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

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Abstract

Several studies suggest a link between acute changes in inflammatory parameters due to an endotoxin or (psychological) stressor and the brain's stress response. The extent to which basal circulating levels of inflammatory markers are associated with the brain's stress response has been hardly investigated so far. In the present study, plasma levels of the cytokine interleukin (IL)-6 were obtained as a baseline measure of peripheral inflammatory processes and linked to neural markers of psychosocial stress using a modified version of the Montreal Imaging Stress Task in a sample of N=65 healthy subjects. Of three a-priori defined regions of interest – the amygdala, anterior insula, and anterior cingulate cortex – baseline IL-6 was significantly and negatively associated with stress-related neural activation in the right amygdala and left anterior insula. Our results suggest that baseline inflammatory physiology might be related to differences in the neural stress response and that this relationship could be inverse to that previously shown by studies on acutely induced inflammation.

Keywords: interleukin-6; stress; Montreal Imaging Stress Task; fMRI; anterior insula, amygdala

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). 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 positive 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 or endotoxin was applied, no fMRI 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 fMRI studies contradict this assumption by reporting negative associations between baseline pro-inflammatory cytokines 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 (task-evoked) inflammatory responses and basal circulating (not task-evoked) inflammatory markers. It is thus possible that heightened neural reactivity to threats may be true during acute immune activation, while the link between basal 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). 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, only participants with complete fMRI scans who provided plasma samples were included (N=86). Unfortunately, one plate of the IL-6 measurement did not meet the quality standards in terms of variation to the other two plates (inter-assay coefficient of variation > 15%), so the values of this plate were not included in the analyses (N=21). The resulting sample consisted of N= 65 participants (39 female). Participants' age ranged from 28 to 54 years (mean age 42.6 ± 7.47 years), the mean Body Mass Index (BMI) was 26.4 ± 4.95. For questionnaire measures, there were missing values (see S1 for details) so that analyses on these parameters were performed for slightly smaller samples sizes (N= 63 - 64).

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 25.7 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 approximately 5 minutes after scanning (average time after onset of the stress task = 30.6 ± 2.9 minutes). 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 in duplicate using a commercially available high-sensitive enzyme-linked immunosorbent assay (ELISA, R&D Systems, Minneapolis) with a minimum detection threshold of 0.2 pg/ml. Within-assay coefficients of variation were <9%, the between-assay coefficient was 13.6%. IL-6 data were positively skewed, so raw values were log transformed to normalize the distribution prior to statistical testing.

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 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 (Wellcome 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, two one-sample t-tests were calculated to assess stress task-related neural activation (stress conditions > control condition) and deactivation (control condition > stress condition). Second, to investigate whether IL-6 levels were associated to stress-related neural activation, IL-6 was included as a covariate in the one-sample t-test of stress task-related activation and positive as well as negative correlation contrasts were calculated.

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 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 (Kelly et al., 2012).

If not mentioned otherwise, all results were corrected for multiple comparisons applying family-wise error (FWE) correction as implemented in SPM12. 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).

First, to investigate whether the MIST task induced stress in the current sample, one-tailed Student's t-tests were computed to test for changes in cortisol as well as negative and positive affect (before to after stress induction). With regard to behavioral performance measures of the MIST, two-tailed Student's t-tests were calculated to compare reaction times and percentage of correctly answered tasks of the 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

associations between IL-6 and change in cortisol, negative and positive affect as well as stress reactivity and chronic stress using partial correlations. The time of the day of cortisol collection was included as covariate in the correlation analysis involving cortisol, because cortisol plasma levels have a circadian rhythm (Lee et al., 2015). 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 29.8% (see Table 1). However, this increase was not significant ($t(64) = -1.13$, $p = 0.132$) due to great individual differences: only 43.1% of the participants showed increases in their cortisol levels ('cortisol responders'), the remaining 56.9% 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). Positive affect significantly decreased after stress induction ($t(62) = 5.61$, $p < 0.001$), but changes in negative affect did not reach significance ($t(62) = -1.60$, $p = 0.057$, see also Table 1). A decrease in positive affect was seen in 73.0 % of participants, an increase in negative affect in 49.2%. Regarding task performance, there was a significant decrease from control to stress condition in reaction time ($t(64) = 16.7$, $p < 0.001$) and correctly answered tasks ($t(64) = 20.5$, $p < 0.001$, see also Table 1).

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

IL-6 did not correlate with questionnaire measures of stress reactivity ($r = -0.05$, $p = 0.70$), chronic stress ($r = -0.02$, $p = 0.88$), or change of affect or cortisol after stress induction (positive affect: $r = .06$, $p = 0.65$; negative affect: $r = .05$, $p = 0.71$; cortisol: $r = -0.17$, $p = 0.19$).

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 bilateral middle temporal gyrus, superior medial and inferior orbital frontal gyrus, cuneus, cerebellum, temporal pole, right insular cortex, and mid cingulum (FWE corrected for multiple comparisons; see Table 2 and S3). In addition, analyses within our previously specified ROIs revealed significant effects in the bilateral amygdala and ACC and bilateral anterior insula (see Table 2). The opposite contrast (control condition > stress) revealed activation in the superior parietal and inferior occipital lobule, angular gyrus and supramarginal gyrus (see Table 3 and S4). In the previously specified ROIs no significant effects were found for this contrast.

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

There was no positive association of stress task-associated neural activity (stress > control condition) with IL-6 levels, but ROI analyses revealed negative correlations with the activity of the left anterior insula and right amygdala (FWE corrected, see Figure 1 and Table 4).

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. Our main finding is that baseline IL-6 levels correlated negatively with left anterior insula and right amygdala activity during stress exposure.

Regarding the activation of the amygdala and the anterior insula during stress exposure, our study replicates previous findings that compared evaluative stress to a non-stressful control condition (e.g., Lederbogen et al., 2011, Chung et al., 2016; Orem et al., 2019; Zschucke et al., 2015). However, there have also been studies reporting no stress-related effects in these regions or even deactivations and the reasons for this heterogeneity of results are still unclear (see van Oort et al., 2017 for review).

To our knowledge, this is the first study showing an association between baseline IL-6 levels and neural markers of 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 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. Furthermore, previous studies may have been underpowered to detect an effect because samples were rather small (*N*=31 in the study by Slavich et al., 2010) or heterogeneous (*N*= 101 comprising women who had a history of suicide attempt (*N*=42), a major depressive episode without suicidal acts (*N*=40) as well as healthy subjects (*N*=19) in the study by Conejero et al., 2019). It should be noted here that in

the current study only small clusters of voxels survived FWE correction. Future studies with high statistical power are needed, which also allow to detect small effects and possibly explain the inconsistent results.

Notably, our finding of a negative association between IL-6 levels and insula as well as amygdala activity 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 inflammatory responses (e.g., induced with a bacterial endotoxin or stress-induction paradigm) and basal circulating levels of inflammatory markers. 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 thus 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, circulating baseline levels of IL-6 were investigated. Although the sympathetic nervous system is also involved in basal inflammatory processes

(Pongratz and Straub, 2014), we assume that the HPA axis is another important player in this process. In conditions of 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). Cytokines can 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 a previous study that has found a negative association of baseline pro-inflammatory cytokine IL-1 beta with neural activity during social exclusion (Conejero et al., 2019). Furthermore, a differential association between acute (increase in IL-6 and TNF levels to endotoxin) and basal (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 basal inflammatory physiology may exert a different effect on neural social stress responses.

Regarding the defined regions of interest of the current study, the AI is assumed to integrate viscerosensory signals (including inflammatory viscerosensation) with representations of motivational, social and cognitive conditions (Craig, 2009). Moreover, a PET study by Rosenkranz and colleagues (2016) suggests that greater stress-evoked activity in the AI may reflect regulation of the stress effect on inflammation. 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, the present data could not detect any effects with regard to basal inflammatory processes.

The present findings should be interpreted in the light of some important limitations. First, 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 low. One may speculate that non-responders were not stressed by the MIST, however they reported a significant change in affect from before to after stress exposure (positive affect: $t(35) = 4.69$, $p < 0.001$; negative affect: $t(35) = -2.46$, $p = 0.02$), suggesting that they were subjectively stressed by the stress task. There are other possible explanations, which could underlie the lack of cortisol response to the task, for example a chronic activation and subsequent dysfunction of the HPA axis (Young et al., 2020) or an anticipatory cortisol response because the scanner environment itself was perceived as stressful (Gossett et al., 2018). The measurement of another stress parameter such as heart rate would be desirable to complement the cortisol results in future studies. Second, 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. 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.

5. Conclusion

In conclusion, the present study suggests that circulating basal IL-6 levels in healthy participants are negatively associated with activity in the AI and amygdala in response to a social evaluative stress task. 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 basal inflammatory physiology. 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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Figure Caption

Figure 1. Correlation of log transformed baseline interleukin-6 (IL-6) levels and stress task-related neural activity A) Cluster of significant correlation between IL-6 and neural activity for the contrast stress > control condition in the right amygdala and left anterior insula (p=0.05, FWE corrected, clusters are displayed on the average T1 image of the sample). B) Scatter plot of the correlation between IL-6 and parameter estimates of the activation in the left anterior insula and right amygdala. For visualization purposes, parameter estimates were extracted from a sphere of 6 mm around the peak activation (anterior insula: -40, 4, -16; amygdala: 34, -4, -28).

Table 1. Descriptive statistics of acute stress task response and stress questionnaires

Mean values ± standard deviation	
Cortisol	
Cortisol level baseline [nmol/l]	5.98±3.86
Cortisol levels post stress [nmol/l]	6.83±5.86
Affective Reaction (PANAS, N=64)	
Positive affect baseline	54.8±16.1
Positive affect post stress	47.2±18.1
Negative affect baseline	11.0±10.3
Negative affect post stress	12.9±10.9
Task performance	
Reaction time control condition [s]	8.02±2.22
Reaction time stress [s]	5.63±1.28
Correct responses control condition [%]	80.0±12.3
Correct responses stress [%]	54.2±8.73
Stress questionnaires (N=64)	
Stress reactivity (SRS)	55.6±9.38
Chronic stress (TICS)	15.3±7.13

PANAS= Positive and Negative Affect Schedule, SRS= Stress Reactivity Scale, TICS= Trier Inventory of Chronic Stress; N=65 if not otherwise specified

Table 2. Activation at contrast stress > control condition

	side	MNI coordinates			Cluster size	T	p(peak)
		x	y	z			
Whole brain analysis							
Middle temporal gyrus	R	62	-18	-10	3064	9.34	0.000
		62	-46	14		8.14	0.000
		56	-48	28		7.97	0.000
(Superior) medial frontal gyrus	R	2	52	2	3579	9.24	0.000
		2	38	4		7.80	0.000
		14	30	56		7.60	0.000
Cuneus	R	8	-84	28	4863	8.83	0.000
		10	-74	-4		8.32	0.000
		-8	-46	34		8.07	0.000
Middle temporal gyrus	L	-62	-20	-12	2176	8.76	0.000
		-56	-58	22		8.12	0.000
		-52	-26	-10		7.49	0.000
Cerebellum	L	-24	-80	-36	260	7.09	0.000
Cerebellum	R	30	-82	-34	144	6.76	0.000
Temporal pole (frontal lobe)	L	-42	20	-18	85	6.44	0.000
Insula	R	34	22	-18	41	5.69	0.004
Thalamus	R	4	0	4	29	5.21	0.017
Mid Cingulum	L	-12	-32	46	13	5.12	0.023
Inferior orbital frontal gyrus	L	-50	30	-8	5	4.95	0.039

Region of interest analyses

Amygdala	L	-30	6	-18	182	4.11	0.002
		-24	2	-16		3.65	0.007
	R	30	6	-20	161	3.45	0.014
		22	0	-14		3.24	0.024
Anterior Cingulate Cortex	L/R	2	52	2	2349	9.24	0.000
		2	38	4		7.80	0.000
		4	52	22		6.96	0.000
		2	14	28		3.86	0.029
Anterior Insula	R	34	12	-18	292	4.55	0.001
		42	16	-12		4.38	0.002
		38	20	-8		4.25	0.003
		32	16	-12		4.22	0.003
		40	-2	-14		3.32	0.040
	L	-38	12	-16	369	4.43	0.002
		-40	14	-12		4.32	0.002
		-30	16	-12		3.89	0.008

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

Table 3. Activation at contrast control > stress (whole brain analysis)

	side	MNI coordinates			Cluster size	T	p(peak)
		x	y	z			
Superior parietal lobule	L	-26	-60	52	1066	7.72	0.000
		-38	-40	42		6.86	0.000
		-22	-62	62		6.17	0.000
Inferior occipital lobule	R	34	-88	-2	244	7.41	0.000
		28	-94	4		6.48	0.000
Angular gyrus	R	28	-60	50	417	6.94	0.000
		30	-52	50		6.26	0.000
Inferior occipital lobule	L	-30	-90	-10	129	6.27	0.000
		-30	-92	0		6.19	0.000
		-22	-96	4		5.85	0.000
Supramarginal gyrus	R	42	-36	46	61	5.87	0.002

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

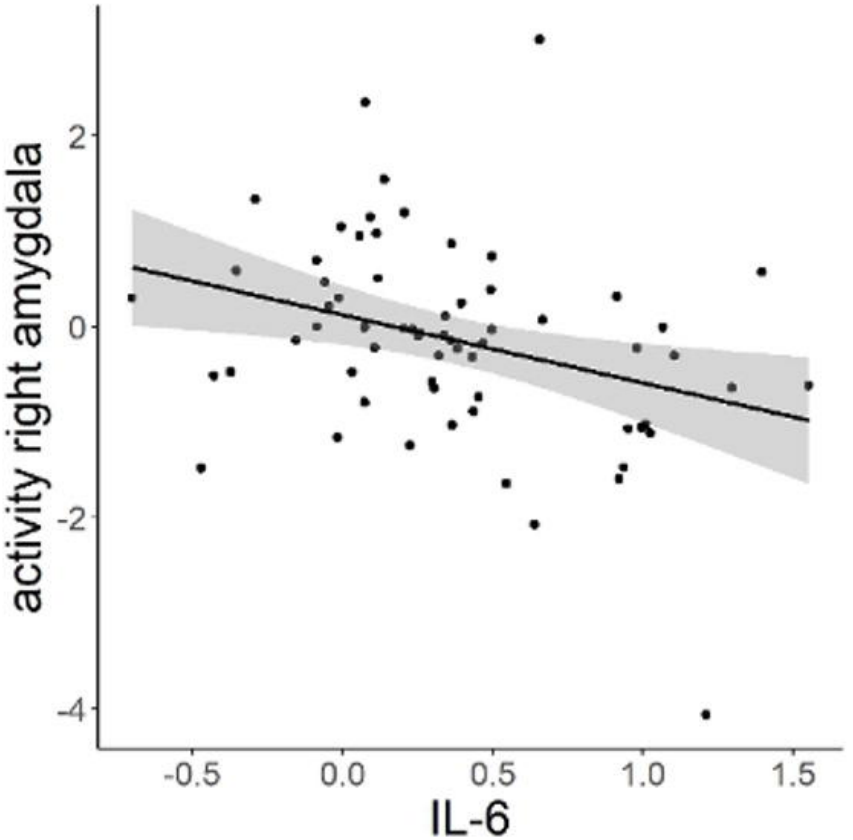
Table 4. Correlations of IL-6 with neural activation at contrast stress > control condition in regions of interest.

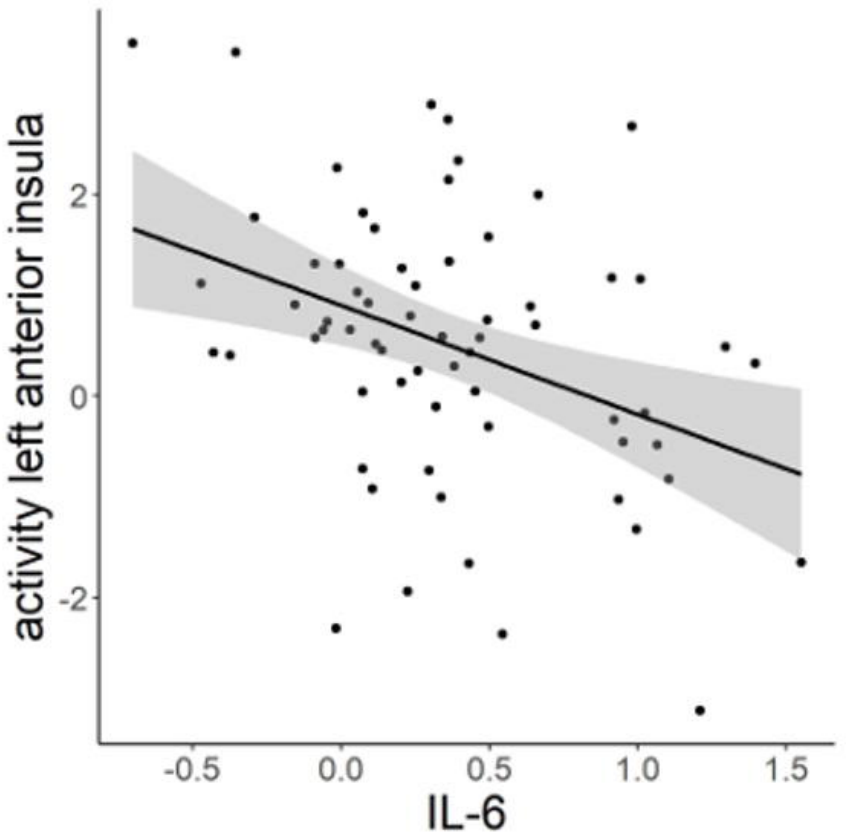
	side	MNI coordinates			Cluster size	T	p (peak)
		x	y	z			
Amygdala	R	34	-4	-28	5	3.14	0.030
		28	-6	-16	6	2.97	0.046
Anterior Insula	L	-40	4	-16	3	3.41	0.029

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

A

B


activity right amygdala
2
0
-2
-4
-0.5
0.0
0.5
1.0
1.5
IL-6


activity left anterior insula
2
0
-2
-0.5
0.0
0.5
1.0
1.5
IL-6