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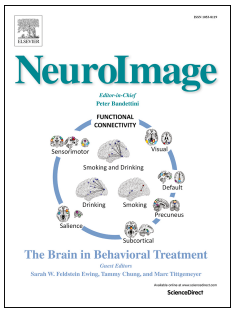

Altered temporal variance and functional connectivity of BOLD signal is associated with state anxiety during acute systemic inflammation

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Altered temporal variance and functional connectivity of BOLD signal is associated with state anxiety during acute systemic inflammation

Short title: Neural variance and state anxiety during acute inflammation

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Abstract

Systemic inflammation is accompanied by complex behavioral changes and disturbed emotion regulation that have been related to the pathophysiology of mood disorders including depression and anxiety. However, the causal role of systemic inflammation on mood disorders is still unclear. We herein investigated neural resting state patterns of temporal variance of the amygdala and functional connectivity within the salience network underlying changes in state anxiety during experimentally-induced systemic inflammation. In this randomized, double-blind study, N = 43 healthy men received an intravenous injection of either low-dose lipopolysaccharide (LPS, 0.4 ng/kg body weight) or saline. Resting state functional magnetic resonance imaging was assessed before and 3.5h after injection. State anxiety, assessed with a standardized questionnaire, and plasma cytokine concentrations were repeatedly measured. LPS administration induced a transient systemic inflammatory response reflected in increases in plasma Interleukin (IL)-6 and Tumor Necrosis Factor (TNF)-α concentration. Compared to placebo, state anxiety and temporal variance in the amygdala significantly increased while functional connectivity in the salience network decreased during LPS-induced systemic inflammation. Together, these data indicate that acute systemic inflammation alters temporal variance of the BOLD signal as well as functional connectivity in brain regions and networks implicated in emotion processing and regulation. These results are of translational importance to encourage further research on the role of inflammatory pathways in the pathophysiology of neuropsychiatric conditions including anxiety disorders.

Keywords: systemic inflammation, lipopolysaccharide, state anxiety, resting state variability, functional connectivity, amygdala, salience network

Introduction

Systemic inflammation and immune dysregulation have been identified as risk factors in the pathophysiology of mood disorders including depression and conceivably anxiety (Hou and Baldwin, 2012; O'Donovan et al., 2010; Vogelzangs et al., 2013). However, while the pivotal role of afferent immune-to-brain communication in depression or depression-like behaviors is well-documented through human and animal research, the putative connection between inflammation and anxiety has received little attention thus far. Yet, animal studies demonstrated increased anxiety-like behavior in response to experimentally induced systemic inflammation (Bassi et al., 2012; Goehler et al., 2007; Prager et al., 2013), mediated through brain structures implicated in emotion regulation including the amygdala, insula and prefrontal cortex (Doenlen et al., 2011; Engler et al., 2011). In humans alike, first studies support increased state anxiety in response to experimental immune challenge reflected in increased pro-inflammatory cytokines (Lasselin et al., 2016a; Reichenberg et al., 2001; Wegner et al., 2014), but neural mechanisms remain essentially unknown. However, brain mechanisms underlying states of acute anxiety and physiological arousal are crucial elements in the transition and maintenance of stress-related health conditions and anxiety disorders – which constitute unresolved clinical challenges affecting a large proportion of the population (Bandelow and Michaelis, 2015).

A major development in neuroscience has been the introduction of resting state functional magnetic resonance imaging (rs-fMRI) which measures spontaneous neuronal fluctuations in the blood oxygen level-dependent (BOLD) signal (Fox and Raichle, 2007). This approach allows a comprehensive characterization of the brains intrinsic functional architecture that is essential for a more conscientious depiction of emotional and cognitive dysfunctions in the psychopathology of neurological and psychiatric disorders. By clustering spatially separated but functionally linked brain regions, resting state networks were identified in the human brain that demonstrate high functional connectivity through synchronous BOLD signal fluctuations and enforce strong biases in

information processing. Herein, three core networks are of particular relevance for understanding affective and cognitive disturbances (Menon and Levitin, 2005). The central executive network is distributed across fronto-parietal areas that demonstrate co-activation during cognitively demanding tasks and are therefore implicated in actively maintaining and shaping information in the context of goal-directed behavior. By contrast, the default-mode network is typically active under rest and shows deactivation during stimulus-driven tasks implicating a role in rumination and self-referential processes. The salience network is anchored in the anterior insula and dorsal anterior cingulate cortex and includes key structures of the amygdala, prefrontal cortex and parietal lobe. The salience network is involved in a variety of brain functions through the integration of sensory, emotional, and cognitive information. Importantly, salience network abnormalities are commonly observed in mood and anxiety disorders (Menon and Uddin, 2010). Furthermore, intrinsic functional connectivity analyses have demonstrated alterations within the salience network in patients with anxiety disorders (Menon and Uddin, 2010).

Changes in resting state brain activity have been shown to predict several psychiatric conditions including depression, schizophrenia and autism (Broyd et al., 2009), but received only little attention in the context of clinical or experimental anxiety. So far, only three fMRI studies documented effects of experimentally-induced systemic inflammation on resting state activity in healthy humans (Labrenz et al., 2016; Lekander et al., 2016; Marsland et al., 2017). These studies, including the one from our research group (Labrenz et al., 2016) however, did not investigate psychological components of the sickness response like anxiety. Furthermore, none of these studies have systematically investigated how experimentally-induced systemic inflammation impacts on functional connectivity in the salience network. Moreover, besides possible anxiety-related alterations of this specific *spatial* feature of the resting state brain activity, also the effects of experimentally-induced systemic inflammation on the *temporal* features of the resting state brain activity are currently unknown. The human brain exhibits, indeed, a complex temporal organization (Buzsaki & Draguhn,

2004). Several resting state fMRI studies focused on the temporal variability that describes the change in the amplitude of neural activity fluctuations (He, 2011; Huang et al., 2014b; Tagliazucchi et al., 2013; Zou et al., 2008). Temporal variability is operationalized as the standard deviation of BOLD signal amplitude fluctuations. Importantly, it was found to be altered in Alzheimer disease (Han et al., 2011; Xi et al., 2012), brain injury (Raja Beharelle et al., 2012), vegetative state (Huang et al., 2014a), anesthesia (Huang et al., 2014b), schizophrenia (Fernández et al., 2013; Hoptman et al., 2010) and bipolar disorders (Martino et al., 2016). Hence, such variability seems to be an index of neural activity with high neuropsychiatric relevance (Huang et al., 2014a; Huang et al., 2014b ; Fernández et al., 2013; Hoptman et al., 2010; Martino et al., 2016).

Therefore, we herein aimed to test alterations of temporal variance of resting state activity in the amygdala and salience network as well as of functional connectivity within the salience network as putative neural mechanisms underlying inflammation-induced anxiety.

In a double-blind placebo-controlled study, we administered low-dose lipopolysaccharide (LPS) as a model that demonstrably induces a transient immune activation characterized by increases in cytokine concentrations in blood and cerebrospinal fluid (Engler et al., 2017), as well as increased state anxiety (Grigoleit et al., 2010; Lasselin et al., 2016a; Wegner et al., 2014). In this current study, we therefore expected significant changes in the temporal variance of resting state activity in the amygdala and salience network, as well as in functional connectivity within the salience network in response to LPS-induced systemic inflammation. The specificity of these effects was explored by testing the same parameters in the central executive network as control network primarily not involved in emotion processing (Sylvester et al., 2012).

Material and Methods

Participants

The sample of the present study included healthy male volunteers aged 18 - 45 years and with a body mass index (BMI) >18 or <29 kg/m^2 (Table 1). As

previously described in detail (Benson et al., 2015; Labrenz et al., 2016), volunteers underwent an in-depth screening process including a personal interview, completion of a standardized questionnaire battery, physical examination and repeated laboratory analyses of blood samples. Exclusion criteria included any previous or current medical or psychological condition, intake of medication, increased questionnaire scores on the Hospital Anxiety and Depression Scale (HADS) (Herrmann-Lingen et al., 2005) above a sum score > 11, smoking or any abnormality upon laboratory analyses of blood samples (i.e., complete blood cell count, liver enzymes, renal parameters, electrolytes, coagulation factors, C-reactive protein). The study protocol was approved by the Institutional Ethics Review Board of the Medical Faculty of the University of Duisburg-Essen (Approval No. 09-4271), and followed the Declaration of Helsinki. All study participants gave informed written consent and were paid for their participation.

Study design

In this randomized, double-blind, placebo-controlled study performed at the University Hospital Essen (Germany), participants were assigned either to the control group receiving an intravenous injection of saline or to the experimental group receiving an injection of LPS at a dose of 0.4 ng per kilogram body weight (Reference Standard Endotoxin, lot G3E069; United States Pharmacopeia, Rockville, MD) dissolved in sterile water as previously described (Grigoleit et al., 2010). Both, the participants and investigator who was involved in the screening process, assessment of resting state and questionnaire data were blinded to the application of either LPS or saline in the respective groups. Acquisition of resting state fMRI data was accomplished at baseline, i.e. before injection, and 3.5h post-injection of LPS or placebo as described in detail in (Labrenz et al., 2016). Anxiety symptoms and mood changes were assessed with validated questionnaires at baseline, 3h and 6h post-injection (see below). For analyses of plasma concentrations of pro-inflammatory cytokines, blood samples were collected at baseline, and subsequently at 1h, 2h, 3h, 4h and 6h post-injection

concomitantly with assessments of vital parameters including body temperature, blood pressure and heart rate (see (Benson et al., 2015; Labrenz et al., 2016)).

Plasma Cytokines

Systemic inflammation evokes the release of pro-inflammatory cytokines mediating information transfer between the peripheral immune system and central nervous system. Of these, interleukin (IL)-6 and tumor necrosis factor (TNF)-α are consistently implicated also in psychiatric conditions including mood and anxiety disorders (Felger and Lotrich, 2013; Furtado and Katzman, 2015). In an experimental setting with healthy participants, administration of endotoxin induces a similar response of increased IL-6 and TNF-α in blood concentrations (Harrison et al., 2009; Wright et al., 2005) and cerebrospinal fluid accompanied by behavioral symptoms of e.g., deteriorating mood (Engler et al., 2017).

Based on this previous work, we therefore focused plasma cytokine analyses on IL-6 and TNF-α. Plasma cytokine concentrations were analyzed using enzyme-linked immunosorbent assays (Quantikine® IL-6 and high-sensitive TNF-α ELISA, R&D Systems, Minneapolis, MN) according to the manufacturer's instructions.

Assessment of anxiety and mood symptoms

To measure the presence and severity of anxiety symptoms, the German version of the State Trait Anxiety Inventory (STAI) (Laux et al., 1981) was used. The Trait Anxiety scale (STAI-T) was assessed prior to the study to cover a general propensity of anxiety and evaluating stable states of confidence, security and calmness with items, e.g., "I feel like crying", "I have disturbing thoughts" and "I am content". The State anxiety scale (STAI-S) measures current symptoms of anxiety including for instance "I am presently worrying over possible misfortunes", "I feel nervous" and "I feel comfortable". To assess changes in state anxiety throughout the experiment, the STAI-S was repeatedly administered before injection and 1h, 2h, 3h and 6h post injection. In addition, we measured changes

in positive vs. negative mood, alertness vs. tiredness and calmness vs. restlessness using the Multidimensional Mood Questionnaire (MDBF) (Steyer et al., 1997). Items of the MDBF include judgments about how the individual feels right now, e.g. “superb”, “alert”, “unhappy”. This questionnaire was provided together with the STAI-S during the experiment at baseline, 3h and 6h post-injection. Changes in mood in response to LPS induced systemic inflammation have repeatedly been reported (Benson et al., 2015; Engler et al., 2017; Wegner et al., 2014). Herein, we used the positive-negative mood subscale of the MDBF in addition to the STAI-S to assess associations between fMRI modeling and negative mood during LPS-induced systemic inflammation, aiming to test the specificity of findings for state anxiety.

Resting State fMRI acquisition and preprocessing

Resting state data were acquired at baseline and 3.5h post-injection. Participants were instructed to keep their eyes closed during the 10-minute scanning session. All MRI data were acquired on a 3T whole-body scanner (Magnetom Skyra 3T, Siemens, Erlangen, Germany) equipped with a 32-channel head coil (Siemens, Erlangen, Germany). Structural images were acquired using a magnetization-prepared rapid gradient-echo T_1-weighted sequence with repetition time TR = 1900 ms, echo time TE = 2.13 ms, TI = 900 ms, flip angle 9°, FOV = 239 × 239 mm^2, 192 slices, slice thickness 0.9 mm, voxel size 0.9 × 0.9 × 0.9 mm^3, matrix 256 × 256 mm^2 and GRAPPA r = 2 as previously described (Benson et al., 2015; Labrenz et al., 2016). For resting state analyses, overall 295 scans were acquired. Functional images were aligned parallel to the AC-PC line using a T_2-weighted multi-echo EPI sequence with repetition time TR = 2000 ms, echo times TE_1: 13.0 ms, TE_2: 28.9 ms, TE_3: 44.8 ms, flip angle 90°, FOV 220 × 220 mm^2, 36 slices, slice thickness 3 mm, voxel-size 2.8 × 2.8 × 3 mm^3, slice gap 0.6 mm, matrix 80 × 80 mm^2 and GRAPPA r = 3 (Poser et al., 2006). The three echoes were combined using weighted summation implemented within the parallel-acquired inhomogeneity-desensitized (PAID) method (Poser et al., 2006).

Functional MRI data were preprocessed with the AFNI software package (Cox, 1996). Functional images from each scan were aligned (head motion correction), slice timing corrected, temporally standardized, resampled to 3 × 3 × 3 mm^3 and spatially smoothed by a Gaussian kernel (FWHM 5mm). Subsequently, images were transformed into Talairach space (Talairach and Tournoux, 1988), and linear trends were removed. The data were then band-pass filtered preserving signals between 0.01 and 0.1 Hz which reported to reflect fluctuations of spontaneous brain activity (Fox and Raichle, 2007; Zhang and Raichle, 2010). The estimated six parameters of head motion and mean time series from the white matter (WM) and cerebrospinal fluid (CSF) were regressed out by linear regression to control for non-neural noise (Fox et al., 2005). Masks of WM and CSF were eroded by one voxel to minimize partial volume effects with gray matter (Chai et al., 2012). To exclude head motion as potential confounder it was used as covariate in the GLM analyses.

Spherical regions-of-interest (ROI) were located in nodes of the salience network (Raichle, 2011; Seeley et al., 2007a), including the dorsal anterior cingulate cortex, anterior prefrontal cortex, lateral parietal lobe, anterior insula and amygdala. Exact coordinates of all ROI are given in Table 2. For the amygdala, coordinates were derived from (Kober et al., 2008) and for the other nodes of the salience network coordinates were derived from (Raichle, 2011). In agreement with previous resting state studies (Baldassarre et al., 2016, 2014; Hacker et al., 2013), ROIs had a radius of 6 mm. ROIs positioning was visually inspected in order to avoid misalignment with respect to the target regions (See figure 1).

Further analyses were then performed to retrieve temporal variance or the standard deviation (SD) of the BOLD signal (Garrett et al., 2011, 2010) as well as resting state functional connectivity (rs-FC). The SD across the time series for each voxel was calculated to yield an SD map for each subject. Subject-level voxel-wise SD maps were then standardized into subject-level Z-score maps per brain volume by subtracting the mean voxel-wise SD obtained for the entire brain (global mean of SD) and then divided by the standard deviation across voxels (Zuo et al., 2010). Finally, we extracted the standardized SD in the standard

frequency range of all the nodes of the salience network and amygdala bilaterally. Within nodes of the salience network and amygdala, rs-FC maps were computed between the averaged time series of the left insula and all voxels in the brain for the standard frequency range (0.01–0.1 Hz). These maps from individual subjects were transformed to Z values with Fisher's transformation for group-level analyses. Finally, we extracted the time series from all ROIs and calculated the rs-FC between all nodes of the salience network and amygdala bilaterally.

Figure 1

Statistical analyses of non-fMRI data

All non-fMRI data were initially tested for normal distribution using the Kolmogorov-Smirnov-Test. In case that the assumption of normal distribution was violated, data were log-transformed prior to analysis (i.e., cytokine data). To investigate the treatment effects of LPS versus saline injection (placebo), a repeated measures ANOVA was conducted on cytokines with within-subject factor time (baseline, 1h, 2h, 3h, 4h, and 6h post-injection) and the between-group factor treatment (LPS, placebo). Moreover, delta values from baseline to peak increase were calculated and compared using independent samples t-tests. For state anxiety and mood scores, independent t-tests were conducted with delta values from baseline to 3h post-injection. A power analysis using G*Power (version 3.1.9.2., http://www.gpower.hhu.de/) was carried out to determine the power for repeated measures ANOVA addressing interaction effects in plasma cytokine concentrations as well as for paired t-tests for anxiety and mood questionnaires. Power analyses for the final sample size of thirty-eight participants revealed a power of $1-\beta = 0.97$ and a critical F of 2.26 for interaction effects in plasma cytokine concentrations, and a power of $1-\beta = 0.73$ and critical t of 1.68 for anxiety and mood scores.

Statistical analyses of fMRI data

Data analysis aimed at testing differences in the temporal variance (SD) and functional connectivity (rs-FC) in the regions of the salience network and the amygdala. Regarding SD, for each ROI we carried out a repeated measures ANOVA with time (pre-injection, post-injection) and side (left, right) as within-subject factors, and group (LPS, placebo) as between-subject factor. For unilateral ROIs (e.g. dorsal anterior cingulate cortex) the factor side was not included. Subsequent t-tests were carried out when necessary. Finally, we calculated the mean SD of all the nodes within the salience network, and compared them between groups and between treatments using repeated measures ANOVA.

Regarding rs-FC, first the aggregated rs-FC values in the salience network, including the amygdala, were submitted to a repeated measures ANOVA with time (pre-injection, post-injection) and group (LPS, placebo) as within- and between-subject factors respectively. To test for the specificity of our results, the same analysis was carried out for the Central Executive Network (CEN) as control. This network has been chosen because, along with the default mode network (DMN) (Coutinho et al., 2016), the CEN is one of the two most prominent networks in the human brain, and unlike for the DMN its contributions to anxiety is still debated (Sylvester et al., 2012).

Finally, with the aim to clarify possible relationships between measures of state anxiety and neural activity after LPS injection at rest, we carried out correlation analyses between SD values in the amygdala, rs-FC in the salience network and inflammation-induced anxiety scores. Since correlation analyses are particularly sensitive to deviant observations (Rousselet and Pernet, 2012), we ran robust correlation analysis. Specifically, we computed skipped parametric (Pearson) correlations (Wilcox, 2004) using the Robust Correlation toolbox (Pernet et al., 2012) and conducted null hypothesis statistical significance testing using the nonparametric percentile bootstrap test (2000 resamples; one-sided 95% confidence interval, corresponding to an alpha level of 0.05), which is more robust against heteroscedasticity compared with the traditional tests (Pernet et al., 2012).

RESULTS

Sample characteristics

Forty-three healthy male volunteers with a mean age of 26.2 ± 3.6 (M ± standard deviation) years and a mean body mass index of 23.5 ± 2.8 kg/m^2 were randomly assigned to the LPS (N = 20) or control (N = 23) group (placebo group). Groups did not differ in age, body mass index, or in psychological characteristics including trait anxiety (STAI-T scores) (for details, see Table 1).

For fMRI analysis, two participants from the LPS group and three participants from the placebo group were discarded due to failure in image processing or values exceeding two standard deviations from the group mean (one participant from the placebo group). These participants were also excluded from analyses of cytokine and anxiety measurements.

LPS-induced changes in plasma cytokine concentrations

As previously reported (Labrenz et al., 2016), administration of LPS induced a transient systemic inflammatory response, reflected by significant increases in plasma TNF-α and IL-6 (all ANOVA time x group interaction effects $F > 23.47$, $p < .001$, $\eta^2 > .57$). Peak concentrations in response to LPS were observed for TNF-α at 2h post-injection (change from baseline within LPS group given as mean ± standard deviation: 1.27 ± 0.22 pg/ml, within control group: -0.02 ± 0.09 pg/ml, $t_{(34)} = 23.99$, $p < .001$) and for IL-6 at 3h post injection (change from baseline within LPS group: 2.01 ± 0.55 pg/ml, within control group: 0.78 ± 0.36 pg/ml, $t_{(34)} = 7.65$, $p < .001$). Additional information on non-normalized cytokine data and vital signs are given in Supplemental information and Supplemental Figure 1.

LPS-induced changes in state anxiety and mood

The distribution of state anxiety and mood scores can be found in Supplemental Figure 1.

For state anxiety scores, the increase from baseline to 3h post-injection was significantly higher in the LPS (1.35 ± 6.77) compared to the placebo group (-3.04 ± 4.31) ($t_{(41)}$ = 2.57, p = .014). Comparably, the LPS group (-2.05 ± 2.96) demonstrated a stronger decrease in positive mood scores from baseline to 3h post-injection than the placebo group (-0.17 ± 2.76) ($t_{(41)}$ = 2.21, p = .033). This effect was no longer evident when comparing decreases from 3h to 6h post-injection when anxiety (LPS group: -3.52 ± 5.31; placebo group: 2.88 ± 3.01) and mood (LPS group: -2.10 ± 2.95; placebo group: -1.52 ± 1.59) scores returned to the initial baseline (STAI-S: $t_{(41)}$ = 1.64, p = .124; MDBF: $t_{(41)}$ = 0.79, p = .434).

Temporal variance of BOLD signal

Initially, we tested for group differences in temporal variability of the BOLD signal at baseline. The analysis revealed no significant effect indicating comparable values before the injection (p = .53; [-.12 .30], Figure 2).

Among the ANOVAs on each ROI carried out on standard deviation (SD) values, the higher order interaction (i.e. time by side by group) was significant only in the amygdala ($F_{(1,37)}$ = 4.5, p = .041). Simple effect analysis showed that SD values in the left amygdala were significantly higher after LPS administration compared to SD values before LPS administration (bootstrap for paired samples t-test: $t_{(17)}$ = 2.4; p = .028; [-.81 -.10]). Moreover, SD values in the amygdala after LPS administration were also significantly higher compared to the placebo group after saline administration (bootstrap for independent samples t-test: $t_{(37)}$ = 3.4; p = .002; [.33 1.39]). For all other ROIs, no significant differences between treatments or groups were observed (all *p*s > .10). Temporal variance of BOLD signal in the salience network did not show differences between treatments or groups (all *p* > .34).

Figure 2

Resting state functional connectivity in the salience network

Analysis on resting state functional connectivity (rs-FC) values revealed a significant interaction ($F_{(1,37)}$= 7.2, p = .011). Simple effect analyses revealed lower connectivity values in the salience network after LPS administration compared to baseline level, i.e. before the injection (bootstrap for paired samples t-test: $t_{(17)}$ = 3.4; p = .007; [.06 .21]). Moreover, rs-FC values after LPS administration were also significantly lower compared to the placebo group after saline injection (bootstrap for independent samples t-test: $t_{(37)}$ = 3.0; p = .005; [-.17 -.03]). The two groups did not differ from each other at baseline, i.e. before injection (p = .82; [-.07 .08], Figure 3). The same analysis on the CEN control network revealed no significant effects (p = .68; [-.02 .10]).

Figure 3

Correlation analyses on temporal variance, resting state functional connectivity and state anxiety

To investigate the putative role of changes in state anxiety during acute inflammation and intrinsic brain activity at rest, correlation analyses were carried out. Herein, analyses revealed a positive correlation between SD values in the amygdala and changes in state anxiety, at 3h post injection, in response to LPS administration (r = .58, $t_{(17)}$ = 2.8, p = .010). Conversely, rs-FC in the salience network was not correlated with changes in state anxiety during inflammation (r = .08, $t_{(17)}$ = -0.4, p = .730). Moreover, SD values in the amygdala and rs-FC in the salience network correlated negatively with each other (r = -.54, $t_{(17)}$ = -2.56, p = .020, Figure 4). Finally, a significant correlation was observed between the peak value of TNF-α and changes in state anxiety (r = .66, $t_{(17)}$ = 2.6, p = .010, Figure 4). To explore the specificity for state anxiety, the same correlation analyses were also carried out with positive mood scores which yielded no significance (data not shown).

Figure 4

DISCUSSION

In an effort to close the research gap regarding neural mechanisms underlying the connection between acute inflammation and increased anxiety in humans, we analyzed temporal variance of resting state activity and functional connectivity within the amygdala and salience network during experimental endotoxemia. In this double-blind and placebo controlled study in healthy men, low-dose LPS induced a systemic and transient immune activation reflected by increased plasma TNF-α and IL-6 concentrations, paralleled by significant changes in anxiety. Consistent with previous findings (Lasselin et al., 2016a; Reichenberg et al., 2001; Wegner et al., 2014), greater anxiety during endotoxemia was significantly correlated with concentrations of pro-inflammatory cytokines, supporting the role of inflammatory mediators in anxiety changes. These findings resemble the magnitude and time course of increased plasma cytokine levels induced through acute experimental endotoxemia as previously shown by our group (Engler et al., 2017; Grigoleit et al., 2010; Lasselin et al., 2016b; Wegner et al., 2014). Moreover, our findings demonstrated increased temporal variance specifically within the amygdala and decreased resting state functional connectivity between distinct nodes of the salience network.

As a powerful index of neural efficiency, increased temporal variance constitutes a proxy measure of system functioning efficiency. Indeed, it is positively associated to enhancement of long-distance functional connectivity, improvement of signal detection and information transfer as well as superior cognitive processing (Garrett et al., 2013; Garrett et al., 2018; McIntosh et al., 2010). Our finding of increased temporal variance specifically within the amygdala during inflammation could reflect increased preparedness of the organism to react to biologically-relevant threat requiring enhanced allocation of neural resources for restoring homeostasis. The amygdala plays a well-characterized role in emotion regulation as well as in the coordination of autonomic, behavioral and endocrine responses to threat (Tovote et al., 2015). Together with other subcortical and limbic structures, the amygdala is considered a major neural hub in the pathophysiology of anxiety- and fear-based disorders (Etkin and Wager, 2007;

Taylor and Whalen, 2015). Moreover, our results demonstrated that only the left, but not right amygdala resulted in higher temporal variance. Functional neuroimaging studies often report lateralization differences in amygdala activity subserving emotional functioning with a strong dominance of the left amygdala (Baas et al., 2004; Wager et al., 2003). However, these meta-analyses showed that the observed predominance of left amygdala activation is not associated with the task instructions, stimulus type or differential habituation rates and should therefore be interpreted with caution. The present data support a role of the amygdala in states of anxiety induced by acute inflammation in humans, complementing evidence from animal studies that pro-inflammatory cytokines released during inflammation impact amygdala function (Doenlen et al., 2011; Goehler et al., 2007; Prager et al., 2013), as evidenced by enhanced neuronal activity and cytokine mRNA expression in the amygdala in rodents (Engler et al., 2011).

Thus far, only three resting state fMRI studies have addressed changes in functional connectivity associated with inflammatory markers (Labrenz et al., 2016; Lekander et al., 2016; Marsland et al., 2017), but none of them addressed the putative role of the salience network in anxiety. We now document a decrease in functional connectivity between nodes of the salience network and amygdala during inflammation, including reduced connectivity between amygdala and anterior insula. The salience network represents a set of mainly subcortical brain regions implicated in emotion, reward and homeostatic regulation (Craig, 2009; Menon and Levitin, 2005; Ongür and Price, 2000). These regions work well-synchronized to identify relevant events, flexibly guide and adapt behavior and together with interconnected brain networks integrate sensory, cognitive and emotional information (Menon and Uddin, 2010; Seeley et al., 2007b), all of which are demonstrably sensitive to acute inflammatory events (Craig, 2009). More specifically, two distinct functions are ascribed to the salience network involving different neural circuits. Salience processing is triggered by amygdala-related circuits and the dorsal anterior cingulate cortex (dACC) (Baur et al., 2013; Geng et al., 2015; Rabany et al., 2017). A distinct circuit encompassing the

anterior insula has been implicated in top-down directed control and the mediation of dynamic interactions between networks aimed to integrate externally oriented attention from bottom-up channels with internally oriented cognitions (Menon and Uddin, 2010). Likewise, the anterior insula is also linked to emotional awareness and has been implicated in interoceptive predictive coding. Considering our finding of increased temporal variance in the amygdala, it does not appear likely that concurrent salience network decoupling indicates a decrease in salience processing. Instead, our results may rather reflect a decrease in control initiated by the anterior insula during acute inflammation, which could conceivably contribute to greater anxiety. Moreover, neural communication between anterior insula and associative cortices could converge to an interoceptive neural representation within the amygdala-insula circuit that may evoke increased state anxiety. Similar to the result of a lateralization effect in amygdala temporal variance, we also observed a predominance of left amygdala-anterior insula functional connectivity decreasing under acute immune challenge. Functional lateralization of the anterior insula has often been observed for interoception, autonomic activity and cognitive processes (Kann et al., 2016). Neuroimaging studies demonstrated that the left anterior insula is implicated in behavioral modulation through increased top-down control (Ham et al., 2013; Späti et al., 2014) and that the left anterior insula demonstrated higher neural activation in men in response to emotional stimuli (Duerden et al., 2013; Wager et al., 2003). Together, our results support the notion that inflammatory markers may contribute to the maintenance and exacerbation of anxiety-related symptoms by affecting the neural activity and connectivity of the amygdala at the center of the brain's fear and salience circuitry. Functional neuroimaging studies revealed that increased inflammation is associated with enhanced activation of the threat- and anxiety-related brain circuitry and specifically the amygdala, e.g. in response to negative social cues (Inagaki et al., 2012; Redlich et al., 2015), social and early life stress (Muscatell et al., 2015; Redlich et al., 2015) but also to positive feedback as a form of social reward (Muscatell et al., 2016). While disturbances in the intrinsic connectivity of the salience network were reported in

anxiety patients (Peterson et al., 2014), we could not establish an association between functional connectivity in the salience network and inflammation-induced changes in state anxiety in our sample of healthy volunteers.

This study should be interpreted in the light of its strength and limitations. We implemented a combination of innovative techniques to address neural mechanisms involved in changes of state anxiety in a translational model of acute systemic inflammation. Using a randomized, double-blind study design, the effects of inflammation on BOLD signal variability and functional connectivity were confirmed both intra-individually against a baseline condition as well as inter-individually against a placebo group supporting the robustness of findings. Furthermore, we tested the specificity of results for anxiety and anxiety-related brain networks that allowed us to exclude associations between neural measures and negative (depression-like) mood as a common, but not anxiety-related psychological state during systemic inflammation. However, a limitation of the study includes the comparably small sample size bearing the risk of false negative results especially for correlation analyses. Further, we could only include male volunteers. Given the evidence of sex differences in the prevalence of anxiety disorders and the role of sex differences in the interaction between serotonin and the amygdala affecting the expression of anxiety symptoms, e.g. (Cerasa et al., 2013), including women would have provided more insights into the proposed impact of pro-inflammatory responses onto state anxiety during acute immune challenge. Finally, the LPS model we used herein is a translational model of acute inflammation and effects may not reflect processes engaged during chronic inflammation and therefore call for further research.

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Conflict of interest statement

All authors declare no conflicts of interest.

References

Baas, D., Aleman, A., Kahn, R.S., 2004. Lateralization of amygdala activation: A systematic review of functional neuroimaging studies. Brain Res. Rev. 45, 96–103. https://doi.org/10.1016/j.brainresrev.2004.02.004

Baldassarre, A., Ramsey, L., Hacker, C.L., Callejas, A., Astafiev, S. V, Metcalf, N. V, Zinn, K., Rengachary, J., Snyder, A.Z., Carter, A.R., Shulman, G.L., Corbetta, M., 2014. Large-scale changes in network interactions as a physiological signature of spatial neglect. Brain 137, 3267–83. https://doi.org/10.1093/brain/awu297

Baldassarre, A., Ramsey, L., Rengachary, J., Zinn, K., Siegel, J.S., Metcalf, N. V, Strube, M.J., Snyder, A.Z., Corbetta, M., Shulman, G.L., 2016. Dissociated functional connectivity profiles for motor and attention deficits in acute right-hemisphere stroke. Brain 139, 2024–38. https://doi.org/10.1093/brain/aww107

Bandelow, B., Michaelis, S., 2015. Epidemiology of anxiety disorders in the 21st century. Dialogues Clin. Neurosci. 17, 327–35.

Bassi, G.S., Kanashiro, A., Santin, F.M., de Souza, G.E.P., Nobre, M.J., Coimbra, N.C., 2012. Lipopolysaccharide-Induced Sickness Behaviour Evaluated in Different Models of Anxiety and Innate Fear in Rats. Basic Clin. Pharmacol. Toxicol. 110, 359–369. https://doi.org/10.1111/j.1742-7843.2011.00824.x

Baur, V., Hänggi, J., Langer, N., Jäncke, L., 2013. Resting-state functional and structural connectivity within an insula-amygdala route specifically index state and trait anxiety. Biol. Psychiatry 73, 85–92. https://doi.org/10.1016/j.biopsych.2012.06.003

Benson, S., Rebernik, L., Wegner, A., Kleine-Borgmann, J., Engler, H., Schlamann, M., Forsting, M., Schedlowski, M., Elsenbruch, S., 2015. Neural circuitry mediating inflammation-induced central pain amplification in human experimental endotoxemia. Brain. Behav. Immun. 48, 222–31. https://doi.org/10.1016/j.bbi.2015.03.017

Broyd, S.J., Demanuele, C., Debener, S., Helps, S.K., James, C.J., Sonuga-Barke, E.J.S., 2009. Default-mode brain dysfunction in mental disorders: A systematic review. Neurosci. Biobehav. Rev. 33, 279–296. https://doi.org/10.1016/j.neubiorev.2008.09.002

Buzs_aki, G., & Draguhn, A. (2004). Neuronal oscillations in cortical networks. Science (New York, N.Y.), 304, 1926–1929.

Cerasa, A., Quattrone, A., Piras, F., Mangone, G., Magariello, A., Fagioli, S., Girardi, P., Muglia, M., Caltagirone, C., Spalletta, G., 2013. 5-HTTLPR, anxiety and gender interaction moderates right amygdala volume in healthy subjects. Soc. Cogn. Affect. Neurosci. 9, 1537–1545. https://doi.org/10.1093/scan/nst144

Chai, X.J., Castañón, A.N., Ongür, D., Whitfield-Gabrieli, S., 2012. Anticorrelations in resting state networks without global signal regression. Neuroimage 59, 1420–8. https://doi.org/10.1016/j.neuroimage.2011.08.048

Coutinho, J.F., Fernandesl, S.V., Soares, J.M., Maia, L., Gonçalves, Ó.F., Sampaio, A., 2016. Default mode network dissociation in depressive and

anxiety states. Brain Imaging Behav. 10, 147–57. https://doi.org/10.1007/s11682-015-9375-7

Cox, R.W., 1996. AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. Comput. Biomed. Res. 29, 162–73. https://doi.org/10.1006/cbmr.1996.0014

Craig, A.D.B., 2009. How do you feel--now? The anterior insula and human awareness. Nat. Rev. Neurosci. 10, 59–70. https://doi.org/10.1038/nrn2555

Doenlen, R., Krugel, U., Wirth, T., Riether, C., Engler, A., Prager, G., Engler, H., Schedlowski, M., Pacheco-Lopez, G., 2011. Electrical activity in rat cortico-limbic structures after single or repeated administration of lipopolysaccharide or staphylococcal enterotoxin B. Proc. R. Soc. B Biol. Sci. 278, 1864–1872. https://doi.org/10.1098/rspb.2010.2040

Duerden, E.G., Arsalidou, M., Lee, M., Taylor, M.J., 2013. Lateralization of affective processing in the insula. Neuroimage 78, 159–175. https://doi.org/10.1016/j.neuroimage.2013.04.014

Engler, H., Brendt, P., Wischermann, J., Wegner, A., Röhling, R., Schoemberg, T., Meyer, U., Gold, R., Peters, J., Benson, S., Schedlowski, M., 2017. Selective increase of cerebrospinal fluid IL-6 during experimental systemic inflammation in humans: association with depressive symptoms. Mol. Psychiatry 1–7. https://doi.org/10.1038/mp.2016.264

Engler, H., Doenlen, R., Engler, A., Riether, C., Prager, G., Niemi, M.-B., Pacheco-López, G., Krügel, U., Schedlowski, M., 2011. Acute amygdaloid response to systemic inflammation. Brain. Behav. Immun. 25, 1384–1392. https://doi.org/10.1016/j.bbi.2011.04.005

Etkin, A., Wager, T.D., 2007. Functional Neuroimaging of Anxiety: A Meta-Analysis of Emotional Processing in PTSD, Social Anxiety Disorder, and Specific Phobia. Am. J. Psychiatry 164, 1476–1488. https://doi.org/10.1176/appi.ajp.2007.07030504

Felger, J.C., Lotrich, F.E., 2013. Inflammatory cytokines in depression: Neurobiological mechanisms and therapeutic implications. Neuroscience 246, 199–229. https://doi.org/10.1016/j.neuroscience.2013.04.060

Fernández, A., Gómez, C., Hornero, R., López-Ibor, J.J., 2013. Complexity and schizophrenia. Prog. Neuro-Psychopharmacology Biol. Psychiatry 45, 267–276. https://doi.org/10.1016/j.pnpbp.2012.03.015

Fox, M.D., Raichle, M.E., 2007. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. Nat. Rev. Neurosci. 8, 700–11. https://doi.org/10.1038/nrn2201

Fox, M.D., Snyder, A.Z., Vincent, J.L., Corbetta, M., Van Essen, D.C., Raichle, M.E., 2005. The human brain is intrinsically organized into dynamic, anticorrelated functional networks. Proc. Natl. Acad. Sci. U. S. A. 102, 9673–8. https://doi.org/10.1073/pnas.0504136102

Furtado, M., Katzman, M.A., 2015. Neuroinflammatory pathways in anxiety, posttraumatic stress, and obsessive compulsive disorders. Psychiatry Res. 229, 37–48. https://doi.org/10.1016/j.psychres.2015.05.036

Garrett, D.D., Kovacevic, N., McIntosh, A.R., Grady, C.L., 2011. The importance of being variable. J. Neurosci. 31, 4496–503.

https://doi.org/10.1523/JNEUROSCI.5641-10.2011
Garrett, D.D., Kovacevic, N., McIntosh, A.R., Grady, C.L., 2010. Blood oxygen level-dependent signal variability is more than just noise. J. Neurosci. 30, 4914–21. https://doi.org/10.1523/JNEUROSCI.5166-09.2010
Garrett, D.D., Samanez-Larkin, G.R., MacDonald, S.W.S., Lindenberger, U., McIntosh, A.R., Grady, C.L., 2013. Moment-to-moment brain signal variability: A next frontier in human brain mapping? Neurosci. Biobehav. Rev. 37, 610–624. https://doi.org/10.1016/j.neubiorev.2013.02.015
Garrett, D.D., Epp, S.M., Perry, A., Lindenberger, U. 2018. Local temporal variability reflects functional integration in the human brain. Neuroimage. 2018 Aug 24. pii: S1053-8119(18)30717-1. doi: 10.1016/j.neuroimage.2018.08.019. [Epub ahead of print]
Geng, H., Li, X., Chen, J., Li, X., Gu, R., 2015. Decreased Intra- and Inter-Salience Network Functional Connectivity is Related to Trait Anxiety in Adolescents. Front. Behav. Neurosci. 9, 350. https://doi.org/10.3389/fnbeh.2015.00350
Goehler, L.E., Lyte, M., Gaykema, R.P.A., 2007. Infection-induced viscerosensory signals from the gut enhance anxiety: Implications for psychoneuroimmunology. Brain. Behav. Immun. 21, 721–726. https://doi.org/10.1016/j.bbi.2007.02.005
Grigoleit, J., Oberbeck, J.R., Lichte, P., Kobbe, P., Wolf, O.T., Montag, T., Rey, A. del, Gizewski, E.R., Engler, H., Schedlowski, M., 2010. Lipopolysaccharide-induced experimental immune activation does not impair memory functions in humans. Neurobiol. Learn. Mem. 94, 561–567. https://doi.org/10.1016/j.nlm.2010.09.011
Hacker, C.D., Laumann, T.O., Szrama, N.P., Baldassarre, A., Snyder, A.Z., Leuthardt, E.C., Corbetta, M., 2013. Resting state network estimation in individual subjects. Neuroimage 82, 616–633. https://doi.org/10.1016/j.neuroimage.2013.05.108
Ham, T., Leff, A., de Boissezon, X., Joffe, A., Sharp, D.J., 2013. Cognitive Control and the Salience Network: An Investigation of Error Processing and Effective Connectivity. J. Neurosci. 33, 7091–7098. https://doi.org/10.1523/JNEUROSCI.4692-12.2013
Han, Y., Wang, J., Zhao, Z., Min, B., Lu, J., Li, K., He, Y., Jia, J., 2011. Frequency-dependent changes in the amplitude of low-frequency fluctuations in amnestic mild cognitive impairment: A resting-state fMRI study. Neuroimage 55, 287–295. https://doi.org/10.1016/j.neuroimage.2010.11.059
Harrison, N.A., Brydon, L., Walker, C., Gray, M.A., Steptoe, A., Critchley, H.D., 2009. Inflammation Causes Mood Changes Through Alterations in Subgenual Cingulate Activity and Mesolimbic Connectivity. Biol. Psychiatry 66, 407–414. https://doi.org/10.1016/j.biopsych.2009.03.015
He, B. J. (2011). Scale-free properties of the functional magnetic resonance imaging signal during rest and task. Journal of Neuroscience, 31, 13786–13795
Herrmann-Lingen, C., Buss, U., Snaith, R., 2005. Hospital Anxiety and

Depression scale - Deutsche Version, Second. ed. Hans Huber, Bern.
Hoptman, M.J., Zuo, X.N., Butler, P.D., Javitt, D.C., D'Angelo, D., Mauro, C.J., Milham, M.P., 2010. Amplitude of low-frequency oscillations in schizophrenia: A resting state fMRI study. Schizophr. Res. 117, 13–20. https://doi.org/10.1016/j.schres.2009.09.030
Hou, R., Baldwin, D.S., 2012. A neuroimmunological perspective on anxiety disorders. Hum. Psychopharmacol. Clin. Exp. 27, 6–14. https://doi.org/10.1002/hup.1259
Huang, Z., Dai, R., Wu, X., Yang, Z., Liu, D., Hu, J., Gao, L., Tang, W., Mao, Y., Jin, Y., Wu, X., Liu, B., Zhang, Y., Lu, L., Laureys, S., Weng, X., Northoff, G., 2014a. The self and its resting state in consciousness: An investigation of the vegetative state. Hum. Brain Mapp. 35, 1997–2008. https://doi.org/10.1002/hbm.22308
Huang, Z., Wang, Z., Zhang, J., Dai, R., Wu, J., Li, Y., Liang, W., Mao, Y., Yang, Z., Holland, G., Zhang, J., Northoff, G., 2014b. Altered temporal variance and neural synchronization of spontaneous brain activity in anesthesia. Hum. Brain Mapp. 35, 5368–5378. https://doi.org/10.1002/hbm.22556
Inagaki, T.K., Muscatell, K.A., Irwin, M.R., Cole, S.W., Eisenberger, N.I., 2012. Inflammation selectively enhances amygdala activity to socially threatening images. Neuroimage 59, 3222–6. https://doi.org/10.1016/j.neuroimage.2011.10.090
Kann, S., Zhang, S., Manza, P., Leung, H.-C., Li, C.-S.R., 2016. Hemispheric Lateralization of Resting-State Functional Connectivity of the Anterior Insula: Association with Age, Gender, and a Novelty-Seeking Trait. Brain Connect. 6, 724–734. https://doi.org/10.1089/brain.2016.0443
Kober, H., Barrett, L.F., Joseph, J., Bliss-Moreau, E., Lindquist, K., Wager, T.D., 2008. Functional grouping and cortical-subcortical interactions in emotion: a meta-analysis of neuroimaging studies. Neuroimage 42, 998–1031. https://doi.org/10.1016/j.neuroimage.2008.03.059
Labrenz, F., Wrede, K., Forsting, M., Engler, H., Schedlowski, M., Elsenbruch, S., Benson, S., 2016. Alterations in functional connectivity of resting state networks during experimental endotoxemia - An exploratory study in healthy men. Brain. Behav. Immun. 54, 17–26. https://doi.org/10.1016/j.bbi.2015.11.010
Lasselin, J., Elsenbruch, S., Lekander, M., Axelsson, J., Karshikoff, B., Grigoleit, J.-S., Engler, H., Schedlowski, M., Benson, S., 2016a. Mood disturbance during experimental endotoxemia: Predictors of state anxiety as a psychological component of sickness behavior. Brain. Behav. Immun. 57, 30–37. https://doi.org/10.1016/j.bbi.2016.01.003
Lasselin, J., Elsenbruch, S., Lekander, M., Axelsson, J., Karshikoff, B., Grigoleit, J., Engler, H., Schedlowski, M., Benson, S., 2016b. Mood disturbance during experimental endotoxemia: Predictors of state anxiety as a psychological component of sickness behavior. Brain. Behav. Immun. 57, 30–37. https://doi.org/10.1016/j.bbi.2016.01.003
Laux, L., Glanzmann, P., Schaffner, P., Spielberger, C., 1981. Das State-Trait-Angstinventar. Theoretische Grundlagen und Handanweisung. Beltz,

Weinheim.
Lekander, M., Karshikoff, B., Johansson, E., Soop, A., Fransson, P., Lundström, J.N., Andreasson, A., Ingvar, M., Petrovic, P., Axelsson, J., Nilsonne, G., 2016. Intrinsic functional connectivity of insular cortex and symptoms of sickness during acute experimental inflammation. Brain. Behav. Immun. 56, 34–41. https://doi.org/10.1016/j.bbi.2015.12.018
Marsland, A.L., Kuan, D.C.-H., Sheu, L.K., Krajina, K., Kraynak, T.E., Manuck, S.B., Gianaros, P.J., 2017. Systemic inflammation and resting state connectivity of the default mode network. Brain. Behav. Immun. 62, 162–170. https://doi.org/10.1016/j.bbi.2017.01.013
Martino, M., Magioncalda, P., Huang, Z., Conio, B., Piaggio, N., Duncan, N.W., Rocchi, G., Escelsior, A., Marozzi, V., Wolff, A., Inglese, M., Amore, M., Northoff, G., 2016. Contrasting variability patterns in the default mode and sensorimotor networks balance in bipolar depression and mania. Proc. Natl. Acad. Sci. U. S. A. 113, 4824–9. https://doi.org/10.1073/pnas.1517558113
McIntosh, A.R., Kovacevic, N., Lippe, S., Garrett, D., Grady, C., Jirsa, V., 2010. The development of a noisy brain. Arch. Ital. Biol. 148, 323–37.
Menon, V., Levitin, D.J., 2005. The rewards of music listening: response and physiological connectivity of the mesolimbic system. Neuroimage 28, 175–84. https://doi.org/10.1016/j.neuroimage.2005.05.053
Menon, V., Uddin, L.Q., 2010. Saliency, switching, attention and control: a network model of insula function. Brain Struct. Funct. 214, 655–67. https://doi.org/10.1007/s00429-010-0262-0
Muscatell, K.A., Dedovic, K., Slavich, G.M., Jarcho, M.R., Breen, E.C., Bower, J.E., Irwin, M.R., Eisenberger, N.I., 2015. Greater amygdala activity and dorsomedial prefrontal-amygdala coupling are associated with enhanced inflammatory responses to stress. Brain. Behav. Immun. 43, 46–53. https://doi.org/10.1016/j.bbi.2014.06.201
Muscatell, K.A., Moieni, M., Inagaki, T.K., Dutcher, J.M., Jevtic, I., Breen, E.C., Irwin, M.R., Eisenberger, N.I., 2016. Exposure to an inflammatory challenge enhances neural sensitivity to negative and positive social feedback. Brain. Behav. Immun. 57, 21–9. https://doi.org/10.1016/j.bbi.2016.03.022
O'Donovan, A., Hughes, B.M., Slavich, G.M., Lynch, L., Cronin, M.-T., O'Farrelly, C., Malone, K.M., 2010. Clinical anxiety, cortisol and interleukin-6: Evidence for specificity in emotion–biology relationships. Brain. Behav. Immun. 24, 1074–1077. https://doi.org/10.1016/j.bbi.2010.03.003
Ongür, D., Price, J.L., 2000. The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys and humans. Cereb. Cortex 10, 206–19. https://doi.org/10.1093/cercor/10.3.206
Pernet, C.R., Wilcox, R., Rousselet, G.A., 2012. Robust correlation analyses: false positive and power validation using a new open source matlab toolbox. Front. Psychol. 3, 606. https://doi.org/10.3389/fpsyg.2012.00606
Peterson, A., Thome, J., Frewen, P., Lanius, R.A., 2014. Resting-state neuroimaging studies: a new way of identifying differences and similarities among the anxiety disorders? Can J Psychiatry. 59(6):294-300. https://doi.org/10.1177/070674371405900602

Poser, B.A., Versluis, M.J., Hoogduin, J.M., Norris, D.G., 2006. BOLD contrast sensitivity enhancement and artifact reduction with multiecho EPI: parallel-acquired inhomogeneity-desensitized fMRI. Magn. Reson. Med. 55, 1227–35. https://doi.org/10.1002/mrm.20900

Prager, G., Hadamitzky, M., Engler, A., Doenlen, R., Wirth, T., Pacheco-López, G., Krügel, U., Schedlowski, M., Engler, H., 2013. Amygdaloid Signature of Peripheral Immune Activation by Bacterial Lipopolysaccharide or Staphylococcal Enterotoxin B. J. Neuroimmune Pharmacol. 8, 42–50. https://doi.org/10.1007/s11481-012-9373-0

Rabany, L., Diefenbach, G.J., Bragdon, L.B., Pittman, B.P., Zertuche, L., Tolin, D.F., Goethe, J.W., Assaf, M., 2017. Resting-State Functional Connectivity in Generalized Anxiety Disorder and Social Anxiety Disorder: Evidence for a Dimensional Approach. Brain Connect. 7, 289–298. https://doi.org/10.1089/brain.2017.0497

Raichle, M.E., 2011. The restless brain: how intrinsic activity organizes brain function. Philos. Trans. R. Soc. B Biol. Sci. 370, 20140172–20140172. https://doi.org/10.1098/rstb.2014.0172

Raja Beharelle, A., Kovačević, N., McIntosh, A.R., Levine, B., 2012. Brain signal variability relates to stability of behavior after recovery from diffuse brain injury. Neuroimage 60, 1528–1537. https://doi.org/10.1016/j.neuroimage.2012.01.037

Redlich, R., Stacey, D., Opel, N., Grotegerd, D., Dohm, K., Kugel, H., Heindel, W., Arolt, V., Baune, B.T., Dannlowski, U., 2015. Evidence of an IFN-γ by early life stress interaction in the regulation of amygdala reactivity to emotional stimuli. Psychoneuroendocrinology 62, 166–73. https://doi.org/10.1016/j.psyneuen.2015.08.008

Reichenberg, A., Yirmiya, R., Schuld, A., Kraus, T., Haack, M., Morag, A., Pollmächer, T., 2001. Cytokine-Associated Emotional and Cognitive Disturbances in Humans. Arch. Gen. Psychiatry 58, 445. https://doi.org/10.1001/archpsyc.58.5.445

Rousselet, G.A., Pernet, C.R., 2012. Improving standards in brain-behavior correlation analyses. Front. Hum. Neurosci. 6, 119. https://doi.org/10.3389/fnhum.2012.00119

Seeley, W.W., Menon, V., Schatzberg, A.F., Keller, J., Glover, G.H., Kenna, H., Reiss, A.L., Greicius, M.D., 2007a. Dissociable intrinsic connectivity networks for salience processing and executive control. J. Neurosci. 27, 2349–56. https://doi.org/10.1523/JNEUROSCI.5587-06.2007

Seeley, W.W., Menon, V., Schatzberg, A.F., Keller, J., Glover, G.H., Kenna, H., Reiss, A.L., Greicius, M.D., 2007b. Dissociable intrinsic connectivity networks for salience processing and executive control. J. Neurosci. 27, 2349–56. https://doi.org/10.1523/JNEUROSCI.5587-06.2007

Shew, W.L., Yang, H., Petermann, T., Roy, R., Plenz, D., 2009. Neuronal Avalanches Imply Maximum Dynamic Range in Cortical Networks at Criticality. J. Neurosci. 29, 15595–15600. https://doi.org/10.1523/JNEUROSCI.3864-09.2009

Späti, J., Chumbley, J., Brakowski, J., Dörig, N., Grosse Holtforth, M., Seifritz, E.,

Spinelli, S., 2014. Functional lateralization of the anterior insula during feedback processing. Hum. Brain Mapp. 35, 4428–4439. https://doi.org/10.1002/hbm.22484

Steyer, R., Schwenkmezger, P., Notz, P., Eid, M., 1997. Der Mehrdimensionale Befindlichkeitsfragebogen (MDBF). Handanweisung. Hogrefe, Göttingen.

Sylvester, C., Corbetta, M., Raichle, M.E., Rodebaugh, T., Schlaggar, B.L., Sheline, Y., Zorumski, C.F., Lenze, E.J., 2012. Functional network dysfunction in anxiety and anxiety disorders. 35, 527–535. https://doi.org/10.1016/j.tins.2012.04.012.Functional

Tagliazucchi, E., von Wegner, F., Morzelewski, A., Brodbeck, V., Jahnke, K., & Laufs, H. (2013). Breakdown of long-range temporal dependence in default mode and attention networks during deep sleep. Proceedings of the National Academy of Sciences of the United States of America, 110, 15419–15424.

Talairach, J., Tournoux, P., 1988. Co-planar stereotaxic atlas of the human brain. Thieme, New York.

Taylor, J.M., Whalen, P.J., 2015. Neuroimaging and Anxiety: the Neural Substrates of Pathological and Non-pathological Anxiety. Curr. Psychiatry Rep. 17, 49. https://doi.org/10.1007/s11920-015-0586-9

Tovote, P., Fadok, J.P., Lüthi, A., 2015. Neuronal circuits for fear and anxiety. Nat. Rev. Neurosci. 16, 317–331. https://doi.org/10.1038/nrn3945

Vogelzangs, N., Beekman, A.T.F., de Jonge, P., Penninx, B.W.J.H., 2013. Anxiety disorders and inflammation in a large adult cohort. Transl. Psychiatry 3, e249. https://doi.org/10.1038/tp.2013.27

Wager, T.D., Phan, K.L., Liberzon, I., Taylor, S.F., 2003. Valence, gender, and lateralization of functional brain anatomy in emotion: A meta-analysis of findings from neuroimaging. Neuroimage 19, 513–531. https://doi.org/10.1016/S1053-8119(03)00078-8

Wegner, A., Elsenbruch, S., Maluck, J., Grigoleit, J.-S., Engler, H., Jäger, M., Spreitzer, I., Schedlowski, M., Benson, S., 2014. Inflammation-induced hyperalgesia: Effects of timing, dosage, and negative affect on somatic pain sensitivity in human experimental endotoxemia. Brain. Behav. Immun. 41, 46–54. https://doi.org/10.1016/j.bbi.2014.05.001

Wilcox, R., 2004. Inferences Based on a Skipped Correlation Coefficient. J. Appl. Stat. 31, 131–143. https://doi.org/10.1080/0266476032000148821

Wright, C.E., Strike, P.C., Brydon, L., Steptoe, A., 2005. Acute inflammation and negative mood: Mediation by cytokine activation. Brain. Behav. Immun. 19, 345–350. https://doi.org/10.1016/j.bbi.2004.10.003

Xi, Q., Zhao, X., Wang, P., Guo, Q., Jiang, H., Cao, X., He, Y., Yan, C., 2012. Spontaneous brain activity in mild cognitive impairment revealed by amplitude of low-frequency fluctuation analysis: A resting-state fMRI study. Radiol. Medica 117, 865–871. https://doi.org/10.1007/s11547-011-0780-8

Zhang, D., Raichle, M.E., 2010. Disease and the brain's dark energy. Nat. Rev. Neurol. 6, 15–28. https://doi.org/10.1038/nrneurol.2009.198

Zuo, X.-N., Di Martino, A., Kelly, C., Shehzad, Z.E., Gee, D.G., Klein, D.F., Castellanos, F.X., Biswal, B.B., Milham, M.P., 2010. The oscillating brain: complex and reliable. Neuroimage 49, 1432–45.

https://doi.org/10.1016/j.neuroimage.2009.09.037

Figure legends

Figure 1 Regions of interest were located in the nodes of the salience network and the amygdala.

Figure 2 Changes in temporal variance in the amygdala due to LPS induced inflammation. Results from planned bootstrapped t-tests. *p < .05.

Figure 3 Strength of resting state functional connectivity in the salience network in the placebo and LPS group before and after injection. Connections that changed significantly (p < .05, FDR corrected) are shown in red; connections that did not change are shown in dashed grey. ROIs: amygdala (Amy), parietal lobe (Par), anterior insula (Ins), and anterior prefrontal cortex (aPFC) bilaterally, and the dorsal anterior cingulum (dACC). (A) Placebo vs LPS pre injection; (B) Placebo vs LPS post injection; (C) Placebo pre vs post injection; (D) LPS pre vs post injection; (E) aggregated values of resting-state functional connectivity (rs-FC) for all ROIs.

Figure 4 Correlation analyses within LPS group. (A) Correlation between temporal variance in the amygdala and changes in state anxiety during inflammation. (B) Correlation between rs-FC in the salience network and changes in state anxiety during inflammation. (C) Correlation between rs-FC in the salience network and temporal variance in the amygdala during inflammation. (D) Correlation between peak value of TNF-α and changes in state anxiety during inflammation.

Table 1 Sample characteristics

Group	Age	BMI	HADS A	HADS D	STAI T
LPS	26.15 ± 4.13	23.18 ± 2.89	2.80 ± 2.38	1.25 ± 1.33	29.85 ± 5.54
Placebo	26.30 ± 3.13	23.71 ± 2.73	3.13 ± 2.34	0.96 ± 0.98	32.96 ± 7.25

Sample characteristics for the LPS and placebo group separately. All values are given as mean ± standard deviation (M ± SD). BMI, body mass index; HADS, Hospital Anxiety and Depression Scale, anxiety (A) and depression (D) score, STAI T, State Trait Anxiety Inventory, trait subscale. LPS and placebo groups did not differ in any variable.

Table 2 ROI coordinates

Brain region	ROI name	MNI (x, y, z)
Left Amygdala	LH Amy	-22, -6, -20
Right Amygdala	RH Amy	20, -4, -20
Dorsal Anterior cingulate	dACC	0, 21, 36
Left Anterior Prefrontal Cortex	LH aPFC	-35, 45, 30
Right Anterior Prefrontal Cortex	RH aPFC	32, 45, 30
Left Insula	LH Ins	-41, 3, 6
Right Insula	RH Ins	41, 3, 6
Left Lateral Parietal	LH Par	-62, -45, 30
Right Lateral Parietal	RH Par	62, -45, 30

Coordinates for left and right amygdala were derived from (Kober et al., 2008), other ROI coordinates were derived from (Raichle, 2011).