Neural Origins of Human Sickness in Interoceptive Responses to Inflammation

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Background: Inflammation is associated with psychological, emotional, and behavioral disturbance, known as sickness behavior. Inflammatory cytokines are implicated in coordinating this central motivational reorientation accompanying peripheral immunologic responses to pathogens. Studies in rodents suggest an afferent interoceptive neural mechanism, although comparable data in humans are lacking.

Methods: In a double-blind, randomized crossover study, 16 healthy male volunteers received typhoid vaccination or saline (placebo) injection in two experimental sessions. Profile of Mood State questionnaires were completed at baseline and at 2 and 3 hours. Two hours after injection, participants performed a high-demand color word Stroop task during functional magnetic resonance imaging. Blood samples were performed at baseline and immediately after scanning.

Results: Typhoid but not placebo injection produced a robust inflammatory response indexed by increased circulating interleukin-6 accompanied by a significant increase in fatigue, confusion, and impaired concentration at 3 hours. Performance of the Stroop task under inflammation activated brain regions encoding representations of internal bodily state. Spatial and temporal characteristics of this response are consistent with interoceptive information flow via afferent autonomic fibers. During performance of this task, activity within interoceptive brain regions also predicted individual differences in inflammation-associated but not placebo-associated fatigue and confusion. Maintenance of cognitive performance, despite inflammation-associated fatigue, led to recruitment of additional prefrontal cortical regions.

Conclusions: These findings suggest that peripheral infection selectively influences central nervous system function to generate core symptoms of sickness and reorient basic motivational states.

Key Words: Cytokines, fatigue, fMRI, insula, interoception, peripheral inflammation

In healthy mammals, systemic infection triggers a set of behavioral, psychological, and physiological changes collectively known as "sickness behavior" (1,2). Symptoms include decreased motivation (e.g., fatigue, lethargy, adipsia, and anorexia), psychomotor retardation, fever, cognitive and affective change, poor concentration, confusion, depression, and impaired memory (3–5). The same stereotyped pattern of sickness behaviors is evoked across a range of infectious and inflammatory conditions, suggesting a highly coordinated reorganization of the body's physiological and motivational state to prioritize adaptive responses to pathogens and preserve bodily integrity (6). These behaviors might also be induced iatrogenically by the therapeutic administration of interferon-α (IFN-α) for the treatment of chronic viral infections and cancer (7,8). The latter observation provides direct evidence that cytokines are central to the etiology of human sickness behaviors and represents a clinical impetus for determining their underlying neurobiological basis.

Studies in rodents, using experimentally induced peripheral inflammation, emphasize a role for pro-inflammatory cytokines in mediating the production of sickness behaviors (6,9). Both early central communication of peripheral inflammatory signals (10) and subsequent motivational reorientation seem dependent upon the integrity of interoceptive vagus nerve afferents, the visceral terminals of which express cytokine binding sites (11). Early in the inflammatory response, antigen-presenting cells cluster in the vicinity of vagus nerve afferents and act as immune chemosensory elements signaling to vagal neurons via cytokine-dependent (12) and -independent mechanisms (13).

Immunohistochemical studies using the immediate early gene c-Fos to index neural activation confirm that peripheral inflammation and specifically binding of pro-inflammatory cytokines to vagus nerve receptors activate brain structures implicated in homeostasis and the representation of internal bodily state (interoception) (10). This afferent signaling is rapid; peripheral inflammation induces c-Fos expression in the primary projection nucleus of the vagus nerve (nucleus tractus solitarius [NTS]) and secondary projection regions (including parabrachial, paraventricular and supraoptic nuclei, central amygdala, and bed nucleus of the stria terminalis) within 60 min of peripheral inflammatory challenge (10). In rodents, damage to the vagus nerve attenuates specific sickness behaviors, including changes in motivation (14) and social behavior (15).

Afferent interoceptive information conveyed via the spinal cord might also contribute to central signaling of peripheral inflammation. Information traveling through spinal lamina I is predominantly tuned to motivationally salient sensations, including pain (16,17), temperature (18), itch (19), and sensual touch (20), and converges with vagus nerve afferent information within brainstem and thalamus (21). In humans, the central terminus of convergent afferent vagus and spinal interoceptive pathways within anterior insula cortex might support a consciously accessible representation of physical wellbeing (22) that is implicated as a neural substrate for subjective emotional feelings (17,22). Whether these interoceptive neural afferent pathways also pro-

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vide the principle channel for rapid central signaling of peripheral inflammation in humans has not been established previously.

We combined functional magnetic resonance imaging (fMRI) with an experimental model of peripheral inflammation to address the question of how inflammatory signals are represented in the brain and to describe the neurobiological mechanisms that underlie an early shift in motivational behavior. The complementary question of how peripheral inflammation induces a change in mood and emotional processing is the focus of another publication in this journal (23). We used *Salmonella typhi* vaccination (Typhim Vi) as our inflammatory challenge. This inflammatory model has been shown previously to stimulate a low-grade peripheral inflammatory response with associated cognitive, behavioral, and emotional components of sickness (24,25). Importantly, we have also previously reported that peripheral inflammation induced with this model in this subject population does not cause a general change in the coupling between neural activity and blood-oxygen-level-dependent (BOLD) signal, the basis of inferences made in fMRI studies (26). We predicted that the central communication of peripheral inflammation is associated with pro-inflammatory cytokine release and achieved through direct modulation of interoceptive pathways engendering core cognitive and motivational components of sickness behavior, including subjective fatigue and lethargy. We used the color-word Stroop as a high-demand cognitive task that induces mental stress through marked attentional load and response-control demands. The Stroop task is frequently employed to assess high-demand cognitive processes including attentional and executive control, stimulus conflict, response monitoring, and inhibition with well described behavioral effects and neural basis (27,28). These processes are typically compromised by sickness and are a focus of the current report. We have previously reported that inflammatory influences on low-level psychomotor responses with this task are modulated via an action on the substantia nigra (26).

Methods and Materials

Subjects and Study Design

The study design was a randomized, double-blind crossover trial. Sixteen healthy male participants, mean age (± SD) 24.9 years (± 4.8), were tested twice a mean of 7 days apart. All were medication-free with no use of nonsteroidal or steroidal anti-inflammatory drugs in the preceding 2 weeks or any vaccination in the preceding 6 months or typhoid vaccination in the 3 years preceding study enrollment. Participants were blind to the injection order. Typhim is a standard vaccination for travel to regions with poor sanitation; other than mild sickness symptoms, local soreness, and erythema, serious reactions are rare. Procedures were approved by the joint University College London (UCL)/University College London Hospitals (UCLH) Ethics Committee. Of note, these are the same group of subjects that were reported on in our previous study (26) and in (23).

Generation and Measurement of Inflammatory Response

Participants received injection with either .025 mg Typhim Vi (Aventis Pasteur, MSD, Maidenhead, Berkshire, United Kingdom) or .9% sodium chloride into the nondominant deltoid muscle, receiving the other injection on their return visit. Venesection was performed at baseline and 3 hours after vaccination immediately after MRI scanning. Plasma interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α) were assessed with high-sensitivity two-site enzyme-linked immunosorbent assays (R&D Systems, Oxford, United Kingdom). Salivary cortisol was collected with cotton dental rolls at baseline and at 2 and 3 hours (Salivettes, Sarstedt, Leicester, United Kingdom) and analyzed with a time resolved immunoassay with fluorescence detection. Body temperature, heart rate, and resting blood pressure were measured at baseline, 2 hours, and 3 hours with sublingual digital thermometer and an electronic sphygmomanometer (A&D UA779, Tokyo, Japan), respectively.

Behavioral and Mood Ratings

Mood and other psychological symptoms were assessed with a modified version of the Profile of Mood States (POMS) (29). This consisted of five to six items from each of five scales (vigor, fatigue, depression, tension-anxiety, and mental confusion), together with four somatic symptom items. Each was rated from 0 to 4, and scores were computed by summing ratings on individual items. Paired *t* test was used to compare responses to vaccine and placebo conditions.

Color Word Stroop Task

The target color word was presented with the four possible response words (red yellow green blue) below. Target words and order of response words were displayed randomly. Subjects were instructed to respond as rapidly as possible to the color of the target word with a four-button response pad corresponding to the response words below. In the incongruent condition all words were printed in a color incongruent with the target word; in the congruent condition the font color of all words matched the read target word. Both targets and possible response words were displayed for 3000 msec preceded by a central fixation cross presented for 2000 msec.

Functional Imaging and Imaging Data Analysis

Functional MRI data were acquired on a 1.5-T Siemens Sonata MR scanner (Siemens, Malvern, Pennsylvania) equipped with a standard head coil. Heart rate was continuously recorded with a pulse oximeter (Nonin 8600, Nonin Medical, Plymouth, Minnesota), probe on the left index finger. The fMRI datasets were analyzed with SPM5 (http://www.fil.ion.ucl.ac.uk/spm). The first five volumes were discarded. Individual scans were realigned and unwarped, time-corrected, normalized, and spatially smoothed with an 8-mm full-width-at-half-maximal Gaussian kernel with standard SPM methods. A high-pass frequency filter (cut off 120 sec) and corrections for auto-correlation between scans (AR1) were applied to the time series.

Each event was modeled by a standard synthetic hemodynamic response function. Congruent and incongruent trials and commission and omission errors were modeled as separate regressors in first-level multiple regression analyses. Null events were included to facilitate identification of differential hemodynamic responses to stochastically ordered stimuli. First-level design matrices for each participant were estimated within the General Linear Model. Effects of task (incongruent and congruent vs. implicit baseline) were computed on a voxel-wise basis for each participant for both vaccination and placebo conditions in the form of statistical parametric maps. Subsequent second-level paired *t* test analyses were performed on the SPM contrast images for formal inference about population effects.

Results for the main effects of task and inflammatory state were thresholded at the conservative $p < .05$ false detection rate (FDR) corrected. The main effect of inflammation was calculated as the effect of all stimulus events versus implicit baseline. Interactions and