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Mild inflammation causes a reduction in resting-state amplitude of low-frequency fluctuation in healthy adult males

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Abstract

Systemic inflammation has been associated with negative mood states and human sickness behaviour. Previous studies have shown an association between systemic inflammation and changes in task-related blood-oxygen-level-dependent activity and functional connectivity within large-scale networks. However, no study has examined the effect of inflammation on the magnitude of blood-oxygen-level-dependent low-frequency fluctuations at rest. We used a double-blind placebo-controlled crossover design to randomise 20 male subjects (aged 20–50 years) to receive either a *Salmonella typhi* vaccine or a placebo saline injection at two separate sessions. All participants underwent a resting-state functional magnetic resonance scan and a measure of inflammation (interleukin 6) and mood (Profile of Mood States) 3 h after injection. We compared the whole brain amplitude of low-frequency fluctuations between the vaccine and placebo conditions using a repeated measures design. Vaccine condition was associated with greater interleukin 6 levels ($p < 0.001$). Vaccine condition was also associated with lower amplitude of low-frequency fluctuations in the right and left frontal pole, superior frontal gyrus, paracingulate gyrus (Cluster 1) and the right mid and inferior frontal gyrus (Cluster 2) ($p < 0.001$, false discovery rate corrected). Lower amplitude of low-frequency fluctuations pertaining to first cluster correlated with greater total Profile of Mood States score (worse mood) ($r = -0.38$; $p = 0.04$). These results imply possible excitation/inhibition imbalance mechanisms during inflammation that may be a relevant target in psychiatric disease, especially mood disorders.

Keywords

Inflammation, mood disorders, functional neuroimaging

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Introduction

Systemic inflammation has been associated with negative mood states and human sickness behaviour. Cross-sectional and longitudinal studies have shown increased inflammatory markers in blood, cerebrospinal fluid (CSF) and the brain (positron emission tomography) of patients with several psychiatric conditions, including major depression (Krishnadas and Harrison, 2016). Systemic inflammation co-varies with brain structure and elicits negative mood states through task-induced changes in blood-oxygen-level-dependent (BOLD) functional magnetic resonance imaging (fMRI) activity and resting-state large-scale and local network properties derived from low-frequency (0.01–0.09 Hz) BOLD fluctuations (Kraynak et al., 2018; Krishnadas et al., 2013; Stefanov et al., 2020). These studies have investigated spatio-temporal synchronisation of low-frequency fluctuations (functional connectivity) but have largely ignored the magnitude of regional activity during resting state. One such measure of regional brain activity is the amplitude of low-frequency fluctuations (ALFF), which indexes the power (amplitude) of the low-frequency ranges of spontaneous neuronal activity (Yang et al., 2007). ALFF correlates with glutamatergic and GABAergic (gamma-aminobutyric acid) activity, the balance of which seems to be disrupted in chronic stress and several psychiatric conditions (Duman et al., 2019; Kurcyus et al., 2018). Inflammation is known to affect glutamatergic and GABAergic activity, and chronic inflammatory conditions have been shown to disrupt ALFF (McIntosh et al., 2018; Vezzani and Viviani, 2015). However, no study has yet shown a causal relationship between low-grade peripheral inflammation and changes in the magnitude of ALFF (Krishnadas and Harrison, 2016). This article utilised a double-blind placebo-controlled crossover experiment to examine the

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effect of inducing a low-grade inflammatory stimulus using a *Salmonella typhi* vaccine on ALFF compared to a placebo saline injection using resting-state fMRI. We hypothesised that ALFF will be significantly disrupted in the vaccination condition, compared to the placebo condition, and will be associated with the absolute mood state at the time of scan. This analysis was an exploratory secondary analysis using the same dataset presented elsewhere (Stefanov et al., 2020).

Methods and materials

The methods have been published in detail previously (Stefanov et al., 2020). Briefly, ethical approval was obtained from the NHS Regional Ethics Committee and all subjects provided informed consent. We used a double-blind crossover design, in which participants were randomised to two conditions: *S. typhi* vaccine or placebo saline injection (6–8 weeks apart) (NCT02653235). We recruited 20 healthy males aged 20–50 years with no history of medical or Axis I *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition (DSM-IV) diagnosis, who did not have a *S. typhi* vaccination over the last 3 years or any other vaccinations over the last 6 months, who had not taken antibiotics or anti-inflammatory agents over the 2 weeks leading to the study and who did not have contraindications for *S. typhi* vaccination or MRI.

At the first visit, participants were randomly assigned to receive either the vaccine or placebo at the initial visit, using a 1:1 allocation as per a randomisation schedule. A nurse, independent of the study, ensured allocation concealment and intramuscularly administered the vaccine/placebo from a pre-filled syringe. The code for allocation was broken only at the end of the study. All participants were first seen at 9 am, when baseline measures and fasting plasma were obtained. Following this, they were administered the placebo/vaccine on the same arm. All participants were then scanned, and plasma was once again collected at around 3 h after the injection.

The vaccine

S. typhi (Typhim Vi) vaccine consisted of the Vi capsular polysaccharide typhoid vaccine, 50 mg/mL virulence polysaccharide antigen of formaldehyde-inactivated *S. typhi*. The placebo was 0.5 mL of normal saline.

Clinical and interleukin 6 measurement

All participants were screened using the Structured Clinical Interview for DSM-IV (SCID). Participants also completed the Profile of Mood State Questionnaire (POMS) at baseline and 3 h post-injection. Body temperature, blood pressure and plasma IL-6 levels were measured at baseline and 3 h after the injection. Plasma was collected from 10 mL of venous blood drawn into BD vacutainers containing K2EDTA by centrifuging at 8000 r/min for 10 min and stored at −80°C. IL-6 was measured in duplicate using Human IL-6 Quantikine High-Sensitivity ELISA kits (R and D systems). Optical densities were read and converted into concentrations against a 7-point standard curve. The kit sensitivity was 0.11 pg/mL, and the intra- and inter-assay coefficients of variation were 10% and 11%, respectively.

Image acquisition and preprocessing

All scans were acquired 3 h after receiving the vaccine/placebo on a GE, 3T Signa Excite HD system (Milwaukee, USA) with a 32-channel head coil. A structural T1 scan (repetition time = 2300 ms, echo time = 2.96 ms, 192 sagittal slices, 1 mm^3 isotropic voxels and image resolution 256 × 256) was obtained for co-registration purposes. A resting-state fMRI scan (10 min; eyes open) was acquired with single-shot full k-space echo-planar imaging with ramp sampling correction using the intercommissural line (AC–PC) as a reference (repetition time = 2000 ms, echo time = 29 ms, matrix size = 64 × 64, 32 slices, voxel size = 3 × 3 × 4 mm^3, axial plane – series ascending – multi-slice mode; interleaved; 300 volumes). Preprocessing was carried out using Statistical Parametric Mapping software (SPM12; Wellcome Department of Cognitive Neurology, London, UK). Briefly, this included the removal of the first five volumes, manual reorientation, T1 co-registration to functional scans, segmentation and normalisation by DARTEL, and spatial smoothing (6 mm full width at half maximum), and band-pass filters (0.01–0.09 Hz). To correct for head movement, 24 motion parameters (6 head motion parameters, 6 head motion parameters one time point before, and the 12 corresponding squared items), along with signals from the white matter and CSF were regressed for each subject and session at the voxel level.

Analysis

ALFF maps represent a measure of BOLD signal power within the frequency band (0.01–0.09 Hz) and are defined as the root mean square (RMS) of BOLD signal at each individual voxel (Yang et al., 2007). Whole brain ALFF was compared between the vaccine condition and placebo condition using a repeated measures design, with age and BMI as covariates using the CONN toolbox (Whitfield-Gabrieli and Nieto-Castanon, 2012). Regions of significant difference were defined by the clusters surviving the voxel-level height threshold of uncorrected $p < 0.001$ and the cluster-level extent threshold of $p < 0.05$ that was corrected for multiple comparisons using the false discovery rate. Change in IL-6 levels between vaccine/placebo conditions was measured using the repeated measures analysis of variance (ANOVA). Total mood (POMS) score was calculated by adding the POMS sub-scores for all dimensions (tension, depression, anger, fatigue, confusion and vigour) and then subtracting the score for 'vigour'. Fisher's Z-transformed signal intensity values of the significant clusters were extracted, and the weighted average of each significant cluster (corrected for mean RMS displacement) was correlated with the absolute total mood (POMS) score at the time of scan (3 h after the injection) using Pearson's r correlations.

Results

The mean age of the sample was 25.63 (SD = 6.51) years and a mean BMI of 22.76 (SD = 2.17). One participant dropped out and one had excess movement (>3 mm) in the scanner. We, therefore, analysed data from 18 subjects who had complete data. Neither placebo nor vaccination was associated with a significant change in systolic/diastolic BP ($p > 0.05$) or temperature ($p > 0.05$) or any movement parameters on resting-state fMRI.

None of the participants exhibited any injection site discomfort, pain or swelling at 3 h.

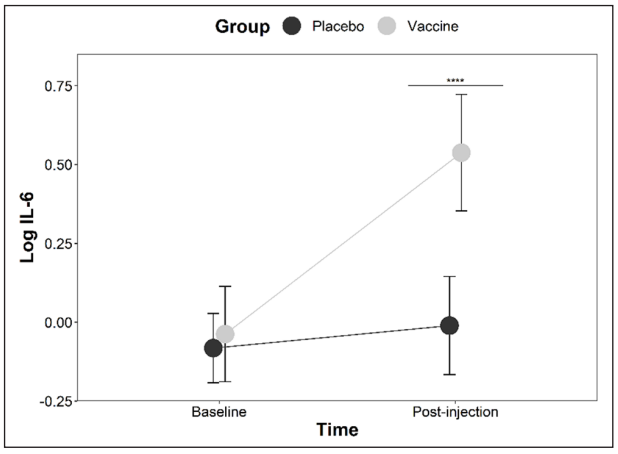

Figure 1. Interleukin (IL)-6 levels (log transformed) as measured prior to and 3 h after injection. The vaccine condition induced significantly higher IL-6 levels than placebo using a repeated-measures analysis of variance. ****$p < 0.001$ level.

Vaccine condition was associated with increase in inflammatory markers

Using a repeated measures ANOVA, we found that there was a significant effect of group ($F(1,17) = 33.69$; $p < 0.001$), time ($F(1,17) = 24.31$; $p < 0.001$) and a group × time interaction ($F(1,17) = 35.99$; $p < 0.001$) on IL-6 levels (Figure 1).

ALFF

Vaccine condition was associated with lower ALFF pertaining to two clusters in the frontal regions (Figure 2). The first cluster included the right and left frontal pole, superior frontal gyrus and paracingulate gyrus (peak MNI coordinates +10 +48 +38; $t = -7.62$; $p < 0.001$ (false discovery rate corrected)). The second cluster included the right mid and inferior frontal gyrus (peak MNI coordinates +34 +18 +20; $t = -8.67$; $p < 0.001$ (false discovery rate corrected)). Lower mean weighted ALFF pertaining to first cluster (peak MNI coordinates +10 +48 +38) was associated with greater total POMS score ($r = -0.34$; $p = 0.04$) at the time of scan. The mean weighted ALFF pertaining to second cluster (peak MNI coordinates +34 +18 +20) did not show any significant correlation ($r = 0.01$; $p = 0.99$).

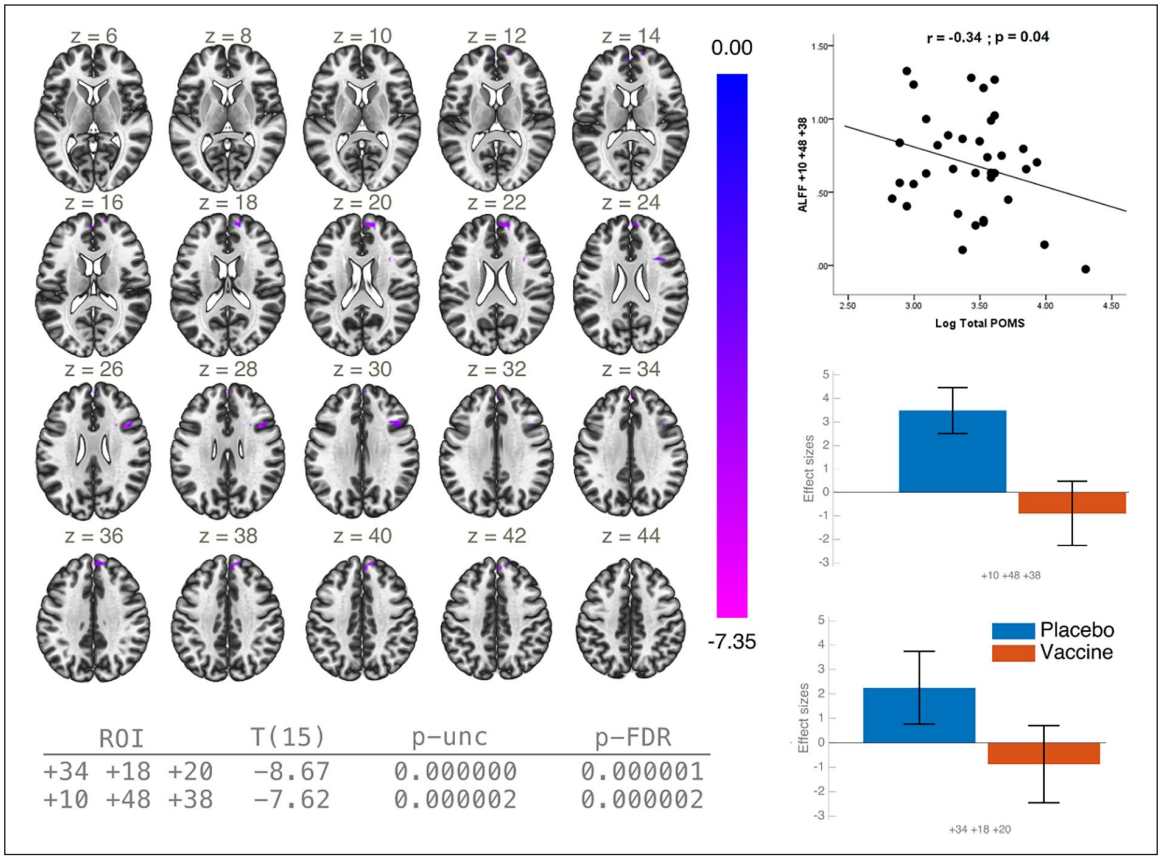

Figure 2. Vaccine-associated changes in ALFF. Vaccine condition was associated with a reduction in ALFF with two major clusters, with peaks in MNI coordinates +10 +48 +38 and +34 +18 +20. The difference in mean ALFF in each cluster is shown. Lower ALFF in the first cluster was associated with greater total POMS score at the time of scan.

Discussion

Using a double-blind placebo-controlled crossover design, we have provided novel and preliminary evidence implying that inducing a mild systemic inflammatory state (as reflected by an increase in IL-6) in healthy adult males is associated with a reduction of ALFF in frontal regions of the brain, previously implicated in psychiatric conditions, including major depressive illness. Crucially, lower ALFF was associated with greater absolute mood score (worse mood) at the time of scan in these individuals.

Our findings were centred around prefrontal regions of the brain previously associated with inflammation-related disrupted activity and connectivity (Kraynak et al., 2018). While these previous investigations have shown a relationship between systemic inflammation and neuronal activity and large-scale brain network organisation, our results for the first time suggest that a mild inflammatory stimulus may affect a more fundamental property that represents the magnitude/amplitude of resting-state BOLD low-frequency fluctuations – a measure of spontaneous neural activity.

The neurological processes underlying ALFF are thought to be associated with glutamatergic and GABAergic neurotransmitter systems (Kurcyus et al., 2018). Inflammatory cytokines have been shown to have a significant effect on the above systems. For example, interleukin 1 beta (IL-1β), IL-6 and tumour necrosis factor alpha (TNF-α) have been shown to have an excitatory effect, mediated by N-methyl-D-aspartate receptor (NMDAR)-induced neuronal calcium influx, subunit expression and phosphorylation, synaptic scaling and increased glutamate release, and an inhibitory effect on GABAergic neurotransmission (Vezzani and Viviani, 2015). This may indeed affect the cortical excitatory/inhibitory balance – a pathophysiological mechanism that is proposed to underlie chronic stress and depression (Duman et al., 2019). We speculate that mild inflammation induced by the *S. typhi* vaccine signals the brain through afferent mechanisms and affects glutamatergic and GABAergic neurotransmission, thus affecting ALFF. Given that proinflammatory cytokines have an excitatory effect on glutamatergic receptors, our finding of lower ALFF in the vaccine condition, compared to the placebo condition, seems counterintuitive. However, we speculate that an increase in inflammation will lead to a generalised disruption in the cortical glutamate and GABA neurotransmitter systems (possibly due to an increase in glutamatergic activity at the NMDAR on GABAergic neurons).

Using a similar experimental paradigm, Harrison et al have previously shown an inflammation-induced increase in metabolism, associated with a disruption in tissue microstructure (Harrison et al., 2015). They speculate that these changes may have been influenced by the accumulation of metabolically active macromolecule, such as lactate, as a result of increased neuronal activity. Lactate is indeed thought to have a differential effect on glutamatergic activity, compared to GABA (Magistretti and Allaman, 2018). This generalised disruption in glutamate/GABA activity would, in turn, affect the signal-to-noise ratio and result in degradation of signal integrity pertaining to resting-state spontaneous neuronal activity, a finding that has been seen in chronic stress and major depression and reversed by recent treatments, such as ketamine (that also has significant anti-inflammatory effects) (Duman et al., 2019). However, the precise mechanism underlying the immunological influences on GABA/glutamate neurotransmission that lead directly to changes in ALFF is not clear.

The conclusions that we can draw in this report are limited by the specific focus on ALFF. We can only indirectly link the effects of immune modulation on excitation/inhibition balance as this study did not record any direct measures of GABA or glutamate turnover, and the interpretation of our findings remains largely speculative. Nevertheless, we show some preliminary evidence to suggest that the change in ALFF associated with inflammation also leads to some negative mood states. The present report is a secondary analysis of a previously published dataset, and therefore, future studies should replicate our finding using imaging methods to confirm that a temporary inflammatory state can alter this glutamatergic/GABA balance in the brain.

In summary, we demonstrated that inducing a mild inflammatory state is associated with reductions in ALFF of frontal regions that are correlated with overall absolute total mood score. These results imply possible excitation/inhibition imbalance during inflammation that may be a relevant target in psychiatric disease, especially mood disorders.

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