholm, Sweden
[e] Laboratory of Neuronal Structure and Function, The Salk Institute for Biological Studies, La Jolla, CA 92037, USA

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ABSTRACT

Lipopolysaccharide (LPS) administration is a well-established model to assess afferent immune-to-brain communication and behavioral aspects of inflammation. Nevertheless, only few studies in comparatively small samples have assessed state anxiety as a psychological component of sickness behavior despite possible clinical implications for the pathophysiology of neuropsychiatric conditions. Thus, the goal of the present analyses carried out in a large, pooled dataset from two independent study sites was to analyze the state anxiety response to LPS administration and to investigate predictors (i.e., cytokine changes; pre-existing anxiety and depression symptoms assessed with the Hospital Anxiety and Depression Scale) of the LPS-induced state anxiety changes at different time points after LPS administration. Data from 186 healthy volunteers who participated in one of six randomized, placebo-controlled human studies involving intravenous administration of LPS at doses of 0.4–0.8 ng/kg body weight were combined. State anxiety as well as circulating interleukin (IL)-6, tumor necrosis factor (TNF)-α and IL-10 concentrations were significantly increased 2 h and 3 h after LPS administration, with a peak at 2 h, and returned to baseline 6 h after administration. Greater changes in IL-6 from baseline to 3 h after LPS administration significantly and independently predicted a more pronounced LPS-induced state anxiety response. In addition, higher pre-existing subclinical anxiety symptoms significantly predicted a lower increase in state anxiety 3 h and 6 h after LPS-administration, which was mediated by TNF-α changes. In conclusion, our findings give additional support for a putative role of inflammatory mechanisms in the pathophysiology of stress-related and anxiety disorders and give new insight on the potential role of pre-existing subclinical affective symptoms.

1. Introduction

Translational research implementing experimental administration of endotoxin in humans has sparked a growing interest over the past decades (Santos and Wilmore, 1996; Bahador and Cross, 2007). Arguably one of the greatest advantages of experimental endotoxemia as a model lies in the complex set of behavioral and physiological responses that can reliably and safely be induced by the administration of low doses of endotoxin. This transient "sickness response" encompasses different facets including physical symptoms (e.g., moderate rise in body temperature or fever, nausea, chills, headache, pain) as well as behavioral manifestations (e.g., fatigue, difficulties concentrating, social withdrawal, mood impairments) (DellaGioia and Hannestad, 2010; Schedlowski et al., 2014). As such, it is a unique model to study afferent immune-to-brain communication and behavioral aspects of inflammation. Importantly, the central effects of peripheral pro-inflammatory cytokines during endotoxemia are relevant not only in the context of understanding normal, adaptive brain functions and behavioral changes during acute inflammation, but also to elucidate behavioral aspects that play a role in a range of neuropsychiatric conditions (Miller et al., 2008; Capuron et al., 2014; Castanon et al., 2014; Schedlowski et al., 2014).

* Corresponding author at: Institute of Medical Psychology and Behavioral Immunobiology, University Hospital Essen, University of Duisburg-Essen, Hufelandstr 55, 45147 Essen, Germany.
E-mail address: sven.benson@uk-essen.de (S. Benson).

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Mood impairments constitute one important psychological aspect of the sickness response. Previous work conducted in the context of LPS as a putative model for affective disorders reported increases in depression-like symptoms (e.g., negative mood, fatigue, motivational changes, social disconnection) (Eisenberger et al., 2010; Hannestad et al., 2012; DellaGioia et al., 2013). Increases in state anxiety have also been reported by a few groups (Reichenberg et al., 2001), including our own (Grigoleit et al., 2011; Wegner et al., 2014; Karshikoff et al., 2015), but have received less attention despite possible clinical implications for the pathophysiology of stress-related disorders and neuropsychiatric conditions. There is evidence supporting a possible role of inflammatory processes in the pathophysiology of clinical anxiety (Pitsavos et al., 2006; O'Donovan et al., 2010; Vogelzangs et al., 2013; Liukkonen et al., 2011, for review, see Hou and Baldwin, 2012), as well as altered immune responses to infection, immune stimulation or psychological stress in stress-related conditions (for review, see Godbout and Glaser, 2006). Findings in experimental animals support the relevance of afferent immune-to-brain communication for anxiety-like behavior (Bassi et al., 2012; Prager et al., 2013; for review see Goehler et al., 2007). However, it remains unclear if this translates to healthy individuals with subclinical anxiety and/or depression symptoms, i.e., symptom scores on clinical screening questionnaires that are below the respective cut-offs but at the upper end of the normal range. Furthermore, experimental findings in the field of endotoxemia research remain scarce and partially inconsistent with respect to the reliability of the state anxiety response and to associations with pro-inflammatory cytokine responses. Existing work, usually carried out in small samples, supports large inter-individual variations, which calls for analyses in larger samples to increase statistical power, as well as for the characterization of predictors.

The goal of the present analyses carried out on a large, pooled dataset from two independent study sites was to analyze the state anxiety response to LPS administration with the following specific objectives: (1) to analyze state anxiety changes in response to LPS *versus* placebo, along with circulating pro- and anti-inflammatory cytokines (i.e., IL-6, TNF-α, IL-10), body temperature and heart rate; (2) to investigate predictors of the LPS-induced state anxiety changes, in particular (a) cytokine changes and (b) pre-existing subclinical (non-pathological) anxiety and depression symptoms; and (3) to test whether a possible effect of pre-existing anxiety or depression symptoms is mediated by the changes in inflammatory marker concentrations. The hypotheses were that cytokine changes significantly predict the LPS-induced state anxiety response and that pre-existing anxiety and depression symptoms modulate the state anxiety response to LPS challenge.

2. Methods

2.1. Participants and protocol

For the purpose of this pooled analysis, data from 186 healthy adults who participated in one of six randomized, placebo-controlled human studies involving intravenous administration of LPS performed either at the Institute of Medical Psychology and Behavioral Immunobiology, University Hospital Essen (Essen, Germany) or at Karolinska Institutet/Stress Research Institute (Stockholm, Sweden) were combined. The sample included in this analysis consisted of 141 men (75.8%) and 45 women (24.2%), with a mean age of 27 (±5) years and an average BMI of 23.3 (±2.8) kg/m^2. Inclusion criteria were being between 18 and 50 years of age, a non-smoker, a non-excessive alcohol consumer, without physical or neuropsychiatric conditions, and free of medication (except for hormonal contraceptives in women). All subjects underwent a medical examination before inclusion and blood CRP concentration was measured to exclude any ongoing infection (cut-off CRP concentration $\geqslant$0.5 mg/dl). For detailed description of the rigorous and highly-standardized screening processes and safety procedures, see (Grigoleit et al., 2011; Karshikoff et al., 2015).

Three studies with a total of 80 volunteers were accomplished using a double-blinded, placebo-controlled, cross-over design (Grigoleit et al., 2011; Benson et al., 2012; Wegner et al., 2015). Herein, volunteers received LPS on one occasion, and placebo (saline: 0.9% NaCl) on another occasion in a counterbalanced order. Study days were separated by at least one week washout period, which is sufficient to allow normalization of cytokine concentrations (Grigoleit et al., 2011), mood parameters, and CRP concentrations (unpublished data). In three between-subject studies, a total of 106 volunteers were randomized to either LPS (N = 74) or placebo (N = 32) in a double-blinded fashion (Wegner et al., 2014; Benson et al., 2015; Karshikoff et al., 2015). In all studies, LPS from *Escherichia coli* (United States Pharmacopeia Rockville, MD) at doses of 0.4, 0.6 or 0.8 ng/kg body weight dissolved in sterile water was used, as described in detail herein (Grigoleit et al., 2011; Wegner et al., 2014; Benson et al., 2015; Karshikoff et al., 2015). Together, merging of data from 186 participants yielded 154 injections with LPS and 112 injections with saline (Supplementary Table). Specifically, 94 subjects (61.0%) received 0.4 ng/kg, 28 subjects (18.2%) received 0.6 ng/kg and 32 subjects (20.8%) received 0.8 ng/kg of LPS.

During the screening visit (approximately seven days before the study day), subjects completed a questionnaire battery on sociodemographic and health-related variables as well as relevant psychological traits. The Hospital Anxiety and Depression Scale (HADS) was used to screen for ongoing affective disturbances (using published cut-offs of $\geqslant$11) and to quantify subclinical symptoms of depression and anxiety (Zigmond and Snaith, 1983). HADS was available for 155 subjects (N = 122 in the LPS condition and N = 78 in the placebo condition) (Supplementary Table).

Blood samples for the analysis of plasma cytokine concentrations were obtained *via* an indwelling catheter before (=baseline) as well as 1.5–2 h (=2 h), 3–3.5 h (=3 h) and 5–6 h (=6 h) after the injection of LPS or saline. At each of these time points, state anxiety was measured with the state version of the State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1979) along with body temperature (intra-aurical) and heart rate (radial pulse).

To avoid unblinding in the original studies, "higher" LPS-doses (i.e., 0.6 or 0.8 ng/kg body weight) were only administered in between-group study designs, while in cross-over (or within-subject) studies only low-dose LPS (i.e., 0.4 ng/kg) was given. To control for potential order effects on state anxiety in cross-over studies (which also would suggest unblinding of subjects), we assessed if changes in state anxiety (STAI) scores differed between subjects who received LPS during the first vs. the second visit. Supplementary repeated measures ANOVA did not indicate any evidence for order effects (data not shown).

All studies were approved by the local ethics committee of the University Hospital Essen, Germany (permit numbers 07-3479, 09-4271) and the Regional Ethical Board in Stockholm, Sweden (permit numbers 2008/955-31, 2009/1273-32, 2010/1629-32, 2010/1362-32). All subjects provided written informed consent and were paid between 275€ and 400€ (depending on the design of the primary study) for their participation.

2.2. Cytokines

Plasma concentrations of TNF-α, IL-6, and IL-10 were measured by multiplexed bead-based assays (MILLIPLEX MAP Assay,

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Millipore Corporation, Billerica, MA, USA; Bio-Plex Pro Cytokine Assay, Bio-Rad Laboratories, Munich, Germany) or enzyme-linked immunosorbent assays (Quantikine ELISA, R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Depending of the method used, the sensitivity of the assays ranged between 0.11 – 0.70 pg/ml for IL-6, 0.11–0.20 pg/ml for TNF-α, and 0.09–0.51 pg/ml for IL-10. Comparison of ELISA and bead-based assays in a subset of samples (119 samples from $N = 17$ subjects collected at baseline and all post-injection time points) revealed that the results obtained by the two methods were highly correlated ($r = 0.87$, $p < .001$), although absolute values were different. Thus, cytokine data from individual studies were normalized by z–transformation to allow comparison across experiments, assay systems, and study sites. Outliers with respect to at least one inflammatory marker (i.e., baseline value higher than mean + 3SD) were excluded. In the present sample of $N = 186$ subjects, two subjects were considered as outliers in the LPS condition and three subjects in the placebo condition.

2.3. Statistical analyses

(1) Linear mixed regression analyses were computed in a factorial model using time (baseline, 2 h, 3 h, 6 h) and condition (LPS/placebo) as fixed effects and participants as random effect in order to assess the main effects of LPS *versus* placebo on state anxiety, cytokine concentrations, body temperature and heart rate. To address differences between LPS and placebo conditions for specific time points, post hoc Bonferroni-corrected *t*-tests were performed (controlling for inflation of alpha error due to multiple testing). Supplementary linear mixed regression analyses were performed to assess the effect of time and LPS dose (placebo *versus* 0.4 *versus* 0.6 *versus* 0.8 ng/kg) on state anxiety, cytokine concentrations, body temperature and heart rate. In the same way, the interaction effect of sex (men *versus* women) with the effects of condition and time were assessed using linear mixed regression analyses.

(2) Linear regression analyses were computed (all adjusted for sex, LPS dose, body temperature changes, study site and study design) within LPS-treated subjects to predict changes in state anxiety from baseline to each post-injection time point. This was carried out in two steps. First, changes in state anxiety were predicted separately for each time point and predictor variable (i.e., IL-6, TNF-α, IL-10, HADS-A, HADS-D) separately. Second, all predictor variables (except IL-10 due to missing data, see Supplementary Table for exact Ns for each parameter and analysis) were simultaneously entered in one model in order to account for intercorrelations of variables.
In all analyses, changes in state anxiety symptoms were calculated for each time point and corrected for baseline values [changes = (post-injection − baseline)/baseline]. Since standardized scores (i.e., z-scores) were calculated for the merged analysis of cytokine concentrations, z-score changes were computed without baseline correction [changes = (post-injection − baseline)].

(3) Subsequently, a mediation analysis was accomplished to address if changes in inflammatory markers mediated the observed effects of pre-existing anxiety or depression symptoms on LPS-induced changes in state anxiety. To do so, we used the PROCESS SPSS macro provided by A. F. Hayes (version 2.13; downloaded from http://www.processmacro.org/download.html; see Hayes, 2013).

3. Results

3.1. Effects of LPS administration compared to placebo

When compared to placebo, LPS resulted in a significant increase in state anxiety [time × condition effect: type III-$F_{(3,744.6)} = 27.4$, $p < .001$, Fig. 1A; main effects of time and condition: both *p*'s < .001, not shown] with significantly increased levels at 2 h and 3 h (for results of post hoc tests, see Fig. 1A). At 2 h, 77.5% LPS-treated subjects showed an increase in state anxiety (mean STAI change from baseline 15.3 ± 24.2%), while 65.4% of the subjects in placebo conditions showed either a decrease or no change in state anxiety (mean STAI change from baseline 0.0 ± 14.2%). LPS also induced significant increases of circulating IL-6 (Fig. 1B), TNF-α (Fig. 1C) and IL-10 (Fig. 1D), as evidenced by significant time × condition interactions [IL-6: type III-$F_{(3,821.6)} = 114.9$; TNF-α: type III-$F_{(3,840.9)} = 101.8$; IL-10: type III-$F_{(3,792.5)} = 98.3$; all *p*'s < .001] as well as significant main effects of time and condition [all *p*'s < .001]. Body temperature and heart rate also increased significantly in response to LPS when compared to placebo, indicated by significant time × condition interactions [body temperature: type III-$F_{(3,853.5)} = 42.7$, Fig. 1E; heart rate: type III-$F_{(3,705.8)} = 55.9$; both *p*'s < .001, Fig. 1F] and both had significant main effects of time and condition [all *p*'s < .001].

Supplementary analyses were carried out to explore effects of LPS dose and sex of participants. Main effects of LPS dose and time as well as an interaction effect time × dose were observed for all variables [all *p*'s < .001]. Overall, the dose of 0.8 ng/kg LPS led to a stronger increase in state anxiety, IL-6, TNF-α, and body temperature in comparison to the doses of 0.4 and 0.6 ng/kg (Supplementary Fig. 1). LPS administration at a dose of 0.4 and 0.6 ng/kg had similar effect, in particular on state anxiety, and IL-6 and TNF-α concentrations (Supplementary Fig. 1).

An interaction effect of sex with condition and time was found for state anxiety [$p = .031$] but no significant difference was found between men and women in the post hoc comparisons. Similar interaction effects were found for IL-6 and TNF-α concentrations [$p = .003$ and $p < .001$, respectively] and for heart rate [$p = .010$]. Women showed significantly greater IL-6 and TNF-α concentrations 2 h and 3 h after LPS administration, as well as increased heart rates 6 h after LPS injection (Supplementary Fig. 2).

3.2. Pre-existing anxiety and depression scores

Mean HADS anxiety score (HADS-A) was 3.1 (±2.1) and mean HADS depression score (HADS-D) was 1.4 (±1.4). Note that these scores are well-below the published cut-offs (i.e., ⩾11) for clinically-relevant symptoms. Supplementary analyses addressing associations between baseline (pre-injection) values of repeated measures and pre-existing anxiety and depression (HADS) scores, computed over all subjects, indicated significant correlations between baseline state anxiety (STAI state) scores and HADS anxiety ($r = .352$, $p = <.001$) and HADS depression ($r = .229$, $p = .0049$), respectively. HADS depression score was also significantly correlated with baseline IL-6 concentrations ($r = .160$, $p = .047$).

3.3. Predictors of LPS-induced changes in state anxiety

To predict changes in state anxiety, linear regression analyses (adjusted for sex, LPS dose, body temperature changes, study design and study site) were computed in two steps, which differed with respect to the variables that were simultaneously entered as predictors (Table 1).

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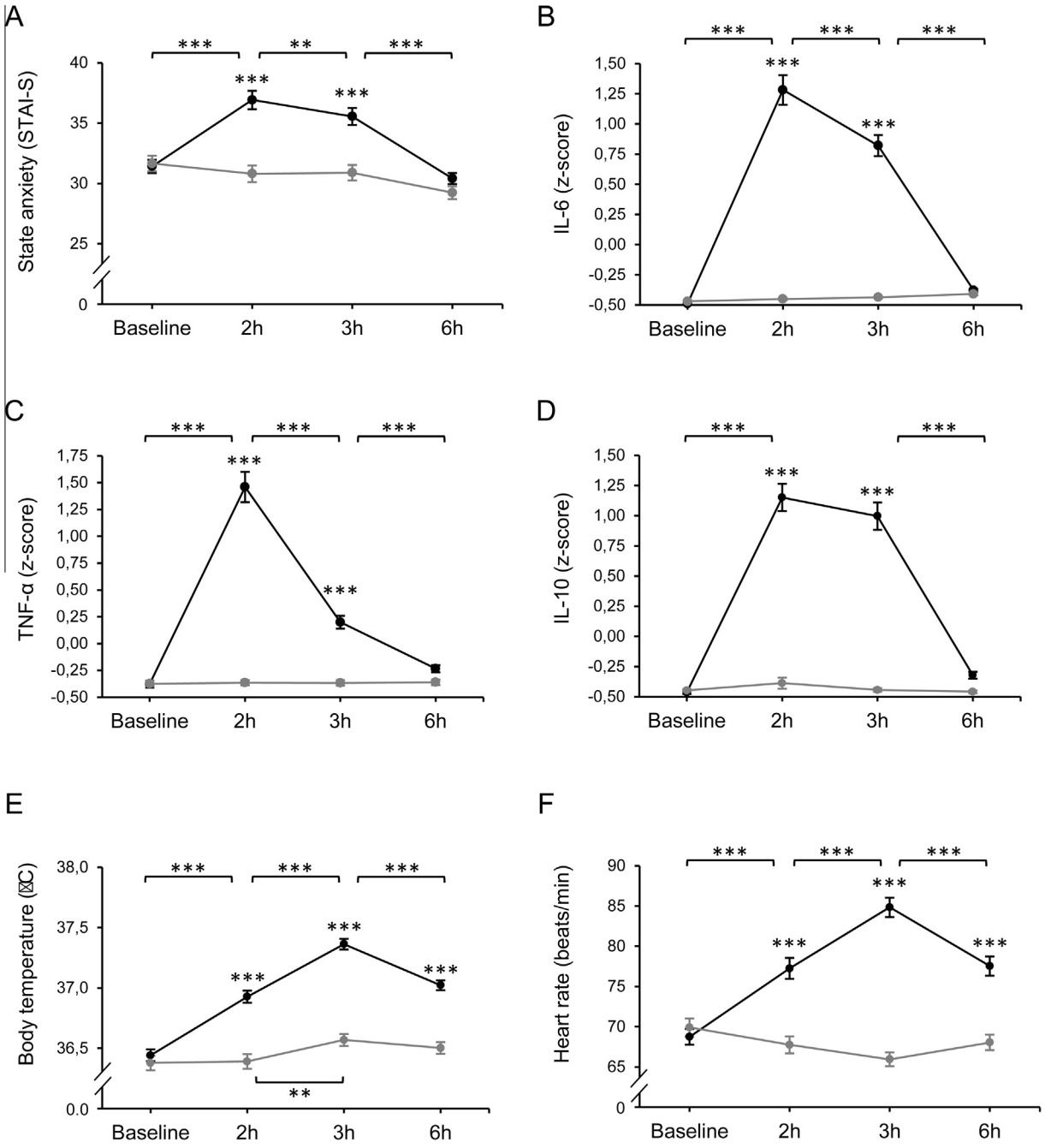

Fig. 1. Effects of LPS administration compared to placebo at baseline and 2 h, 3 h and 6 h after injection. Black lines represent LPS administration (at a dose of 0.4–0.8 ng/kg), grey lines indicate saline administration. (A) State anxiety score measured with the STAI-S, (B–D) cytokine concentration z-scores, (E) body temperature, (F) heart rate. $^{***}p < .001$, $^{**}p < .01$, results from Bonferroni-corrected post hoc *t*-tests comparing LPS and placebo at individual time points or paired *t*-tests (Bonferroni-corrected) comparing changes between two consecutive time points within each group, respectively (for results of linear mixed regression analyses, see text) *Abbreviations:* LPS: lipopolysaccharide; STAI-S: state version of the State-Trait Anxiety Inventory; IL-6: interleukin-6; TNF-α: tumor necrosis factor-α; IL-10: interleukin-10.

In model 1, separate linear regression analyses were computed for each predictor variable (i.e., IL-6, TNF-a, IL-10, HADS-A and HADS-D). These analyses revealed that IL-6 (Fig. 2A), TNF-α (Fig. 2B), and pre-existing anxiety symptoms (HADS-A, Fig. 2C) were significant predictors of LPS-induced change in state anxiety for the time point 3 h after LPS administration. No significant associations were observed for other time points (2 h and 6 h post-injection) with respect to cytokine changes. However, a significant association between pre-existing anxiety symptoms (HADS-A) and changes in state anxiety at 6 h post-injection of LPS was found. Of note, the positive β weights indicated that a greater increase in state anxiety at 3 h was best predicted by higher cytokine release in response to LPS. In addition, negative β weights indicated that higher pre-existing anxiety symptoms (HADS-A scores) predicted lower state anxiety changes at both 3 h and 6 h post-injection.

In model 2, all variables (except for IL-10 due to missing data) were simultaneously entered in order to account for inter-correlations of variables and to identify unique and independent predictor variables. This combined model revealed that the associations of TNF-α and pre-existing anxiety symptoms (HADS-A) with state anxiety changes at 3 h were no longer significant, while the association with IL-6 remained borderline significant ($p = .053$)

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Table 1
Linear regression models.

	2 h						3 h						6 h					
	Model				Estimates		Model[a]				Estimates		Model				Estimates	
	n	R^2	*F*	*p*	*β*	*p*	*n*	R^2	*F*	*p*	*β*	*p*	*n*	R^2	*F*	*p*	*β*	*p*
Model 1																		
IL-6	117	.073	2.5	.03	.165	.10	94	.197	5.6	<.001	**.385**	**<.001**	115	.057	2.1	.06	.092	.34
TNF-α	117	.052	2.1	.06	.054	.59	94	.169	4.8	.001	**.361**	**.001**	115	.052	2.0	.07	.072	.52
IL-10	104	.032	1.6	.17	.070	.51	81	.092	2.6	.03	.118	.33	102	.054	2.0	.08	−.011	.93
HADS-A	117	.061	2.6	.04	−.105	.25	94	.124	3.6	.005	**−.242**	**.02**	117	.081	2.7	.02	**−.199**	**.03**
HADS-D	117	.050	2.0	.07	.006	.95	94	.082	2.7	.03	−.136	.19	117	.041	1.8	.10	.016	.86
Model 2																		
IL-6	117	.065	1.9	.06	.224	.10	94	.203	4.0	<.001	.246	.05	115	.086	2.2	.03	.057	.56
TNF-α					−.113	.40					.182	.16					.051	.66
HADS-A					−.133	.22					−.106	.36					**−.256**	**.02**
HADS-D					.055	.61					−.017	.88					.150	.16

Linear regression analyses, adjusted for sex, LPS dose, body temperature changes, study design and study location. The dependent variable is the change in state anxiety (STAI-S) from baseline to 2 h, 3 h or 6 h, respectively. The independent variables are the changes in cytokine concentration z-scores or pre-existent anxiety and depression symptoms (measured with the Hospital Anxiety and Depression Scale). *Model 1* assessed each independent variable separately. *Model 2* assessed the effect of IL-6 and TNF-α concentration z-score changes and pre-existent anxiety and depression symptoms together. Bold font represents significant values of estimates.
Abbreviations: LPS: lipopolysaccharide; 2 h: values at 1.5–2 h after LPS administration; 3 h: values at 3–3.5 h after LPS administration; 6 h: values at 5–6 h after LPS administration; STAI-S: state version of the State-Trait Anxiety Inventory; IL-6: interleukin-6; TNF-α: tumor necrosis factor-α; IL-10: interleukin-10; HADS-A: anxiety subscale of the Hospital Anxiety and Depression Scale; HADS-D: depression subscale of the Hospital Anxiety and Depression Scale.
[a] Study location was removed from the model (STAI-S was not assessed at 3 h in studies conducted in Stockholm).

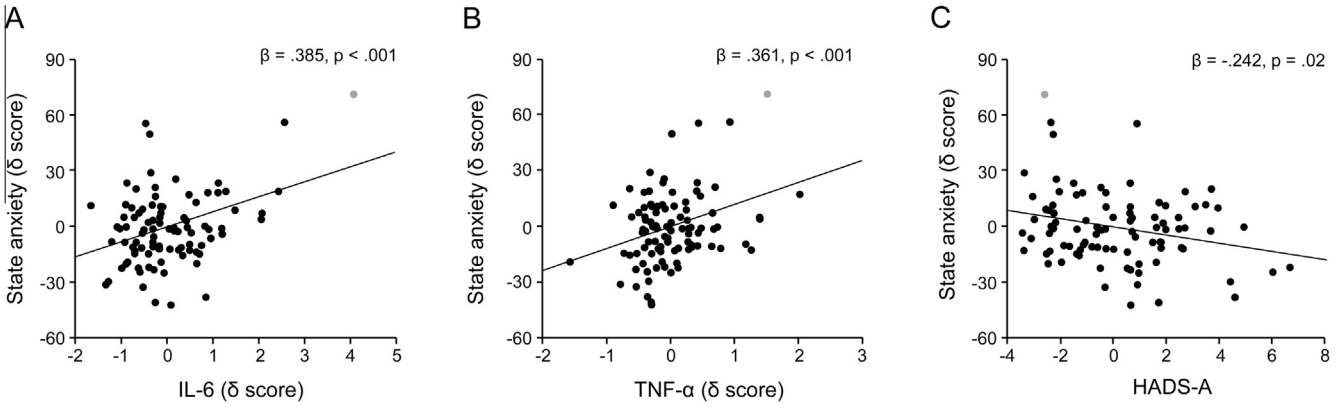

Fig. 2. Associations between the state anxiety response to LPS and cytokine concentration changes (A-B) and pre-existing anxiety symptoms (HADS-A scores, C) 3h after LPS administration. Linear regression analyses adjusted for sex, LPS dose, body temperature changes, study design and study site. For statistical details, see Table 1, model 1. The state anxiety changes are presented in percentage of change from baseline. The grey dot represents a subject outlier for state anxiety change. Results remained significant when removing this subject from the analyses ((A) $\beta = .244$, $p = .026$; (B) $\beta = .270$, $p = .017$; (C) $\beta = -.212$, $p = .036$). *Abbreviations:* LPS: lipopolysaccharide; IL-6: interleukin-6; TNF-α: tumor necrosis factor-α; STAI-S: state version of the State-Trait Anxiety Inventory; HADS-A: Hospital Anxiety and Depression Scale, anxiety part.

(Table 1, model 2). For state anxiety changes at 6 h, pre-existing anxiety symptoms (HADS-A) remained a significant predictor variable, supporting the result of model 1.

Similar results were observed when removing one subject who was outlier for state anxiety change, although the effects were lower (for details, see Fig. 2).

No significant associations with HADS depression scores were observed in either model. Moreover, no significant effects of LPS dose or sex on state anxiety changes were found in any of the models. Changes in body temperature were positively associated with state anxiety changes 3 h after LPS administration in the first models assessing separately the predicting effects of pre-existing anxiety and depression ($\beta = .227$, $p = .031$ and $\beta = .213$, $p = .047$, respectively), but this effect was no longer significant in the model 2 ($\beta = .120$, $p = .249$).

3.4. Mediation analysis

Mediation analyses, illustrated in Fig. 3A, revealed that the change in IL-6 from baseline to 3 h was a significant mediator of the association of pre-existing anxiety symptoms (HADS-A) with LPS-induced state anxiety changes. Indeed, (a) lower pre-existing anxiety symptoms were significantly associated with greater increases in state anxiety; (b) pre-existing anxiety symptoms and the changes in IL-6 from baseline to 3 h were significantly and negatively associated; (c) the changes in IL-6 concentrations was significantly associated with the changes in state anxiety from baseline to 3 h, when adjusting for pre-existing anxiety; (d) pre-existing anxiety symptoms were no longer significantly associated with the changes in state anxiety from baseline to 3 h when entering together with IL-6 concentrations. The indirect effect was −0.0105 (95% Confidence Interval (CI) = [−0.0257; −0.0001], suggesting that the association between higher pre-existing anxiety symptoms and lower state anxiety responses 3 h hours after LPS administration was mediated through IL-6. A similar effect was found for TNF-α concentrations (Fig. 3B; indirect effect = −0.0069, 95% CI = [−0.0170; −0.0012]), which indicates that TNF-α production mediated the association between pre-existing anxiety symptoms and lower state anxiety response 3 h after LPS administration. However, when removing the outlier subject for

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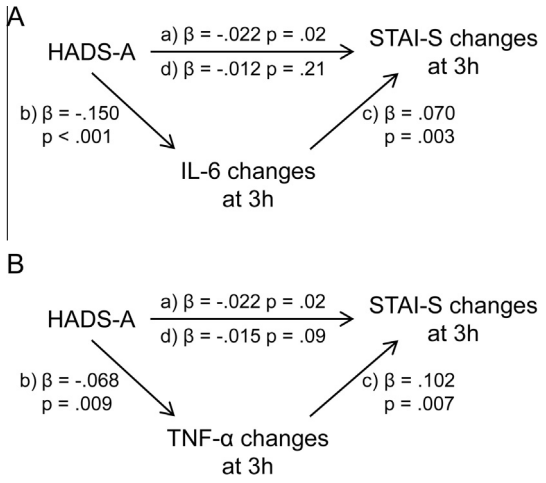

Fig. 3. Visualization of mediation analysis. Mediation analysis revealed that the change in interleukin (IL)-6 (A) and tumor necrosis factor (TNF)-α (B) from baseline to 3 h was a significant mediator of the association of pre-existing anxiety symptoms (assessed with the anxiety subscale of the Hospital anxiety and Depression Scale, HADS-A) with LPS-induced state anxiety changes. For details, see text.

state anxiety, the mediating effect of IL-6 was no longer significant (indirect effect = −0.0051, 95% CI = [−0.0146; 0.0013]), while the mediating effect of TNF-α remained significant (indirect effect = −0.0043, 95% CI = [−0.0105; −0.0006]).

4. Discussion

Mood impairments constitute one important psychological aspect of the sickness response. The present analyses were based on a merged dataset of six studies with the aim to describe the etiology of state anxiety during experimental endotoxemia. We observed increased levels of state anxiety 2 h and 3 h post-injection of LPS and a full recovery after 6 h when compared to placebo. This finding in our comparatively large sample supports that transient increases in state anxiety constitute one aspect of the sickness response that can be induced by administration of low to moderate LPS doses as accomplished herein, in line with previous independent human studies on much smaller samples that also assessed state anxiety (Reichenberg et al,. 2001; Kullmann et al., 2013), and animal studies showing reduced exploration and increased anxiety-like behavior in response to immune challenges (Bassi et al., 2012; Engler et al., 2011; Prager et al., 2013; for review see Goehler et al., 2007). On the other hand, negative findings have also been reported in humans by our own (Benson et al., 2012) and one other group (Hannestad et al., 2011), which may be due to limited statistical power given much smaller sample sizes. Supplementary analyses revealed that increases in state anxiety were greater in response to higher doses (i.e., 0.8 ng/kg) of LPS, suggesting a dose-dependent LPS effect, which was also observed for cytokine concentrations and body temperature. Moreover, women demonstrated greater cytokine responses to LPS compared to men, as previously found in some (van Eijk et al., 2007; Wegner et al., 2015) but not all (Coyle et al., 2006) endotoxemia studies, and tended to show greater LPS-induced state anxiety increases.

In a second analytic step, we tested the associations between the LPS-induced state anxiety changes at different time points with the cytokine responses. Our results support that greater state anxiety responses correlated significantly with LPS-induced increases in the concentrations of IL-6 and TNF-α three hours after LPS administration, in line with a previous report (Reichenberg et al., 2001). Interestingly, IL-6 was the main predictor in a multiple regression model accounting for the influence of other factors (including LPS-induced TNF-α response), explaining 20% of the variance of the state anxiety response. The cytokine driven anxiety response found in our dataset complements evidence that endotoxin-induced cytokine responses are associated with increased depressive symptoms (Reichenberg et al., 2001; Wright et al., 2005; Grigoleit et al., 2011; Wegner et al., 2014), although negative findings showing no correlations between cytokine concentrations and depressive symptoms have also been reported (Brydon et al., 2008; Harrison et al., 2009; Hannestad et al., 2011; Moieni et al., 2015). Of note, studies in smaller samples may have been underpowered to detect small correlations, which may explain some of the inconsistencies in this field of human endotoxin research.

Studies in humans and animals have provided insight into the possible mechanisms involved in the connection between peripheral cytokines and sickness symptoms, including neural and humoral afferent pathways. Peripheral cytokines demonstrably sensitize vagal afferent neurons, which terminate at the nucleus of the solitary tract (NTS) with projections to brain regions involved in autonomic, emotional and behavioral aspects of the sickness response such as the hypothalamus, amygdala, insula, locus coeruleus and periaqueductal gray (Konsman et al., 2002; Goehler et al., 2007). In an animal study from our group, we could demonstrate that LPS led to increased amygdaloid neuronal activity and *de novo* synthesis of pro-inflammatory cytokines in the amygdala. Interestingly, the changes in amygdala activation were related to increased anxiety-related behavior (Engler et al., 2011). In humans, a brain imaging study involving an experimental endotoxin application also supports IL-6 to modulate central processes underlying negative mood during the sickness response (Harrison et al., 2009).

Our third goal was to explore the putative role of pre-existing subclinical anxiety and depression symptoms, respectively, on LPS-induced changes in state anxiety. Our results revealed negative associations indicating that higher HADS anxiety scores predicted *lower* increases in state anxiety 3 h and 6 h (but not 2 h) after LPS-administration. This finding appears counter-intuitive and is indeed difficult to explain since one might have expected worse emotion regulation with increasing anxiety symptoms. Future studies in the field should incorporate psychological trait measures even when conducting endotoxemia research in carefully-screened healthy individuals, and could focus on interactions between inter-individuals variability in psychological traits, emotion regulation and peripheral immune responses.

Interestingly, the association between anxiety symptoms (HADS scores) and state anxiety response was mediated by changes in IL-6 and TNF-α, as shown by mediation analysis. While we were not able to include measures of hypothalamus–pituitary–adrenal (HPA) axis mediators, such as cortisol, in our analyses, it is tempting to hypothesize that this mediating effect could be explained by stronger negative feedback from the HPA axis or a modulation of the autonomic nervous system response (Kadmiel and Cidlowski, 2013; Kenney and Ganta, 2014). Activation of the sympathetic nervous system through meditation techniques and body ice immersion has been shown to result in lower cytokine responses after LPS administration (Kox et al., 2012, 2014), underscoring the role of efferent CNS-immune pathways in experimental endotoxemia.

Clearly, this is the first attempt to delineate a possible role of pre-existing, sublinical affective symptoms and psychological aspects of the sickness response to acute inflammation and the present study should be interpreted in the light of its limitations. First, we pooled data from different studies and study sites, which has a number of drawbacks including different populations, slight variations in protocols or the lack of matched measurements other than anxiety and cytokines. Nevertheless, the strategy provides

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high power for analyses of individual differences. In addition, results that apply across variations in protocols and study sites speak for the stability as well as generalizability of the relation between LPS-induced inflammation and anxiety. Importantly, state anxiety as primary outcome variable was assessed with the same method, and potentially confounding variables were adjusted for in the analyses. Second, the protocols of the primary studies differed with respect to the measurement time points, which only allowed inclusion of data from selected time points, which were not fully identical. Third, while we explored state anxiety as an indicator of the behavioral sickness response to LPS, we did not include other emotional or behavioral aspects of sickness behavior, especially depressive symptoms, which could be differently affected by LPS and interact with state anxiety. It would be important to know whether the effects are specific to state anxiety. Moreover, we cannot fully exclude that subjects who participated in those primary studies with a crossover design might have been unblinded by LPS-induced sickness symptoms during the first visit. However, in separate (unpublished) analyses, no order effect of state anxiety responses was found. Another important consideration is to emphasize that our study participants were carefully screened for inflammatory as well as psychiatric symptoms, limiting transfer to patients with (low-grade) inflammatory conditions and/or overt mood disturbances. Additional studies are therefore needed to further disentangle the complex relationships between peripheral inflammation and behavioral consequences in healthy humans as well as in individuals with disturbed affectivity or inflammatory conditions. Herein, brain imaging techniques will be instrumental to further improve knowledge about neural mechanisms underlying different behavioral and especially emotional aspects of human sickness behavior and extend existing brain imaging studies in humans (e.g., by Harrison et al., 2009; Karshikoff et al., 2015; Labrenz et al., 2015), and to translate previous findings on immune-to-brain pathways from animal research (Goehler et al., 2007; Engler et al., 2011) to humans.

In conclusion, our findings provide additional support for a putative role of inflammatory mechanisms in the pathophysiology of stress-related and anxiety disorders and give new insight on the potential role of pre-existing subclinical affective symptoms.

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Conflict of interest

All authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbi.2016.01.003.

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