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Neural Predictors of Inflammatory Responses to Social Stress

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy

in Psychology

by

Keely Ann Muscatell

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ABSTRACT OF THE DISSERTATION

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by

Keely Ann Muscatell

Doctor of Philosophy in Psychology

University of California, Los Angeles, 2013

Professor Naomi I. Eisenberger, Chair

Psychological stress is associated with a variety of negative physical and mental health outcomes. Recent research suggests that inflammation may be a key biological mediator of the relationship between stress and health, though the neurocognitive pathways engaged during stressors that underlie inflammatory response are largely known. Therefore, the present dissertation project examined the neural systems that are related to inflammatory responses to social stress. This is the first known study to examine how the brain processes social stress *and* how the inflammatory system responds to the same stressor.

To accomplish this goal, healthy, female participants (n = 31) were exposed to a social evaluative stressor while they underwent a functional MRI (fMRI) scan. Blood samples taken before and after the scan were assayed for levels of the pro-inflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α). Additional blood samples were used to measure

gene expression in immune cells. Changes in pro-inflammatory cytokines and pro-inflammatory gene expression from pre- to post-scan were then linked with neural activity during the social evaluation task.

Paper 1 reports results linking neural activity during negative social feedback (compared to neutral feedback) and stressor-evoked changes in pro-inflammatory cytokine levels. Data indicated that exposure to the social stressor was associated with significant increases in IL-6, and greater increases in IL-6 were associated with greater neural activity in the amygdala and the dorsomedial prefrontal cortex (DMPFC) during negative social feedback. Functional connectivity analyses revealed that individuals who showed heightened inflammatory responses to the stressor also showed greater coupling of the DMPFC and amygdala during negative feedback, compared to those who showed a lower inflammatory response. Paper 2 explores the neural systems associated with changes in pro-inflammatory gene expression in response to social evaluation. As predicted, exposure to the social stressor was associated with increases in pro-inflammatory gene expression. Results also indicated that greater neural activity in the DMPFC and the dACC in response to the social evaluation was associated with greater upregulation of pro-inflammatory genes following the stressor. Together, these studies provide initial empirical evidence for the neurocognitive mechanisms that may link social stress with inflammation and disease.

The dissertation of Keely Ann Muscatell is approved.

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INTRODUCTION

Chronic diseases such as cardiovascular disease, diabetes, arthritis, and stroke afflict nearly 133 million Americans, and are the cause of 7 out of every 10 deaths in the United States each year (Center for Disease Control, 2005). Given these staggering numbers, understanding the causes and consequences of chronic disease is a significant population health issue. Extensive research over the past thirty years has shown that many of these common and costly diseases involve dysregulation of the immune system, and, particularly, elevated levels of inflammation (Choy & Panayi, 2001; Dowlati et al., 2010; The Emerging Risk Factors Collaboration, 2010). Although the microbiological regulators of inflammation have been known for some time, exciting new research in psychology demonstrates that social-environmental factors can also influence inflammatory activity (Kiecolt-Glaser et al., 2003; Seeman et al., 2010; Steptoe et al., 2007). These findings suggest that psychological and biological factors may interact to influence the onset and course of chronic disease. Thus, the research presented in this dissertation aimed to understand how the brain responds to the social environment in ways that may lead to increases in inflammation, thereby possibly affecting the development and progression of chronic disease.

Social stressors, such as interpersonal rejection or racial discrimination, are among the most potent psychological activators of inflammatory processes (Kemeny, 2009). However, the neural mechanisms by which social stressors get translated into inflammatory responses are largely unknown (cf. Slavich et al., 2010). Thus, the overarching goal of this dissertation was to elucidate the neurocognitive processes that link social stress and inflammation. Understanding these neural systems is important for at least two reasons. First, the brain is the primary organ of stress perception and appraisal that begins the cascade of physiological activation we typically characterize as “stress”; yet we know relatively little about how this important organ responds to

social stressors and influences peripheral physiology (McEwen & Gianaros, 2011). Thus, examining the neural activity during social stress that is linked with inflammation may provide clues into the neurocognitive processes that allow the external social world to get "under the skin" to affect health. Though we cannot directly infer what people are thinking or feeling by examining their brain activity, the patterns of activation that emerge when people are experiencing social stress can lead to clues about the types of cognitive and affective processes that may be engaged during these experiences and, therefore, possibly involved in inflammation. From these initial clues, we can design additional experiments to directly manipulate the social cognitive or affective processes we think are reflected by those patterns of neural activity, to directly examine if they are one of the relevant components of social stress that is associated with increases in inflammation.

A second important reason to investigate neurocognitive systems linking social stress and inflammation is that examining patterns of neural activity when people are experiencing social stressors may help us to unpack the relatively amorphous, non-specific construct that is "psychological stress." For example, behavioral studies that examine psychological and physiological responses to social stressors have mostly relied on retrospective self-reports of what participants were thinking and feeling during experiences of stress, given that it is difficult to interrupt people during a laboratory stressor to ask them what they are thinking or how they are feeling. Although these studies have yielded important insights into the types of cognitive and affective processes that people may engage during a socially stressful experience, they are subject to biases associated with retrospective recall (e.g., "How were you feeling when you were being evaluated?") and demand characteristics associated with self-reporting on thoughts and feelings. Examining neural activity during a social stressor, therefore, allows for a more

"real time" window into what neurocognitive processes people may be engaging when they experience social stress, without having to disrupt the experience or rely on self-report. These neural processes may thus extend our current understanding of the cognitive and affective processes that are related to inflammatory responses to stress, without being subject to the same biases as retrospective self-report measures.

It is against this backdrop that the present dissertation project combined methods from experimental social psychology, cognitive neuroscience, and psychoneuroimmunology to examine neural systems that are related to inflammatory responses to social stress. To accomplish this goal, I exposed a sample of healthy, female participants (n =31) to a social evaluative stressor while they underwent a functional Magnetic Resonance Imaging (fMRI) scan of their brains to provide an index of neural activity. Blood samples taken before and after the fMRI scan were assayed for levels of the pro-inflammatory cytokines interlukein-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), which provided an index of circulating markers of inflammation. Additional blood samples were used to measure gene expression in immune cells (peripheral blood mononuclear cells, or PBMCs), which provided another index of inflammatory activity, measured at a different level of analysis. Changes in pro-inflammatory cytokines and inflammatory gene expression from pre- to post-scan were then linked with neural activity during the social evaluation task, in an effort to elucidate neurocognitive processes that may influence inflammatory responses to social stress. This is the first known study to examine how the brain processes social stress *and* how the inflammatory system responds to the same stressor.

What follows in this dissertation are two empirical papers that emerged from the aforementioned project. In Study 1, I explored neural correlates of stressor-evoked increases in pro-inflammatory cytokines, with a focus on how the brain responded to negative social

evaluative feedback, compared to neutral feedback. In Study 2, I report on the neural systems that predicted upregulation of pro-inflammatory gene expression in response to the social stressor. Specifically, I explored if neural activity in regions involved with thinking about others and responding to threats would be associated with social stress-evoked increases in pro-inflammatory gene expression. Together, it is my hope that these studies provide some initial clues regarding the neurocognitive mechanisms that may link social stress with inflammation and disease.

PAPER 1:

Engagement of Dorsomedial Prefrontal Cortex and Amygdala is Associated with Inflammatory Responses to Social Stress

Abstract

Psychological stress is associated with a variety of negative physical and mental health outcomes. Recent research suggests that inflammation may be a key biological mediator of the relationship between stress and health, though the neurocognitive pathways engaged during stressors that underlie inflammatory response are largely known. Thus, the present study examined the association between neural activity during a social stressor and stressor-evoked inflammatory responses. To investigate this issue, healthy female participants (n = 31) were exposed to a social-evaluative stressor while they underwent an fMRI scan. Blood samples were taken before and after the stressor, and plasma was assayed for markers of inflammatory activity. Results indicated that exposure to the social stressor was associated with significant increases in the pro-inflammatory cytokine interleukin-6 (IL-6), and greater increases in IL-6 were associated with greater neural activity in the amygdala and the dorsomedial prefrontal cortex (DMPFC) during negative social feedback. Functional connectivity analyses revealed that individuals who showed heightened inflammatory responses to the stressor also showed greater coupling of the DMPFC and amygdala during negative feedback, compared to those who showed a lower inflammatory response. These data thus provide evidence that activity in and coupling between regions often activated during tasks that involve processing threat and thinking about the thoughts and feelings of others is associated with increases in inflammation. This neurocognitive account may help shed light on possible neural mechanisms linking social stress with inflammatory-related diseases.

Engagement of Dorsomedial Prefrontal Cortex and Amygdala is Associated with Inflammatory Responses to Social Stress

Psychological stress is associated with the onset or progression of a number of major physical and mental health problems (e.g., cardiovascular disease, chronic pain conditions, major depressive disorder; Cohen et al., 2007; Cutulo & Straub, 2007; Kendler et al., 1999; Steptoe & Kivimaki, 2012). An emerging body of evidence suggests that inflammation may be a key mediator linking stress and disease (Miller, Chen, & Cole, 2009; Slavich et al., 2010). Markers of inflammation are elevated in a number of physical and mental health disorders (Choy & Panayi, 2001; Dowlati et al., 2010; The Emerging Risk Factors Collaboration, 2010), and exposure to an inflammatory challenge is associated with increases in depressive symptoms (Eisenberger et al., 2010; Reichenberg et al., 2001). Furthermore, both naturalistic chronic stress and laboratory manipulations of acute stress are associated with increases in pro-inflammatory cytokines, proteins that are the key orchestrators of the inflammatory response (Kiecolt-Glaser et al., 2003; Steptoe et al., 2007). Taken together, these findings suggest that inflammation may be a key biological mediator of the relationship between stress and physical and mental health. Yet despite this growing literature linking stress, inflammation, and disease, little is known about the neurocognitive systems that translate psychological stressors into downstream inflammatory responses.

Although we know relatively little about the neurocognitive systems that link stress and inflammation, some research has focused on the psychological characteristics of stressors that may be most likely to elicit increases in inflammatory activity. Along these lines, accumulating evidence from both animal and human research suggests that stressors that are social in nature may be especially potent activators of the inflammatory response. For example, animals exposed

to social defeat or subordination are more likely to show increases in inflammatory processes, compared to animals exposed to non-social stressors (e.g., physical restraint; Sheridan et al., 2000; Stefanski & Engler, 1998). In humans, experiencing a social rejection life event is associated with greater increases in pro-inflammatory transcription factors than exposure to non-social life events (e.g., failing an exam; Murphy et al., 2013), and laboratory stressors that involve social evaluation are related to greater stimulated production of pro-inflammatory cytokines, compared to non-social stressors (Dickerson et al., 2009). Indeed, upregulating inflammation in response to social threats may be an adaptive response, preparing the organism to deal with potential wounding or infection that could occur following an antagonistic social exchange (Dhabhar, 1998; Kemeny, 2009) or preparing the organism to deal with the increased likelihood of wounding that may occur as a function of social ostracism (e.g., due to predation, environmental threats; Eisenberger, in press).

Insofar as mounting a swift inflammatory response to social threats confers potential survival benefits, neural systems that involve: 1) monitoring the environment for potential social threats and 2) decoding if an individual has antagonistic or benevolent intentions may be associated with inflammatory responding. First, neural regions involved in processing basic survival-related or social threats may be important drivers of inflammatory activity. These neural regions include (but are not limited to) the amygdala, the dorsal anterior cingulate cortex (dACC), the anterior insula (AI), and the periaqueductal gray (PAG). Prior research has demonstrated that these neural regions are often engaged when people are processing survival-relevant stimuli (Morrison & Salzman, 2010), such as threatening faces (Whalen et al., 2001) and impending pain (Mobbs et al., 2010), or when they are experiencing pain, be it physical or social (i.e., social rejection; Eisenberger, 2012; Kross et al., 2011). Second, given that socially

threatening situations often involve attending to the feelings, opinions, and intentions of others to determine if they deem to harm, it is possible that activity in neural regions involved in thinking about the mental states of others (i.e., "social cognition") may also relate to inflammatory responses. These social cognition-related regions include the dorsomedial and medial prefrontal cortex (DMPFC, MPFC), the temporal-parietal junction (TPJ), the posterior-superior temporal sulcus (pSTS), the temporal poles, and the posterior cingulate cortex (PCC)/precuneus (Frith & Frith, 2006; Lieberman, 2010; Mitchell, 2009).

As further evidence that these regions may be involved in eliciting inflammatory responses to social stressors, prior work has shown that greater activity in several of these neural regions (i.e., amgydala, dACC, AI, DMPFC, MPFC) is associated with increases in indices of sympathetic nervous system (SNS) activation (Critchley, 2005; Gianaros et al., 2009; Muscatell & Eisenberger, 2012; Wager et al., 2009a, 2009b). Activation of the SNS has been shown to drive inflammatory responses (Grebe et al., 2010; Sloan et al., 2007) and thus neural regions involved in promoting SNS activity may also be critical for driving the inflammatory response. Finally, a growing body of evidence suggests that certain cortical neural regions (e.g., DMPFC) may play an important role in sustaining activity in threat-related neural regions (e.g., amygdala). In animal work, stimulation of a region analogous to human DMPFC/dACC is associated with increases in amygdala activity and behavioral indicators of fear (Sierra-Mercado et al., 2011; Vidal-Gonzalez et al., 2006), and activity in such regions has also shown to amplify transient amygdala responses to threat (Burgos-Robles et al., 2009). This raises the intriguing possibility that connectivity between threat and social cognition regions during social stress may also be related to changes in inflammation.

Given the gaps in our knowledge of the neurocognitive systems that are related to inflammation, the present study examined neural and inflammatory responses to a social stressor. Elucidating the neural systems that link social stress and inflammation is important, as these studies may help us to unpack the precise neurocognitive processes that are engaged during stress and that have downstream physiological implications that are relevant for health. To examine the neurocognitive systems linking social stress and inflammation, healthy young women (*N*=31) were scanned using fMRI while they underwent an episode of social evaluation. Blood samples taken before and after the stressor were assayed for levels of the pro-inflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), which provide an index of inflammatory activity. We hypothesized that greater activity in neural regions often associated with processing threat and social cognition would be associated with greater inflammatory responses to the stressor. In addition to this primary analysis, we also conducted exploratory functional connectivity analyses, to examine the functional coupling of threat and social cognition regions during social stress. Specifically, we explored the possibility that there may be stronger functional coupling of threat-related neural regions (i.e., the amygdala) and other regions implicated in sustaining threat responses (i.e., DMPFC) for individuals who show larger inflammatory responses to social stress, compared with those who smaller changes in inflammatory activity.

Materials & Methods

Participants

Participants were 31 female, undergraduate students at UCLA (*M* age = 19 years; range = 18-22 years). The final sample self-identified as 32% Asian/Asian American, 23% Hispanic/Latina, 22% Mixed/Other, 13% African American, and 10% White. All participants

provided written informed consent, and all procedures were approved by the UCLA Institutional Review Board. Participants were paid $135 for completing all study procedures.

Eligibility for the study was first assessed via an initial phone screening, during which time we confirmed that prospective participants were free from a number of factors thought to influence levels of inflammation (O'Connor et al., 2009), including: current or prior chronic physical illness, including allergies and autoimmune diseases; major sleep disturbance in the past six weeks (i.e., working nightshifts, major time zone changes); tobacco use; current prescription medication use, including hormonal birth control; excessive caffeine use (i.e., >8 caffeinated beverages per day); and Body Mass Index (BMI) over 30. In addition, participants had to be right-handed, not claustrophobic, and not have any metal in their body (with the exception of dental fillings), all inclusionary criteria for MRI studies. Finally, participants had to be free from current or prior Axis-I psychiatric diagnosis, which was assessed via the Structured Clinical Interview for DSM-IV Axis 1 Disorders (SCID; First et al., 1995) during an in-person session (see below).

Procedure

Interested participants were screened via telephone to see if they met the inclusionary criteria stated above. Then, they were invited to the lab for an in-person session, during which time psychiatric status was assessed with the SCID. Next, participants completed a video recorded "impressions interview" that lasted ten minutes, in which they responded to questions such as "What would you most like to change about yourself?" and "What are you most proud of that you've done in your life thus far?" Participants were told that in the next session for the study, they would be meeting another participant, and the experimenters would choose one

person to form an impression of the other based on the video of the interview. Meanwhile, the other person would be scanned while they viewed the impression being formed of them.

The fMRI session occurred within 2 days of this initial interview session, and was always completed in the afternoon from 12:30 PM to 4:30 PM, to control for potential diurnal variation in basal levels of pro-inflammatory cytokines (Petrovsky et al., 2003). Participants were asked to refrain from exercising and taking non-prescription medication the morning of the session, and were also instructed not to eat or drink caffeine within two hours of the start of the session. Upon arrival at the scanner, participants met a female confederate, whom they believed was also participating in the study. After a brief two-minute introduction to one another and the protocol, the participant and confederate were taken to separate testing rooms. There, a nurse inserted an indwelling catheter into the participant's left (non-dominant) forearm, through which blood samples were taken. A sham catheter was also taped to the confederate's non-dominant forearm, to increase believability of the cover story. Participants were given at least 45 minutes to acclimate to the presence of the catheter, and then a baseline blood sample was taken.

Following the first baseline blood sample, participant and confederate were reunited and told that the experimenters had determined that the confederate was going to be watching the participant's video, and forming an impression of her, while the participant would undergo the fMRI scan and view the confederate's impressions. Both participant and confederate were familiarized with the impression formation task (see below for more detail), and then a second baseline blood sample was drawn. Next, the confederate was seated in front of a computer screen in the fMRI scanner control room, while the participant was set-up in the scanner, which included stabilizing the head using foam padding to minimize motion. Following structural scans, the confederate supposedly evaluated the participant's interview, and the participant

received feedback about how she was supposedly coming across (see below). Participants also viewed the confederate's feedback about a nature video (not included in the present study). Once the scan ended, both participant and confederate returned to their testing rooms, during which time additional blood samples were collected from the participant. After the final blood sample, participants were probed regarding any suspicions they may have had about the cover story, and then they were fully debriefed, and dismissed. No participants indicated that they thought the feedback was fake, or that the confederate was a member of our research team.

fMRI Social Evaluation Task

During the scan, participants were given MRI-compatible goggles, though which they viewed a video that was believed to be a live interface of the confederate's impressions of the participant's interview. The video showed a mouse cursor moving around a screen that displayed 24 "adjective buttons". The cursor selected a new adjective every 11-12 seconds. Feedback adjectives were divided into one-third positive (e.g., "intelligent," "interesting"), one-third neutral ("practical," "talkative"), and one-third negative words (e.g., "annoying," "insecure"). Adjectives were selected based on pilot testing with an independent sample of UCLA undergraduates (n = 74), who were asked to indicate on a 1-7 Likert scale how they would feel if someone described them using each one of a list of adjectives (1= *highly rejected*, 7 = *highly accepted*). Based on these pre-ratings, we selected 8 words that were rated highly rejecting (M = 2.07, SD = 1.0) to form the negative feedback condition, 8 words that were rated as neither rejecting or accepting (M = 4.65, SD = 1.10) to form the neutral feedback condition, and 8 words that were rated as highly accepting (M = 5.88, SD = .97) to form the positive feedback condition. Each time an adjective was selected, participants were asked to respond to the question "How do you feel?" using a 1-4 scale (1= *really bad*, 4 = *really good*), which they

indicated using a scanner-compatible button box with four buttons. Over the course of the evaluation, participants viewed 15 presentations of each type of adjective (thus, certain words were repeated) in a pseudorandom order with the constraint that no more than two adjectives of the same valence could be presented consecutively. The feedback task was preceded and followed by a fixation crosshair (10 sec), which formed the implicit baseline. For the current study, we focused on neural activity during the negative feedback trials compared to the neutral feedback trials, given that prior work has demonstrated that negative social experiences are especially likely to elicit an inflammatory response (Chiang et al., 2012).

Manipulation Checks

Participants were asked three single-item questions before and after the scan, which served as manipulation checks. Immediately preceding and following the evaluation task (while still in the MRI scanner), participants were asked to respond to the question, "How do you feel right now?" on a four-point scale (1 = *really bad*, 4 = *really good*). Prior to entering the scanner and after returning to the testing room following the scan, participants also indicated the extent to which they felt evaluated and judged by the confederate, on a seven-point scale (1 = *not at all*, 7 = *very much*). Responses to these two items were combined to form an overall measure of feelings of evaluation (α= .84).

Inflammatory Activity

Inflammatory responses to the social stressor were assessed at two baseline time points prior to the stressor (once prior to receiving instructions, approximately 55 minutes before the social evaluation, and once immediately after the participant was notified that she would be evaluated by the confederate, approximately 35 minutes prior to the evaluation), and at 30, 60, and 90 minutes after the termination of the evaluation. At each time point, 12 mL of blood was

drawn into EDTA Vacutainer blood tubes, which were placed on ice immediately after draw until the completion of the session. After the blood had been centrifuged for 15 minutes at 3000 RPM, plasma was divided into 1 mL aliquots and frozen at -80°C until study completion. Concentrations of IL-6 and TNF-α were measured in duplicate using commercially available high sensitivity enzyme-linked immunosorbent assays (ELISAs; R& D Systems, Minneapolis, MN). The lower limit of detection for these assays is 0.2 pg/mL and 0.5 pg/mL for IL-6 and TNF- α, respectively. Two TNF- α samples were below the detectable limit and were thus excluded from all analyses. Furthermore, we were unable to obtain a blood sample for one participant at the 60 min. time point due to difficulty with the indwelling catheter. All cytokine data were positively skewed, so raw values were log transformed to normalize the distribution for statistical testing. All analyses involving the inflammatory data control for BMI, given the known association between BMI and inflammation (O'Connor et al., 2009).

fMRI Image Acquisition

Imaging data were acquired using a Siemens Trio 3.0 Tesla MRI scanner at the UCLA Staglin Center for Cognitive Neuroscience. First, we acquired a T1-weighted MPRAGE anatomical image for functional image registration and normalization (slice thickness = 1 mm, 176 slices, TR = 2300ms, TE=2.98ms, flip angle = 9 degrees, matrix = 256x256, FOV = 256mm). Then, we acquired 288 functional T2-weighted EPI volumes, during the social evaluation task (slice thickness = 3mm, gap =1mm, TR=2000ms, TE=25ms, flip angle = 90 degrees, matrix =64x64, FOV=200mm.

Data Analysis

Neuroimaging data were pre-processed and analyzed using Statistical Parametric Mapping (SPM8; Wellcome Department of Cognitive Neurology, London, UK). Pre-processing

included image realignment to correct for head motion, normalization into Montreal Neurologic Institute space (resampled at 3 x 3 x 3 mm), and spatial smoothing using an 8 mm Gaussian kernel, full width at half maximum, to increase signal-to-noise ratio. All imaging coordinates are presented as MNI coordinates.

Following pre-processing, we set up a general linear model for each participant. The presentations of each feedback word and the subsequent 11-12 seconds (until the next word was selected) were modeled as a block, and were convolved with a canonical hemodynamic response function. Our regressor-of-interest coded for the type of feedback presented (positive, neutral, or negative), and we included the six motion parameters as covariates. For each model, the timeseries was high-pass filtered using a 128 hz function, and serial autocorrelation was modeled as an AR(1) process. Following estimation, we computed linear contrasts for each participant that compared BOLD signal during the negative feedback trials to BOLD signal during neutral feedback. Contrast images for each participant were then entered into random effect analyses at the group level for statistical inference. First, we examined the main effect of receiving negative feedback, compared to receiving neutral feedback, which involved exploring neural activation when participants saw negative words selected compared to when they saw neutral words selected. We then performed a whole-brain regression analysis to explore the neural activity during negative feedback (compared to neutral feedback) that was correlated with changes in inflammation. Thus, we entered in each participant's IL-6 change score (T90-AvgBL) as a regressor in the contrast of negative feedback > neutral feedback, and examined neural activity that was positively and negatively correlated with change in IL-6.

To examine the main effect (ignoring inflammatory activity) of negative feedback compared to neutral feedback, we used a threshold of $p < .005$, 40 voxels, which corresponds to

a .05 false-discovery rate as determined by Monte Carlo simulations conducted in the AFNI program 3dClustSim (parameters: individual voxel *p*-value = 0.005; 10,000 simulations; FWHM 8 mm in each direction *x*, *y*, and *z*; whole-brain mask including 44,428 resampled voxels). For analyses involving the inflammatory data, we used a more liberal threshold of $p < .005$, 10 voxels. Given that this is the first study to examine neural and inflammatory responses to a social stressor, and the difficulty of comparing neural activity at one point in time with peripheral biological responses collected hours later, using a more liberal threshold allowed us to increase sensitivity for detecting any relationships between the neural activity and inflammatory responses.

Finally, we wanted to examine the functional connectivity of the amygdala with other regions of the brain, given that differential functional connectivity of such regions has been related to negative mental health outcomes (e.g., Robinson et al., 2012). To do so, we conducted a generalized psychophysiological interaction analysis (PPI), using the left and right amygdala anatomical ROIs as seeds. This analysis allowed us to determine the neural regions that showed a change in correlation with the amygdala, as a function of the type of feedback (negative vs. neutral). At the individual subject level, we extracted, separately for left and right amygdala, a deconvolved time course averaged across each seed region. This time course was then included in a generalized PPI model, together with a PPI regressor for each of the variables of interest (negative feedback and neutral feedback), as well as motion parameters. The resulting PPI connectivity estimates were then taken to the group level, where we conducted an independent samples *t*-test, in which we grouped participants into "high IL-6 responder" and "low IL-6 responder" groups (based on a median split of the difference between IL-6 levels at T90-baseline), and examined differences in PPI connectivity between the groups. This analysis

allowed us to determine which neutral regions were correlated with the time course of activity in the amygdala, during negative feedback > neutral feedback, for those who showed a higher IL-6 responses to the social stressor compared to those who showed a smaller change in IL-6.

Results

Manipulation Checks

First, as a manipulation check, we examined if exposure to the social stressor led to changes in feelings of evaluation and mood. We found significant increases in self-reported feelings of evaluation from pre-scan (M = 2.87, SD = 1.85) to post-scan (M = 4.97, SD = 1.41; $t(30) = -8.14$, $p < .001$). Participants also reported feeling significantly worse immediately following the evaluation (pre-stress M = 3.29, SD = .53; post-stress M = 2.83, SD = .75; $t(29) = 4.20$, $p < .001$).

Inflammatory Data

Next, we examined if exposure to the social evaluation task was associated with increases in pro-inflammatory cytokines. We found a significant increase in IL-6 over time, $F(4, 116) = 46.76$ $p < .001$, but no significant change in TNF-α, $F(4, 108) = 1.42$, $p = .23$. Follow-up pairwise-comparisons of the IL-6 data indicated that there was no significant difference between the two baseline measures (M baseline 1 = .0027, SD = .27; M baseline 2 = .0032, SD = .27; $p = .95$); thus, these two measures were combined to form an average baseline for the remainder of the analyses. Additional pairwise-comparisons revealed significant increases in IL-6 for each post-stress timepoint compared to the combined baseline (T30: $t(30) = -6.15$, $p < .001$, T60: $t(29) = -6.30$, $p < .001$, T90: $t(30) = -8.07$, $p < .001$). For the remainder of our analyses, we focus on the change in IL-6 from the 90 min time point compared to the combined baseline, as IL-6 levels were at their highest at this time point.

To ensure that the observed increases in IL-6 were not simply due to anxiety associated with being in the neuroimaging environment, we ran a separate sample of 10 participants through the identical experimental procedure outside the MRI scanner (though we did not include a 90 minute post-stress sample, as in the present study). Results from this pilot study also showed a significant increase in IL-6 in response to the social evaluation $F(3, 27) = 9.70$, $p < .001$, which was of similar magnitude to the increase we observed in the present study (partial eta squared for pilot study = .52; partial-eta squared for current study, only including time-points that match pilot study= .54). These data suggest that the increases in IL-6 observed in the present study were not simply due to being in the neuroimaging environment.

fMRI Data

Turning to the fMRI data, we first wanted to examine the neural regions that were active when participants were getting negative feedback compared to neutral feedback, regardless of inflammatory response. Results from this contrast revealed significant clusters of activation in DMPFC and MPFC (extending into pregenual anterior cingulate cortex [pACC] and dACC), bilateral ventrolateral prefrontal cortex (VLPFC), bilateral temporal parietal junction (TPJ), bilateral posterior superior temporal sulcus (pSTS), bilateral temporal poles, occipital lobe, and cerebellum (for a full list of activations, see Supplementary Table 1 and Supplementary Figure 1). Thus, when receiving negative feedback (compared to neutral feedback), participants showed greater activity in regions commonly activated during tasks that involve (a) thinking about other people (DMPFC, MPFC/pACC, TPJ, pSTS, temporal poles), (b) processing threat or distress (dACC, AI), and (c) regulating emotion (VLPFC).

Linking fMRI and Inflammatory Data

The main goal of the present study was to examine the neural regions that were associated with inflammatory responses to social stress. To accomplish this goal, we regressed participants' change in IL-6 from baseline to T90 into the contrast of negative feedback > neutral feedback. Results of this whole-brain regression analysis revealed significant, positive correlations between IL-6 responses and neural activity in the DMPFC (-9, 50, 34), left amygdala (-21, -4, 11), and right inferior frontal gyrus extending into right amygdala (24, 8, -17; see Figure 1, and Table 1 for a complete list of activations). In other words, participants who showed greater activity in regions often active during tasks that involve processing threat, and thinking about others, during negative evaluations also showed greater IL-6 responses to the stressor. No neural activity was negatively correlated with IL-6 responses.

Functional Connectivity

In our last set of analyses, we explored the functional connectivity of the amygdala with other regions of the brain during negative feedback, to examine if individuals who showed a high IL-6 response to the evaluation would show greater functional coupling of the amygdala and DMPFC, compared to individuals who showed a low IL-6 response. A PPI analysis revealed that high IL-6 responders (compared to low responders) showed greater functional connectivity of the left amygdala with bilateral temporal poles, and with DMPFC (but only when the cluster extent threshold was lowered to 9 voxels), both regions that are often active during tasks that involve thinking about the thoughts and feelings of others. Using the right amygdala as a seed, we found that high IL-6 responders (compared to low responders) also showed greater functional connectivity of the right amygdala and the DMPFC, among other regions (see Figure 2 and Table 2 for a complete list of regions).

Discussion

Social stressors, including being negatively evaluated by others or rejected from importation social groups, are among the most noxious events we encounter in daily life. Far from being a mere nuisance, exposure to social stress is associated with a variety of negative physical and mental health outcomes. Inflammation may be a critical mediator of the relationship between social stress and disease, as levels of inflammation are elevated in a number of physical and psychological disorders, and exposure to social stress is associated with increases in inflammatory markers. The present study is the first to investigate how neural activity during social stress is linked with inflammatory responses to the same stressor. Our results demonstrated that greater neural activity in the DMPFC and the amygdala in response to negative social feedback is related to greater stressor-evoked changes in inflammation. Functional connectivity analyses revealed that individuals who showed greater changes in inflammation in response to the social stressor also had more tightly coupled activity of the amygdala with the DMPFC during negative feedback. Taken together, these results suggest that, when receiving negative feedback from another person, greater activity in and coupling between brain regions involved in processing threat and thinking about the thoughts and feelings is associated with heightened inflammatory responses.

Results from the present study are consistent with data from animal and human studies of fear and anxiety, which suggest that the DMPFC may provide top-down influence on the amygdala to create an "aversive amplification" circuit during conditions of threat or stress (Robinson et al., 2012). For example, electrical stimulation of the prelimbic cortex, the rodent analog of human DMPFC/dACC (Milad et al., 2007, 2009), has been shown to increase activity in the amygdala and subsequent behavioral indicators of fear (Sierra-Mercado et al., 2011; Vidal-

Gonzalez et al., 2006). Other work from animals suggests that prelimbic cortex is responsible for sustaining transient amygdala responses to threat (Burgos-Robles et al., 2009), providing further evidence that it is the *co-activation* of DMPFC and amygdala during threat that drives increases in behavioral and physiological stress responses. Finally, accumulating evidence in human neuroimaging studies suggests that DMPFC-amygdala connectivity is observed in many negative emotional states (Etkin et al., 2001), and that co-activation of these regions during threat is associated with increases in anxiety, negative affect and greater attention to threatening cues (Ochsner & Gross, 2005; Robinson et al., 2012). Results from the current study suggest, for the first time, that activity in this DMPFC-amygdala circuit and functional connectivity between DMPFC and amygdala may also drive inflammatory responses to social stress, providing additional evidence for the importance of these regions in orchestrating affective, behavioral, and physiological responses to stress.

Evidence for a potential DMPFC-amygdala "aversive amplification" circuit begs the question of what precise neurocognitive processes might be engaged during social stress that are associated with activation of these regions and subsequent inflammatory responses. Numerous studies have observed increased activity in DMPFC during tasks that involve thinking about the traits, mental states, and intentions of others (Frith & Frith, 2006; Lieberman, 2010; Mitchell, 2009), and the body of animal work reviewed above suggests that DMPFC-analogous regions in rats may drive increases in threat-related neural activity in other regions, such as the amygdala. Together with data from the current study, these lines of research point to the possibility that paying greater attention to the thoughts and feelings of others during a social stressor may amplify threat-related neural activity, and thus lead to greater inflammation. Although currently speculative, it would be interesting for future studies to directly explore this possibility by

experimentally manipulating the extent to which individuals are asked to think about others when they are being evaluated, to examine if this heightened attention is associated with greater activation of DMPFC, tighter coupling of DMPFC and amygdala, and greater inflammatory responses. Future research could also explore if thinking about the thoughts and feelings of others during social stress and attempts to increase feelings negative feelings during a stressor activate overlapping or nearby neural regions. Thus, much more research is needed to disentangle the precise neurocognitive processes that lead to greater coupling of DMPFC and amygdala, and how such processes are translated into increases in inflammation.

The present study represents an important step in elucidating the neurocognitive systems that are related to inflammatory responses to social stress (see also Slavich et al., 2010). However, the study is not without limitations. First, all participants were healthy, young adult women, which decreases the generalizability of the findings. It will be important for future work to explore if the same neurocognitive processes are associated with increases in inflammation in men, in younger and older populations, and in individuals at-risk for physical and psychiatric disorders. However, given that women are more likely than men to develop some inflammatory-related diseases (e.g., major depression, rheumatoid arthritis; Nolen-Hoeksema, 2001; Tengstrand et al., 2004), and are more sensitive to the negative effects of social stress (Stroud et al., 2002), the present findings are relevant for a number of critical public health issues. A second limitation of the present study is that all participants were exposed to the same stressor, which limits the conclusions that can be drawn about the precise features of the procedure that are most relevant for inflammation. Furthermore, our analyses focused on linking neural activity during negative evaluation and inflammatory responses, and we cannot be certain that this negative feedback is what was driving increases in inflammatory activity. Future studies could manipulate

the type of feedback presented and the specific characteristics of the stressor in a between-groups design, which would enable a more careful examination of the social environmental characteristics and neurocognitive processes that are most strongly linked with inflammation. Finally, the present study cannot address the precise physiological mechanisms by which activity in DMPFC and amygdala may lead to increases in inflammation. It is possible that efferent projections from DMPFC to the amygdala (Gabbott et al., 2005), and from the amygdala to brainstem regions such as the locus coeruleus (LeDoux et al., 1998), may lead to increases in SNS activity (Herman et al., 2003; Ulrich-Lai & Herman, 2009), which has been shown to drive contribute to increases in levels of inflammation (Dhabhar et al., 2012). Given the timing of measurement of inflammatory markers in the present study, it is likely that the observed increases in IL-6 reflect release of existing cytokines from vesicular stores in monocytes, for example, and adipose tissue, as well as skeletal muscle, and not de-novo production of inflammatory cytokines (Steptoe et al., 2007). Thus, future research is needed to examine if similar or different neurocognitive systems are involved in stressor-evoked production of inflammatory cytokines (e.g., proinflammatory-related gene expression).

In conclusion, the present study provides novel evidence for the role of the DMPFC and amygdala in inflammatory responses to social stress. Across correlational and connectivity analyses, it appears that activity in and coupling between DMPFC and amygdala during negative social feedback is related to increases in levels of the pro-inflammatory cytokine IL-6. These findings represent an exciting first step in understanding the neurocognitive processes that are engaged during social stress, and that translate features of the external social environment into physiological changes that can affect health. This window into the neural correlates of inflammatory responses to a social stressor will hopefully lead to the identification of novel,

modifiable risk factors that can be targeted to reduce psychological and inflammatory responses to stress and improve the health and well-being of the population.

Table 1. Regions that were significantly positively correlated with IL-6 response (T90-baseline) during the contrast of negative social feedback > neutral social feedback.

Anatomical Region	BA	*x*	*y*	*z*	*t*	*k*
DMPFC	9	-9	50	34	3.36	10
amygdala	n/a	-21	-4	-11	3.47	15
IFG/ amygdala	n/a	27	11	-20	3.30	13
TPJ?	39	-48	-73	25	3.51	13
Cerebellum	n/a	-3	-82	-38	3.69	11

Notes. All activations thresholded at $p < .005$, 10 voxels. BA refers to putative Broadmann's Area; *x*, *y*, and *z* refer to MNI coordinates in the left-right, anterior-posterior, and inferior-superior dimensions, respectively; *t* refers to the *t* score at those coordinates (local maxima); *k* refers to the number of voxels in each cluster. The following abbreviations are used for specific brain regions: DMPFC = dorsomedial prefrontal cortex; IFG = inferior frontal gyrus; TPJ = temporal parietal junction

Table 2. Regions that were positively correlated with the amygdala seeds in the psychophysiological interaction analysis

Anatomical Region	BA	*x*	*y*	*z*	*t*	*k*
Left Amygdala Seed						
High IL-6 Responders > Low IL-6 Responders						
DMPFC	9	-9	50	25	3.62	9
Temporal Pole	38	36	14	-20	5.13	106
	38	-36	17	-23	3.87	23
IFG	45	-54	20	16	3.69	14
Low IL-6 Responders > High IL-6 Responders						
VLPFC	11	-21	41	-17	4.75	11
Hippocampus	n/a	18	-22	-11	3.45	10
Occipital		-30	-82	-8	4.04	26
		-33	-73	22	3.41	12
Right Amygdala Seed						
High IL-6 Responders > Low IL-6 Responders						
DMPFC	9	-15	50	31	4.03	79
	8/9	9	44	46	3.54	57
SMA	6	-6	20	64	3.47	28
IFG	47	-33	26	14	3.40	14
	47/11	-36	35	-5	3.69	61
	45	-54	20	16	4.00	57
	n/a	45	26	-11	3.43	16
Superior Temporal Gyrus	38	-51	-1	-11	3.92	12
Middle Temporal Gyrus	n/a	-45	-25	-8	3.67	10
	39	-51	-76	22	4.37	30
Low IL-6 Responders > High IL-6 Responders						
No sig. activity						

Notes. All activations thresholded at $p < .005$, 10 voxels. BA refers to putative Broadmann's Area; *x*, *y*, and *z* refer to MNI coordinates in the left-right, anterior-posterior, and inferior-superior dimensions, respectively; *t* refers to the *t* score at those coordinates (local maxima); *k* refers to the number of voxels in each cluster. The following abbreviations are used for specific brain regions: DMPFC = dorsomedial prefrontal cortex; IFG = inferior frontal gyrus; SMA = supplementary motor area

Supplementary Table 1. Regions that were more active during the contrast of negative social feedback > neutral social feedback.

Anatomical Region	BA	*x*	*y*	*z*	*t*	*k*
DMPFC[2]	8/9	0	53	34	8.71	370
MPFC[2]	10	12	56	13	6.56	296
dACC/pACC[2]	32	0	41	16	5.83	99
TPJ	40	57	-46	31	4.23	72
		-54	-55	34	4.27	96
pSTS	22	54	-31	-8	4.37	133
	22/21	-51	-25	-8	3.45	46
Temporal Pole[1]	38	48	27	-29	4.11	26
	38	-45	20	-29	3.67	22
VLPFC[1]	47/45	-33	17	-20	5.54	274
	11/47	-36	50	-11	4.89	84
	47/45	51	26	-2	4.63	158
Anterior Insula[1]	13/47	-27	11	-17	5.43	53
	13/47	30	20	-17	7.48	37
Middle Frontal Gyrus	9/8	45	17	43	3.83	96
Middle Temporal Gyrus	22/21	-63	-46	4	3.94	56
Cuneus	18/19	-3	-85	19	10.54	520
Cerebellum	n/a	-24	-82	-35	9.26	466
		27	-76	-35	7.29	333
		3	-55	-41	6.53	91

Notes. All activations thresholded at $p < .05$, FDR corrected. BA refers to putative Broadmann's Area; *x*, *y*, and *z* refer to MNI coordinates in the left-right, anterior-posterior, and inferior-superior dimensions, respectively; *t* refers to the *t* score at those coordinates (local maxima); *k* refers to the number of voxels in each cluster. The following abbreviations are used for specific brain regions: DMPFC = dorsomedial prefrontal cortex; MPFC = medial prefrontal cortex; dACC= dorsal anterior cingulate cortex; pACC = pregenual anterior cingulate cortex; TPJ = temporal parietal junction; pSTS = posterior superior temporal sulcus; VLPFC = ventrolateral prefrontal cortex

[1-2]These sub-regions are encompassed within the same large cluster

Figure Captions

Figure 1. Relations between inflammatory responses to the social stressor as measured by log-transformed IL-6 responses from T90-baseline (in pg/ml) and neural activity in the (A) DMPFC, (B) left amygdala, and (C) right amygdala, from the contrast negative social evaluation > neutral social evaluation. The left side of each panel depicts the clusters that were positively correlated with IL-6 responses from a whole-brain regression analysis, and the right side shows a scatter plot of parameter estimates in each active cluster and IL-6 response.

Figure 2. Panel A depicts the anatomical ROI of the left amygdala that was used as a seed region in the PPI analysis (left), and the region in DMPFC that was more strongly correlated with left amygdala activity for high IL-6 responders (compared to low IL-6 responders) during negative feedback (compared to neutral feedback; right). Panel B depicts the anatomical ROI of the right amygdala that was used as a seed region in the PPI analysis (left), and the region in DMPFC that was more strongly correlated with right amygdala activity for high IL-6 responders (compared to low IL-6 responders) during negative feedback (compared to neutral feedback; right).

Supplementary Figure 1. Regions that were more active when participants were receiving negative feedback, compared to receiving neutral feedback ($p < .05$, FDR corrected)

Figure 1.

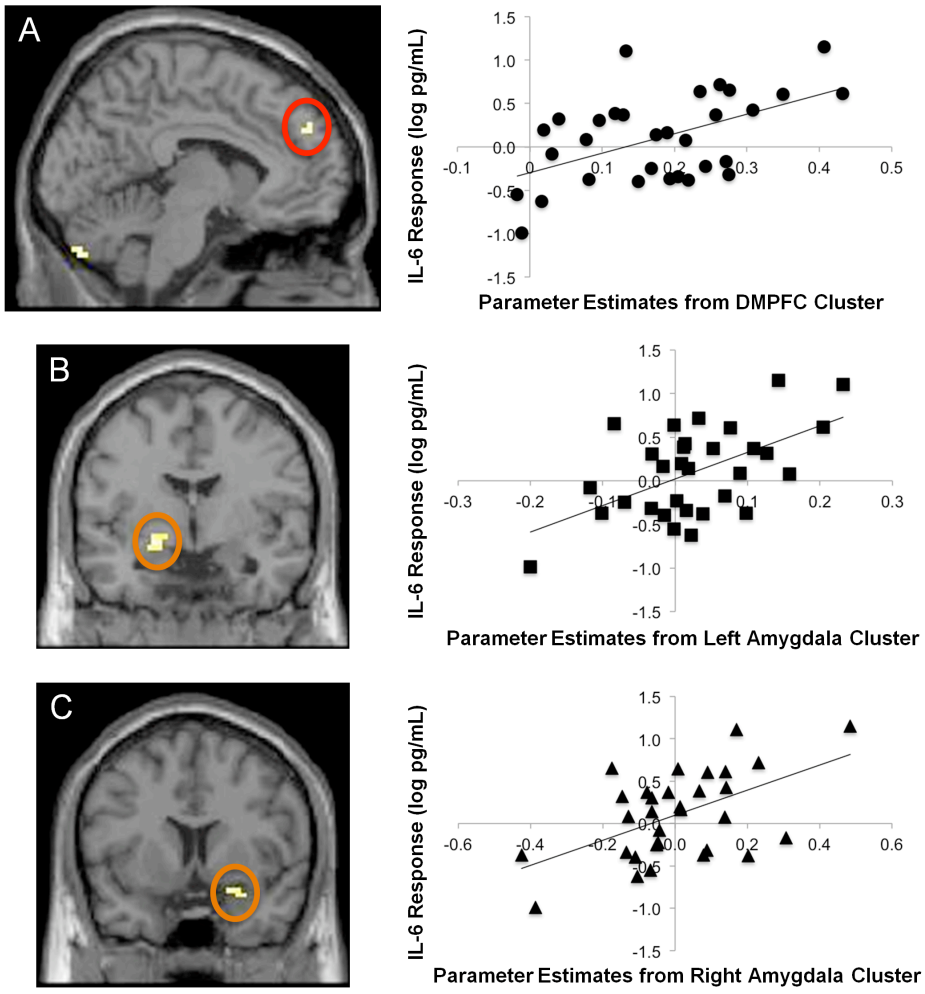
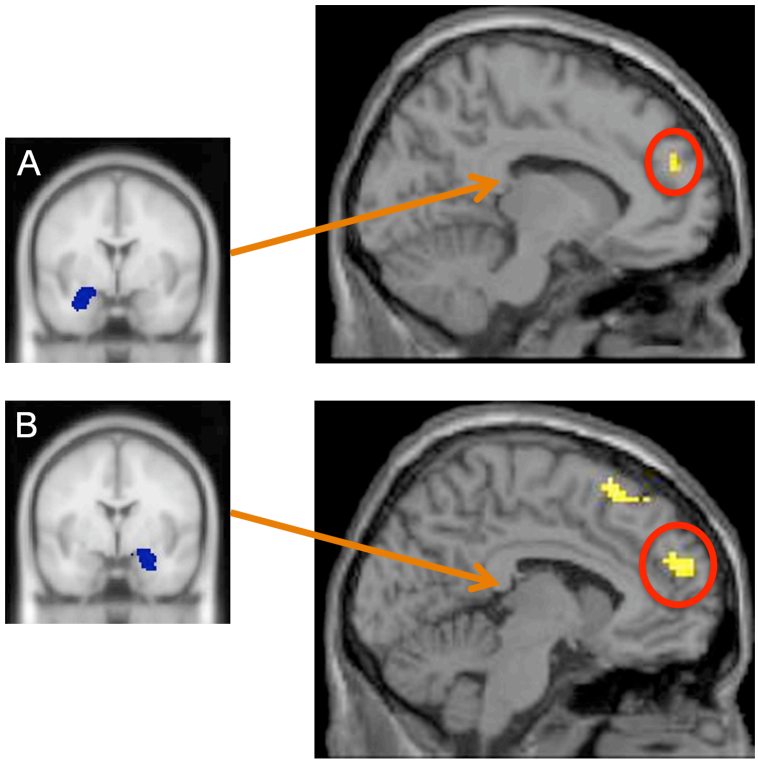
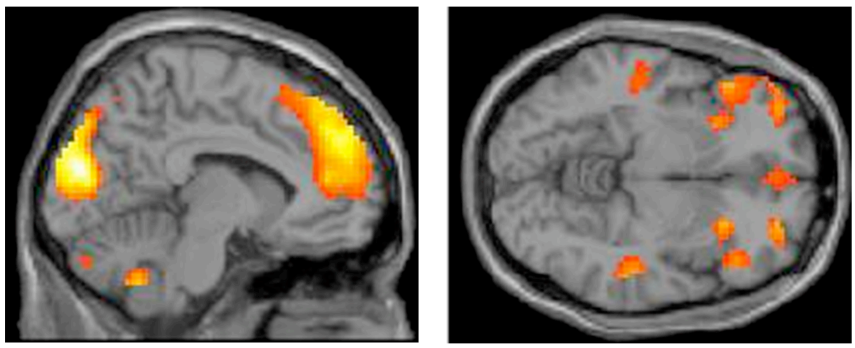


Figure 2.



Supplementary Figure 1.



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Paper 2:

Neural Activity During Social Evaluation is Associated with Increases in Pro-Inflammatory Gene Expression

Abstract

Social stress is related to many inflammatory-related diseases (e.g., cardiovascular disease, depression), perhaps in part because experiences of social stress can lead to increases in inflammation. A growing literature in "social genomics" suggests that socially stressful life circumstances (e.g., early separation from caregivers, chronic social isolation) are associated with heightened expression of genes involved in inflammation. However, the neurocognitive pathways that underlie social stress-induced changes in pro-inflammatory gene expression remain unknown. Thus, in the present study, we investigated if neural activity during a socially stressfully experience was associated with increases in pro-inflammatory gene expression. We exposed healthy, female participants (n = 30) to a social-evaluative stressor while they underwent an fMRI scan. Gene expression in immune cells was measured before and after the scan. As predicted, exposure to the social stressor was associated with increases in pro-inflammatory gene expression. Results also indicated that greater neural activity in the dorsomedial prefrontal cortex (DMPFC) when participants were getting negative feedback (compared to neutral feedback) was associated with greater upregulation of pro- inflammatory genes following the stressor. This is the first study to investigate how neural activity during stress links with stressor-evoked changes in pro-inflammatory gene expression and thus provides initial evidence that activity in the DMPFC during social stress may be part of a pathway involved in upregulating pro-inflammatory genes in response to stress.

Neural Activity During Social Evaluation is Associated with Increases in Pro-Inflammatory Gene Expression

Psychological stress is associated with a variety of negative outcomes, including the development of diseases of aging (e.g., cardiovascular disease, arthritis) and psychiatric disorders (e.g., major depression). Social stress in particular is associated with the onset and progression of many major diseases, with chronic experiences of social isolation and acute episodes of social rejection being especially likely to lead to ill health (Keller et al., 2007; Lutgendorf et al., 2012; Steptoe et al., 2013). Recent research in "social genomics" suggests that one of the ways in which social stress may impact health is via increased expression of genes involved in inflammation (for a review, see Slavich & Cole, 2013). For example, separation from caregivers early in life, disruptions in social hierarchies, and subjective reports of social isolation have both been associated with the upregulation of the expression of genes involved in inflammation (Cole et al., 2007, 2012; Tung et al., 2012). Although potentially adaptive in the short term, these increases in inflammatory gene expression could, over time, lead to chronic, systemic inflammation, which has been implicated in the pathophysiology of many major diseases (Choy & Panayi, 2001; Dowlati et al., 2010; The Emerging Risk Factors Collaboration, 2010). Despite these important links between social stress, inflammatory gene expression, and disease, very little experimental work in humans has examined if exposure to social stress leads to changes in pro-inflammatory gene expression (cf. Nater et al., 2009). Furthermore, the neurocognitive mechanisms that may link social stress and pro-inflammatory gene expression are currently unknown.

Although no known studies have examined how neural activity during social stress is linked with changes in pro-inflammatory gene expression, a growing literature has established

the neural systems that are engaged during stressors and associated with increases in other stress-related physiological systems (Eisenberger & Cole, 2012; Muscatell & Eisenberger, 2012). Most relevant to the current project, two known studies have examined how neural activity during a socially stressful experience is related to increases in circulating levels of pro-inflammatory cytokines (Muscatell et al., Paper 1 this Dissertation; Slavich et al., 2010). Results from these studies indicate that neural activity in the amygdala, the dorsal anterior cingulate cortex (dACC), the anterior insula, and the dorsomedial prefrontal cortex (DMPFC) during social stress is related to increases in levels of pro-inflammatory cytokines. Interestingly, the amygdala, dACC and anterior insula are key regions in a neural system generally thought to be active during experiences of threat and pain, while the DMPFC is a core region of the "mentalizing network", a neural system often engaged during tasks that involve thinking about the thoughts and feelings of others. Thus, results from these prior studies suggest the possibility that paying greater attention to the intentions of others and experiencing more threat during a socially stressful experience is related to greater increases in inflammation. However, to date the role that these regions play in relating to changes in pro-inflammatory gene expression are yet unknown.

The main goals of the present study were two-fold: First, we examined if exposure to a laboratory-based social stressor would be associated with changes in pro-inflammatory gene expression. Given that most prior human work in this area has examined how self-reported experiences of socially stressful life conditions relate to gene expression (e.g., Cole et al., 2007; Miller et al., 2009) or whether stress-reduction interventions can alter gene expression (Antoni et al., 2012; Creswell et al., 2012), we first wanted to establish if exposure to an acute social stressor was associated with changes in pro-inflammatory gene expression. Consistent with this previous work, we hypothesized that social stress would be associated with upregulation of pro-

inflammatory genes. Second, we examined the neurocognitive processes elicited during a social stressor that were associated with changes in gene expression. Based on prior research examining the neural systems related to increases in pro-inflammatory cytokines, we hypothesized that activity in threat-related neural regions (i.e., amygdala, dACC, anterior insula) and the DMPFC would be related to increases in pro-inflammatory gene expression. To address these questions, we exposed a sample of healthy, young women (n = 30) to a social evaluative stressor that involved being given feedback from a confederate about how they came across in a video-recorded interview while they underwent an fMRI scan to assess neural activity. Changes in immune-cell gene expression were measured in blood samples taken before and after the stressor, and neural responses to receiving negative social feedback (compared to neutral feedback) was correlated with changes in pro-inflammatory gene expression. This is the first known study to examine the neurocognitive mechanisms that may link social stress and pro-inflammatory gene expression.

Materials & Methods

Participants

Participants were 30 female, undergraduate students at UCLA (M age = 19; range = 18-22)[1], who were participating in a large study on "how the brain and the body respond to first impressions". The final sample was 33% Asian or Asian American, 23% Mixed/Other, 20% Hispanic/Latina, 13% African American, and 10% White. All participants provided written informed consent, and all procedures were approved by the UCLA Institutional Review Board. Participants were paid $135 for completing all study procedures.

Eligibility for the study was first assessed via an initial phone screening, during which time we confirmed that prospective participants were free from a number of factors thought to

influence activity of stress-related physiological systems (O'Connor et al., 2009), including: current or prior chronic physical illness, including allergies and autoimmune diseases; major sleep disturbance in the past six weeks (i.e., working nightshifts, major time zone changes); tobacco use; current prescription medication use, including hormonal birth control; excessive caffeine use (i.e., >8 caffeinated beverages per day); and Body Mass Index (BMI) over 30. In addition, participants had to be right-handed, not claustrophobic, and not have any metal in their body (with the exception of dental fillings), all inclusionary criteria for MRI studies. Finally, participants had to be free from current or prior Axis-I psychiatric diagnosis, which was assessed via the Structured Clinical Interview for DSM-IV (SCID; First et al., 1995) during an in-person session (see below).

Procedure

Interested participants were screened via telephone to see if they met the inclusionary criteria stated above. Then, they were invited to the lab for an in-person session, during which psychiatric status was assessed with the SCID. Next, participants completed a video recorded "impressions interview" that lasted ten minutes, in which they responded to questions such as "What would you most like to change about yourself?" and "What are you most proud of that you've done in your life thus far?" Participants were told that in the next session for the study, they would be meeting another participant, and the experimenters would choose one person to form an impression of the other based on the video of the interview. Meanwhile, the other person would be scanned while they viewed the impression being formed of them.

The fMRI session occurred within 2 days of this initial interview session, and was always completed in the afternoon from 12:30 PM to 4:30 PM, to control for any diurnal variation in levels of stress hormones and pro-inflammatory cytokines, which may affect inflammatory gene

expression (Petrovsky et al., 2003). Participants were asked to refrain from exercising and taking non-prescription medication the morning of the session, and were also instructed not to eat or drink caffeine within two hours of the start of the session. Upon arrival at the scanner, participants met a female confederate, whom they believed was also participating in the study. After a brief two-minute introduction to one another and the protocol, the participant and confederate were taken to separate testing rooms. There, a nurse inserted an indwelling catheter into the participant's left (non-dominant) forearm, through which blood samples were taken. A sham catheter was also taped to the confederate's non-dominant forearm, to increase believability of the cover story. Participants were given at least 45 minutes to acclimate to the presence of the catheter, and then a baseline blood sample was taken.

Following the baseline blood sample, participant and confederate were reunited and told that the experimenters had determined that the confederate was going to be watching the participant's video and forming an impression of her, while the participant would undergo the fMRI scan and view the confederate's impressions. Both participant and confederate were familiarized with the impression formation task (see below for more detail), following which the confederate was seated in front of a computer screen in the fMRI scanner control room, and the participant was set-up in the scanner, which included stabilizing the head using foam padding to minimize motion. Following structural scans, the confederate supposedly evaluated the participant's interview, and the participant received feedback about how she was supposedly coming across (see below). Participants also viewed the confederate's feedback about a nature video (not included in the present study). Once the scan ended, the participant and confederate returned to their testing rooms and a post-task blood sample was collected from participants 30 minutes after the completion of the social evaluation task. Finally, participants were probed

regarding any suspicions they may have had about the cover story, and then they were fully debriefed and dismissed. No participants indicated that they thought the feedback was fake, or that the confederate was a member of our research team.

Social Evaluation Task

During the scan, participants were given MRI-compatible goggles, though which they viewed a video that was believed to be a live interface of the confederate's impressions of the participant's interview. The video showed a mouse cursor moving around a screen that displayed 24 "adjective buttons". The cursor selected a new adjective every 11-12 seconds. Feedback adjectives were divided into one-third positive (e.g., "intelligent," "interesting"), one-third neutral ("practical," "talkative"), and one-third negative words (e.g., "annoying," "insecure"). Adjectives were selected based on pilot testing with an independent sample of UCLA undergraduates (n = 74), who were asked to indicate on a 1-7 Likert scale (1= *highly rejected*, 7 = *highly accepted*) how they would feel if someone described them using each one of a list of adjectives. Based on these pre-ratings, we selected 8 words that were rated highly rejecting (M = 2.07, SD = 1.0) to form the negative feedback condition, 8 words that were rated as neither rejecting or accepting (M = 4.65, SD = 1.10) to form the neutral feedback condition, and 8 words that were rated as highly accepting (M = 5.88, SD = .97) to form the positive feedback condition. Each time an adjective was selected, participants were asked to respond to the question "How do you feel?" using a 1-4 scale (1= *really bad*, 4 = *really good*), which they indicated using a scanner-compatible button box with four buttons. Over the course of the evaluation, participants viewed 15 presentations of each type of adjective (thus, certain words were repeated) in a pseudorandom order with the constraint that no more than two adjectives of

the same valence could be presented consecutively. All tasks were preceded and followed by a fixation crosshair (10 sec), which formed the implicit baseline.

Manipulation Checks

Participants were asked three single-item questions, which served as manipulation checks. Immediately preceding and following the evaluation in the scanner, participants were asked to respond to the question, "How do you feel right now?" on a four-point scale (1= *really bad*, 4 = *really good*). Prior to entering the scanner and after returning to the testing room following the scan, participants also indicated the extent to which they felt evaluated and judged by the confederate (prior to the scan, and during the social evaluation task), on a seven-point scale (1 = *not at all*, 7 = *very much*). Responses to these two items were combined to form an overall measure of feelings of evaluation ($\alpha = .74$).

Gene Expression Profiling and Analysis

Participants provided 8 ml of blood at baseline (approximately 55 minutes before the start of the social evaluation task), and 30 minutes following the completion of the social evaluation task. The specific details of the genome-wide transcriptional profiling procedure used have been described elsewhere (Cole et al., 2010, 2011). Briefly, peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation and total RNA was extracted (RNeasy; Qiagen, Valencia, CA), tested for suitable mass (Nanodrop ND1000) and integrity (Agilent Bioanalyzer), and converted to fluorescent cRNA for hybridization to human HT-12 BeadChips (Illumina, San Diego, CA) following the manufacturer's standard protocol in the UCLA Southern California Genotyping Consortium Core Laboratory. Quantile normalized gene expression values were transformed to log2 for genome-wide general linear model analysis. To ensure that results were not confounded by individual differences in the prevalence of specific

leukocyte subtypes within the PBMC pool, analyses controlled for variation in the prevalence of gene transcripts marking T lymphocytes subsets (CD3, CD4, CD8), B lymphocytes (CD19), and NK cells (CD16/FCGR3A, CD56/NCAM1; Cole et al., 2007).

To examine if exposure to the stress task was associated with significant upregulations in pro-inflammatory genes, we first used examined if there was a greater than 20% increase in expression of six key pro-inflammatory genes (IL1A, IL1B, IL6, TNF, IL8, and PTGS2) from pre- to post-stress. Then, we examined if any additional genes showed a greater than 20% increase from pre-to post stress. Finally, we identified downregulated genes as those that showed a > 20% decrease from baseline to post-stress. For analyses that examine how neural activity is related to changes in pro-inflammatory gene expression, we computed a differential gene expression contrast measure for each individual. This continuous measure was defined as the individual's average baseline-to-post scan change in expression of the 4 pro-inflammatory genes that showed significant upregulation across the group as a whole, minus the individual's average change in expression of genes downregulated across the group as a whole (i.e., the extent to which the individual's pro-inflammatory transcriptome shift is greater than, similar to, or less extreme that that observed across the sample as a whole). This individual-difference contrast score was used as a regressor in whole-brain analyses examining neural activity related to changes in pro-inflammatory gene expression (see below).

In addition to these primary analyses, we also wanted to examine the prevalence of specific transcription factor binding motifs in the promoter regions of the genes that showed differential expression from baseline to post-stress. To accomplish this goal, genes that showed > 15% change in expression from baseline to post-stress were analyzed by the transcription factor search engine TELiS (Cole et al., 2005). We employed a more liberal threshold for inclusion in

these ancillary analyses (15% change vs. 20% change for the primary analyses presented above) given that the number of genes showing > 20% expression was too few to be examined using TELiS.

fMRI Image Acquisition

Imaging data were acquired using a Siemens Trio 3.0 Tesla MRI scanner at the UCLA Staglin Center for Cognitive Neuroscience. First, we acquired a T1-weighted MPRAGE anatomical image for functional image registration and normalization (slice thickness = 1 mm, 176 slices, TR = 2300ms, TE=2.98ms, flip angle = 9 degrees, matrix = 256x256, FOV = 256mm). Then, the social evaluation scan was completed (288 functional T2-weighted EPI volumes; slice thickness = 3mm, gap =1mm, TR=2000ms, TE=25ms, flip angle = 90 degrees, matrix =64x64, FOV=200mm).

fMRI Data Analysis

Neuroimaging data were pre-processed and analyzed using Statistical Parametric Mapping (SPM8; Wellcome Department of Cognitive Neurology, London, UK). Pre-processing included image realignment to correct for head motion, normalization into Montreal Neurologic Institute space (resampled at 3 x 3 x 3 mm), and spatial smoothing using an 8 mm Gaussian kernel, full width at half maximum, to increase signal-to-noise ratio.

Following pre-processing, we setup a general linear model for each participant. The presentations of each feedback word and the subsequent 11-12 seconds (until the next word was selected) were modeled as a block, and were convolved with a canonical hemodynamic response function. Our regressors-of-interest coded for the type of feedback presented (positive, neutral, or negative), and we also included the six motion parameters as covariates. For each model, the timeseries was high-pass filtered using a 128 hz function, and serial autocorrelation was modeled

as an AR(1) process. Following estimation, we computed linear contrasts for each participant that compared BOLD signal during the negative feedback trials to BOLD signal during neutral feedback. Contrast images for each participant were then entered into random effect analyses at the group level for statistical inference. We then performed a whole-brain regression analysis to explore the neural activity during negative feedback (compared to neutral feedback) that was correlated with changes pro-inflammatory gene expression. Thus, we entered in each participant's change in expression of the 4 key pro-inflammatory genes that showed > 20% differential expression from pre-scan to post-scan (pro-inflammatory gene expression at T30-pro-inflammatory gene expression at BL) as a continuous regressor in the contrast of negative feedback > neutral feedback, and examined neural activity that was positively and negatively correlated with change in pro-inflammatory gene expression. Initial whole-brain regression analyses were evaluated using the threshold of p <.005 (uncorrected), 20 voxels; follow-up exploratory analyses were evaluated using the threshold of p <.05 (uncorrected), 20 voxels (see below for more details).

Results

Manipulation Checks

First, as a manipulation check, we examined if exposure to the social evaluation led to changes in mood and self-reported feelings of evaluation. Participants reported feeling significantly worse immediately following the evaluation compared to immediately preceding it (i.e., in response to the question "How do you feel right now" where 1 = *really bad* and 4 = *really good*; pre-stress M = 3.30, SD = .53; post-stress M = 2.83, SD = .76; $t(29) = 4.20$, p< .001).

We also found significant increases in self-reported feelings of evaluation from pre-scan (M = 3.33, SD = 1.56) to post-scan (M = 5.33, SD = 1.20; $t(29) = 7.68$, $p < .001$), suggesting that participants felt more evaluated during the social evaluation task (compared to baseline).

Changes in Gene Expression Following Social Stress

Consistent with the hypothesis that exposure to social stress is associated with increases in inflammatory gene expression, we found that 4 of 6 key pro-inflammatory genes showed ≥ 20% greater expression at 30 minutes post-stress compared to baseline, controlling for variation in the prevalence of specific leukocyte subtypes within the PBMC pool. The upregulated pro-inflammatory genes included those that code for IL1B, TNF, IL8, and PTGS2. The probability that these 4 genes would be among the 15 total genes that were upregulated as a function of the social stressor simply due to chance (out of all 34,681 transcripts analyzed) is < .01%. Several other genes involved in inflammation were substantially upregulated as a function of exposure to social stress, including other key regulatory cytokines involved in cellular immune responses (INFG), and defensins involved in antimicrobial responses (DEFA1, DEFA3, DEFA1B). Additional upregulated genes included PDK4, which is involved in decreasing metabolism and conserving glucose; ID3, which inhibits DNA binding; and DNAJ89, which protects stressed cells from apoptosis. Four un-named genes with unknown function were also upregulated (LOC1000085, LOC1001323, LOC441763, and one completely unnamed transcript). Finally, three genes showed ≥ 20% decreased expression at 30 minutes post-stress compared to baseline: HIST1H2AC, which is involved in transcription regulation and DNA repair; HBA1 which is involved in oxygen transport from lungs to peripheral tissues; and CLC, involved in stabilizing plasma membranes, and transport of salt and fluid.

In addition to these 18 genes that showed differential expression from baseline to post-stress (15 upregulated, 3 downregulated), we examined the specific transcription factor binding motifs that were present in the promoter regions among genes that showed greater than 15% change in expression over the course of the task. TELiS bioinformatics analyses revealed greater prevalence of genes targeted by the transcription factors nuclear-factor kappa B (NF-κB) and the cyclic-AMP response element-binding protein (CREB) following the social evaluation task (compared to baseline). Given that NF-κB is a key pro-inflammatory transcription factor, these ancillary analyses provide additional evidence that exposure to an acute episode of social stress leads to upregulation of pro-inflammatory genes.

Neural Responses to Negative vs. Neutral Social Feedback

Neural responses to receiving negative social feedback (compared to neutral feedback) during the social evaluation task are reported elsewhere (see Muscatell et al., Paper 1 of this Dissertation). Briefly, the results from this contrast revealed significant clusters of activation in regions associated with mentalizing, including the DMPFC and MPFC (extending into pregenual anterior cingulate cortex [pACC] and dACC), bilateral ventrolateral prefrontal cortex (VLPFC), bilateral temporal parietal junction (TPJ), bilateral posterior superior temporal sulcus (pSTS), bilateral temporal poles, occipital lobe, and cerebellum (for a full list of activations, see Muscatell et al., Paper 1, Supplementary Table 1 and Supplementary Figure 1).

Correlation Between Neural Activity and Changes in Pro-Inflammatory Gene Expression

Our main goal in the present study was to examine how neural activity during social evaluation is associated with changes in pro-inflammatory gene expression. To accomplish this goal, we regressed participants' change in pro-inflammatory gene expression from baseline to post-stress into the contrast of negative feedback > neutral feedback. Results of this whole-brain

regression analysis revealed a significant, positive correlation between changes in pro-inflammatory gene expression and neural activity in the DMPFC (12, 29, 49, *t*= , *k*=; see Figure 2). In other words, participants who showed greater activity in a region often active during tasks that involve thinking about others during negative evaluations also showed greater pro-inflammatory gene expression changes in responses to the stressor. No neural activity was negatively correlated with pro-inflammatory gene expression change.

We did not find evidence that activity in threat-related neural regions (amygdala, dACC, anterior insula) was correlated with changes in pro-inflammatory gene expression at our a priori defined statistical threshold ($p < .005$, 20 voxels). However, given our interest in the role these regions may play in relating to pro-inflammatory gene expression based on prior research in this area, we conducted follow-up analyses at a more liberal statistical threshold ($p < .05$, 20 voxels) to explore if there was any relationship between activity in threat-related neural regions and changes in pro-inflammatory gene expression. When we examined the neural activity during negative evaluation (compared to neutral) that was correlated with change in pro-inflammatory gene expression at this new threshold, two clusters of neural activity within the dACC (-10, 38, 31; -9, 13, 32) emerged as positively correlated with change in pro-inflammatory gene expression (see Figure 2). Even at this more liberal threshold we found no evidence of a correlation between neural activity in the amygdala or the anterior insula and change in pro-inflammatory gene expression. Thus, these data suggest some preliminary evidence that activity in the dACC during negative social evaluation may be related to changes in pro-inflammatory gene expression, though this relationship does not survive standard statistical thresholding techniques.

Discussion

Exposure to social stress is associated with changes in pro-inflammatory gene expression that may have implications for physical and mental health (Cole et al., 2007, 2012; Miller et al, 2009; Tung et al., 2012). Recent experimental evidence also suggests that interventions designed to reduce stress and improve mental health lead to reductions in pro-inflammatory gene expression (Antoni et al., 2012; Creswell et al., 2012). Despite this growing literature in the field of human social genomics (Slavich & Cole, 2013), the neurocognitive mechanisms that link social stress and changes in the expression of pro-inflammatory genes are unknown. Results from the present study show for the first time that neural activity during social evaluation is associated with the upregulation of pro-inflammatory gene expression. Specifically, greater activity in the DMPFC during negative social feedback (compared to neutral feedback) was related to increased pro-inflammatory gene expression. These data suggest that engagement of a neural region commonly activated in response to a variety of tasks involving thinking about the feelings and intentions of others may lead to increases in the expression of genes involved in inflammation.

Why might DMPFC activity during negative social feedback be associated with greater increases in pro-inflammatory gene expression? One possibility is that greater DMPFC activity may reflect heightened effort to decode *why* the evaluator was providing negative feedback to the participant, given that DMPFC activity is often observed during tasks that involve thinking about the thoughts and feelings of others (Frith & Frith, 2006; Lieberman, 2010; Mitchell, 2009). A second possibility is that the DMPFC may be involved in sustaining responses in more basic threat-related neural regions, including the nearby dACC and the amygdala (Burgos-Robles et al., 2009), which are connected to brainstem and hypothalamic regions that can induce SNS activation, thus starting a physiological cascade that may result in increased pro-inflammatory

gene expression. However, given that this is the first study to link activity in DMPFC and changes in pro-inflammatory gene expression, much more research is needed to disentangle the precise role the DMPFC may play in driving changes in pro-inflammatory gene expression.

In addition to the findings relating neural activity in the DMPFC to changes in pro-inflammatory gene expression, we also found some preliminary evidence that activity in a threat-related neural region, specifically the dACC, may also be related to stress-induced changes in pro-inflammatory gene expression. Given that the dACC was only related to gene expression changes at a more liberal statistical threshold, it is difficult to determine how critical a role this region is in driving changes in gene expression. However, these tenuous findings are consistent with one prior study that found a relationship between activity in the dACC during a social rejection task and stressor-evoked changes in levels of pro-inflammatory cytokines (Slavich et al., 2010), providing additional evidence that the dACC may be activated during social stressors and involved with inflammatory responding to such events. More broadly, the dACC plays an important role in responding to both physically and socially painful experiences (Eisenberger, 2012; Kross et al., 2011), and given that inflammation is one of they body's key defenses against wounding and infection, it makes sense that activity in this region would be related to a key physiological process that helps us recover from potential insults (Eisenberger & Cole, 2012; Slavich et al., 2010). It is also interesting to note that we did not find any evidence that activity in the amygdala or the anterior insula was related to changes in pro-inflammatory gene expression. While it is difficult to know what to make of these null effects, one possibility is that dACC activity represents a more "subjective" appraisal or experience of the negative feedback (given that dACC activity is often correlated with self-reports of distress during social rejection tasks; Eisenberger et al., 2003; Spunt et al., 2012), while activity in the amygdala is observed more

consistently during tasks that involve processing external cues of negative outcomes (e.g., angry faces, electric shocks), rather than internally generated, complex emotional states (Eisenberger & Cole, 2012). Prior research suggests that levels of inflammatory gene expression may be more strongly correlated with subjective appraisals of the social environment than objective indicators of social environmental circumstances (Slavich & Cole, 2013), so it is possible that dACC activity may be more strongly correlated with changes in pro-inflammatory gene expression (compared to other threat-related regions) given its possible role in representing the subjectively distressing nature of social stressors. However, this possibility is currently speculative and much more research is needed to disentangle how activity in different regions of the threat network are related to different components of the inflammatory response.

Beyond these findings relating neural activity to changes in pro-inflammatory gene expression, it is important to note that the present study is one of the first to show that exposure to an experimentally-induced social stressor is associated with the upregulation of pro-inflammatory gene expression (see also Nater et al., 2009). Prior human work that has examined social-environmental influences on gene expression has focused almost exclusively on how self-reports of social stress map on to patterns of gene expression (Cole et al., 2007; Miller et al., 2009), while some work in non-human primates has examined how experimental manipulations of social-environmental conditions affect transcriptional responses (Cole et al., 2012; Tung et al, 2012). Thus, the present study provides an important extension of this prior work by demonstrating that exposure to a laboratory-based social stressor is associated with the upregulation of key pro-inflammatory genes, and more broadly, of genes containing binding motifs for the transcription factor NF-κB (a key pro-inflammatory transcription factor). It will be interesting for future studies in this area to manipulate various social psychological features of

laboratory stressors, to examine the "active ingredients" of stress that lead to changes in gene expression. For example, in the present study, all participants were exposed to the same social experience, making it impossible to determine if the observed changes in gene expression were due to being given negative feedback specifically, or if just the experience of being evaluated is sufficient to elicit upregulation of inflammatory genes (for a similar discussion, see Taylor et al., 2010). Now that we have established that acute, relatively minor social stressors can lead to changes in pro-inflammatory gene expression, it will be important for additional research to determine the boundary conditions of these effects, and to examine how individual differences in psychosocial factors, early life stress exposure, and social status (among other characteristics) may moderate stressor-evoked changes in pro-inflammatory gene expression (Chen et al., 2011; Danese et al., 2011; Taylor et al., 2008).

There are a number of caveats to the present study that will need to be addressed by future research. First, all participants were healthy, young females, which limits the conclusions that can be drawn about the neurocognitive mechanisms that may link social stress and pro-inflammatory gene expression in other populations. Second, it is unclear if the relatively acute changes in pro-inflammatory gene expression seen here have relevance for long-term mental or physical health. Given the importance of inflammatory processes for a variety of diseases, however, it is possible that repeatedly upregulating pro-inflammatory gene activity in the face of social stress may be an important mechanism linking social stress and health (McEwen, 1998). Finally, results from the current study provide evidence that activation in the DMPFC is associated with increases in pro-inflammatory gene expression, but the precise mechanisms for *how* DMPFC activity leads to changes in gene expression are still unknown. More careful mechanistic work is needed to map the neural pathways by which cortical activation in DMPFC

leads to activation of basic limbic and brainstem structures that are capable of starting the physiological cascade to elicit changes in inflammatory gene expression.

In sum, results from the present study provide evidence for the role of the DMPFC in the upregulation of pro-inflammatory gene expression in response to social stress. These are the first data to link neural activity with changes in patterns of gene expression, and are also among the first to provide experimental evidence that exposure to social stress can lead to transcriptional shifts in immune cell gene expression. Together, these data provide an initial neurocognitive account for how social stress is represented in the brain and translated into physiological processes that may affect health.

Footnotes

[1] The number of participants in Paper 2 (n = 30) differs from that of Paper 1 (n = 31) in this dissertation owing to the fact that we were unable to get a T30 sample for gene expression assays from one participant, due to difficulties with the blood draw.

Figure Captions

Figure 1. The left panel depicts the cluster in DMPFC from the contrast negative evaluation > neutral evaluation that was positively correlated with change in pro-inflammatory gene expression ($p < .005$, 20 voxels). The right panel displays the scatter plot of the parameter estimates from this DMPFC cluster on the x-axis, and the change in pro-inflammatory gene expression on the y-axis.

Figure 2. The left panel depicts the clusters in dACC from the contrast negative evaluation > neutral evaluation that were positively correlated with change in pro-inflammatory gene expression ($p < .05$, 20 voxels). The right panel displays the scatter plot of the parameter estimates from these dACC clusters on the x-axis, and the change in pro-inflammatory gene expression on the y-axis.

Figure 1.

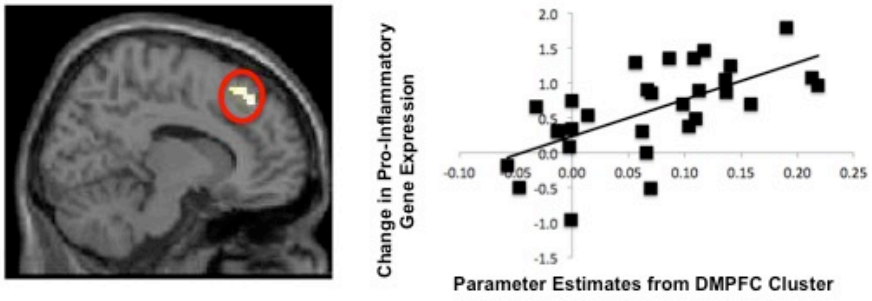
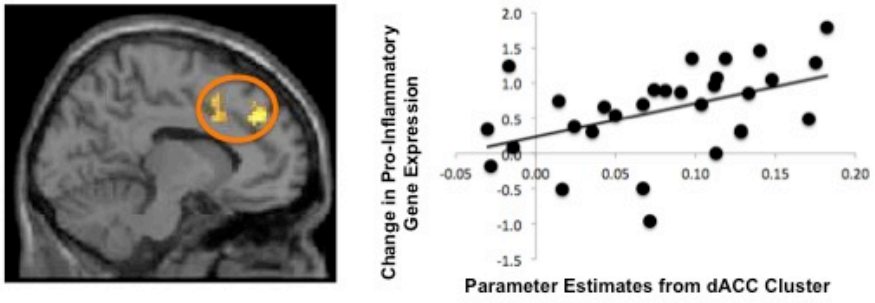


Figure 2.



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CONCLUSIONS

Stress-related health disorders are a serious social and economic problem, and a growing body of literature suggests that inflammation may be a key biological mechanism linking stress and disease. However, we know relatively little about the neurocognitive processes that are engaged during stressful experiences that link stress with inflammation and poor health. The present project was designed to address this gap in the literature by examining the neural correlates of stress-related increases in inflammation. Two papers that resulted from the project examined how neural activity during a social evaluation task was related to changes in two indices of inflammatory activity. In Paper 1, I showed that exposure to a social evaluative stressor in the neuroimaging environment was associated with increases in plasma levels of the pro-inflammatory cytokine IL-6 (but not TNF-α). I also found that neural activity in the DMPFC and the amygdala during negative social feedback was associated with changes in IL-6, such that individuals who showed more activity in these neural regions showed corresponding greater increases in IL-6. Interestingly, I also reported that individuals who showed high IL-6 responses to the stressor showed greater functional connectivity between the amygdala and DMPFC, compared to individuals who showed low IL-6 responses. This is the first known paper to examine how both the brain and inflammatory system respond to the same stressor. In Paper 2, I demonstrated that exposure to an acute, social evaluative stressor was related to upregulated expression of pro-inflammatory genes measured in immune cells. As with Paper 1, I found that neural activity in the DMPFC during negative social feedback (compared to neutral feedback) was associated with greater levels of pro-inflammatory gene expression in response to the stressor. I also found that activity in the dACC was related to greater levels of pro-inflammatory gene expression, although these analyses were conducted at a more liberal threshold and should

thus be considered only preliminary at this time. This is the first paper to examine neural correlates of stressor-evoked changes in pro-inflammatory gene expression, and is among the first to investigate if acute stressors lead to upregulation of pro-inflammatory genes.

Taken together, these papers provide the initial empirical basis for a neurocognitive account for how social stress influences inflammatory activity. In particular, it appears that neural activity in the DMPFC in response to negative social feedback is related both to increases in levels of the pro-inflammatory cytokine IL-6, and to increases in the expression of four key pro-inflammatory genes. This converging evidence suggests that the DMPFC may be a critical part of neurocognitive system that responds to social evaluation and is associated with increases in inflammation. Given its well-established role as a key region engaged during tasks that involve thinking about the mental states of others, one potential interpretation of this activation is that individuals who are more focused on the thoughts and feelings of an evaluator when they receive negative feedback from that person may be more likely to show increases in inflammation. This interpretation dovetails nicely with some behavioral work suggesting that being evaluated by another person is a key component of social stressors that leads to increases in physiological activation (Dickerson & Kemeny, 2004; Dickerson et al., 2009), and is also consistent with a body of animal research suggesting that the rat-analog of DMPFC plays an important role in sustaining neural responses in threat-related regions (Burgos-Robles, 2009). Although we need much more research to fully understand the precise neurocognitive processes reflected by this DMPFC activation, the converging evidence presented in two papers here suggests that the DMPFC may be a key player in the neural system involved in inflammatory responding to social stress.

Beyond the context of the present study, the findings that DMPFC activation is associated with increases in two measures of inflammation may help advance our understanding of the "social brain" more generally. Most research in social neuroscience and social psychology more broadly has focused on the fact that thinking about the thoughts and feelings of others (and presumably corresponding activation in the "mentalizing system", including the DMPFC) is associated with a variety of positive social outcomes, including decreased stereotyping (Galinsky & Moskowitz, 2000), lower levels of aggression (Miller & Eisenberg, 1988), and increased empathic accuracy (Zaki et al., 2009). While these data are compelling, results from the current dissertation suggest that, perhaps especially in evaluative contexts, activating DMPFC and attending to the thoughts and feelings of others may have negative consequences for health and well-being, insofar as inflammatory activation remains elevated over time. Thus, it will be important for future investigations of the consequences of mentalizing to consider the context in which the mentalizing is occurring (e.g., evaluative vs. non-evaluative) to provide a more complete understanding of the role these brain regions and social cognitive processes play in our social lives and health outcomes.

Although this dissertation provides initial converging evidence regarding the neural correlates of stress-related increases in inflammation, results from the present project also raise many additional questions about how the brain responds to stress and influences inflammatory activity. For example, it is interesting to note that activity in different neural regions within the "threat network" (amygdala vs. dACC) were related to increases in IL-6 compared to pro-inflammatory gene expression, respectively. While it is difficult to speculate on null effects, one possible explanation for why amygdala activity was associated with IL-6 responses and dACC activity to pro-inflammatory gene expression changes relates to the types of tasks in which

activation in these regions is typically observed. For example, amygdala activation is often observed in response to viewing negative facial expressions, viewing negative scenes, or experiencing electric shocks in the context of fear conditioning; all stimuli that could all be considered "cues" of negative outcomes (Eisenberger & Cole, 2012). On the other hand, dACC activation is often observed during tasks in which the participant is experiencing some form of internally-generated distress (i.e., being socially rejected, frustrated with a demanding cognitive task; Eisenberger et al., 2003; Spunt et al., 2012). Together with findings from the present study, one interpretation of our divergent findings is that the amygdala may be more tightly linked with changes in IL-6 possibly because changes in circulating levels of inflammation are driven more strongly by cues of negative outcomes (rather than subjective negative experiences), whereas the dACC may be more tightly linked with changes in pro-inflammatory gene expression because this index of inflammatory activity is driven more strongly by subjective experiences of the social environment. This interpretation is highly speculative at this point in time, however, and should be considered tenuous, at best. Much more research is needed to fully map the neurocognitive systems that underlie stress-related increases in these different inflammatory processes, and to establish their differences and similarities.

It is also interesting to note that although the anterior insula is often active during socially stressful experiences and plays an important role in representing changes in physiological states that typically accompany stressors, we did not find any evidence that activity in the anterior insula was related either to changes in IL-6, or in pro-inflammatory gene expression. These null findings point to the possibility that individuals' awareness of changes in their physiological state may not be important in driving changes in inflammation; in other words, while participants may have experienced increases in autonomic nervous system activation during the social

evaluation, the conscious representation of these changes may not be necessary for increases in inflammation to occur. Once again, future research should continue to follow-up on these findings to further establish if the anterior insula plays a role in the neurocognitive system underlying inflammatory responses to social stress.

The results presented in this dissertation suggest that neural regions involved in processing threat and survival-relevant stimuli (i.e., amygdala, dACC) and a region often active when people are thinking about the thoughts and feelings of others (i.e., DMPFC) may be important for translating experiences of social stress into inflammatory activity. Interestingly, all three of these regions are also thought to play a role in driving autonomic responses to threat and fear-inducing stimuli, raising the possibility that neural systems related to SNS responses during stress may be key drivers of inflammatory activity. This conclusion is speculative based on the results of these first two studies, but future work could measure SNS activation to examine its role as a mediator of the relation between neural activity and inflammation, or employ pharmacological blockers of SNS activity to see if this "breaks the link" between activity in these neural regions and inflammatory responses.

Many additional questions can be explored in the context of the current study to advance this line of research. It will be especially interesting to explore individual difference factors that may moderate the observed associations between neural activity and inflammatory activity. I have collected data on participants' health behaviors (e.g., sleep quantity and quality), psychosocial resources (e.g., social support, self-esteem, optimism), early life environment (e.g., socioeconomic status, parental relationships), and cumulative stress exposure, all of which may be moderators of neural reactivity to social evaluation, inflammatory responses to the social stressor, or both. Furthermore, I am currently completing one-year follow-ups with participants,

which will enable me to examine how neural and inflammatory responses to this laboratory social stressor may relate to longitudinal changes in psychosocial factors (e.g., self-esteem, social support seeking) and affective processes (e.g., internalizing and externalizing symptoms). In sum, the two papers presented in the present dissertation reflect the primary aims of the study, but many additional questions will hopefully be answered by continued analysis of these data over the next few years.

Beyond the present study, this dissertation project provides an important jumping off point for future research exploring the neural correlates of inflammatory responses to stress. It is challenging to study complex social situations within the confines of the neuroimaging environment, and thus one of the major innovations of the present investigation is that we have developed a novel approach to inducing social-evaluative threat in the fMRI scanner, and have shown that it is associated with increases in pro-inflammatory cytokines (at least IL-6) and pro-inflammatory gene expression. Moving forward, we can explore how different features of the social stressor relate to inflammatory activity, by perhaps employing between-subjects manipulations that vary the type of social feedback provided (e.g., all positive feedback vs. mixed feedback) and/or characteristics of the individual supposedly performing the evaluation (e.g., in-group vs. out-group members). It will also be important for future research to further explore physiological mechanisms that may link neural activity with inflammatory responding, such as studies indexing or manipulating SNS activation mentioned previously. Finally, future research could also focus on establishing the relevance of these neural systems and inflammatory responses for actual health outcomes, in an effort to link these relatively circumscribed, acute responses to a laboratory stressor to outcomes in the "real world."

Finally, it is my hope that knowledge gained from this dissertation project and the program of research more broadly will one day be used to more effectively target modifiable risk factors to reduce health disparities and improve population health. As we begin to understand the neurocognitive systems that link social stress and inflammation, we may be able to use this information to design more effective stress-reduction intervention and prevention programs, which could improve health. For example, if additional research continues to converge on the importance of neural regions involved in processing threat and thinking about the thoughts and feelings of others, we could focus stress-reduction programs on attentional re-training to reduce threat perception, or on helping individuals to reframe their negative thoughts about the appraisals of others. Though much more research is needed to fully establish the relevance of these neurocognitive processes for inflammatory responding, the line of inquiry established in this dissertation will hopefully one day provide knowledge that can be used to improve health.

References for Introduction and Conclusions

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