

# Neural mechanisms linking social status and inflammatory responses to social stress

Keely A. Muscatell,<sup>1,2</sup> Katarina Dedovic,<sup>2,3</sup> George M. Slavich,<sup>4</sup>  
Michael R. Jarcho,<sup>4,5</sup> Elizabeth C. Breen,<sup>4</sup> Julienne E. Bower,<sup>2,4</sup>  
Michael R. Irwin,<sup>2,4</sup> and Naomi I. Eisenberger<sup>2</sup>

<sup>1</sup>Robert Wood Johnson Foundation Health and Society Scholars Program, University of California, San Francisco and University of California, Berkeley, CA 94118, USA, <sup>2</sup>Department of Psychology, University of California, Los Angeles, CA 90095-1563, USA, <sup>3</sup>Department of Psychiatry, Research Centre of the Douglas Mental Health University Institute, McGill University, Montreal, Quebec, Canada H4H1R3, <sup>4</sup>Department of Psychiatry & Biobehavioral Sciences and Cousins Center for Psychoneuroimmunology, University of California, Los Angeles, CA 90095, USA and <sup>5</sup>Department of Neuroscience, Loras College, Dubuque, IA, USA 52011

Correspondence should be addressed to Keely A. Muscatell, RWJF Health and Society Scholars Program, UCSF Center for Health and Community, 3333 California Street Suite 465, San Francisco, CA 94118, USA. E-mail: kmuscatell@berkeley.edu.

## Abstract

Social stratification has important implications for health and well-being, with individuals lower in standing in a hierarchy experiencing worse outcomes than those higher up the social ladder. Separate lines of past research suggest that alterations in inflammatory processes and neural responses to threat may link lower social status with poorer outcomes. This study was designed to bridge these literatures to investigate the neurocognitive mechanisms linking subjective social status and inflammation. Thirty-one participants reported their subjective social status, and underwent a functional magnetic resonance imaging scan while they were socially evaluated. Participants also provided blood samples before and after the stressor, which were analysed for changes in inflammation. Results showed that lower subjective social status was associated with greater increases in inflammation. Neuroimaging data revealed lower subjective social status was associated with greater neural activity in the dorsomedial prefrontal cortex (DMPFC) in response to negative feedback. Finally, results indicated that activation in the DMPFC in response to negative feedback mediated the relation between social status and increases in inflammatory activity. This study provides the first evidence of a neurocognitive pathway linking subjective social status and inflammation, thus furthering our understanding of how social hierarchies shape neural and physiological responses to social interactions.

**Key words:** subjective social status; stress; dorsomedial prefrontal cortex; inflammation; interleukin-6; fMRI

## Introduction

The social structures of many species, from insects (Yan *et al.*, 2015) and fish (Fernald and Maruska, 2012) to primates (Ghazanfar and Santos, 2004) and human beings (Hill and Dunbar, 2003), are characterized by their profound hierarchical organization. This social stratification has important implications for health

and well-being, as animals and humans lower in social status are often found to have worse outcomes than those with relatively higher standing in the social hierarchy (Adler *et al.*, 1994; Sapolsky, 2005).

Interestingly, alterations in immune system processes, and particularly heightened levels of inflammation, may provide a biological link between lower social status and poor physical

and emotional outcomes (Kemeny, 2009). Indeed, mice that are consistently subjected to social defeat (a rodent model of low social status) show greater inflammatory dysregulation (Blanchard *et al.*, 1993; Powell *et al.*, 2009), and lower-ranking female macaques have been shown to have greater expression of genes involved in inflammation than higher-ranking females (Tung *et al.*, 2012). In humans, subjective ratings of social status have been associated with increases in stressor-evoked inflammation, such that lower-status individuals show a more pronounced inflammatory response to a laboratory stressor than individuals who perceive themselves as higher in status (Brydon *et al.*, 2004; Derry *et al.*, 2013). While short-term increases in inflammation in response to injury or infection are an integral part of the innate immune system's response to physical insults, exaggerated inflammatory activation in response to purely psychological threats (Slavich and Cole, 2013) and systemic elevations in inflammation are associated with the development of a number of chronic diseases (Hansson, 2005; Miller *et al.*, 2009), thus providing a possible physiological mechanism linking social status and poor physical and mental health outcomes. However, to date no known studies have investigated the neurocognitive systems that are engaged by those lower in subjective social status during a stressor that may lead to increases in inflammation.

Although no studies have directly investigated the neural mechanisms linking social status and stress-related increases in inflammation, a few studies have explored how status affects neural responses to social threat. For example, subordinate animals have been shown to have greater functional activation of the amygdala following social stress, relative to dominant animals (Kollack-Walker *et al.*, 1997). Results from two human studies have also demonstrated that lower-status individuals show greater neural activity in the amygdala, a key brain region in responding to salience cues and threat, when processing external social threats such as angry facial expressions (Gianaros *et al.*, 2008; Muscatell *et al.*, 2012). Given that the amygdala plays a key role in initiating activation of the sympathetic nervous system during stress (LeDoux *et al.*, 1988), and sympathetic activation is thought to drive inflammatory responses (Powell *et al.*, 2013), the tendency of low status individuals to activate the amygdala during social threat processing may lead to increases in inflammation.

Activity in the dorsomedial prefrontal cortex (DMPFC), a key node of the 'mentalizing network' that is often active during tasks that involve thinking about the thoughts and feelings of others, has also been associated with social status. Specifically, individuals lower in subjective status show greater activity in the DMPFC in response to social information, compared to their higher-status counterparts (Muscatell *et al.*, 2012). Furthermore, research in mice suggests that the prelimbic cortex (the mouse analog of human DMPFC/dorsal anterior cingulate cortex) may play a causal role in establishing social rank (Wang *et al.*, 2014). Combined with behavioral research showing that lower-status individuals tend to be more engaged during social interactions (Kraus and Keltner, 2009) and are better at reading the emotions of others (Kraus *et al.*, 2010), these patterns suggest that DMPFC-related attention to others' thoughts and feelings may also track with lower perceived social status. The DMPFC has strong connections with the amygdala and other brainstem regions whose activity can drive stress-related changes in the cardiovascular system and the hypothalamic-pituitary-adrenal axis (Gianaros and Sheu, 2009; Eisenberger and Cole, 2012; Muscatell and Eisenberger, 2012), and as such, it is possible that the DMPFC may also play a key role in linking social status and inflammation. To date, however, no known research has tested this possibility.

With this background in mind, the aim of this study was to explore neural activity in the amygdala and the DMPFC in response to negative social information as a neural mechanism linking social status and stress-related inflammatory responses. To investigate this, 31 healthy, female participants were exposed to a social stressor while they underwent a functional magnetic resonance imaging (fMRI) scan. We focused on females in this study given that women have been shown to be more reactive than men to social stressors (Rohleder *et al.*, 2001; Stroud *et al.*, 2002) and are at greater risk for some inflammatory-related conditions, such as major depressive disorder (Nolen-Hoeksema, 2001). Blood samples were taken before and after the scan, and plasma was assayed for two inflammatory markers commonly studied in the acute stress literature: interleukin-6 (IL-6) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ; Steptoe *et al.*, 2007). Participants also completed a measure of subjective social status, and reported their affective responses to the social stressor. Consistent with prior research, we hypothesized that lower subjective social status would be associated with greater stressor-evoked increases in inflammation. We also hypothesized that lower subjective status would be related to greater neural activity in the amygdala and the DMPFC in response to negative social feedback, replicating prior research. Finally, we explored whether the relationship between social status and inflammatory responses was mediated by neural activity in the amygdala and/or DMPFC in response to negative social feedback. This is the first known study to examine the potential neurocognitive mechanisms linking social status and inflammatory responses to stress.

## Materials and methods

### Participants

Participants were 31 healthy young-adult females ( $M$  age = 19 years; range = 18–22 years). The sample self-identified as 32% Asian/Asian American, 23% Hispanic/Latina, 22% Mixed/Other, 13% African American and 10% White (non-Hispanic/Latina). The socioeconomic background of participants was varied: 45.2% ( $n = 14$ ) of participants' mothers had completed high school education or less, whereas 32.3% ( $n = 10$ ) of the sample had fathers who had completed high school education or less. All participants provided written informed consent, and procedures were approved by the UCLA Institutional Review Board. Participants were paid \$135 for participating.

### Procedure

Complete details of the experimental procedure have been previously reported (Muscatell *et al.*, 2015). In brief, prospective participants were excluded during phone screening if they endorsed a number of criteria known to influence levels of inflammation (e.g. acute infection, chronic illness, BMI over 30) or contraindications for the MRI environment (e.g. left-handedness, claustrophobia, metallic implants). Participants were also excluded if they endorsed any current or lifetime history of Axis-I psychiatric disorder, as confirmed by the Structured Clinical Interview for DSM-IV Axis 1 Disorders (First *et al.*, 1995). Individuals who met all inclusion criteria completed a video-recorded 'impressions interview' in the laboratory, in which they responded to questions such as 'What would you most like to change about yourself?' and 'What are you most proud of in your life so far?' Participants were told that in the next session for the study, they would meet 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 saw the impression being formed of them.

Within 2 days of completing the initial interview, the scan session occurred. At the MRI scanning center, participants were introduced to 'the other participant' (actually a confederate), an indwelling catheter was inserted for blood sampling, followed by at least 45 min of acclimation time and collection of a first baseline (BL) sample. During the acclimation time, questionnaire measures, including the subjective social status measure, were completed (see below for detail).

Following the blood collection, the participant and confederate were told that the experimenters had randomly assigned the confederate to watch the participant's video and form an impression of her, while the participant would undergo the fMRI scan and view the confederate's impressions. After being familiarized with the impression formation task, a second BL blood sample was drawn. During the scan, the participant completed the social stress task, in which she viewed the confederate's feedback about how she was supposedly coming across in the video (see below for more detail). After the scan, additional blood samples were collected 30, 60 and 90 min after the termination of the stressor. During this time, participants were given neutral reading material to read. We specifically asked they not use their cell phones, go on the internet or study during this time, as we wanted to ensure that any changes in inflammation that were observed were not due to engagement in these other activities and were most likely due to the social stress task. After the final blood sample was collected, participants were probed regarding any suspicion about the cover story, and were fully debriefed. No participants indicated that they thought the feedback was fake, or that the confederate was a member of our research team.

### fMRI social stress task

We induced social stress using procedures similar to those employed in a prior study (Eisenberger et al., 2011; see Supplementary Materials for full details). Briefly, during the scan, participants viewed a video of a mouse cursor moving around a screen that displayed 24 'adjective buttons', which they believed was a live interface of the confederate's impressions of their interview. Feedback adjectives were divided into one-third positive (e.g. 'intelligent'), one-third neutral (e.g. 'practical') and one-third negative words (e.g. 'annoying'). The cursor selected a new adjective button every 11–12 s. Over the course of the scan, participants received 15 each of positive, neutral and negative feedback; every time an adjective was selected, participants responded to the question 'How do you feel?' using a button box with four buttons (1 = really bad, 4 = really good). The feedback task was preceded and followed by a fixation crosshair (10 s each), which formed the implicit BL.

### Affective responses

To measure participants' affective responses to the social stress task, four different self-report measures were examined. Participants' ratings during the scan of how they felt in response to each type of feedback (1 = really bad, 4 = really good). Responses to this measure were reverse-coded (so higher numbers indicate greater negative feelings), and responses to each type of feedback (positive, negative, neutral) were averaged to form a measure of 'in-the-moment' affective responses to each

type of feedback. Participants were also asked to indicate how they felt overall (1 = really bad, 4 = really good) immediately prior to the social evaluation (i.e. while in the scanner, but before being evaluated) and immediately following the conclusion of the evaluation (i.e. while still in the scanner). Responses to this measure were also reverse-coded (so higher numbers indicate greater negative feelings) and formed a measure of overall change in negative affect in response to the evaluation. We also examined participants' self-reports of feelings of social evaluation by averaging their scores on two items ('I feel evaluated by the other participant'; 'I feel judged by the other participant';  $\alpha = 0.84$ ) measured on a seven-point scale (1 = not at all, 7 = very much) prior to going in the scanner and after returning to the testing room following the scan. Finally, participants' perceptions of social rejection were measured with three items (i.e. 'I feel like the other participant likes me'; 'I feel like the participant has a positive impression of my interview'; 'I feel the other participant accepts me';  $\alpha = 0.88$ ) also measured on a seven-point scale before and after the scan, which were averaged to create an index of social rejection. Responses to the social rejection items were reverse coded so higher numbers indicate greater feelings of rejection.

### Inflammatory responses

Circulating levels of pro-inflammatory cytokines were assessed at two BL time points prior to the stressor and three time points after the stressor as previously described (Muscatell, et al., 2015). Briefly, EDTA plasma samples were assayed for IL-6 and TNF- $\alpha$  (Quantikine High Sensitivity ELISAs, R&D Systems, Minneapolis, MN) according to the manufacturer's protocols; all samples from a single participant were assayed on the same plate. Within- and between-assay coefficients of variation were < 9%. All cytokine data were positively skewed, so raw values were log transformed to normalize the distribution prior to statistical testing. A prior paper from this dataset established that there were significant increases in levels of IL-6, but not TNF- $\alpha$ , following the stressor (Muscatell et al., 2015); as such, in this article, inflammatory responses are calculated as the change in plasma cytokine concentration from BL (average of two BL measures) to the 90-min post-stress time point, given that levels of inflammation were at their highest at this final time point.

### Subjective social status measure

To measure subjective social status, participants completed the MacArthur Scale of Subjective Social Status (Adler et al., 2000). Participants were shown a picture of a ladder with 10 rungs with the description: 'The ladder represents where people stand in society: At the top of the ladder are the people who are the best off (most money, most education, best jobs), and at the bottom of the ladder are people who are the worst off (least money, least education and worst jobs or no jobs)'. Participants were asked to place an X on the rung that best represented where they thought they stood on the ladder. Scores ranged from 3 to 9 (mean = 6.2, s.d. = 1.5).

### 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 = 2300 ms, TE = 2.98 ms, flip angle = 9°,

matrix = 256 × 256, FOV = 256 mm). Then, we acquired 288 functional T2-weighted EPI volumes, during the stress task (slice thickness = 3 mm, gap = 1 mm, TR = 2000 ms, TE = 25 ms, flip angle = 90°, matrix = 64 × 64, FOV = 200 mm).

### Data analysis

Neuroimaging data were pre-processed and analysed 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 × 3 × 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 reported in Montreal Neurological Institute (MNI) format.

Following pre-processing, a general linear model was constructed for each participant. The selection of each feedback word (lasting 3 s) and the subsequent 8–9 s (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, negative), and we included the six motion parameters as covariates. For each model, the time series was high-pass filtered using a 128 Hz function, and serial autocorrelation was modeled as an AR(1) process. For this study, we focused on neural activity during the negative feedback trials compared with the neutral feedback trials. 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.

Given our *a priori* hypotheses regarding the associations between social status and neural activity in the amygdala and the DMPFC, we conducted region-of-interest (ROI) analyses focusing on these brain regions. Amygdala ROIs were defined anatomically based on the Automated Anatomical Labeling atlas (left amygdala:  $-32 < x < -12$ ,  $-12 < y < 4$ ,  $-24 < z < -8$ ; right amygdala:  $12 < x < 32$ ,  $-21 < y < 4$ ,  $-24 < z < -8$ ). The DMPFC ROI was defined functionally based on a prior paper that explored the neural underpinnings of person impression formation (Mitchell et al., 2005). We focused on this region given that we were specifically interested in targeting a sub-region of DMPFC that has been shown to be involved in mentalizing, given prior studies showing that lower status individuals tend to engage in more mentalizing (e.g. Kraus et al., 2010). Using Marsbar, we created a 10 mm spherical ROI around the peak coordinate in DMPFC that was associated with person impression formation in this prior study (−9, 54, 36). Mean parameter estimates were extracted from the resulting ROIs for each participant using Marsbar, and entered into SPSS for further analysis with the social status and inflammatory measures (see Supplementary Figure S1 for images of the ROIs). Self-reported race was not related to scores on the social status measure or inflammatory responses to the social stressor, and thus we do not control for race in subsequent analyses.

In addition to these primary ROI analyses, we also conducted exploratory whole-brain regression analyses to examine if activity in any other neural regions besides the amygdala and DMPFC was associated with social status. For these analyses, participants' rating on the ladder was entered as a regressor into the contrast of negative feedback > neutral feedback.

Resulting images were thresholded at  $P < 0.005$ , 20 voxels. Given that these analyses were primarily exploratory in nature to supplement our *a priori* ROI analyses, we used this somewhat liberal threshold to decrease the likelihood of type-2 error (Lieberman and Cunningham, 2009).

Finally, we conducted path analyses to test whether neural activity in the *a priori* ROIs described above mediated any observed relations between social status and inflammatory responses to the social stressor. Statistical testing of mediation was performed using the SPSS macro, PROCESS (Preacher and Hayes, 2004). Specifically, we conducted nonparametric bootstrapping (10 000 iterations) with 90% confidence intervals (CIs) for the indirect mediation effect ( $a \times b$  effect) generated by the bias-corrected method.

## Results

### Social status and affective responses to social stress

First, we examined if subjective social status was related to participants' self-reported affective responses to the social stressor. There was no relationship between status and self-reported feelings in response to the negative words ( $r = 0.05$ ,  $P = 0.79$ ); there was a marginally significant, positive relationship between status and responses to positive words ( $r = 0.33$ ,  $P = 0.07$ ), and there was a significant, positive correlation between status and self-reported feelings in response to the neutral words ( $r = 0.46$ ,  $P = 0.009$ ). These data suggest that individuals reporting higher social status more negative in response to the neutral and positive feedback, compared to those reporting lower standing. We also examined the relation between social status and overall changes in affect, perceptions of social evaluation, and perceptions of social rejection from pre- to post-stress. There was a significant, negative relationship between social status and overall change in self-reported negative feelings ( $r = -0.43$ ,  $P = 0.02$ ), suggesting that individuals reporting higher status felt worse following the stressor. There was no relationship between social status and perceptions of evaluation ( $r = -0.07$ ,  $P = 0.71$ ) or rejection ( $r = 0.10$ ,  $P = 0.59$ ).

### Social status and social stressor-evoked inflammatory responses

Next, we examined if subjective social status was associated with social-stressor evoked changes in the inflammatory markers IL-6 and TNF- $\alpha$ . Consistent with hypotheses and previous research (Brydon et al., 2004; Derry et al., 2013), we found a significant, negative correlation between social status and stressor-evoked IL-6 responses,  $r = -0.38$ ,  $P < 0.05$  (Figure 1). Specifically, participants who ranked themselves lower in social status showed greater increases in IL-6 in response to the stressor. There was no relationship between status and stressor-evoked TNF- $\alpha$  responses ( $P > 0.80$ ); as such, subsequent analyses focus exclusively on IL-6. As indicated in a prior report on this dataset (Muscatell et al., 2015), there was also no correlation between any of the affective responses to the task (i.e. responses to the words, overall affect, social evaluation or social rejection) and inflammatory responses (all  $P > 0.77$ ).

### Social status and neural responses to social stress

Next, we explored if subjective social status was related to neural activity in the amygdala and/or the DMPFC in response to

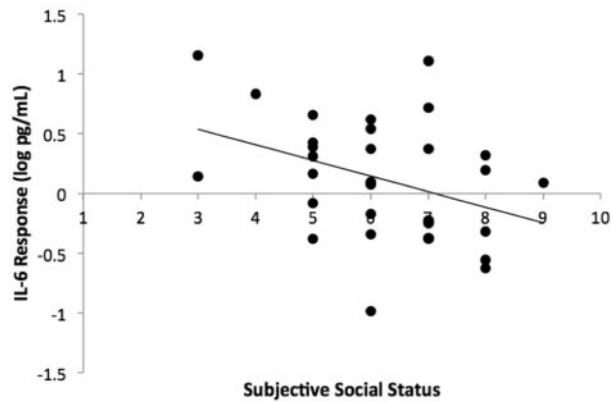


Fig. 1. Lower subjective social status is associated with greater IL-6 responses to the social stressor.

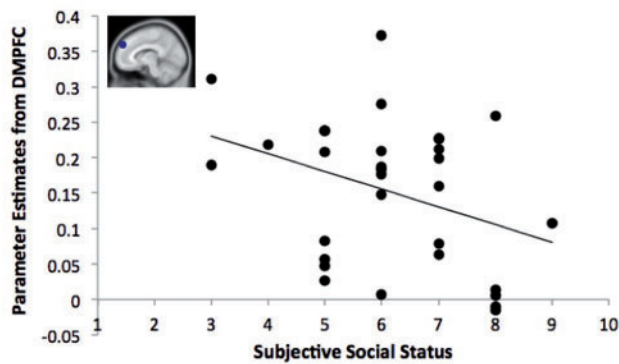


Fig. 2. Lower subjective social status is associated with greater neural activity in the DMPFC ROI in response to negative feedback.

receiving negative social feedback (compared with neutral feedback). As hypothesized and consistent with previous research, there was a significant, negative correlation between status and neural activity in the DMPFC ROI ( $r = -0.35$ ,  $P < 0.05$ ; Figure 2). Thus, individuals who ranked themselves lower on the ladder showed greater activity in the DMPFC in response to receiving negative feedback. Contrary to hypotheses, there was no relation between social status and neural activity in the left or right amygdala ( $P_s > 0.4$ )<sup>1</sup>.

We also conducted ancillary whole-brain regression analyses to explore if neural activity in any other regions in response to negative feedback (vs neutral feedback) was related to subjective social status. Results revealed that activity in two clusters within DMPFC were negatively correlated with status; status was also negatively correlated with activity in bilateral inferior frontal gyrus (all  $P < 0.005$ , 20 voxels; see Supplementary Figure S2 and Table S1). Neural activity in the right fusiform gyrus was positively correlated with social status.

<sup>1</sup> Because activity in the amygdala was not related to social status in this sample, we did not explore amygdala activity as a potential mediator of the relation between status and inflammatory responses. For the sake of completeness, however, we did examine if activity in the amygdala ROIs (for the contrast negative > neutral feedback) was related to changes in IL-6 from pre- to post-stress; these correlations were not significant ( $r$  for left amygdala = 0.09,  $r$  for right amygdala = 0.14, both  $P > 0.05$ ).

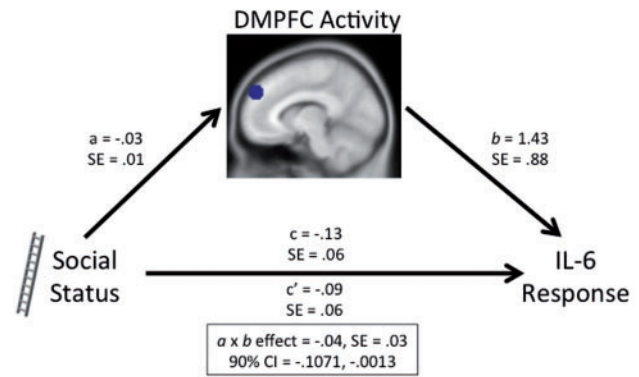


Fig. 3. Mediation model linking subjective social status and inflammatory responses via activation in the DMPFC.

Note:  $a$ ,  $b$ ,  $c$  and  $c'$  refer to the unstandardized coefficients for each path in the model; SE refers to the standard error for each effect.

### Mediation testing of subjective social status, DMPFC responses to negative feedback and inflammatory reactivity

Finally, we tested if neural activity in the DMPFC ROI in response to negative (vs neutral) feedback mediated the observed relation between social status and inflammatory reactivity. (Given that activity in the amygdala was not related to status, and IFG activity was not related to inflammatory responses, we did not test these regions as mediators.) Results indicated that the 90% CI for the indirect effect of subjective social status on inflammatory reactivity via DMPFC activation did not include 0 (point estimate for  $a \times b$  effect =  $-0.036$ ,  $SE = 0.03$ , 90% CI =  $-0.1071$ ,  $-0.0013$ ), thus suggesting that DMPFC activation is a significant mediator linking social status and inflammatory responses to stress (Figure 3). Indeed, the effect size for the indirect effect of status on inflammatory responses via DMPFC activation was medium ( $\kappa^2 = 0.104$ , CI = 0.0145, 0.2745; Preacher and Kelley, 2011), further suggesting that lower subjective social status may lead to stress-related increases in inflammation via activation in the DMPFC in response to negative social feedback.

## Discussion

This study examined the neural mechanisms linking social status and inflammatory responses to social stress, in an effort to understand how social hierarchies may influence health and well-being. As hypothesized, lower subjective social status was associated with greater increases in the pro-inflammatory cytokine IL-6 in response to a social stressor. In addition, neuroimaging data revealed that status was related to neural responses in a key mentalizing-related brain region (DMPFC), with individuals lower in subjective social status showing greater activity in the DMPFC in response to receiving negative social feedback. Finally, we found that activity in the DMPFC in response to negative feedback mediated the relation between social status and inflammatory responses, suggesting a possible neurocognitive pathway by which lower subjective social status may lead to greater inflammation. To our knowledge, this is the first study to find mediational evidence linking perceptions of social status, neural activation and stress-induced increases in inflammation.

Why might DMPFC activation in response to negative social feedback mediate the relation between social status and inflammation? On a psychological level, it is possible that heightened DMPFC activation reflects increased 'mentalizing' on the part of

lower subjective status individuals (given that the DMPFC is a key node of the 'mentalizing network' that is often engaged during tasks that involve thinking about the minds of others; Lieberman, 2007). In other words, those who perceive they have low social status may be more focused on trying to understand what an evaluator is thinking about or why an evaluator is giving them negative feedback, compared to those with higher subjective status, which would be reflected in greater DMPFC activation (see also Muscatell *et al.*, 2012). This type of heightened attention to others has been linked with greater inflammatory responses to stress in a prior study (Dickerson *et al.*, 2009), thus suggesting the possibility that DMPFC-supported mentalizing under social evaluation may contribute to lower status individuals' increases in inflammation. While we did not find a relationship between social status and self-reports of feeling socially evaluated, it is possible that demand characteristics or post-task recall biases may have influenced participants' reports of how evaluated they felt. To deal with these discrepancies between the self-report and neural data, future research should experimentally manipulate mentalizing processes by asking individuals (particularly those reporting low status) to direct their attention toward or away from the evaluator and examining how this affects DMPFC activity and inflammatory responses.

At an anatomical level, the DMPFC has strong anatomical connections to other neural regions that play a role in physiological stress responding, including the amygdala, hypothalamus and periaqueductal gray (Ongur and Price, 2000), making it ideally situated to link social status and inflammatory responses. Indeed, recent research has suggested that the DMPFC may be part of an 'aversive amplification' circuit, in which DMPFC activity may sustain and amplify activation in limbic regions during threatening, stressful experiences (Muscatell *et al.*, 2015; Robinson *et al.*, 2012). It will be interesting for future research using Diffusion Tensor Imaging (DTI) and other techniques to examine if there is greater white-matter connectivity between DMPFC and limbic regions among individuals lower in subjective social status.

Somewhat surprisingly, there was no relationship between social status and neural activity in the amygdala, a key threat-related neural region that has been associated with social status in prior studies (Gianaros *et al.*, 2008; Muscatell *et al.*, 2012). It is possible that differences in the stimuli used in this study compared with prior investigations may explain these divergent findings. For example, the past studies by Gianaros *et al.* (2008) and Muscatell *et al.* (2012) both examined how status influenced amygdala reactivity to external images of threatening facial expressions that were largely context-free, whereas in this study we created a more personal threat experience by using an elaborate cover story that provided context for the evaluative words. Given that the amygdala is hypothesized to be important for the detection of emotion and salience in the environment, but not sufficient for the generation of emotional 'feelings' (Wager *et al.*, 2008), it is possible that the use of an emotion-generation task (compared to an emotion-detection task) contributed to the differences in findings between the present investigation and past work in this area. Another possibility is that threatening faces may be more salient than written words, and thus may lead to greater amygdala activation (Adolphs, 2010; Cunningham and Brosch, 2012). Much more research is needed to fully specify the precise neural underpinnings of reactivity to different types of social threats, and how social status may affect these responses.

The present finding that social status is related to inflammatory responses to stress replicates two prior studies showing

that individuals lower in subjective social status have a greater stressor-evoked increase in IL-6 (Brydon *et al.*, 2004; Derry *et al.*, 2013). Importantly, while the stress tasks used in these previous experiments involved both cognitive effort and some degree of social evaluation, the stressor used in this study isolated the social evaluative component. Therefore, it appears that the effects of status on inflammatory responses to stress are not simply due to performing a cognitively demanding task on which lower status individuals may perform more poorly (Noble *et al.*, 2007). Rather, it may be that the social-evaluative component of the previously used stress tasks drives the observed increases in inflammation, as lower-status individuals are more sensitive to social cues in the environment (Kraus and Keltner, 2009) and may thus be especially sensitive to the effects of social stress. Future studies could directly test this possibility by assigning participants to complete either a social or a cognitive stressor and examining how social status influences inflammatory responses to these two different tasks.

It should also be noted that in this study, social status was associated with stressor-evoked increases in IL-6, but not TNF- $\alpha$ , another pro-inflammatory cytokine often studied in the context of stress research (Steptoe *et al.*, 2007). It is possible that a restricted range or floor effect in TNF- $\alpha$  responses may have limited our ability to detect associations between social status and this inflammatory marker, as the overall group did not show changes in TNF- $\alpha$  after exposure to the stressor (Muscatell *et al.*, 2015). It will be important for future studies to clarify the potential specificity in the relation between social status and measures of inflammatory activity.

Interestingly, while the neural and inflammatory data suggest that individuals reporting lower status may be more reactive to a social stressor, a very different pattern emerged when examining how social status was related to affective responses to the stressor. Specifically, higher subjective status individuals reported greater increases in negative feelings from pre- to post-stress, compared with lower subjective status individuals. Higher subjective social status was also related to less positive feelings in response to the positive and neutral feedback words, though status was unrelated to affective responses to the negative feedback. These data are somewhat consistent with literature showing that individuals from higher socioeconomic status (SES) backgrounds are more likely than lower SES individuals to respond to stressors with extreme emotional distress (Kessler, 1979), as well as some data suggesting that higher-status individuals show greater cortisol responses to acute stress (Gruenewald *et al.*, 2006). It is possible that, because of their relatively greater social standing, individuals reporting higher social status are less accustomed to receiving evaluative feedback and thus experience it as more distressing when they do. However, these in-the-moment negative feelings do not appear to translate into downstream changes in inflammatory activity. Given that physiological and experiential responses to social threat have been shown to have dissociable neural substrates (Wager *et al.*, 2009), it makes sense that biological and self-report responses do not often cohere (Mauss *et al.*, 2005). Much more research is needed to fully understand the complex relationship between social status and psychological vs physiological responses to stress.

Data from this study should be interpreted in light of some important limitations. First, all participants in the present sample were female, and it is thus not clear if the findings generalize to males. Second, given that participants were all students at a public university, they have achieved a relatively high level of objective SES, which limits generalizability of the findings to other populations. More research is needed to explore if these

same neural and inflammatory processes operate at the tails of the objective SES distribution, such as among those living in poverty and/or individuals with extremely high SES. It will also be important for future research to examine if subjective perceptions of social status or objective indicators of SES are stronger predictors of neural and inflammatory responses to social stress, or if there are dissociable neural and physiological stress responses as a function of subjective vs objective SES. Third, given that participants in this study received negative, neutral and positive feedback, we cannot determine if it was specifically the negative feedback that was driving the observed increases in inflammation among those reporting lower social status. It will also be important for future studies to examine if simply being socially evaluated (even if the feedback one receives is positive) is sufficient to increase inflammation among lower subjective status individuals, or if the presence of negative feedback is necessary. Finally, we note that although the effect size for the mediation analysis indicated a medium effect, only the 90% CI did not include 0, possibly due to a small sample size for detecting these sorts of complex relationships between social status, neural activity and physiological responses. More research in larger samples will be needed to replicate this effect, but given that this is the first known study to link social status, neural and inflammatory reactivity data, we believe it is an important first step in exploring the neural mechanisms linking social status and inflammatory responses to stress.

Despite these limitations, data from the present study are the first to show a neural mediator of the relation between social status and inflammatory responses to stress. Furthermore, we replicate prior work showing that lower subjective status is related to greater neural activation in the DMPFC, and extend this previous work by using a novel social stress task. Finally, we also replicate a number of studies showing that lower subjective social status is related to greater stress-related increases in inflammation, and demonstrate for the first time that this is true even when there is no cognitive, effortful component to the stressor. Together, these findings shed light on possible neurocognitive and immune mechanisms that may contribute to the negative health consequences of low social status, and further our knowledge of how social standing shapes our brain and bodily responses in social interactions.

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## Supplementary data

Supplementary data are available at SCAN online.

Conflict of interest. None declared.

## References

- Adler, N.E., Boyce, T., Chesney, M.A., et al. (1994). Socioeconomic status and health: the challenge of the gradient. *American Psychologist*, **49**, 15–24.
- Adler, N.E., Epel, E.S., Castellazzo, G., Ickovics, J.R. (2000). Relationship of subjective and objective social status with psychological and physiological functioning: preliminary data in healthy, White women. *Health Psychology*, **19**, 586–92.
- Adolphs, R. (2010). What does the amygdala contribute to social cognition? *Annals of the New York Academy of Sciences*, **1191**, 42–61.
- Blanchard, D.C., Skai, R.R., McEwen, B., Weiss, S.M., Blanchard, R.J. (1993). Subordination stress: behavioral, brain, and neuroendocrine correlates. *Behavioural Brain Research*, **58**, 113–21.
- Brydon, L., Edwards, S., Mohamed-Ali, V., Steptoe, A. (2004). Socioeconomic status and stress-induced increases in interleukin-6. *Brain, Behavior, and Immunity*, **18**, 281–90.
- Cunningham, W.A., Brosch, T. (2012). Motivational salience amygdala tuning from traits, needs, values, and goals. *Current Directions in Psychological Science*, **21**, 54–9.
- Derry, H.M., Fagundes, C.P., Andridge, R., Glaser, R., Malarkey, W.B., Kiecolt-Glaser, J.K. (2013). Lower subjective social status exaggerates interleukin-6 responses to a laboratory stressor. *Psychoneuroendocrinology*, **38**, 2676–85.
- Dickerson, S.S., Gable, S.L., Irwin, M.R., Aziz, N., Kemeny, M.E. (2009). Social-evaluative threat and proinflammatory cytokine regulation: an experimental laboratory investigation. *Psychological Science*, **20**, 1237–44.
- Eisenberger, N.I., Cole, S.W. (2012). Social neuroscience and health: neurophysiological mechanisms linking social ties with physical health. *Nature Neuroscience*, **15**, 669–74.
- Eisenberger, N.I., Inagaki, T.K., Muscatell, K.A., Haltom, K.E.B., Leary, M.R. (2011). The neural sociometer: a mechanism for translating interpersonal appraisals into state self-esteem. *Journal of Cognitive Neuroscience*, **23**, 3448–55.
- Fernald, R.D., Maruska, K.P. (2012). Social information changes the brain. *Proceedings of the National Academy of Sciences of the United States of America*, **109**, 17194–9.
- First, M.B., Gibbon, M., Spitzer, R.L., Williams, J.B.W. (1995). *User's Guide for the Structured Clinical Interview for DSM-IV Axis I disorders (SCID-I, Version 2.0, Final Version)*. New York (NY): New York State Psychiatric Institute.

- Ghazanfar, A.A., Santos, L.R. (2004). Primate brains in the wild: the sensory bases for social interactions. *Nature Reviews Neuroscience*, *5*, 603–16.
- Gianaros, P.J., Horenstein, J.A., Hariri, A.R., et al. (2008). Potential neural embedding of parental social standing. *Social Cognitive and Affective Neuroscience*, *3*, 91–6.
- Gianaros, P.J., Sheu, L.K. (2009). A review of neuroimaging studies of stressor-evoked blood pressure reactivity: emerging evidence for a brain-body pathway to coronary heart disease risk. *NeuroImage*, *47*, 922–36.
- Gruenewald, T.L., Kemeny, M.E., Aziz, N. (2006). Subjective social status moderates cortisol responses to social threat. *Brain, Behavior, and Immunity*, *20*, 410–9.
- Hansson, G.K. (2005). Inflammation, atherosclerosis, and coronary artery disease. *New England Journal of Medicine*, *352*, 1685–95.
- Hill, R.A., Dunbar, R.I.M. (2003). Social network size in humans. *Human Nature*, *14*, 53–72.
- Kemeny, M.E. (2009). Psychobiological responses to social threat: evolution of a psychological model in psychoneuroimmunology. *Brain, Behavior, and Immunity*, *23*, 1–9.
- Kessler, R.C. (1979). Stress, social status, and psychological distress. *Journal of Health and Social Behavior*, *20*, 259–72.
- Kollack-Walker, S., Watson, S.J., Akil, H. (1997). Social stress in hamsters: defeat activates specific neurocircuits within the brain. *The Journal of Neuroscience*, *17*, 8842–55.
- Kraus, M.W., Cote, S., Keltner, D. (2010). Social class, contextualism, and empathic accuracy. *Psychological Science*, *21*, 1716–32.
- Kraus, M.W., Keltner, D. (2009). Signs of socioeconomic status a thin-slicing approach. *Psychological Science*, *20*, 99–106.
- LeDoux, J.E., Iwata, J., Cicchetti, P., Reis, D.J. (1988). Different projections of the central amygdaloid nucleus mediate autonomic and behavioral correlates of fear. *The Journal of Neuroscience*, *8*, 2517–29.
- Lieberman, M.D. (2007). Social cognitive neuroscience: a review of core processes. *Annual Review of Psychology*, *58*, 259–89.
- Lieberman, M.D., Cunningham, W.A. (2009). Type I and Type II error concerns in fMRI research: re-balancing the scale. *Social Cognitive and Affective Neuroscience*, *4*, 423–8.
- Mauss, I.B., Levenson, R.W., McCarter, L., Wilhelm, F.H., Gross, J.J. (2005). The tie that binds? Coherence among emotion experience, behavior, and physiology. *Emotion*, *5*, 175–90.
- Miller, G.E., Chen, E., Fok, A.K., et al. (2009). Low early-life social class leaves a biological residue manifested by decreased glucocorticoid and increased proinflammatory signaling. *Proceedings of the National Academy of Sciences*, *106*, 14716–21.
- Miller, A.H., Maletic, V., Raison, C.L. (2009). Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biological Psychiatry*, *65*, 732–41.
- Mitchell, J.P., Macrae, C.N., Banaji, M.R. (2005). Forming impressions of people versus inanimate objects: social-cognitive processing in the medial prefrontal cortex. *NeuroImage*, *26*, 251–7.
- Muscattell, K.A., Dedovic, K., Slavich, G.M., et al. (2015). Greater amygdala activity and dorsomedial prefrontal-amygdala coupling are associated with enhanced inflammatory responses to stress. *Brain, Behavior, and Immunity*, *43*, 46–53.
- Muscattell, K.A., Eisenberger, N.I. (2012). A social neuroscience perspective on stress and health. *Social and Personality Psychology Compass*, *6*, 890–904.
- Muscattell, K.A., Morelli, S.A., Falk, E.B., et al. (2012). Social status modulates neural activity in the mentalizing network. *NeuroImage*, *60*, 1771–7.
- Noble, K.G., McCandliss, B.D., Farah, M.J. (2007). Socioeconomic gradients predict individual differences in neurocognitive abilities. *Developmental Science*, *10*, 464–80.
- Ongur, D., Price, J.L. (2000). The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys, and humans. *Cerebral Cortex*, *10*, 206–19.
- Powell, N.D., Bailey, M.T., Mays, J.W., et al. (2009). Repeated social defeat activates dendritic cells and enhances toll-like receptor dependent cytokine secretion. *Brain, Behavior, and Immunity*, *23*, 225–31.
- Powell, N.D., Sloan, E.K., Bailey, M.T., Arevalo, J.M.G., Miller, G.E., Cole, S.W. (2013). Social stress up-regulates inflammatory gene expression in the leukocyte transcriptome via  $\beta$ -adrenergic induction of myelopoiesis. *Proceedings of the National Academy of Sciences of the United States of America*, *110*, 16574–9.
- Preacher, K.J., Hayes, A.F. (2004). SPSS and SAS procedures for estimating indirect effects in simple mediational models. *Behavioral Research Methods*, *36*, 717–31.
- Preacher, K.J., Kelley, K. (2011). Effect size measures for mediation models: quantitative strategies for communicating indirect effects. *Psychological Methods*, *16*, 93–115.
- Robinson, O.J., Charney, D.R., Overstreet, C., Vytal, K., Grillon, C. (2012). The adaptive threat bias in anxiety: amygdala-dorsomedial prefrontal cortex coupling and aversive amplification. *NeuroImage*, *60*, 523–9.
- Rohleder, N., Schommer, N.C., Hellhammer, D.H., Engel, R., Kirschbaum, C. (2001). Sex differences in glucocorticoid sensitivity of proinflammatory cytokine production after psychosocial stress. *Psychosomatic Medicine*, *63*, 966–72.
- Sapolsky, R.M. (2005). The influence of social hierarchy on primate health. *Science*, *308*, 648–52.
- Slavich, G.M., Cole, S.W. (2013). The emerging field of human social genomics. *Clinical Psychological Science*, *1*, 331–48.
- Steptoe, A., Hamer, M., Chida, Y. (2007). The effects of acute psychological stress on circulating inflammatory factors in humans: a review and meta-analysis. *Brain, Behavior, and Immunity*, *21*, 901–12.
- Stroud, L.R., Salovey, P., Epel, E.S. (2002). Sex differences in stress responses: social rejection versus achievement stress. *Biological Psychiatry*, *52*, 318–27.
- Tung, J., Barreiro, L.B., Johnson, Z.P., et al. (2012). Social environment is associated with gene regulatory variation in the rhesus macaque immune system. *Proceedings of the National Academy of Sciences of the United States of America*, *109*, 6490–5.
- Wager, T.D., Barrett, L.F., Bliss-Moreau, E., et al. (2008). The neuroimaging of emotion. *The Handbook of Emotion*, *3*, 249–71.
- Wager, T.D., van Ast, V.A., Hughes, B.L., Davidson, M.L., Lindquist, M.A., Ochsner, K.N. (2009). Brain mediators of cardiovascular responses to social threat, Part II: prefrontal-subcortical pathways and relationship with anxiety. *NeuroImage*, *47*, 836–51.
- Wang, F., Kessels, H.W., Hu, H. (2014). The mouse that roared: neural mechanisms of a social hierarchy. *Trends in Neurosciences*, *37*, 674–82.
- Yan, H., Bonasio, R., Simola, D.F., Liebig, J., Berger, S.L., Reinberg, D. (2015). DNA methylation in social insects: how epigenetics can control behavior and longevity. *Annual Review of Entomology*, *60*, 435–52.