Association of stress-related limbic activity and baseline interleukin-6 plasma levels in healthy adults

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Abstract

There is a bidirectional interaction between peripheral inflammatory processes and the brain. In the context of stress, top-down effects on the immune system have already been studied, but bottom-up effects of peripheral inflammation on neural processes are still unclear. In the present study, baseline plasma levels of the cytokine interleukin (IL)-6 were obtained as a measure of peripheral inflammatory processes and psychosocial stress was induced in 78 healthy adults during functional magnetic resonance imaging. The association of IL-6 with stress-related neural activation in the amygdala, anterior insula, and anterior cingulate cortex as regions of interest was explored. In order to account for individual differences in the stress response, salivary cortisol increase was also considered in the analyses. Stress task-related neural activity in the amygdala and anterior insula was significantly and negatively associated with IL-6 levels only in participants showing a cortisol response. These results suggest that peripheral inflammatory processes might affect the evaluation of a social stressor and highlight that individual differences in stress task responses should be considered in future studies.

Keywords: cytokine; interleukin-6; inflammation; social threat; stress; Montreal Imaging Stress Task; fMRI; cortisol; amygdala; anterior insula

1. Introduction

Peripheral inflammatory processes interact with the brain bidirectionally (Kraynak et al., 2018): Bottom-up pathways signal changes in peripheral inflammatory activity to the brain, particularly via the vagus nerve and cytokines (Dantzer et al., 2008) – immune mediators that cross the blood-brain barrier (Banks, 2015; Vitkovic et al., 2000). These inflammatory signals influence neural processes such as synaptic plasticity or neurotransmitter metabolism (Miller et al., 2009). On the other hand, the brain regulates peripheral inflammatory processes via top-down pathways – mainly via the sympathetic nervous system (SNS) and the hypothalamus-pituitary-adrenal cortex axis (HPA axis) (Eisenberger and Cole, 2012; Irwin and Cole, 2011). The current study aims to investigate bottom-up effects of subclinical peripheral inflammatory processes on neural stress processing.

While these bottom-up effects are not well understood yet, top-down effects of stress on the immune system have already been investigated in numerous studies. When confronted with an external stressor (e.g., psychosocial stress), the brain evaluates the potential danger and coordinates an evolutionary developed reaction (McEwen, 2007), which involves an activation of the immune system (Slavich and Irwin, 2014). This immune activation in response to perceived acute threat is mainly exerted by the SNS initiating a release of cytokines into the blood which coordinate a subsequent immune response (Bierhaus et al., 2003). Consequently, short-term laboratory psychosocial stress protocols have reliably shown to produce an increase in peripheral cytokine levels, especially of interleukin-6 (IL-6) and interleukin-1 beta (IL-1 beta) (Marsland et al., 2017; Steptoe et al., 2007). In contrast, the HPA axis, which (in healthy conditions) is also activated in response to an external stressor, acts predominantly anti-inflammatory (Strehl et al., 2019). Since its effects have a slower onset than SNS effects, acute inflammation is downregulated and an excessive immune response is prevented (Kunz-Ebrecht et al., 2003; Thoma et al., 2017).

On the level of neural systems, it is assumed that the same brain areas are involved in both bottom-up and top-down immune pathways – forming a network that is critical for feedback loops between the brain and the body (Kraynak et al., 2018). It is not yet clear which specific brain regions are involved in this network, but in recent years, a number of studies have investigated the neural correlates of peripheral inflammatory processes. Most of these studies either induced an inflammatory response or correlated baseline inflammatory parameters with task-related brain activity or measures of connectivity during resting-state, using either functional magnetic resonance imaging (fMRI) or positron emission tomography (PET) (see Kraynak et al., 2018 for a meta-analysis of previous studies). Only very few studies have directly induced acute psychosocial stress during scanning and linked the brain's stress response to inflammatory parameters. One fMRI study examined the neural response to negative social feedback by an alleged interaction partner (who evaluated the participant with negative, positive, and neutral adjectives based on an a-priori recorded interview) and related IL-6 changes (Muscatell et al., 2015). The authors found significant correlations between the activity of the left amygdala, subgenual anterior cingulate cortex (ACC), and left middle temporal gyrus and IL-6 increases from before to after scanning. Other studies have used bacterial endotoxins to induce peripheral inflammation before fMRI scanning and reported increased activity in the bilateral amygdala, dorsal ACC, and dorsomedial prefrontal cortex (dmPFC) during processing of negative feedback (Muscatell et al., 2016) and increased amygdala activity in response to fearful faces (Inagaki et al., 2012). Furthermore, endotoxin-induced increases of IL-6 were reported to correlate with the activity of the anterior insula, amygdala, and dmPFC during negative feedback (Muscatell et al., 2016) and activity of the anterior insula and dorsal ACC during a game simulating social exclusion (Eisenberger et al., 2009). In addition to these studies using a bacterial endotoxin, one study experimentally induced acute social stress to induce peripheral inflammation before fMRI scanning and examined the relationship between stress-associated increases in inflammatory parameters and

neural activity in a social exclusion setting (Slavich et al., 2010). The authors found that increases in the soluble cytokine receptor sTNFαRII correlated significantly with the activity in the dorsal ACC and anterior insula and changes in IL-6 were marginally related to the activation of the right anterior insula.

While the studies mentioned above are all concerned with the acute changes of inflammatory parameters after a stressor was applied, no study has investigated the association between baseline cytokine levels and social stress task-related neural activity. There are nevertheless reasons to assume a relationship between basal IL-6 levels and stress-related brain activity. Constantly elevated IL-6 levels might alter the brain's perception of the social context (Eisenberger et al., 2017), since they enter the brain directly (Banks, 2015) and interact with a variety of neural processes (Dantzer et al., 2008). For instance, Eisenberger and colleagues suggested that pro-inflammatory cytokines such as IL-6 (via several mechanisms) may evoke increases in the brain's sensitivity for information from the direct social environment (Eisenberger et al., 2017). In the course of an infection, this could help to distinguish between persons who can provide care and help to recover more easily and others who are more likely to harm and delay recuperation. However, two previous studies contradict this assumption by reporting negative associations between baseline pro-inflammatory cytokines (IL-6 and IL-1 beta) and neural activity to pleasant pictures (IL-6 (Alvarez et al., 2020)) and social exclusion (IL-1 beta (Conejero et al., 2019)). In this context, it is important to note that the theory of Eisenberger and coworkers does not distinguish between acute and chronic inflammatory processes. It is thus possible that heightened neural reactivity to threats may be true during acute immune activation, while the link between chronic inflammatory processes and threat responses may be of a different nature.

In conclusion, there is a lack of knowledge regarding the relationship between peripheral inflammatory processes and neural stress reactivity. The current study addresses this gap by examining baseline blood levels of IL-6 and neural stress reactivity. Acute social stress was

induced in healthy adults using the Montreal Imaging Stress Task (Dedovic et al., 2005). To investigate associations between chronic inflammatory processes and neural stress processing it is important to consider that the stress response depends on the context: It cannot simply be assumed that a given stress task is equally stressful for all participants. For this reason, it is of critical importance to consider individual, task-specific stress responses (for example SNS or HPA responses) when interpreting the neural correlates of the stress task and linking them to chronic inflammatory processes. Thus, we obtained cortisol measurements as an marker of inter-individual differences in stress reactivity (Hellhammer et al., 2009) and were thus able to distinguish HPA axis 'responders' from 'non-responders'.

The amygdala, anterior insula, and ACC were defined as regions of interest as they were found most consistently in previous studies on neural correlates of peripheral inflammatory processes and are also part of the networks involved in the neurobehavioral response to acute stress (see van Oort et al., 2017 for review). Thus, correlations of stress-associated brain activity in these regions with basal IL-6 levels as markers of peripheral inflammatory processes were explored.

2. Methods

2.1 Participants

Prior to the experiments, all participants gave informed and written consent. The study was approved by the local ethics committee at the University of Lübeck. Participants were recruited through local advertisement and contact addresses provided by the residents' registration office. As the current study was part of an interdisciplinary project which also examined ingestive behavior and obesity, participants had to meet the following inclusion criteria: scanner compatibility, no metabolic disorders, no current episode of major depression, mania or schizophrenia, no eating disorder, no alcohol misuse classified as ≥ 8 points in the alcohol use disorder identification test (Saunders et al., 1993), and no intake of antidepressants, antipsychotics, antihistamines, beta blockers, corticosteroids, valproate, lithium, antiemetics,

laxatives, amphetamines, or other stimulants. For the present analysis, all participants (N=100) who took part in fMRI scanning and who provided plasma samples were included. Eighteen participants had to be excluded from the analysis due to premature termination of the task, technical problems during the task, or very slow responses (three standard deviations slower than the mean of all participants). Additionally, three participants were excluded who suspected that the task aimed at inducing stress and thus did not feel stressed. Furthermore, one participant was excluded from further analyses because double measurements of IL-6 deviated more than 30 percent from each other. The resulting sample consisted of N= 78 participants (53 female). Participants' age ranged from 29 to 54 years (mean age 41.6 ± 7.63 years), the mean Body Mass Index (BMI) was 26.2 ± 4.83. For cortisol and questionnaire measures, there were missing values (see S1 for details) so that analyses on these parameters were performed for slightly smaller samples sizes (N= 74 - N= 77). Regarding IL-6 levels, one subject was found to be an extreme outlier (more than seven SD from the mean of all subjects) and was thus excluded from all analyses involving IL-6, resulting in a sample size of N= 77 for these analyses.

2.2 Procedure

The study took place on two different days and consisted of several experiments that are not all part of this publication (e.g., tests on eating behavior). On one of the days, fasting blood samples were drawn and body fat was determined. This was always scheduled in the morning (between 8 am and 10.30 am) to reduce circadian influences. On the other day, psychosocial stress was induced during fMRI scanning. For organizational reasons, this investigation could not always be performed at the same time of day. Before and immediately after stress induction, saliva samples were obtained for cortisol measurement and participants filled in the Positive and Negative Affect Schedule (PANAS, Watson et al., 1988) as a measure of the affective response to stress. Furthermore, participants completed the Trier Inventory for Chronic Stress (TICS, Schulz et al., 2004) and the Stress Reactivity Scale (SRS, Schulz et al., 2005) to obtain measures

for chronic stress in the last three months and stress reactivity in the sense of a disposition that underlies individual differences in stress reaction (see below for details).

2.3 Stress induction

For stress induction, a modified version of the Montreal Imaging Stress Task (MIST) by Dedovic and colleagues was used (Dedovic et al., 2005). Participants were supposed to solve mental arithmetical problems of varying difficulty. The task was performed in three runs, in which two blocks of stress conditions alternated with a control condition. In the stress conditions, the time limit for solving the questions was adapted to the participant's performance in order to reduce the success rate in comparison to the control condition. A bar on the screen visualized the time that had already elapsed while processing the tasks. Arrows indicated the participant's own performance in comparison to a reference group (please see supplementary section S2 for an example of the screen). To increase pressure, the experimenter provided negative feedback on the participants' performance by telling them via speaker: 'You unfortunately still make many mistakes.' If a participant solved a row of tasks correctly, the feedback was reformulated: 'Unfortunately you are not fast enough.' Time constraints of the task and especially the social evaluative component were supposed to induce psychosocial stress in the stress condition (Dickerson and Kemeny, 2004). During the control condition, there was no time limit and no feedback on task performance. The two blocks of stress condition were initially planned to consider differences between two distinct stress conditions: In one condition, a frame around the screen indicated that an audience of three persons of the study staff was watching the participant's performance live. In the other condition, this component was missing. Since we did not assume that this experimental manipulation would affect the research question followed in this publication, data of these two conditions were collapsed.

The experiment was run in a fixed order and lasted on average 24 minutes. Before scanning, the participants practiced the task in order to become familiar with the procedure.

Two parameters were used as behavioral measures of performance: The mean reaction time required to correctly solve the arithmetic tasks and the percentage of correctly solved tasks. To investigate stress-related changes in performance, we compared the mean of the control condition to the mean of the stress condition for both reaction time and percentage of correctly answered tasks.

2.4 Stress questionnaires

As a measure of subjective distress during the experiment, positive and negative affect was obtained before and after stress induction using the PANAS (Watson et al., 1988). The PANAS comprises ten positive and ten negative adjectives to describe the current affective state on a five-point scale. Positive and negative affect obtained before stress induction was subtracted from the score after scanning to get a measure of affective response to the MIST.

To assess perceived chronic stress during the last three months, participants filled out the TICS (Schulz et al., 2004). The questionnaire consists of 57 items with subscales for different types of stress and is answered to on a five-point Likert scale. The 12-item screening scale of the TICS was used as a global measure for experienced stress.

Dispositional stress reactivity was measured with the SRS, that consists of 29 items with three response options (Schulz et al., 2005). Stress reactivity is herein defined as a constitutional trait that relatively stable constitutes the individual reaction to stressors (Schlotz et al., 2011).

2.5 Cortisol

Cortisol was assessed from saliva samples collected 30 minutes after the participant's arrival and immediately after scanning. Saliva samples were frozen and stored at -20 °C until they were sent to the lab of Dresden LabService GmbH. After thawing, salivettes were centrifuged at 3000 rpm for five minutes, which resulted in a clear supernatant of low viscosity. Salivary

concentrations were measured by commercially available chemiluminescence immunoassay with high sensitivity (IBL International, Hamburg, Germany).

2.6 IL-6

Immediately after collection, blood samples were centrifuged at 2000 g for ten minutes at 4 °C and plasma was then stored at -80 °C until further analysis. Interleukin-6 was measured en-bloc using a commercially available high-sensitive ELISA (R&D Systems, Minneapolis). No samples exceeded the upper or lower limit of detection. Measurement was performed in double value determination. For subsequent analyses, only values with a double value deviation <30% were included.

2.7 fMRI scanning and data processing

Participants were scanned on a 3 Tesla MAGNETOM Skyra MR tomograph (Siemens) equipped with a 64-channel head-coil at the Center of Brain, Behavior and Metabolism (CBBM) in Lübeck, Germany. A BOLD sensitive echo planar imaging (EPI) sequence was used for acquisition of functional volumes during the experiment (TR = 1.5 s, TE = 25.0 ms, flip angle = 80°, 56 interleaved/ascending slices, slice thickness = 3 mm, FOV= 512x512). The number of scans varied between the participants as they needed different amounts of time for the task (mean per run= 309 scans ±22). Additionally, structural images of the whole brain were acquired (TR = 1.9s, TE = 2.44, flip angle = 9°, slice thickness = 1 mm, FOV = 256x256).

Functional MRI data analysis was performed using SPM12 (Welcome Department of Imaging Neuroscience, London, UK; http://www.fil.ion.ucl.ac.uk/spm) implemented in Matlab R2019b (Mathworks Inc., Sherborn, MA, USA). Functional images were slice timed and realigned to the mean image to correct for head motions. Next, they were normalized into MNI space using the standard segmentation as implemented in SPM12 on the mean functional image and tissue probability maps. Normalization parameters were then applied on all images of the time series.

Finally, functional images were spatially smoothed with an 8 mm full width half maximum isotropic Gaussian kernel and high-pass filtered at 1/256 Hz to remove low frequency drifts.

2.8 Statistical analysis of fMRI data

Statistical analysis of the fMRI data was performed in a two-level, mixed-effects procedure. The Generalized linear model (GLM) on the first level included regressors for the three conditions (control condition, two stress conditions) and the six motion parameters as regressors of no interest. Linear contrasts were computed for each participant that compared blood oxygenation level dependent (BOLD) signal during stress to the control condition. These contrast images of each participant were then used for random effects analyses at the group level. First, a one-sample t-test was calculated to assess stress task-related neural activation (stress conditions > control condition) in the whole sample. Second, to focus specifically on stress-related neural processes in individuals who showed a physiological stress response, a two-sample t-test with cortisol responders and non-responders was calculated and stress task-related neural activity (stress conditions > control condition) was compared between the two subgroups. Third, to investigate whether IL-6 levels affected stress-related neural activation, IL-6 was included as a covariate in the one-sample t-test (stress conditions > control condition) in the whole group and the two-sample t-test of cortisol responders and non-responders. The effect of IL-6 was contrasted between the two groups, and in case of significant group differences, the effect was considered separately for each group.

Based on previous literature on neural correlates of peripheral inflammatory processes, region-of-interest (ROI) analyses were performed in the amygdala, insular cortex, and ACC for the contrasts mentioned above. ROIs for the bilateral amygdala and the bilateral ACC were defined anatomically based on the Automated Anatomical Labeling Atlas (Tzourio-Mazoyer et al., 2002) with a dilation factor of one. The bilateral anterior insula ROI was based on the two anterior insula clusters from the three cluster solution by DiMartino and Kelly (2012).

If not mentioned otherwise, all results were corrected for multiple comparisons applying family-wise error (FWE) correction as implemented in SPM12. Overlap between neural activation and cytoarchitectonic areas was analyzed with the SPM ANATOMY toolbox v2.2c (Eickhoff et al., 2005). All results are reported in MNI space.

2.9 Statistical analyses of stress parameters

Statistical analyses were performed using the open source software Jamovi (The jamovi project, 2021). Shapiro-Wilk-tests were computed to test for normal distribution of all dependent variables.

First, to investigate whether the MIST task induced stress in the current sample, Wilcoxon signed-rank tests were computed to test for changes in cortisol and negative affect and a Student's t-test was calculated for changes in positive affect (before to after stress induction). As cortisol samples were not always taken at the same time and cortisol plasma levels have a circadian rhythm (Lee et al., 2015), we decided to not analyze stress-induced cortisol change quantitatively with regard to inter-individual differences but only qualitatively by building groups of cortisol responders (cortisol increase from baseline to post-stress >0) and non-responders as done in previous stress research (e.g., Pruessner et al., 2008). To assess possible circadian effects on cortisol levels, we performed Spearman correlations on the association of baseline levels as well as stress-related cortisol increase with the time of the day of saliva sampling. To explore whether the cortisol response was associated with the affective stress response, a Student's t-test was performed comparing cortisol responders and non-responders with regard to MIST-associated changes in positive affect and a Mann Whitney U test for changes in negative affect. With regard to behavioral performance measures of the MIST, a Wilcoxon signed-rank test (reaction time) and a Student's t-test (percentage of correctly answered tasks) were calculated to compare stress and control conditions.

Next, we investigated, whether IL-6 was related to the acute cortisol and affective reaction to stress as well as chronic stress levels and stress reactivity. To this end, we tested for group differences in IL-6 between cortisol responders and non-responders using Mann Whitney U testing and for associations between IL-6 and stress reactivity and chronic stress using non-parametric partial correlations. Age and percentage of fat mass were included as covariates in the correlational analyses as these variables have been shown to correlate with IL-6 in previous research (Hermsdorff et al., 2011; Himmerich et al., 2006) and in the present sample (for percentage of fat mass: r_s= .325, p= 0.004; for age marginally: r_s= .218, p= 0.055). As we hypothesized that higher IL-6 would be associated with higher levels of stress, we used one-tailed correlations. All reported results were considered to be significant at the $p < 0.05$ level.

3. Results

3.1. Stress task effects

On average, cortisol levels increased from before to after stress induction by 28.6% (see Table 1). However, this increase was not significant (W= 1450, p= 0.794) due to great individual differences: Only 43.6% of the participants showed increases in their cortisol levels ('cortisol responders'), the remaining 56.4% showed no alterations or even a decline in cortisol levels. The observed percentage of cortisol responders is similar to previous studies using the MIST (Khalili-Mahani et al., 2010; Wheelock et al., 2016). Cortisol baseline levels were significantly associated with the time of the day of saliva sampling (r_s = -.66, p< 0.001) but stress-related cortisol increase was not associated with time of the day (r_s = .15, p= 0.20). Furthermore, there was no difference between cortisol responders and non-responders with respect to the time of the day when the investigation took place (see S3 for details).

Positive affect significantly decreased after stress induction (t (73) = 7.12, p< 0.001), but there was no significant effect of stress induction on negative affect (W= 1042, p= 0.322). A decrease in positive affect was seen in the majority of participants (76,7%). Regarding task performance,

there was a significant decrease from control to stress condition in reaction time (W= 3081, $p< 0.001$) and correctly answered tasks ($t (77) = 22.7$, $p< 0.001$).

Stress task-associated change in positive affect was significantly higher in cortisol non-responders than in cortisol responders ($t (71) = 2.243$, $p=0.028$). There was no group difference regarding negative affect (U= 562, p= 0.274). For calculations on the association of task performance with the change in affect or cortisol response see supplement section S3.

3.2. Association of IL-6 with chronic stress levels and stress reactivity

There was no group difference between cortisol responders and non-responders in IL-6 levels (U= 635, p= 0.325). IL-6 did not correlate with questionnaire measures of stress reactivity (r_s= -0.09, p= 1.0), chronic stress (r_s= 0.05, p= 0.353), or change of affect after stress induction (positive affect: r_s=.16, p= 0.089; negative affect: r_s=-.00, p= 1.000).

3.3. Neural correlates of the stress task

When comparing stress to the control condition of the MIST, increased brain activity was found in the superior and middle temporal gyrus, calcarine gyrus, lingual gyrus, right insular cortex, temporal pole, (inferior orbital) frontal gyrus, cuneus, cerebellum, and thalamus (family-wise error (FWE) corrected for multiple comparisons; see Table 2 and S4), replicating previous findings (e.g., Lederbogen et al., 2011). In addition, analyses within our previously specified ROIs revealed significant effects in the bilateral amygdala and ACC and – as already found in the whole brain analysis – in the bilateral anterior insula (see Table 2). Since the focus of our study was the analysis of neural correlates of stress, we further distinguished between participants who showed a physiological stress response in terms of a cortisol increase and those who did not. A comparison of stress task-related activity between cortisol responders and non-responders revealed no difference.

3.4. Association of stress task-related neural activity and IL-6

In the whole group, there was no association of stress task-associated neural activity (stress > control condition) with IL-6 levels. To assess if the relationship between IL-6 and stress task-related activation in the ROIs was more pronounced in participants showing a physiological stress response, we contrasted cortisol responder and non-responder. Results showed a significantly stronger association of IL-6 levels with neural activation in the right amygdala (34, -2, -18, p= 0.046, t= 3.18) and a trend-wise effect for the right AI (40, 2, 4, p= 0.097, t= 3.18) for the cortisol responders compared to the non-responders. In the subgroup of cortisol responders, IL-6 levels were negatively correlated with neural activation in response to stress in the left and right insula (left: -36, 2, 6; right: 42, 2, 6) and the right amygdala (34, -2, -16; all values FWE corrected on a peak level, see Fig. 1 and Table 3), while there was no correlation in cortisol non-responders.

To better understand this group difference, scatterplots of parameter estimates (extracted at the peak activation with a sphere of 6 mm) were generated, which showed that in the non-responder group, three individuals with comparatively high IL-6 levels had a large influence on the group difference in correlation (see S5).

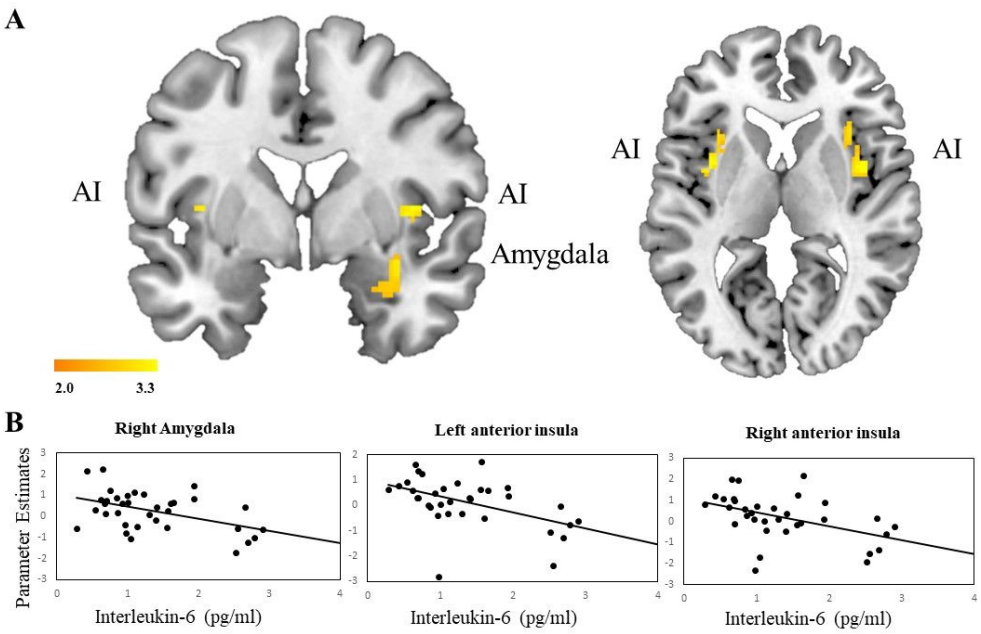

Figure 1. Correlation of baseline interleukin-6 (IL-6) levels and stress task-related neural activity in cortisol responders. A) Clusters of significant correlation between IL-6 and neural activity for the contrast stress > control condition in the anterior insula (AI) and amygdala (p=0.05, FWE corrected). B) Scatter plots of the correlations between IL-6 and parameter estimates extracted from a sphere of 6 mm around the peak activation in the right amygdala (34, -2, -16) and anterior insula (left: -36, 2, 6; right: 42, 2, 6).

4. Discussion

The aim of the present study was to investigate the association of baseline IL-6 levels as a marker of peripheral inflammatory processes and neural responses to psychosocial stress. For this purpose, blood was collected from healthy participants and they performed a validated fMRI stress paradigm. Cortisol samples were taken before and after stress induction as a measure of the physiological stress response. Our main finding is that baseline IL-6 levels correlated negatively with amygdala and anterior insula activity during stress exposure in participants showing a physiological stress response in terms of salivary cortisol increase.

Regarding the involvement of the amygdala and the anterior insula during stress exposure, our study nicely replicated previous findings that compared evaluative stress to a non-stressful control condition (see for example Lederbogen et al., 2011). In the present study we found significant activations for all previously defined ROIs during stress exposure (in line with Chung et al., 2016; Kogler et al., 2017, 2015; Orem et al., 2019).

However, stress task-related activity in these ROIs was not associated to IL-6 levels when the whole sample of participants was considered. Only for the group of participants who showed a physiological stress response to the task (so-called 'cortisolresponders'), IL-6 baseline levels were negatively linked to stress task-related activity of the right amygdala and bilateral anterior insula. To our knowledge, this is the first study showing an association between baseline IL-6 levels and neural markers of a stress response.

Two previous studies linked baseline IL-6 levels to neural activity during a social exclusion paradigm (Conejero et al., 2019; Slavich et al., 2010), but did not find a significant association. One may speculate that differences in the experimental procedures as well as in the target states may account for the potential discrepancy. While social exclusion paradigms have been reported to induce different degrees of social stress, the primary outcome usually is described as feelings of ostracism, social rejection or social pain (Eisenberger, 2012; Wang et al., 2017). Critically, these latter states do not involve the unpleasant component of social evaluation that is key to social stress and was therefore of particular significance in the context of the present study. Second, earlier studies did not consider individual differences in the task response. The present data reveal that for the investigation of associations between chronic inflammatory processes and neural stress responses it is necessary to consider the possibility that the applied task may not induce similar levels of social stress across participants. Thus, we defined two groups of cortisol responders and non-responders in order to distinguish between participants with and without a physiological stress response to the stress paradigm. By doing so, we were able to show that IL-6 levels as a marker of peripheral inflammatory processes negatively

correlated with neural responses to psychosocial stress specifically only in the group showing a cortisol stress response. By collapsing cortisol responders and non-responders (as in previous studies) this result would not have been detectable. The finding of a significant group difference between cortisol responders and non-responders in the relationship between IL-6 levels and stress task-related activation in the amygdala (and a trend-wise group difference in the right AI), highlights that individual differences in stress task responses should be considered in further studies. We assume that in the cortisol non-responder group stress reactivity is not captured as clearly as in the group with a physiologically measurable stress response and therefore there is more noise in the data. It is not clear why cortisol non-responders did not show a stress task-related cortisol increase (e.g. because of not feeling stressed by the specific task or disturbances in the HPA axis) and thus many confounding factors are possible. In this context, it is important to consider that group differences appear to be strongly influenced by cortisol non-responders with comparably high IL-6 values. For future studies, it will be important to consider whether participants with inflammatory parameters above a certain threshold may show disturbed responses to stress tasks.

Notably, our finding of a negative association between IL-6 levels and insula as well as amygdala activity in cortisol responders is in contrast to the theory of Eisenberger and colleagues who assumed that inflammatory markers would correlate positively to neural reactivity underlying social evaluative stress (Eisenberger et al., 2017). However, in their theory Eisenberger and coworkers do not explicitly distinguish between acute (e.g., induced with a bacterial endotoxin or stress-induction paradigm) and chronic inflammatory (e.g., baseline IL-6) processes. Most of their work on neural correlates of inflammation focused on the effect of brief increases of IL-6 or other cytokines after acute stress induction or as a response to an immune stimulant. In these studies, the association between peripheral inflammation and neural activity of the amygdala, anterior insula, and ACC underlying social processes such as exclusion, rejection, or social pain was positive (Eisenberger et al., 2009; Inagaki et al., 2012;

Muscatell et al., 2016, 2015; Slavich et al., 2010). The present findings of an inverted association between inflammatory parameters and neural activity may appear contradictory at first glance. However, it should be noted that IL-6 release to acute stress is mainly driven by a short-term, stress-induced activation of the sympathetic nervous system (Slavich and Irwin, 2014). Likewise, neural (ACC, insula) and physiological (pupil measures) markers of acute stress (e.g., processing of negative emotions or pain) have also been associated with the sympathetic nervous system (Bradley et al., 2008; Geuter et al., 2014; Muscatell et al., 2015; Paulus et al., 2015) and the regulation of homeostasis (Critchley et al., 2005). Thus, a positive association between stress task-induced increase in IL-6 levels and neural activity can be expected, reflecting individual differences in the stress response to the task. In contrast, in the current study, IL-6 baseline levels were investigated as a marker for inflammatory processes of longer duration. Although the sympathetic nervous system is also involved in chronic inflammation (Pongratz and Straub, 2014), we assume that the HPA axis is another important player in this process. In conditions of chronically elevated levels of inflammation, for example due to lifestyle factors (overweight, chronic stress, alcohol etc.), the complex signaling loops between the brain, the HPA axis and inflammatory processes could be altered (Haroon et al., 2012). As a result of chronic HPA axis activity, a state of glucocorticoid resistance can develop, in which the predominant anti-inflammatory effect of the HPA axis is diminished (Hänsel et al., 2010). A reduction of the inhibitory effect of the HPA axis on cytokine transcription (Strehl et al., 2019) results in higher cytokine levels (Hänsel et al., 2010; Silverman and Sternberg, 2012; Slavich and Irwin, 2014). During chronic inflammation, persistent elevated cytokine levels interfere with neurotransmitters (Dantzer, 2016) and slow synaptic transmission (Gruol, 2015; Karpuk et al., 2011) and could distort the evaluation of a social stressor. This notion is in line with two previous studies that have found an association of elevated baseline pro-inflammatory cytokines (IL-6 and IL-1 beta) with reduced neural activity to non-stressful pleasant pictures (IL-6 (Alvarez et al., 2020)) and social exclusion (IL1 beta (Conejero et al.,

2019)). Furthermore, a differential association between acute (increase in IL-6 and TNF levels to endotoxin) and chronic (pro-inflammatory gene expression) immune status and the reaction to social stress could be demonstrated (Moieni et al., 2015). To conclude, Eisenberger's theory on heightened neural reactivity to threats may apply to acute immune processes, whereas chronic inflammatory processes may exert a different effect on neural social stress responses.

Regarding the defined regions of interest of the current study, the AI is a key region for interoceptive awareness (Craig, 2009) also with regard to peripheral immunological processes (Harrison et al., 2009). The amygdala on the other hand is a potent activator of the HPA axis (Jankord and Herman, 2008) and in this is closely connected to the AI (Hermans et al., 2011; van Marle et al., 2010). The AI and amygdala could therefore form a feedback loop in which peripheral inflammation is perceived and regulated via the HPA axis. For the third region of interest, the ACC, which had been reported in several previous studies in the context of acute inflammatory processes, our data suggest a less important role with regard to chronic inflammatory processes.

The present findings should be interpreted in the light of some important limitations. First, due to different time of day of sampling, we cannot rule out circadian influences on cortisol levels (Kudielka et al., 2004). However, we did not find a correlation between time of the day and task-associated cortisol increase. Furthermore, cortisol responders and non-responders did not differ in the time of investigation and therefore the effect of circadian influences should not have had a critical impact on group comparisons. Second, more than half of the participants did not show an increase in cortisol after the stress task. The ratio of cortisol responders to non-responders is in line with previous studies using the MIST (Khalili-Mahani et al., 2010; Wheelock et al., 2016), but it is not clear why the ratio is that high. One may speculate that non-responders were not stressed by the MIST, but they reported an even higher change in affect from before to after stress exposure than cortisol responders, suggesting that they were subjectively stressed by the stress task. Another possible explanation could be that the scanner

environment itself was perceived as stressful and the increase in cortisol levels took place before entering the scanner as an anticipatory stress reaction in some participants (Gossett et al., 2018). Furthermore, different underlying chronic stress levels may also play a role: Chronic activation and subsequent dysfunction of the HPA axis often leads to a blunted cortisol response to acute stress (Young et al., 2020). Third, in view of highly discrepant findings on plasma cytokine levels, we used only the reliable and frequently measured IL-6 (Hänsel et al., 2010; Marsland et al., 2017) as a surrogate for underlying inflammatory processes, being aware that cytokines function as closely coordinated network, with pleiotropic, activating, synergistic, antagonizing, and redundant effects (Himmerich et al., 2019). Thus, we do not know whether cytokines with similar or opposite effects could have mitigated the IL-6 effects observed in our study.

Future studies are needed to replicate and extend the present findings in order to better understand the complex interplay between inflammatory and neural processes in the context of stress. For example, it would be important to examine also chronically inflamed patients and/or chronically stressed individuals. In this context, it would be desirable to establish a more precise definition of low-grade systemic inflammation (e.g., how many and which cytokines should be measured and what are threshold values for different analysis methods, above which one speaks of clinically relevant inflammatory processes). As the underlying mechanisms of the relationship between peripheral inflammation and neural stress processing are still unclear, prospective studies should further attempt to elucidate potentially involved neural systems and signaling pathways, e.g. on the neurotransmitter or genetic level.

Conclusion

In conclusion, the present study suggests that IL-6 levels as markers of peripheral inflammatory processes in healthy participants are negatively associated with activity in the amygdala and AI in response to a social evaluative stress task, when the subgroup of participants is considered

who showed a physiological stress response. Our results extend on the existing literature on the association between the acute immune and neural response to social threat but indicate an opposite correlation for markers of chronic inflammatory processes. Findings from our study could thus help to better understand the complex interplay between inflammation and neural stress processing. This is of particular relevance, because disruption of the feedback loops between the peripheral immune system and the brain could contribute to the development of psychiatric illness (Kraynak et al., 2018). Further research is needed to translate the findings into the clinical setting.

Conflicts of interest: The authors declare no conflicts of interest.

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Table 1. Descriptive statistics (mean and standard deviation) of acute stress task responses and questionnaires

	Whole sample	Cortisol responder	Cortisol non-responder	Group comparison
Cortisol				
Cortisol level baseline [nmol/l]	6.31±3.97	6.03±3.12	6.56±4.59	U=725, p=0.951
Cortisol levels post stress [nmol/l]	8.12±8.25	13.6±9.72	3.78±2.31	U=143, p<0.001
Affective Reaction (PANAS)				
Positive affect baseline	56.3±15.9	59.0±15.7	53.8±15.9	t(73)=-1.2, p=0.236
Positive affect post stress	47.1±17.6	52.5±17.1	42.8±17.0	t(73)=-2.39, p=0.02
Negative affect baseline	9.92±9.4	12.5±11.3	7.98±7.25	U=493, p=0.033
Negative affect post stress	11.8±9.9	12.9±11.1	10.8±8.84	U=628, p=0.465
Task performance				
Reaction time control condition [s]	7.99±2.11	8.31±2.04	7.74±2.18	U=598, p=0.172
Reaction time stress [s]	5.6±1.23	5.72±1.14	5.51±1.31	U=648, p=0.400
Correct responses control condition [%]	80.6±11.7	80.4±11.6	80.6±12.0	U=686, p=0.648
Correct responses stress [%]	55±8.34	55±7.3	54.7±9.25	U=713, p=0.857
Stress questionnaires				
Stress reactivity (SRS)	54.7±9.45	54.6±9.37	54.7±9.72	t(73)=-0.69, p=0.494
Chronic stress (TICS)	14.4±6.93	15.2±5.87	13.7±7.67	t(73)=-0.13, p=0.893

PANAS= Positive and Negative Affect Schedule, SRS= Stress Reactivity Scale, TICS= Trier Inventory of Chronic Stress

Table 2. Activation at contrast stress > control condition

	side	MNI coordinates			Cluster size	T	p(cluster)
		x	y	z			
Whole brain analysis							
Calcarine gyrus (BA 17)	L	4	-84	8	6611	9.53	0.000
Cuneus	L	-4	-92	16		8.39	
Lingual gyrus	R	12	-72	-6		8.04	
Medial frontal gyrus	R	2	54	0	5223	9.28	0.000
Superior medial frontal gyrus	L	0	54	24		8.41	
Medial frontal gyrus	R	14	32	58		8.00	
Middle temporal gyrus	R	62	-20	-10	3125	9.14	0.000
Superior temporal gyrus	R	54	-42	14		7.46	
Middle temporal gyrus	R	54	-56	6		7.33	
Cerebellum	L	-26	-80	-36	519	9.11	0.000
Middle temporal gyrus	L	-60	-24	-12	2751	8.69	0.000
Middle temporal gyrus	L	-52	-26	-8		8.24	
Middle temporal gyrus	L	-54	-14	-12		7.92	
Cerebellum	R	24	-80	-34	290	7.09	0.000
Cerebellum	R	32	-82	-34		6.72	
Temporal pole (frontal lobe)	L	-42	22	-18	327	6.59	0.000
Inferior frontal orbital gyrus	L	-34	24	-22		6.42	
Inferior orbital frontal gyrus	L	-50	28	-8		6.38	
Insula	R	34	22	-18	291	5.79	0.000
Inferior orbital frontal gyrus	R	46	32	-12		5.27	

Thalamus	R	4	0	2	166	6.04	0.000
Cerebellum	L	-46	-62	-44	15	5.49	0.017
Cerebellum	L	-8	-52	-44	54	5.40	0.004
Parahippocampal gyrus	L	-18	-42	-10	19	5.31	0.014
Paracentral gyrus	L	-18	-26	64	12	4.69	0.020
Inferior frontal (triangular) gyrus	R	52	30	6	11	4.99	0.021
Region of interest analyses							
Amygdala	L	-30	6	-18		4.37	0.001
	R	30	6	-20		3.55	0.004
	R	24	0	-14		3.55	0.018
Anterior Cingulate Cortex	R	2	54	0		9.28	0.000
	R	4	52	22		8.01	0.000
	R	0	18	28		4.26	0.007
	R	0	-4	30		3.62	0.048
Anterior Insula	R	32	16	-12		5.34	0.000
	R	40	6	-14		3.35	0.042
	R	40	2	-14		3.51	0.041
	L	-30	12	-16		5.28	0.000

All *p*-values are family-wise error corrected.

Table 3. Correlations of IL-6 with neural activation at contrast stress > control condition in regions of interest in the subgroup of cortisol responders.

	side	MNI coordinates			T	p (peak)
		x	y	z		
Amygdala	R	34	-2	-16	3.33	0.042
Anterior Insula	R	42	2	6	3.63	0.038
	L	-36	2	6	3.5	0.043

All *p*-values are family-wise error corrected.