



## Anterior insula morphology and vulnerability to psychopathology-related symptoms in response to acute inflammation

Kristoffer N.T. Månsson<sup>a,b,c,d,1,\*</sup>, Julie Lasselin<sup>e,f,g,1</sup>, Bianka Karshikoff<sup>f</sup>, John Axelsson<sup>e,f</sup>, Harald Engler<sup>h</sup>, Manfred Schedlowski<sup>f,h</sup>, Sven Benson<sup>h</sup>, Predrag Petrovic<sup>d,i</sup>, Mats Lekander<sup>e,f,g</sup>

<sup>a</sup> Center for Lifespan Psychology, Max Planck Institute for Human Development, Berlin, Germany

<sup>b</sup> Max Planck UCL Centre for Computational Psychiatry and Ageing Research, Berlin/London, Germany/United Kingdom

<sup>c</sup> Centre for Psychiatry Research, Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden

<sup>d</sup> Center for Cognitive and Computational Neuropsychiatry, Karolinska Institutet, Stockholm, Sweden

<sup>e</sup> Stress Research Institute, Department of Psychology, Stockholm University, Stockholm, Sweden

<sup>f</sup> Division of Psychology, Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden

<sup>g</sup> Osher Center for Integrative Medicine, ME Neuroradiologi, Karolinska Universitetssjukhuset, Stockholm, Sweden

<sup>h</sup> Institute of Medical Psychology and Behavioral Immunobiology, Center for Translational Neuro- and Behavioral Sciences, University Hospital Essen, University of Duisburg-Essen, Essen, Germany

<sup>i</sup> Neuro Division, Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden

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### ABSTRACT

**Introduction:** The role of inflammation in common psychiatric diseases is now well acknowledged. However, the factors and mechanisms underlying inter-individual variability in the vulnerability to develop psychopathology-related symptoms in response to inflammation are not well characterized. Herein, we aimed at investigating morphological brain regions central for interoception and emotion regulation, and if these are associated with acute inflammation-induced sickness and anxiety responses.

**Methods:** Systemic inflammation was induced using an intravenous injection of lipopolysaccharide (LPS) at a dose of 0.6 ng/kg body weight in 28 healthy individuals, while 21 individuals received an injection of saline (placebo). Individuals' gray matter volume was investigated by automated voxel-based morphometry technique on T1-weighted anatomical images derived from magnetic resonance imaging (MRI). Plasma concentrations of TNF- $\alpha$  and IL-6, sickness symptoms (SicknessQ), and state anxiety (STAI-S) were measured before and after the injection.

**Results:** A stronger sickness response to LPS was significantly associated with a larger anterior insula gray matter volume, independently from increases in cytokine concentrations, age, sex and body mass index ( $R^2 = 65.6\%$ ). Similarly, a greater LPS-induced state anxiety response was related to a larger anterior insula gray matter volume, and also by a stronger increase in plasma TNF- $\alpha$  concentrations ( $R^2 = 40.4\%$ ).

**Discussion:** Anterior insula morphology appears central in the sensitivity to develop symptoms of sickness and anxiety in response to inflammation, and could thus be one risk factor in inflammation-related psychopathologies. Because of the limited sample size, the current results need to be replicated.

### 1. Introduction

Inflammation has been acknowledged to play a role in the development of many clinical disorders, including psychiatric conditions such as depression (Bell et al., 2017; Dantzer et al., 2008; Khandaker et al.,

2014; Slavich, 2015). The putative mechanisms underlying the effect of inflammation on psychiatric symptoms include the passage of inflammatory cytokines through humoral pathways (diffusion or active transport of cytokines through the blood–brain-barrier and activation of endothelial cells by cytokines), a neuronal pathway (activation of

\* Corresponding author at: Centre for Psychiatry Research, Department of Clinical Neuroscience, Karolinska Institutet, Norra Stationsgatan 69, SE-113 64 Stockholm, Sweden.

E-mail addresses: [kristoffer.mansson@ki.se](mailto:kristoffer.mansson@ki.se), [mansson@mpib-berlin.mpg.de](mailto:mansson@mpib-berlin.mpg.de) (K.N.T. Månsson).

<sup>1</sup> Equal contribution by the first and second author.

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afferent nerves by cytokines), and a cellular pathway (entry of peripheral monocytes into the brain parenchyma) (Dantzer et al., 2008; Wohleb et al., 2014). While experimental studies in humans support that peripheral inflammation causes psychopathology-related symptoms (Lasselin et al., 2020), the symptomatic response to peripheral inflammation differs substantially across individuals. This inter-individual variability holds for the degree of increase in inflammatory cytokines, the neural response, as well as the behavioral changes that follow inflammation (Grigoleit et al., 2015; Karshikoff et al., 2016; Lasselin et al., 2016). Understanding the mechanisms underlying such variability is crucial if we are to understand what makes some individuals more vulnerable to develop psychiatric diseases in presence of inflammation. It also connects to an ongoing quest to understand differences in how bodily cues are interpreted, regulated and responded to in health and disease (Khalsa et al., 2018; Petrovic and Castellanos, 2016; Quadt et al., 2018).

Collectively, the representation of the internal bodily environment in the brain is denoted interoception (Barrett and Simmons, 2015; Craig, 2002; Critchley et al., 2004). In this internal model of the bodily state, predictions are compared to incoming sensory information, so that the model can be updated (Barrett and Simmons, 2015). Dysfunction of interoception is increasingly recognized as an essential component in mental health conditions (Di Lernia et al., 2016; Eggart et al., 2019; Khalsa et al., 2018; Owens et al., 2018a), which is reflected in an increased use of interventions manipulating interoceptive exposure so as to target anxiety-provoking physical sensations (Boettcher et al., 2016; Hedman et al., 2014). This development calls for more knowledge about individual differences in interoceptive sensitivity, for instance during systemic inflammation (Lasselin et al., 2018; Lekander et al., 2016; Quadt et al., 2018).

Brain regions involved in interoceptive processing include insular, mid- and anterior cingulate and orbitofrontal cortices (Khalsa et al., 2018; Lekander et al., 2016; Quadt et al., 2018). It has been suggested that interoceptive information from the body projects to posterior insula (Craig, 2003, 2002). This information is then re-represented in more anterior parts of the insula adding more complex information and expectations (Craig, 2003, 2002). Anterior insula constructs a *meta*-representation of interoceptive information from the body and supports the subjective experience associated with different interoceptive input (sometimes denoted as “feeling states”) (Craig, 2003; Dixon et al., 2014; Singer et al., 2009). It is connected with anterior cingulate cortex, and forms a functional unit with the amygdala and orbitofrontal prefrontal cortex (Quadt et al., 2018). We have previously shown that experimental systemic inflammation induced by administration of lipopolysaccharide (LPS) in healthy individuals is followed by increased connectivity between left anterior insula and left mid cingulate cortex, and this connectivity was partly related to subjective experience of inflammatory activation (Lekander et al., 2016). In addition, heightened pain sensitivity after LPS was paralleled by decreased activity in the ventrolateral prefrontal cortex and the rostral anterior cingulate cortex, and higher activity in the left anterior insular cortex (Karshikoff et al., 2016). These studies confirm that regions involved in interoception are sensitive to changes in peripheral inflammation, and strongly overlap with functional networks involved in inflammation-associated depression (Harrison, 2017) as well as emotional control (Petrovic and Castellanos, 2016).

It has been noted that individuals differ substantially in the awareness or accuracy of perceiving afferent information from within their bodies (Bechara and Naqvi, 2004; Craig, 2004; Ludwick-Rosenthal and Neufeld, 1985). Such investigations have often been based on heartbeat perception (Ainley et al., 2016), but tests related to gastric perception, proprioception, ischemic pain, balancing ability, and perception of bitter taste have also been applied to chart individual differences (Ferentzi et al., 2018). In addition to behavioral differences, there are striking disparities between individuals in the morphology of the insula (Butti and Hof, 2010; Craig and (Bud) Craig, 2010; Craig, 2004; Naidich

et al., 2004). In turn, differences in insula morphology and activity correlate with divergences in interoceptive ability (Critchley et al., 2004) and emotional hyper-reactivity (Petrovic et al., 2016; Petrovic and Castellanos, 2016; Umeda et al., 2015). Further, patients with lesions in anterior insula show disruptions in breathing-related interoceptive accuracy and sensitivity (Wang et al., 2019). Corresponding observations demonstrate high inter-individual variability in the extent and position of microscopically defined Brodmann’s areas of the orbitofrontal cortex (Uylings et al., 2010), with data showing that structural differences correlate with e.g. the emotional regulatory functions in which the orbitofrontal cortex is involved (reviewed in Petrovic and Castellanos, 2016). Specifically, smaller lateral orbitofrontal cortices is associated with emotional hyper-reactivity, as well as with subclinical problems with emotional regulation in healthy subjects (Petrovic et al., 2016; Petrovic and Castellanos, 2016). An argument can also be raised for rostral anterior cingulate cortex, the size of which is smaller in patients with fibromyalgia, displaying reduced capacity for pain inhibition, as compared to healthy controls (Jensen et al., 2013). Along this line, subgenual ACC activations are also associated with pro-inflammatory cytokines, and response to grief related stimuli (O’Connor et al., 2009). Thus, key areas that are involved in interoception and emotion regulation show high inter-individual structural and functional variability, which is related to behavioral outcomes. Activity in these areas, such as insula, amygdala and cingulate cortices, are known to be modulated during inflammatory activation (Benson et al., 2015; Hanestad et al., 2012; Harrison, 2016; Harrison et al., 2009; Karshikoff et al., 2016; Labrenz et al., 2019; Lekander et al., 2016).

In sum, signaling from the immune system to the brain and its behavioral response encompasses key areas involved in interoception and emotional regulation. These areas show high inter-individual variability in morphology which might also be related to variability in the vulnerability to develop short-term psychopathology-related symptoms in response to acute inflammation. To this end, we aimed to investigate the relation between a morphological measure of the brain and subjective responses to experimental inflammatory stimulation. We hypothesized that morphological grey matter volume of the insula (anterior and posterior), the anterior cingulate cortex (ACC; pregenual and subgenual ACC separately), the mid cingulate cortex, the lateral and medial orbitofrontal cortex, as well as the amygdala, will relate to the degree of sickness and anxiety in response to injection of LPS.

## 2. Methods

### 2.1. Study design and participants

The current study is derived from a previous study assessing brain mechanisms underlying pain and sickness malaise during acute inflammation (Karshikoff et al., 2016; Lekander et al., 2016). The experiment followed a double-blind randomized placebo-controlled between-subjects design, in which LPS (*E. coli*, Lot G3E0609, United States Pharmacopeia Rockville, MD) at a dose of 0.6 ng/kg body weight, or placebo (saline) were randomly injected in 52 healthy subjects (29 women and 23 men, mean age  $28.6 \pm 7.1$  years). Sample size was determined based on data from a pilot study that was used for power calculation. Based on effect sizes of 1.24 for general health and 1.96 for current health, a power of 0.80, and an alpha level of 0.05, it was estimated that we needed 19 and 27 participants in the current study for testing current and general health, respectively, in a between-subject design. We oversampled the LPS group (60/40 rate of LPS/placebo) to increase the power for analyzes of inter-individual differences in response to LPS. The relatively low dose of LPS and the between-subjects design were chosen to improve blinding and to facilitate MR scanning by avoiding shivering and nausea. Inclusion criteria included: age 18–50, right handed, normal body mass index (BMI), medication free, non-smokers. Exclusion criteria included: history of drug abuse, inflammatory, psychiatric or sleep disorders, sleep disturbances, chronic pain, and

pregnancy (assessed using a pregnancy test on the morning of the experiment). All participants underwent a complete medical examination including routine blood samples and electrocardiogram before inclusion, and C-reactive protein was assessed on the experimental day to exclude ongoing infection (no subject had  $> 3$  mg/L). Women were tested in the early follicular phase, except if using contraceptives abrogating menses (7 subjects). Participants were asked to refrain from strenuous physical activities and from alcohol the day before the experiment, as well as to sleep regular hours (7–8 h per night during two nights before each study day). Participants were asked to drink their regular amount of coffee in the morning, to avoid withdrawal symptoms. Individuals who were heavy coffee consumers were excluded from the study to avoid withdrawal symptoms during the day, as no coffee was served.

Among the 52 subjects included, thirty-one subjects (18 women) were randomly assigned to receive LPS and twenty-one (11 women) to receive saline (placebo). Within the LPS condition, one subject's cytokine data were missing and two subjects' self-reported questionnaire data were missing. Accordingly, the total number of subjects included in the current analyses was 28 in the LPS condition and 21 in the placebo condition. Anatomical MRI data (scanning performed between 2 h and 3h30min after injection) were available from all 52 participants.

More details on the procedure can be found in (Karshikoff et al., 2016, 2015). The study was approved by the Regional Ethical Review Board in Stockholm (2008/955–31) and all participants provided written informed consent.

## 2.2. Plasma cytokine concentrations

Blood samples were taken before, 1 h 30 min, 3 h 30 min, and 5 h after the LPS or placebo injection. Fasting blood samples were not possible, as the samples were taken throughout the day. All blood samples were processed within an hour and stored in a  $-20$  °C freezer, then transported to a  $-70$  °C freezer at the end of the day. All blood samples were thawed for analysis at the end of data collection. Plasma concentrations of TNF- $\alpha$ , IL-6, and IL-8 were measured using Millipore's MILLIPLEX MAP high sensitivity human cytokine kit (Millipore Corporation, Billerica, MA, USA) according to the manufacturer's instructions, with detection limits of 0.4 pg/ml, 0.9 pg/ml, and 0.4 pg/ml, respectively. Values below detection limits were replaced by the detection limit. TNF- $\alpha$  and IL-6 values were log-transformed. A change score from baseline to 1h30min post LPS injection was calculated. IL-8 concentrations were intended for another (i.e. associations with pain testings, see Karshikoff et al., 2015) and were not used in the current study.

## 2.3. Sickness and anxiety symptoms

Self-report questionnaires were completed before, 1h30min, 3h30min, and 5 h after the LPS or placebo injection. Sickness symptoms were assessed using the Sickness Questionnaire (SicknessQ) (Andreasson et al., 2018). The SicknessQ includes 10 items measuring the intensity of sickness symptoms, with a total score ranging from 0 (no symptom) to 30 (high sickness symptoms). SicknessQ items include somatic symptoms, i.e. "I feel nauseous", "My body feels sore", "I feel shaky", "I have a headache", as well as emotional and fatigue aspects, i.e. "I want to keep still", "I wish to be alone", "I don't wish to do anything at all", "I feel depressed", "I feel drained", and "I feel tired".

State anxiety was assessed with the state version of the State-Trait Anxiety Inventory (STAI-S) (Spielberger et al., 1970), a 20-item questionnaire assessing the intensity of state anxiety symptoms with a score ranging from 20 to 80. For both sickness and anxiety symptoms, a change score from baseline to 1h30min post LPS injection was calculated and defined behavioral outcomes in subsequent analyses.

## 2.4. Magnetic resonance imaging acquisition and preprocessing

Anatomical images were acquired on a 3 Tesla scanner (Discovery MR750, General Electric, GE) with a 32 channel head-coil (MR instruments Inc.). The T1-weighted structural scan was acquired with an axial 3D sequence, flip angle  $12^\circ$ , voxel size  $1 \times 1 \times 1$  mm<sup>3</sup>. The MRI-measurement was performed between 2 h and 3h30min after LPS injection, the subjects were under the influence of the LPS during the scanning session.

The Computational Anatomical Toolbox (CAT12.3) (Gaser and Dahnke, 2016), implemented in MATLAB (Mathworks, Natick, MA, USA) were used for the T1-weighted MRI data preprocessing. The CAT12.3 segmentation for voxel-based morphometry (VBM) was used and spatial registration to the Montreal Neurological Institute coordinate space was performed on all images using the geodesic shooting algorithm (Ashburner and Friston, 2011). Before preprocessing, all raw data were manually reviewed and after preprocessing, sample homogeneity check was performed in accordance with the CAT12 manual. Based on the mahalanobis distance on mean voxel correlations and the weighted average quality measure, no participants were removed from further analysis. The mean weighted overall image quality of the 31 images used for the analyses was  $86.4 \pm 0.5\%$ . Finally, the segmented and spatially normalized T1-weighted MRI data were smoothed using a Gaussian kernel with 8 mm full width at half maximum (FWHM) in SPM12.

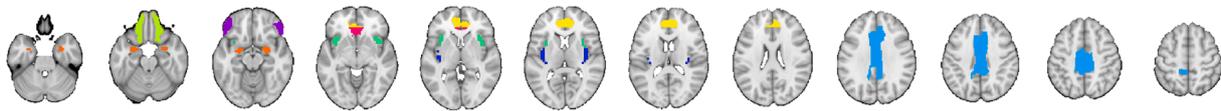
## 2.5. Regions of interest

Departing from a 3-component parcellation of the insular cortex (Kelly et al., 2012), and in accordance with our previous publication (Lekander et al., 2016), the anterior and posterior components of the insular cortex was used as regions of interest (ROI). The mid cingulate cortex ROI was defined in accordance with our previous functional MRI study on LPS (Lekander et al., 2016). The amygdala, subgenual and pregenual anterior cingulate cortex, and medial and lateral orbitofrontal cortex were defined using the recent parcellations in automated anatomical labeling version 3 (AAL3; publicly available on <https://www.oxcns.org/aal3.html>; see also Fig. 1) (Rolls et al., 2020). In SPM12, within each ROI, the average of all voxels was extracted and subject for further statistical analysis. Primary analyses were performed for each brain region bilaterally, and for completeness, also unilateral results are reported for bilateral regions suggesting statistical significance - see Supplementary Material Table S1.

## 2.6. Statistical analyses

Analyses on behavioral data, cytokines, and the extracted gray matter (GM) volumes were performed using STATA (StataCorp LLC. 2017. Stata Statistical Software: release 15.1. College Station, TX, USA). Linear and multiple regressions were conducted in the group of participants having received LPS administration, in two steps: (1) separate linear regression analyses were conducted to assess the association between (a) the change in cytokine concentrations from baseline to 1h30min after LPS administration, and (b) regional GM volumes of the brain regions of interest, with the change in sickness symptoms and anxiety from baseline to 1h30min after LPS administration; and (2) multiple linear regression analyses that included the independent variables that were found to be significantly ( $P < .05$ ) associated with the sickness and anxiety response in the first step. Standardized betas coefficients ( $\beta$ ) and adjusted  $R^2$  values are reported. Age, sex and BMI were added as covariates of no interest in all models, and in analyses including regional brain morphology, each individual's total GM was added as a covariate of no interest.

The multivariate Cook's  $D$  measure was used to identify values of high influence in each regression model, and a value exceeding  $4/N$  was defined as a statistical outlier and removed from the models presented



**Fig. 1. Regions of interest.** The figure depicts defined regions of interest (ROI) in the current study. The average voxel values were extracted from each region and used for further analysis. 1) Light green = Medial orbitofrontal; 2) Purple = Lateral orbitofrontal; 3) Orange = Amygdala; 4) Turquoise = Anterior insula (Kelly et al., 2012); 5) Dark blue = Posterior insula (Kelly et al., 2012); 6) Yellow = Pregenua anterior cingulate; 7) Pink = Subgenual anterior cingulate; 8) Light blue = Mid cingulum (Lekander et al., 2016). If not explicitly stated, the ROI was defined using the Automated Anatomical Labeling version 3 (AAL3) (Rolls et al., 2020). Within each region and across all voxels, the average voxel value was extracted and subject for further statistical analysis. The high-resolution anatomical image from Keuken et al. (2014) is used as a background. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

herein (see also [Supplementary Material Figure S1](#) demonstrating the final multiple linear regressions including the statistical outliers). Further, the variance inflation factor (VIF) was used to review multicollinearity among multiple independent variables (i.e., a VIF above 5 points was considered troublesome).

**3. Results**

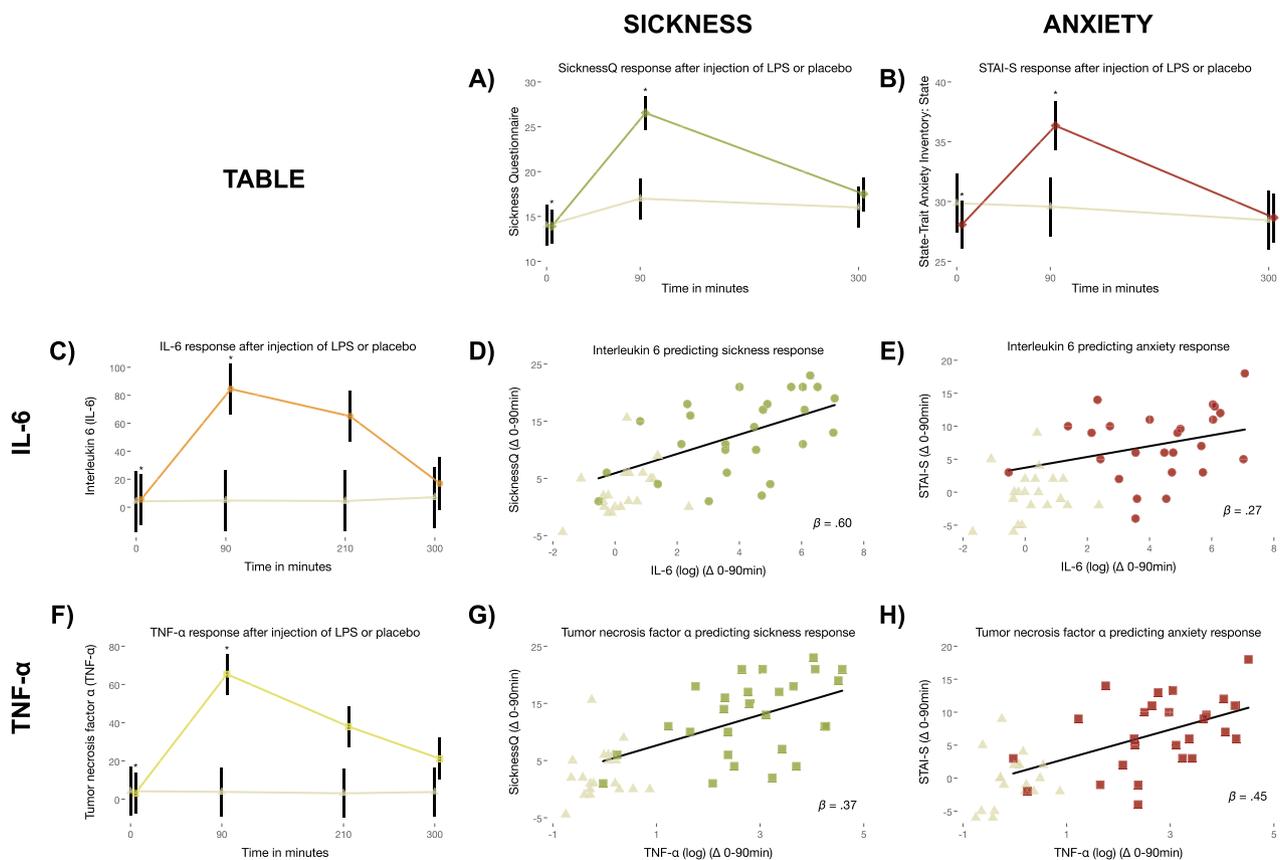
**3.1. Behavioral and cytokine responses to LPS**

Sickness symptoms and state anxiety symptoms increased from 0 to 1h30min after the LPS injection, but not in participants having been

injected with the placebo (Fig. 2AB). LPS-induced sickness response is moderately correlated with the anxiety response (changes from baseline to 1h30min), Pearson’s  $r(28) = 0.57, P = .002$ . Plasma concentrations of IL-6 and TNF- $\alpha$  significantly increased after LPS injection compared to the placebo injection (Fig. 2CF). For details on the kinetic of the changes, see (Lekander et al., 2016).

**3.2. Association between brain morphology, cytokines and behavioral responses to LPS**

As presented in [Table 1](#) and [Fig. 2DG](#), increases in both IL-6 and TNF- $\alpha$  concentrations from baseline to 1h30min after the LPS injection were



**Fig. 2. Changes and associations in cytokine plasma concentrations, sickness symptoms, and state anxiety after lipopolysaccharide (LPS) or placebo injection.** Changes in **A)** sickness symptoms (SicknessQ), **B)** anxiety symptoms (STAI-S), **C)** IL-6 plasma concentrations (pg/mL), and **F)** TNF- $\alpha$  plasma concentrations (pg/mL). Error bars represent 95% confidence intervals. Changes in cytokines and behaviors are calculated as delta scores between baseline and 1h30min after LPS administration (as denoted by \* in [Fig. 2 A,B,C,F](#)). Both **D)** IL-6 (green circles), and **G)** TNF- $\alpha$  (green squares) significantly relates to the sickness response (all  $P$ 's < 0.05). **E)** IL-6 did not (red circles;  $P = .351$ ), but **H)** TNF- $\alpha$  was significantly associated with the anxiety response (red squares;  $P = .036$ ). See also [Table 1](#) for details. All associations between behavior and cytokines ([Fig. 2 D,E,G,H](#)) included age, sex and BMI as covariates of no interest. The placebo group is excluded in all regression models and only included in the figures for illustrative purposes (beige triangles). **Abbreviations:** LPS: lipopolysaccharide, IL-6: interleukin-6, TNF- $\alpha$ : tumor necrosis factor- $\alpha$ , SicknessQ: Sickness Questionnaire, STAI-S: State part of the State-Trait Anxiety Inventory. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Table 1**

Separate linear regression models assessing the association of brain morphology and cytokine response with the behavioral 1) sickness, or 2) anxiety response. Both cytokine and bilateral regional GM volume models included age, sex and BMI as covariates of no interest. In addition, all regional GM volume models included the total GM volume as a covariate of no interest. Subjects answered questionnaires on sickness (measured with the Sickness Questionnaire) and anxiety (STAI-S) before and 1h30min after. Analyses on unilateral (left/right separately) GM volume are presented in Supplementary Material Table S1.

	Sickness			Anxiety		
	$\beta$	Adj $R^2$	P	$\beta$	Adj $R^2$	P
<b>Cytokines</b>						
IL-6 change	0.60	46.1%	0.003 <sup>§</sup>	0.27	1.6%	0.351
TNF- $\alpha$ change	0.37	33.4%	0.047	0.45	10.9%	0.036
<b>Regional and bilateral GM volume</b>						
Amygdala	0.33	19.8%	0.277	0.61	19.2%	0.016
Cingulate, anterior, pregenual	0.06	14.7%	0.846	0.03	0.3%	0.934
Cingulate, anterior, subgenual	0.39	34.9%	0.096	0.36	2.8%	0.204
Cingulate, mid	-0.01	25.4%	0.983	-0.22	0.5%	0.654
Insula, anterior	0.71	57.5%	0.001 <sup>§</sup>	0.84	41.0%	0.001 <sup>§</sup>
Insula, posterior	0.33	30.5%	0.228	0.19	1.3%	0.552
Orbitofrontal, medial	0.02	25.4%	0.952	-0.21	3.1%	0.557
Orbitofrontal, lateral	-0.50	42.6%	0.019	-0.15	3.2%	0.567

<sup>§</sup> denotes significant ( $P < 0.05$ ) after Bonferroni correction.

**Abbreviations:** IL-6, Interleukin 6; GM, gray matter; TNF- $\alpha$ , Tumor necrosis factor alpha; STAI-S: State part of the State-Trait Anxiety Inventory;

associated with higher sickness response from baseline to 1h30min after LPS. Increased TNF- $\alpha$ , but not IL-6, concentration was significantly associated with a stronger state anxiety response to LPS. Among the investigated brain regions, larger anterior insula and smaller lateral orbitofrontal GM volume, respectively, were significantly related to an increased LPS induced sickness response. Furthermore, larger anterior insula and amygdala GM volumes were associated with a stronger anxiety response to LPS. Cytokines and regional GM volume significantly associated with sickness or anxiety were implemented in the final multiple regressions (Table 2). A larger anterior insula GM volume, and lower BMI, were independently and significantly associated with a stronger sickness response ( $R^2 = 65.6\%$ ) (Fig. 3A), whereas both a larger anterior insula GM volume and increased TNF- $\alpha$  concentrations were related to a stronger state anxiety response ( $R^2 = 40.4\%$ ) (Fig. 3BC).

#### 4. Discussion

To obtain a better understanding of phenotypic differences in the

**Table 2**

Multiple regressions assessing the association of brain morphology and cytokine response with the (A) sickness, and (B) anxiety responses after LPS injection. Subjects answered questionnaires on sickness (measured with the Sickness Questionnaire) and anxiety (i.e., STAI-S) before and after injection (1h30min post LPS). Individual's change scores were computed for both behavioral self-reports and cytokines. All regional gray matter volumes are bilaterally defined. Further, multicollinearity was checked and showed that all variance inflation factors (VIF's) were below 4.46 for model (A), and below 3.69 for model (B). Both regression models included 25 subjects.

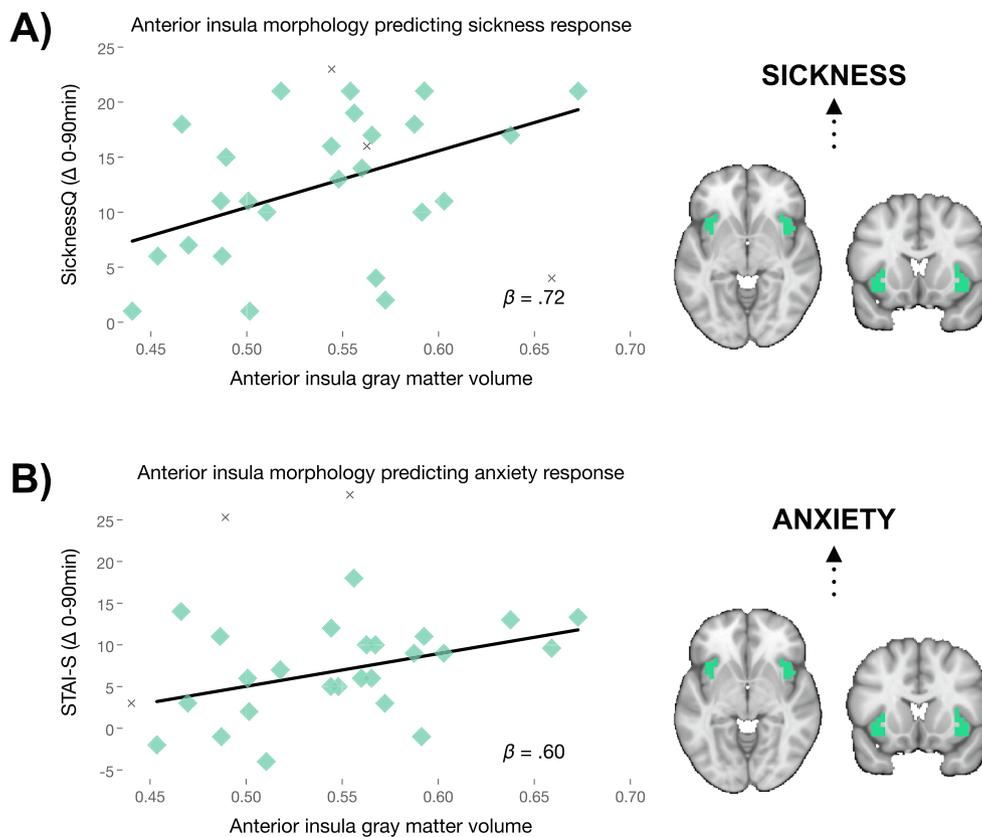
	Sickness			Anxiety		
	$\beta$	t	P	$\beta$	t	P
IL-6 change	0.34	1.42	0.175	–	–	–
TNF- $\alpha$ change	0.02	0.07	0.946	0.40	2.37	0.030
Amygdala	–	–	–	0.24	0.79	0.439
Insula, anterior	0.72	3.64	0.002	0.60	2.34	0.032
Orbitofrontal, lateral	0.08	0.38	0.709	–	–	–
Total GM volume	-0.18	0.70	0.496	-0.25	1.00	0.331
Age	0.02	0.12	0.909	0.25	1.27	0.221
Sex	-0.10	0.61	0.550	-0.28	1.24	0.232
BMI	-0.76	4.48	< 0.001	-0.33	1.46	0.162
<b>Model</b>	<b>(A) Adj <math>R^2 = 65.6\%</math>, <math>P &lt; 0.001</math></b>			<b>(B) Adj <math>R^2 = 40.4\%</math>, <math>P &lt; 0.001</math></b>		

– denotes that the variable was not included in the multiple regression model.

**Abbreviations:** GM, gray matter; BMI, body mass index; IL-6, Interleukin 6; TNF- $\alpha$ , Tumor necrosis factor- $\alpha$ .

vulnerability to short-term behavioral effects of inflammatory activation, this study examined the associations between GM volume of brain regions central for interoception and emotion regulation, and the behavioral response to experimentally-induced inflammation. To summarize the findings, a larger anterior insula volume was associated with greater responses in both sickness and anxiety, independent from other potential variables and covariates of no interest. In addition, a stronger increase in TNF- $\alpha$  was independently related to a stronger increase in anxiety, although it was not significant when correction for multiple testing was considered. These findings support that anterior insula is central for how we interpret immune-activated changes in the body (Benson et al., 2015; Karshikoff et al., 2016; Labrenz et al., 2019; Lekander et al., 2016), and support that differences in GM volume are associated with being more sensitive to develop inflammation-induced sickness. In the univariate regression models, a stronger subjective sickness response to immune activation was indicated as also being associated with smaller lateral orbitofrontal cortex, in addition to higher increases in both TNF- $\alpha$  and IL-6. A stronger increase in anxiety was related to larger amygdala volume. These associations were expected, but were not significant after Bonferroni correction and when entered together in the multivariate model. Notably, neither GM volume of the pregenual, subgenual nor mid cingulate cortices was associated with the subjective response to LPS.

Previous studies have investigated correlations between circulating (unstimulated) levels of pro-inflammatory markers, such as IL-6 and C-reactive protein, and brain morphology, reporting that higher peripheral inflammation was associated with less cortical gray and white matter as well as hippocampal volume (Marsland et al., 2015, 2008). Results from such observational studies of peripheral inflammation and brain structure are likely to reflect longer-term homeostatic perturbations, in contrast to the present study's use of experimental inflammation and focus on the acute behavioral response to immune challenge. Studies using inflammatory activation with functional neuroimaging or fluorodeoxyglucose positron emission tomography (FDG-PET) together point at increased activity in insula, amygdala, hippocampus and cingulate cortices (Benson et al., 2015; Karshikoff et al., 2016; Kraynak et al., 2018; Labrenz et al., 2019; Lekander et al., 2016). The present results extend such studies by indicating that the morphology of some of these regions of interest might modulate the vulnerability to develop acute psychopathology-associated symptoms in response to the inflammatory challenge. Although it remains to be tested with a repeated measure design, the findings may converge with the notion that measures of brain morphology can show short-term changes, such as when viewing complex arousing pictures (Månsson et al., 2020), or two hours after having received pharmacotherapy (dopamine D2 antagonist



**Fig. 3. Anterior insula associated with behavioral responses after LPS administration.** Figure A) displays that anterior insula gray matter volumes are associated with the sickness response (Sickness-Q) from 0 to 1h30min after administration of LPS, B) depicts that anterior insula gray matter volumes also relate to the anxiety response (STAI-S) from 0 to 1h30min after administration of LPS. Statistical outliers (as defined by the influence measure Cook's *D*) were excluded from analyses but displayed (i.e., as marked by the character  $\times$ ) in the figure to ensure transparency. See also Table 2 for details. **Abbreviations:** LPS: lipopolysaccharide, SicknessQ: Sickness Questionnaire, STAI-S: State part of the State-Trait Anxiety Inventory.

haloperidol) (Tost et al., 2010). Although, it remains unclear what T1-weighted MR imaging of the brain's GM volume represents (Tardif et al., 2016 for a review), such macro level assessment reflects several cellular mechanisms, e.g., plasticity in synapses, neurons, and glial cells (Wenger et al., 2017; Zatorre et al., 2012). Thus, we cannot rule out that insular cortex morphology responds to acute inflammation, results which in that case coincide with findings of studies on inflammation induced microstructural changes (Harrison et al., 2015) or neural responses as measured with functional MRI (Lekander et al., 2016). Although different underlying mechanisms may be at play, morphological changes in brain networks parallel their function states (Månsson et al., 2020; Zatorre et al., 2012).

Importantly, the present results point towards immune-to-brain communication as an integral part of interoception (Harrison et al., 2009; Lekander et al., 2016). Specifically, the anterior insula has been argued to provide a consciously accessible representation of inflammation (Harrison et al., 2009; Lekander et al., 2016), and the current findings suggest that the intensity of the conscious experience of malaise is associated with differences in the morphology of the anterior insula. Interestingly, Critchley et al (2004) observed that GM volume of the anterior insula/opercular cortex correlated with accuracy in a heartbeat detection task as well as with subjective ratings of general visceral awareness. As noted, a number of studies have shown that inflammatory activation with LPS modulates interoceptive pathways, including the insular and the anterior and the mid cingulate cortices (Benson et al., 2015; Hannestad et al., 2012; Harrison et al., 2009; Karshikoff et al., 2016; Labrenz et al., 2019; Lekander et al., 2016). Because individuals having a larger insula exhibited both stronger sickness feelings and anxiety after the inflammatory challenge in the present study, the morphology of the anterior insula could be related to vulnerability to inflammatory activation. Again, the cingulate cortices were not associated with sickness and anxiety in the present investigation. The reasons for this are unclear, but if a true relation between cingulate morphology

and the response to inflammatory activation exists, this may be better captured with methods actively engaging top-down regulation, relative to a non-active condition as T1-weighted imaging typically represents. On a related note, volume of the lateral orbitofrontal cortex was negatively related to sickness, in keeping with its suggested role in emotional regulation and mapping of interoceptive needs (Petrovic et al., 2016; Petrovic and Castellanos, 2016). However, volume of the lateral orbitofrontal cortex did not remain a significant predictor in the multivariate regression model.

An influential hypothesis on interoception states that the brain's simulations function as Bayesian filters for incoming sensory input, matching incoming sensory information with top-down predictions created by prior experiences (Barrett and Simmons, 2015; Owens et al., 2018a). This hypothesis is referred to as predictive coding, and corresponds to the concept of top-down modulation. Interestingly, it has been suggested that the lateral orbitofrontal cortex has a pivotal role in such top-down predictions within the interoceptive-emotional domain (Petrovic et al., 2005, 2002). Importantly, such modulation does not only pertain to the subjective experience or other higher-order processing, but also to descending control, as when the anterior cingulate inhibits incoming pain signals through connections with the brain-stem in placebo responses (Fields, 2004; Petrovic et al., 2002), or when interactions between the insula and anterior cingulate cortex together provide descending regulatory influences on peripheral inflammatory pathways (Harrison, 2016). Together with the regions of interest in the present study, these regions constitute nodes involved in integration of interoceptive information and in allostatic predictions (Khalsa et al., 2018). Thus, as part of a hierarchically organized "central autonomic network", regions such as insula and anterior cingulate seem involved in both predicting, representing and regulating somatic/visceral state (Smith et al., 2017). The perspective of predictive coding is therefore relevant in order to understand individual differences in response to inflammation, and how expectations and prior knowledge shape this response.

Following this logic, we previously presented preliminary support for a role of prediction errors, measured as the discrepancy between the immune signal and sickness expectancy, in predicting emotional distress to experimental inflammatory stimulation, suggesting that the emotional response to inflammation might follow a predictive coding model (Lasselín et al., 2018). It is also in keeping with the notion that differences between observed and expected bodily state can be a ‘bottom-up’ source of anxiety (Paulus and Stein, 2006), supported by observations that the anterior insula detects discrepancies in predictions rather than actual changes in physical state (Owens et al., 2018b). Thus, the relation between the expected and the experienced response to an inflammatory stimulus might relate to the degree of anxiety and therefore the propensity of the individual to act on the bodily signal, a process that in speculation could be influenced by the morphology of the anterior insula.

Strengths of the present study were that pre-planned analyses were performed on a restricted number of regions of interest based on theoretically and empirically defined priors in order to contain Type I errors. Another strength is the placebo-controlled experimental design. In addition, the study demonstrates the feasibility of the LPS model to fulfill the need for methods to elicit homeostatic perturbations in controlled settings to chart interoceptive processes (Khalsa et al., 2018). Particularly, two limitations need to be highlighted. First, the statistical power to detect individual differences is restricted. Power analysis was conducted based on a pilot study and the number of included participants allowed a strong power for behavioral and immunological analyses. However, at the time of planning the study, power was unknown for estimates of GMV. To handle this limitation, we included 8 bilaterally defined brain regions and two cytokines, all defined *a priori*, and focused interpretation and conclusions on the final multiple regressions where all variables of interest and covariates were tested at once. Second, MR scans were performed after immune activation. Measures of brain morphology are sensitive to the current mental state and activity (Månsson et al., 2020), providing an alternative explanation to the inferred effects of morphology on the response to inflammation. Given these two main limitations, the current results need to be replicated in independent datasets, especially including designs in which scans are performed both before and after inflammatory activation.

In conclusion, the present study supports that morphological volume of an area central for interoceptive and emotional processes, the anterior insula, is related to how strongly an individual feels sick and becomes anxious after an acute inflammatory activation. Considering the involvement of inflammatory processes in psychiatric disorders and its relation to comorbid problems and outcomes in a range of disease states, the clinical relevance of this phenotypic sensitivity should be studied in clinical groups.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Appendix A. Supplementary data

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#### References

- Ainley, V., Apps, M.A.J., Fotopoulou, A., Tsakiris, M., 2016. “Bodily precision”: a predictive coding account of individual differences in interoceptive accuracy. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 371 (1708), 20160003. <https://doi.org/10.1098/rstb.2016.0003>.
- Andreasson, A., Wicksell, R.K., Lodin, K., Karshikoff, B., Axelsson, J., Lekander, M., 2018. A global measure of sickness behaviour: Development of the Sickness Questionnaire. *J. Health Psychol.* 23 (11), 1452–1463.
- Ashburner, J., Friston, K.J., 2011. Diffeomorphic registration using geodesic shooting and Gauss-Newton optimisation. *Neuroimage* 55 (3), 954–967.
- Barrett, L.F., Simmons, W.K., 2015. Interoceptive predictions in the brain. *Nat. Rev. Neurosci.* 16 (7), 419–429.
- Bechara, A., Naqvi, N., 2004. Listening to your heart: interoceptive awareness as a gateway to feeling. *Nat. Neurosci.*
- Bell, J.A., MRC ImmunoPsychiatry Consortium, Kivimäki, M., Bullmore, E.T., Steptoe, A., Carvalho, L.A., 2017. Repeated exposure to systemic inflammation and risk of new depressive symptoms among older adults. *Transl. Psychiatry* 7, e1208–e1208.
- 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, 222–231.
- Boettcher, H., Brake, C.A., Barlow, D.H., 2016. Origins and outlook of interoceptive exposure. *J. Behav. Ther. Exp. Psychiatry* 53, 41–51.
- Butti, C., Hof, P.R., 2010. The insular cortex: a comparative perspective. *Brain Structure and Function* 214 (5–6), 477–493. <https://doi.org/10.1007/s00429-010-0264-y>.
- Craig, A.D., 2003. Interoception: the sense of the physiological condition of the body. *Curr. Opin. Neurobiol.* 13 (4), 500–505.
- Craig, A.D., 2002. How do you feel? Interoception: the sense of the physiological condition of the body. *Nat. Rev. Neurosci.* 3 (8), 655–666.
- Craig (bud), A.D., Craig (bud), A.D., 2010. The sentient self. *Brain Structure and Function* 214 (5–6), 563–577. <https://doi.org/10.1007/s00429-010-0248-y>.
- Craig, A.D.B., 2004. Human feelings: why are some more aware than others? *Trends Cogn. Sci.* 8 (6), 239–241.
- Critchley, H.D., Wiens, S., Rotshtein, P., Öhman, A., Dolan, R.J., 2004. Neural systems supporting interoceptive awareness. *Nat. Neurosci.* 7 (2), 189–195.
- Dantzer, R., O’Connor, J.C., Freund, G.G., Johnson, R.W., Kelley, K.W., 2008. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat. Rev. Neurosci.* 9 (1), 46–56.
- Di Lernia, D., Serino, S., Riva, G., 2016. Pain in the body. Altered interoception in chronic pain conditions: A systematic review. *Neurosci. Biobehav. Rev.* 71, 328–341.
- Dixon, M.L., Fox, K.C.R., Christoff, K., 2014. Evidence for rostro-caudal functional organization in multiple brain areas related to goal-directed behavior. *Brain Res.* 1572, 26–39.
- Eggart, M., Lange, A., Binsler, M., Queri, S., Müller-Oerlinghausen, B., 2019. Major Depressive Disorder Is Associated with Impaired Interoceptive Accuracy: A Systematic Review. *Brain Sci* 9 (6), 131. <https://doi.org/10.3390/brainsci9060131>.
- Ferentzi, E., Bogdány, T., Szabolcs, Z., Csala, B., Horváth, A., Köteles, F., 2018. Multichannel Investigation of Interoception: Sensitivity Is Not a Generalizable Feature. *Front. Hum. Neurosci.* 12, 223.
- Fields, H., 2004. State-dependent opioid control of pain. *Nat. Rev. Neurosci.* 5 (7), 565–575.
- Gaser, C., Dahnke, R., 2016. CAT—a computational anatomy toolbox for the analysis of structural MRI data. *HBM* 2016, 336–348.
- Grigoleit, J.-S., Engler, H., Schedlowski, M., 2015. Experimental Human Endotoxemia, Sickness Behavior, and Neuropsychiatric Diseases. In: Müller, N., Myint, A.-M., Schwarz, M.J. (Eds.), *Immunology and Psychiatry: From Basic Research to Therapeutic Interventions*. Springer International Publishing, Cham, pp. 63–82.
- Hannestad, J., Subramanyam, K., DellaGioia, N., Planeta-Wilson, B., Weinzimmer, D., Pittman, B., Carson, R.E., 2012. Glucose metabolism in the insula and cingulate is affected by systemic inflammation in humans. *J. Nucl. Med.* 53 (4), 601–607.
- Harrison, N.A., 2017. Brain Structures Implicated in Inflammation-Associated Depression. *Curr. Top. Behav. Neurosci.* 31, 221–248.
- Harrison, N.A., 2016. Commentary on the 2016 named series: Neuroimaging, inflammation and behavior. *Brain Behav. Immun.*
- Harrison, N.A., Brydon, L., Walker, C., Gray, M.A., Steptoe, A., Dolan, R.J., Critchley, H. D., 2009. Neural origins of human sickness in interoceptive responses to inflammation. *Biol. Psychiatry* 66 (5), 415–422.
- Harrison, N.A., Cooper, E., Dowell, N.G., Keramida, G., Voon, V., Critchley, H.D., Cercignani, M., 2015. Quantitative Magnetization Transfer Imaging as a Biomarker for Effects of Systemic Inflammation on the Brain. *Biol. Psychiatry* 78 (1), 49–57.
- Hedman, E., Axelsson, E., Göring, A., Ritzman, C., Ronnheden, M., Alaoui, S.E., Andersson, E., Lekander, M., Ljótsson, B., 2014. Internet-delivered exposure-based cognitive-behavioural therapy and behavioural stress management for severe health anxiety: randomised controlled trial. *Br. J. Psychiatry* 205 (4), 307–314.

- Jensen, K.B., Srinivasan, P., Spaeth, R., Tan, Y., Kosek, E., Petzke, F., Carville, S., Fransson, P., Marcus, H., Williams, S.C.R., Choy, E., Vitton, O., Gracely, R., Ingvar, M., Kong, J., 2013. Overlapping Structural and Functional Brain Changes in Patients With Long-Term Exposure to Fibromyalgia Pain. *Arthritis & Rheumatism* 65 (12), 3293–3303. <https://doi.org/10.1002/art.38170>.
- Karshikoff, B., Jensen, K.B., Kosek, E., Kalpouzos, G., Soop, A., Ingvar, M., Olgart Höglund, C., Lekander, M., Axelsson, J., 2016. Why sickness hurts: A central mechanism for pain induced by peripheral inflammation. *Brain Behav. Immun.* 57, 38–46.
- Karshikoff, B., Lekander, M., Soop, A., Lindstedt, F., Ingvar, M., Kosek, E., Olgart Höglund, C., Axelsson, J., 2015. Modality and sex differences in pain sensitivity during human endotoxemia. *Brain Behav. Immun.* 46, 35–43.
- Kelly, C., Toro, R., Di Martino, A., Cox, C.L., Bellec, P., Castellanos, F.X., Milham, M.P., 2012. A convergent functional architecture of the insula emerges across imaging modalities. *Neuroimage* 61 (4), 1129–1142.
- Keuken, M.C., Bazin, P.-L., Crown, L., Hootsmans, J., Laufer, A., Müller-Axt, C., Sier, R., van der Putten, E.J., Schäfer, A., Turner, R., Forstmann, B.U., 2014. Quantifying inter-individual anatomical variability in the subcortex using 7 T structural MRI. *Neuroimage* 94, 40–46.
- Khalsa, S.S., Adolphs, R., Cameron, O.G., Critchley, H.D., Davenport, P.W., Feinstein, J. S., Feusner, J.D., Garfinkel, S.N., Lane, R.D., Mehling, W.E., Meuret, A.E., Nemeroff, C.B., Oppenheimer, S., Pletzschner, F.H., Pollatos, O., Rhudy, J.L., Schramm, L.P., Simmons, W.K., Stein, M.B., Stephan, K.E., Van den Bergh, O., Van Diest, I., von Leupoldt, A., Paulus, M.P., Ainley, V., Al Zoubi, O., Aupperle, R., Avery, J., Baxter, L., Benke, C., Berner, L., Bodurka, J., Breese, E., Brown, T., Burrows, K., Cha, Y.-H., Clausen, A., Cosgrove, K., Deville, D., Duncan, L., Duquette, P., Ekhtiari, H., Fine, T., Ford, B., Garcia Cordero, I., Gleghorn, D., Guereca, Y., Harrison, N.A., Hassanpour, M., Hechler, T., Heller, A., Hellman, N., Herbert, B., Jarrahi, B., Kerr, K., Kirlic, N., Klabunde, M., Kraynak, T., Kriegsmann, M., Kroll, J., Kuplicki, R., Lapidus, R., Le, T., Hagen, K.L., Mayeli, A., Morris, A., Naqvi, N., Oldroyd, K., Pané-Farré, C., Phillips, R., Poppa, T., Potter, W., Puhl, M., Safron, A., Sala, M., Savitz, J., Saxon, H., Schoenhals, W., Stanwell-Smith, C., Teed, A., Terasawa, Y., Thompson, K., Toups, M., Umeda, S., Upshaw, V., Victor, T., Wierenga, C., Wohlrab, C., Yeh, H.-W., Yoris, A., Zeidan, F., Zotev, V., Zucker, N., 2018. Participants, 2018. Interoception and Mental Health: A Roadmap. *Biol. Psychiatry. Cogn. Neurosci. Neuroimaging* 3 (6), 501–513.
- Khandaker, G.M., Pearson, R.M., Zammit, S., Lewis, G., Jones, P.B., 2014. Association of serum interleukin 6 and C-reactive protein in childhood with depression and psychosis in young adult life: a population-based longitudinal study. *JAMA Psychiatry* 71 (10), 1121. <https://doi.org/10.1001/jamapsychiatry.2014.1332>.
- Kraynak, T.E., Marsland, A.L., Wager, T.D., Gianaros, P.J., 2018. Functional neuroanatomy of peripheral inflammatory physiology: A meta-analysis of human neuroimaging studies. *Neurosci. Biobehav. Rev.* 94, 76–92.
- Labrenz, F., Ferri, F., Wrede, K., Forsting, M., Schedlowski, M., Engler, H., Elsenbruch, S., Benson, S., Costantini, M., 2019. Altered temporal variance and functional connectivity of BOLD signal is associated with state anxiety during acute systemic inflammation. *Neuroimage* 184, 916–924.
- Lasselín, J., Elsenbruch, S., Lekander, M., Axelsson, J., Karshikoff, B., Grigoleit, J.-S., Engler, H., Schedlowski, M., Benson, S., 2016. Mood disturbance during experimental endotoxemia: Predictors of state anxiety as a psychological component of sickness behavior. *Brain Behav. Immun.* 57, 30–37.
- Lasselín, J., Lekander, M., Benson, S., Schedlowski, M., Engler, H., 2020. Sick for science: experimental endotoxemia as a translational tool to develop and test new therapies for inflammation-associated depression. *Mol. Psychiatry*. <https://doi.org/10.1038/s41380-020-00869-2>.
- Lasselín, J., Petrovic, P., Olsson, M.J., Paues Göransson, S., Lekander, M., Jensen, K.B., Axelsson, J., 2018. Sickness behavior is not all about the immune response: Possible roles of expectations and prediction errors in the worry of being sick. *Brain Behav. Immun.* 74, 213–221.
- Lekander, M., Karshikoff, B., Johansson, E., Soop, A., Fransson, P., Lundström, J.N., Andreasson, A., Ingvar, M., Petrovic, P., Axelsson, J., Nilsson, G., 2016. Intrinsic functional connectivity of insular cortex and symptoms of sickness during acute experimental inflammation. *Brain Behav. Immun.* 56, 34–41.
- Ludwick-Rosenthal, R., Neufeld, R.W.J., 1985. Heart beat interoception: a study of individual differences. *Int. J. Psychophysiol.* 3 (1), 57–65.
- Månsson, K.N.T., Cortes, D.S., Manzouri, A., Li, T.-Q., Hau, S., Fischer, H., 2020. Viewing Pictures Triggers Rapid Morphological Enlargement in the Human Visual Cortex. *Cereb. Cortex* 30, 851–857.
- Marsland, A.L., Gianaros, P.J., Abramowitz, S.M., Manuck, S.B., Hariri, A.R., 2008. Interleukin-6 covaries inversely with hippocampal grey matter volume in middle-aged adults. *Biol. Psychiatry* 64 (6), 484–490.
- Marsland, A.L., Gianaros, P.J., Kuan, D.-H., Sheu, L.K., Krajina, K., Manuck, S.B., 2015. Brain morphology links systemic inflammation to cognitive function in midlife adults. *Brain Behav. Immun.* 48, 195–204.
- Naidich, T.P., Kang, E., Fatterpekar, G.M., Delman, B.N., Gultekin, S.H., Wolfe, D., Ortiz, O., Yousry, I., Weismann, M., Yousry, T.A., 2004. The insula: anatomic study and MR imaging display at 1.5 T. *AJNR Am. J. Neuroradiol.* 25, 222–232.
- O'Connor, M.-F., Irwin, M.R., Wellisch, D.K., 2009. When grief heats up: pro-inflammatory cytokines predict regional brain activation. *NeuroImage* 47 (3), 891–896.
- Owens, A.P., Allen, M., Ondobaka, S., Friston, K.J., 2018a. Interoceptive inference: From computational neuroscience to clinic. *Neurosci. Biobehav. Rev.* 90, 174–183.
- Owens, A.P., Friston, K.J., Low, D.A., Mathias, C.J., Critchley, H.D., 2018b. Investigating the relationship between cardiac interoception and autonomic cardiac control using a predictive coding framework. *Autonomic Neuroscience* 210, 65–71. <https://doi.org/10.1016/j.autneu.2018.01.001>.
- Paulus, M.P., Stein, M.B., 2006. An insular view of anxiety. *Biol. Psychiatry* 60 (4), 383–387.
- Petrovic, P., Castellanos, F.X., 2016. Top-Down Dysregulation-From ADHD to Emotional Instability. *Front. Behav. Neurosci.* 10, 70.
- Petrovic, P., Dietrich, T., Fransson, P., Andersson, J., Carlsson, K., Ingvar, M., 2005. Placebo in emotional processing—induced expectations of anxiety relief activate a generalized modulatory network. *Neuron* 46 (6), 957–969.
- Petrovic, P., Ekman, C.J., Klahr, J., Tigerström, L., Rydén, G., Johansson, A.G.M., Sellgren, C., Golkar, A., Olsson, A., Öhman, A., Ingvar, M., Landén, M., 2016. Significant grey matter changes in a region of the orbitofrontal cortex in healthy participants predicts emotional dysregulation. *Soc. Cogn. Affect. Neurosci.* 11, 1041–1049.
- Petrovic, P., Kalso, E., Petersson, K.M., Ingvar, M., 2002. Placebo and opioid analgesia—imaging a shared neuronal network. *Science* 295, 1737–1740.
- Quadt, L., Critchley, H.D., Garfinkel, S.N., 2018. The neurobiology of interoception in health and disease. *Ann. N. Y. Acad. Sci.* 1428, 112–128.
- Rolls, E.T., Huang, C.-C., Lin, C.-P., Feng, J., Joliot, M., 2020. Automated anatomical labelling atlas 3. *Neuroimage* 206, 116189. <https://doi.org/10.1016/j.neuroimage.2019.116189>.
- Singer, T., Critchley, H.D., Preusschoff, K., 2009. A common role of insula in feelings, empathy and uncertainty. *Trends Cogn. Sci.* 13 (8), 334–340.
- Slavich, G.M., 2015. Understanding inflammation, its regulation, and relevance for health: a top scientific and public priority. *Brain Behav. Immun.* 45, 13–14.
- Smith, R., Thayer, J.F., Khalsa, S.S., Lane, R.D., 2017. The hierarchical basis of neurovisceral integration. *Neurosci. Biobehav. Rev.* 75, 274–296.
- Spielberger, C.D., Gorsuch, R.L., Lushene, R.E., 1970. *STAI: Manual for the State-Trait Anxiety Inventory (Self-Evaluation Questionnaire)*. Consulting Psychologist Press, Palo Alto.
- Tardif, C.L., Gauthier, C.J., Steele, C.J., Bazin, P.-L., Schäfer, A., Schaefer, A., Turner, R., Villringer, A., 2016. Advanced MRI techniques to improve our understanding of experience-induced neuroplasticity. *Neuroimage* 131, 55–72.
- Tost, H., Braus, D.F., Hakimi, S., Ruf, M., Vollmert, C., Hohn, F., Meyer-Lindenberg, A., 2010. Acute D2 receptor blockade induces rapid, reversible remodeling in human cortical-striatal circuits. *Nat. Neurosci.* 13 (8), 920–922.
- Umeda, S., Harrison, N.A., Gray, M.A., Mathias, C.J., Critchley, H.D., 2015. Structural brain abnormalities in postural tachycardia syndrome: A VBM-DARTEL study. *Frontiers in Neuroscience*. <https://doi.org/10.3389/fnins.2015.00034>.
- Uylings, H.B.M., Arigita, E.J.S., de Vos, K., Pool, C.W., Evers, P., Rajkowska, G., 2010. 3-D Cytoarchitectonic parcellation of human orbitofrontal cortex. *Psychiatry Research: Neuroimaging* 183 (1), 1–20. <https://doi.org/10.1016/j.pscychres.2010.04.012>.
- Wang, X., Wu, Q., Egan, L., Gu, X., Liu, P., Gu, H., Yang, Y., Luo, J., Wu, Y., Gao, Z., Fan, J., 2019. Anterior insular cortex plays a critical role in interoceptive attention. *eLife*. <https://doi.org/10.7554/eLife.42265>.
- Wenger, E., Brozzoli, C., Lindenberg, U., Lövdén, M., 2017. Expansion and Renormalization of Human Brain Structure During Skill Acquisition. *Trends Cogn. Sci.* 21 (12), 930–939.
- Wohleb, E.S., McKim, D.B., Sheridan, J.F., Godbout, J.P., 2014. Monocyte trafficking to the brain with stress and inflammation: a novel axis of immune-to-brain communication that influences mood and behavior. *Front. Neurosci.* 8, 447.
- Zatorre, R.J., Fields, R.D., Johansen-Berg, H., 2012. Plasticity in gray and white: neuroimaging changes in brain structure during learning. *Nat. Neurosci.* 15 (4), 528–536.