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

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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).

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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,