Psychoneuroendocrinology 62 (2015) 336–342

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

Trait sensitivity to social disconnection enhances pro-inflammatory responses to a randomized controlled trial of endotoxin

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ARTICLE INFO

Article history:
Received 25 March 2015
Received in revised form 18 August 2015
Accepted 24 August 2015

Keywords:
Inflammation
Loneliness
Endotoxin
Cytokines
Rejection sensitivity
Social genomics

ABSTRACT

One proposed mechanism for the association between social isolation and poor health outcomes is inflammation. Lonely or socially disconnected individuals show greater inflammatory responses, including up-regulation of pro-inflammatory gene expression, and people who are sensitive to cues of social disconnection (e.g., high levels of anxious attachment) exhibit greater inflammation in response to psychological stress. However, no studies have examined how sensitivity to social disconnection may influence pro-inflammatory responses to an inflammatory challenge. In the present study, we investigated the impact of sensitivity to social disconnection (a composite score comprised of loneliness, anxious attachment, fear of negative evaluation, and rejection sensitivity) on pro-inflammatory cytokines and gene expression in response to endotoxin, an inflammatory challenge, vs. placebo in a sample of one hundred and fifteen ($n = 115$) healthy participants. Results showed that those who are more sensitive to social disconnection show increased pro-inflammatory responses (i.e., increased levels of tumor necrosis factor-alpha and interleukin-6) to endotoxin, as well as up-regulation of multiple genes related to inflammation. Furthermore, bioinformatics analyses revealed that those in the endotoxin group who are more sensitive to social disconnection exhibited a conserved transcriptional response to adversity (CTRA) regulatory profile, involving up-regulation of beta-adrenergic and pro-inflammatory transcription control pathways and down-regulation of antiviral transcription factors in response to endotoxin. These results may ultimately have implications for understanding the links between social isolation, inflammation, and health.

Clinical Trials Registration: ClinicalTrials.gov NCT01671150

1. Introduction

Decades of research have established a link between social isolation and poor health outcomes (Hawkley and Cacioppo, 2010; House et al., 1988; Uchino et al., 1996). In fact, meta-analysis indicates that social isolation can have a stronger effect on mortality risk than more traditional health risk factors such as obesity and physical inactivity, such that individuals with strong relationships have a 50% greater likelihood of survival than their isolated counterparts (Holt-Lunstad et al., 2010). Interestingly, peoples' subjective perceptions of social isolation (e.g., feelings of loneliness or social disconnection) serve as a stronger predictor of health-related outcomes than objective measures (e.g., number of people in their social network or frequency of social contact; Shor and Roelfs, 2015). While associations between social isolation and health are well-established, the pathways or mechanisms behind this link are less clear.

One proposed pathway for the impact of subjective social isolation on health outcomes involves inflammation (Eisenberger and Cole, 2012; Hawkley et al., 2007). Inflammation, the body's first line of defense against foreign agents and infection, is generally adaptive when it is acute, but chronic inflammation has been linked to a number of diseases, including cardiovascular disease, arthritis, metastatic cancer, and diabetes (Ferrucci et al., 1999; Finch, 2010).

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http://dx.doi.org/10.1016/j.psyneuen.2015.08.020
0306-4530/

Furthermore, a growing body of work has found that subjective social isolation is related to increased inflammation. For example, individuals who report feeling chronically lonely and distant from others, compared to individuals who report feeling more socially connected to others, exhibit up-regulation of genes involved in inflammation (Cole et al., 2007; Creswell et al., 2012). Lonelier individuals also show increased levels of pro-inflammatory cytokines such as interleukin(IL)-6 and tumor necrosis factor-alpha (TNF-α) in response to an acute psychological stressor (Hackett et al., 2012; Jaremka et al., 2013a).

Other related psychological constructs, such as anxious attachment, sensitivity to negative social evaluation, and rejection sensitivity, have also been examined in relation to inflammation. As these constructs tap into an individual's sensitivity to social disconnection (i.e., increased sensitivity to rejection, social evaluation, or abandonment), it is reasonable to expect that these constructs would show similar patterns of inflammatory activity to those observed with loneliness or subjective social isolation. Indeed, individuals who demonstrate high levels of anxious attachment show immune-related alterations (Jaremka et al., 2013b). Similarly, it has been shown that social evaluative threat leads to increases in pro-inflammatory cytokines, whereas equally stressful tasks without a socially evaluative component do not (Dickerson, 2008; Dickerson et al., 2009). Finally, greater neural sensitivity to social rejection has been associated with increased inflammatory responding to a social stressor (Slavich et al., 2010). Together, these findings suggest that subjective social isolation as well as increased sensitivity to cues of social disconnection are associated with increased inflammatory activity.

It has been suggested that this increase in inflammatory responses to social disconnection was once adaptive (Cole, 2013, 2014; Eisenberger and Cole, 2012; Irwin and Cole, 2011). That is, when an individual faced social exclusion or ostracism, the risk of wounding increased (e.g., through hostile social interactions, increased vulnerability to predators), and bacterial infections became more likely. Meanwhile, viral infections, which are typically transmitted through social contact, became less likely. Because of this, it would have been adaptive to temporarily up-regulate pro-inflammatory gene expression and down-regulate anti-viral gene expression during periods of perceived social disconnection (Cole, 2013, 2014; Eisenberger and Cole, 2012; Irwin and Cole, 2011). Indeed, humans appear to have developed what has been called a conserved transcriptional response to adversity (CTRA), which can be activated by perceived social disconnection and involves up-regulation of the transcription of pro-inflammatory genes and down-regulation of the expression of anti-viral genes. These effects are mediated at least in part by threat-related activation of the sympathetic nervous system (SNS), which modulates gene expression by stimulating beta-adrenergic-linked transcription factors such as CREB and pro-inflammatory transcription factors such as NF-κB, and inhibiting anti-viral transcription factors such as Interferon Response Factors (IRFs; Cole, 2013, 2014). As such, activation of this CTRA profile in response to social disconnection may have been adaptive in ancestral times but in modern society may potentially contribute to some of the poor health outcomes associated with subjective social isolation.

In summary, the current literature supports the idea that socially disconnected individuals show greater pro-inflammatory gene expression and that people who are sensitive to cues of social disconnection exhibit greater inflammatory profiles in response to psychological stress. However, it is unclear how sensitivity to social disconnection may impact an individual's pro-inflammatory response to an inflammatory challenge. Thus, in the present study, we build on prior literature by investigating the impact of sensitivity to social disconnection (a composite score comprised of loneliness, anxious attachment, fear of negative evaluation, and rejection sensitivity) on pro-inflammatory cytokines and gene expression in response to endotoxin, a well-controlled inflammatory challenge, in a large sample ($n = 115$) of healthy adults. Using this same sample, we have previously examined the effects of experimental inflammation on affect, social experience, and social cognition. Specifically, we have shown that endotoxin leads to increases in depressed mood and feelings of social disconnection, which are particularly pronounced in women (Moieni et al., 2015b). We have also found that endotoxin leads to decreases in theory of mind capabilities (Moieni et al., 2015a). However, we have *not* explored how baseline differences in psychological traits impact the inflammatory response to endotoxin.

Thus, in this report, we examine how differences in trait sensitivity to social disconnection impacts inflammation in response to endotoxin. We hypothesized that being more sensitive to social disconnection would be associated with increased pro-inflammatory responses (i.e., increased levels of TNF-α and IL-6) to endotoxin. Furthermore, we hypothesized that individuals who were more sensitive to social disconnection would also exhibit greater activation of the CTRA gene expression profile (i.e., increased expression of pro-inflammatory-related genes; decreased expression of anti-viral genes) in response to endotoxin.

2. Methods and materials

2.1. Participants and procedures

One hundred and fifteen healthy participants (69 female; mean age: 24.2 ± 6.6) completed the study. Participants were recruited from UCLA and the greater Los Angeles community, and screened for eligibility using a structured telephone interview and an in-person screening session, as described elsewhere (Moieni et al., 2015a,b). The study was conducted between March 2011 and August 2013 at the UCLA Clinical and Translational Research Center (CTRC) using a randomized, double-blind, placebo-controlled design. Ninety minutes after arrival to the CTRC, each participant was randomly assigned to receive either low-dose endotoxin (0.8 ng/kg of body weight; *Escherichia coli* group O:113: BB-IND 12948 to M.R.I.) or placebo (same volume of 0.9% saline), which was administered by the nurse as an intravenous bolus. The random allocation sequence was generated by a consultant (who was not involved in running participants) and kept by the UCLA Pharmacy to ensure proper drug preparation for each participant. Randomization was done using a computerized uniform random number generator; males and females were randomized separately in permuted blocks of 4. Participants were enrolled in the study by a study coordinator (I.J.).

Details of the study protocol are provided in previous reports (Moieni et al., 2015a,b). Briefly, throughout the study, vital signs and blood draws to assess circulating cytokine levels were collected at baseline (*T0*) and then approximately every hour for the next six hours (*T1–T6*). Because peak RNA response precedes peak protein levels, blood was collected for gene expression analyses only at baseline (*T0*) and approximately 30 min (*T0.5*) after injection with endotoxin or placebo; all gene expression analyses focused on these two timepoints. Participants were discharged from the CTRC following the last blood draw upon approval from the study's physician; approval was granted once self-reported symptoms returned to baseline levels. All subjects provided written consent before participating, and all procedures were approved by the UCLA Human Subjects Protection Committee.

Of the 115 participants, 54 were randomized into the placebo condition and 61 were randomized into the endotoxin condition. For further demographic information about this sample, please see the online supplementary material.

2.2. Composite score for sensitivity to social disconnection

In order to create a composite score that reflected sensitivity to social disconnection, we standardized and summed four reliable measures related to cues or direct measures of feelings of social disconnection. This included measures of loneliness, anxious attachment, fear of negative evaluation, and rejection sensitivity. All four questionnaires were completed by all participants at baseline.

Loneliness was measured using the 20-item UCLA Loneliness Scale, a highly reliable measure of subjective feelings of loneliness, or feeling subjectively isolated from others (Russell, 1996). Participants rate how often they feel certain feelings relevant to loneliness such as how often they feel "alone," "left out," or that they "lack companionship."

Attachment-related anxiety was measured using a short (9-item) version of the anxious attachment subscale of the Experiences in Close Relationships-Revised (ECR-R) questionnaire (Fraley et al., 2000), a reliable and valid measure of adult attachment (Sibley et al., 2005). In order to measure attachment anxiety, which is associated with being overly sensitive to signs of rejection or abandonment by others and having a high need for closeness with or approval from others, participants rate how much they agreed with statements such as "I worry a lot about relationships" and "I often worry that my partner doesn't really love me."

Fear of negative evaluation was measured using the 12-item Brief Fear of Negative Evaluation (BFNE) Scale, a reliable measure of the degree to which people feel apprehension at the idea of being evaluated negatively by others (Leary, 1983), which is a potential cue for being socially rejected or isolated. Participants indicate how characteristic certain statements are of them, such as "I am afraid that others will find fault with me" and "I am usually worried about what kind of impression I make."

Rejection sensitivity was measured using the 24-item Mehrabian Sensitivity to Rejection (MSR) Scale, a reliable scale intended to assess components of sensitivity to rejection such as reluctance to express opinions because of fear of rejection, reluctance to impose on others, and being easily hurt by negative feedback from others and fearing such feedback (Mehrabian, 1970, 1994). Participants indicate the extent to which they agree with statements such as "I am cautious expressing my opinions until I know people quite well," "I seldom contradict people for fear of hurting them," and "I sometimes take criticism too hard."

Together, these four constructs reflect sensitivity to cues or direct measures of feelings of social disconnection. Thus, in order to create a composite score that reflected sensitivity to social disconnection, we standardized and summed the four questionnaires above (UCLA Loneliness, ECR-R, BFNE, MSR). The reliability of the composite scale, comprised of these four scales, was good ($\alpha = .75$). Furthermore, the results of a principal components analysis revealed that the composite of these four scales reflects a single factor or component. Using the commonly-used Kaiser criteria (Field, 2009; Guttman, 1954; Kaiser, 1960), only one component emerged; only one component had an eigenvalue over 1 (eigenvalue = 2.362), and this single component explained nearly 60% of the variance in the indicator variables. Furthermore, all variables had a loading of .58 or better, indicating strongly loading items (Costello and Osborne, 2005). These results were fully replicated with identical variance explained by a factor analysis using principal axis factoring.

2.3. Plasma levels of cytokines

Whole blood samples were collected in pre-chilled EDTA tubes. After collection, the samples were centrifuged at 4 °C, plasma was harvested into multiple aliquots, and then stored in a −70 °C freezer until the completion of the study.

Plasma TNF-a and IL-6 concentrations (assay ranges 0.8–3100 pg/mL and 0.2–3800 pg/mL, respectively) were determined using a Bio-Plex 200 (Luminex) Instrument, and a high sensitivity bead-based multiplex immunoassay (Performance High Sensitivity Human Cytokine, R& D Systems, Minneapolis, MN), as previously described (Moieni et al., 2015a,b). All plasma samples from each subject (baseline and all subsequent time points) were assayed on the same 96-well plate; every subject demonstrated the expected profile of change of cytokine concentrations over time, based on previous studies (Eisenberger et al., 2010, 2009). The mean intra-assay CV% of the standards was <8% for TNF-α and IL-6; the inter-assay CV% of an internal laboratory quality control sample was ≤13% for both analytes.

2.4. Gene expression and bioinformatics

Genome-wide transcriptional profiling was conducted on peripheral blood mononuclear cells (PBMC) isolated from 8 ml venipuncture blood samples by density gradient centrifugation (BD Vacutainer Cell Preparation Tubes). RNA was extracted (Qiagen QIAcube), tested for suitable mass (Nanodrop ND1000) and integrity (Agilent Bioanalyzer), converted to fluorescent cRNA (Ambion TotalPrep) and hybridized to Illumina Human HT-12 v4 BeadArrays following the manufacturer's standard protocol in the UCLA Neuroscience Genomics Core Laboratory. 111 of 115 participants provided blood samples at both baseline and follow-up *T0.5* time points, and all paired samples yielded sufficient RNA for analysis. All samples were assayed in a single batch and yielded valid results according to standard data quality metrics (e.g., median probe fluorescence intensity >100 units).

2.5. Statistical analyses

As reported previously (Moieni et al., 2015b), to establish between-group differences in the effect of endotoxin vs. placebo on cytokines, we used a standard statistical program (SPSS 22.0) in order to conduct analyses of covariance (ANCOVA) examining condition effects controlling for baseline values at each timepoint. Because the cytokine values were not normally distributed at any time point, values were natural log-transformed for analyses, and due to known effects of BMI on cytokines, we controlled for BMI.

We then used the PROCESS macro for SPSS (Hayes, 2012) in order to conduct moderation analyses examining whether inflammatory responses to endotoxin were moderated by sensitivity to social disconnection. The PROCESS macro estimates coefficients using OLS regression and automatically calculates interaction terms for moderation analyses. PROCESS also generates conditional effects by default, which allows for probing of significant interactions in moderation models. Although the peak of the inflammatory response typically occurred at *T2* (Moieni et al., 2015b) several participants also peaked at *T1* or *T3*. Hence, to capture peak responses, we focused on inflammatory responses (TNF-α, IL-6) averaged across *T1* to *T3*. To determine whether sensitivity to social disconnection moderated inflammatory responses to endotoxin (from *T1* to *T3*), we tested the effect of condition (endotoxin vs. placebo), sensitivity to social disconnection, and their interaction on inflammatory responses (TNF-α, IL-6). We also controlled for baseline (*T0*) values of the cytokine, demographic variables (i.e., age, gender, BMI, education), as well as psychological variables that may be theoretically related to the social disconnection sensitivity composite (i.e., depression, anxiety; Beck et al., 1988; Spielberger et al., 1983).

Gene expression data were quantile-normalized (Bolstad et al., 2003) and log2-transformed for general linear model analyses examining the magnitude of within-subject change in transcript

abundance from baseline to follow-up (i.e., a pre-post difference score from *T0* to *T0.5*) as a function of control variables (age, gender, BMI, education, depressive symptoms, and anxiety symptoms), a main effect of experimental condition (endotoxin vs. placebo), a main effect of social disconnection sensitivity (expressed as a *z* score), and a condition x social disconnection sensitivity interaction. To identify transcription control pathways that might mediate differential response to endotoxin as a function of social disconnection sensitivity, TELiS promoter-based bioinformatics analyses (Cole et al., 2005) were performed on all gene transcripts showing a point estimate of ≥1.2-fold in the condition x social disconnection sensitivity interaction term (i.e., magnitude of difference in the endotoxin vs. placebo effect over the range from −2 SD to +2 SD relative to mean level of social disconnection sensitivity). These putatively associated genes were subject to TELiS promoter-based bioinformatic analysis to assess activity of NF-κB (using the TRANSFAC V$NFKB_Q6 transcription factor-binding motif weight matrix), CREB (V$CREB_02), and Type I interferon-activated (V$ISRE_01, V$STAT_01) transcription factor families. Transcription factor activity was assessed by the log-ratio of transcription factor-binding motifs (TFBM) in the promoter sequences of up-regulated genes vs. down-regulated genes (ensuring that all analyzed genes can potentially be expressed in PBMC and avoiding cell type-specific bias in transcript expression patterns), with results averaged across 9 parametric combinations of 3 promoter sequence lengths (−300 bp upstream of the RefSeq gene transcription start site, −600 bp, and −1000 to +200 bp) and 3 stringencies for motif detection (Transfac mat_sim values ≥.80, .90, and .95) and standard errors derived by bootstrapping of residuals (Efron and Tibshirani, 1993) (200 cycles of resampled residual vectors, which controls for any potential correlation among residuals across genes). Low-level transcript-phenotype associations (reported in Supporting Dataset S1) were estimated solely to provide inputs into high-level TELiS gene set analyses and were not tested for statistical reliability at the level of individual genes.

3. Results

3.1. Inflammatory responses to endotoxin

As reported previously (Moieni et al., 2015b), endotoxin (vs. placebo) led to significant increases in the pro-inflammatory cytokines TNF-α and IL-6 over time; condition effects of endotoxin vs. placebo at each timepoint were significant from one hour post-injection through six hours-post injection, controlling for baseline values (all *p*'s < .001). These effects also held after controlling for BMI, age, gender, education, depression, and anxiety (*p*'s < .001).

Consistent with previous work (e.g., Eisenberger et al., 2010), the peak of the cytokine response occurred, on average, at approximately 2 h post-injection (*T2*) in the endotoxin group. However, because several participants peaked on either TNF-α or IL-6 at *T1* or *T3*, all moderation analyses below focus on the inflammatory response averaged across *T1, T2,* and *T3*.

Furthermore, although gender was included as a covariate in all the analyses below, it is important to point out that there were no gender differences in cytokine responses to endotoxin, as reported previously (Moieni et al., 2015b). Additionally, there were no gender differences in the composite measure of sensitivity to social disconnection (*p* > .1).

3.2. Sensitivity to social disconnection and cytokine responses

As hypothesized, there was a significant condition (endotoxin vs. placebo) by social disconnection sensitivity interaction, such that greater sensitivity to social disconnection was associated with a greater TNF-α response to endotoxin (vs. placebo; Fig. 1a; condition x social disconnection sensitivity interaction: *B* = .057, SE = .027, 95% CI = [.003, .111], *t* = 2.08, *p* < .05), controlling for *T0* values, BMI, age, gender, education, depression, and anxiety. Analysis of conditional effects revealed that for the endotoxin group, increased sensitivity to social disconnection significantly predicted heightened TNF-α responses (*B* = .077, SE = .027, 95% CI = [.023, .132], *t* = 2.84, *p* < .01). This effect was not present in the placebo group (*B* = .021, SE = .024, 95% CI = [−.027, .068], *t* = .868, *p* = .39).

In line with the TNF-α results, IL-6 levels showed a significant condition by sensitivity to social disconnection interaction, such that greater sensitivity to social disconnection was associated with a greater IL-6 response to endotoxin (vs. placebo; Fig. 1b; condition x social disconnection sensitivity interaction: *B* = .079, SE = .037, 95% CI = [.006, .151], *t* = 2.16, *p* < .05), controlling for *T0* values, BMI, age, gender, education, depression, and anxiety. Analysis of conditional effects revealed that for the endotoxin group, increased sensitivity to social disconnection predicted heightened IL-6 responses *B* = .074, SE = .037, 95% CI = [.001, .147],

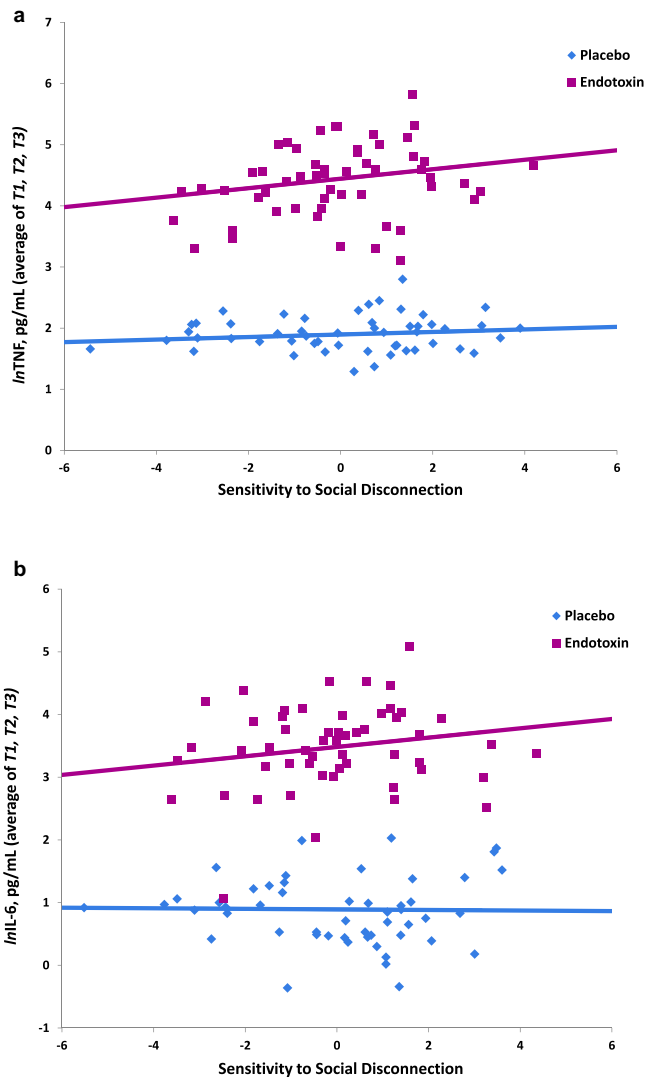

Fig. 1. Relationship between composite scores on sensitivity to social disconnection and (A) TNF-α or (B) IL-6 response to endotoxin (average of *T1, T2,* and *T3*). Cytokine values (pg/mL) were transformed and plotted on a natural log scale; social disconnection sensitivity scores, displayed regression lines, and all statistical analyses adjusted for *T0* cytokine values, BMI, age, gender, education, depression, and anxiety.

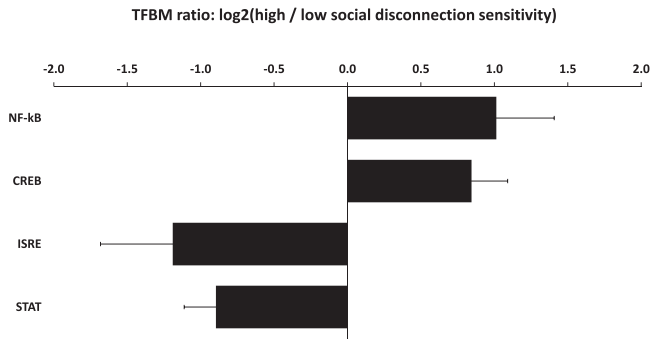

Fig. 2. Bioinformatic analysis of endotoxin-induced activation of pro-inflammatory and anti-viral transcription control pathways in PBMC from people with high vs. low levels of sensitivity to social disconnection. Data represent (log2-transformed) ratio of transcription factor-binding motifs (TFBM) for beta-adrenergic-related (CREB), pro-inflammatory (NF-κB), and antiviral (ISRE and STAT) transcription factors in the promoters of all genes showing >1.2-fold greater magnitude of endotoxin-induced activation over the general range of individual differences in sensitivity to social disconnection (−2SD vs +2SD relative to mean). Differential gene expression was analyzed in continuous (log2) metric, with the 1.2-fold discrete threshold used solely to define a discrete gene set for promoter-based bioinformatics analysis.

$t = 2.02$, $p < .05$). This effect was not present in the placebo group ($B = -.005$, SE = .033, 95% CI = [−.069., .060], $t = -.139$, $p = .89$).

3.3. Sensitivity to social disconnection and gene expression

To identify transcription control pathways that might contribute to the increased inflammatory responses observed in people with high levels of sensitivity to social disconnection, we conducted promoter-based bioinformatics analyses of 667 gene transcripts that showed ≥1.2-fold difference in the magnitude of response to endotoxin (vs. placebo) over a 4-SD range of individual differences in social sensitivity (i.e., point estimate of the condition x social disconnection sensitivity interaction ≥0.065710 log2 expression units/social disconnection z unit; genes listed in supporting information data File S1). Prominent among the 390 named genes showing greater endotoxin response in people with high social disconnection sensitivity were transcripts encoding pro-inflammatory cytokines and chemokines (*IL1B*, *IL6*, *IL8*, *CCL2*, *CCL8*, *CCL11*, *CCL3L1*, *CCL4L2*, *CCL20*, *CXCL10*, *PTGS1*, *PTGS2*) and pro-inflammatory transcription factors (*FOS*, *JUN*, *FOSB*, *EGR1*, *EGR2*). Consistent with these observations at the level of individual gene transcripts, TELiS promoter-based bioinformatics analyses of the entire up-regulated gene set indicated significant elevation in activity of beta-adrenergic-linked CREB family transcription factors and pro-inflammatory NF-κB family transcription factors (Fig. 2; CREB: $B = .844$, SE = .246, 95% CI = [.362, 1.327], $z = 3.428$, $p = .0007$; NF-κB: $B = 1.014$, SE = .394, 95% CI = [.242, 1.786], $z = 2.573$, $p = .0108$).

Among the 277 named genes showing comparatively down-regulated response to endotoxin in people with high social disconnection sensitivity were several interferon-related genes (*IFIT2*, *IFNG*, *IL18RAP*, *IL28A*). Consistent with that observation, TELIS analysis of the entire down-regulated gene set also indicated reduced activation of interferon-responsive transcription factors (Figure 2; interferon-stimulated response element - ISRE: $B = -1.190$, SE = .492, 95% CI = [−2.154, −.226], $z = -2.42$, $p = .0164$; signal transducer and activator of transcription - STAT: $B = -.896$, SE = .217, 95% CI = [−1.320, −.471], $z = -4.137$, $p < .001$).

4. Discussion

Although sensitivity to social disconnection or social isolation has been shown to be associated with increased inflammatory activity, no work has examined how sensitivity to social disconnection impacts inflammatory responses to an inflammatory challenge. We found that people highly sensitive to social disconnection exhibited greater inflammatory responsiveness to endotoxin, an inflammatory challenge, compared to placebo. These effects were seen across multiple pro-inflammatory biomarkers, including TNF-α and IL-6 in plasma, as well as multiple gene transcripts in PBMC.

While this is the first time these effects have been examined in humans, these results mirror what has previously been observed in animal work. For example, mice who experience social disruption (i.e., a period of social isolation followed by regrouping) show enhanced TNF-α and IL-6 activity in response to endotoxin (Gibb et al., 2008). Furthermore, rats who have experienced social threat, compared to rats who have not been exposed to social threat, exhibit increased pro-inflammatory activity in response to endotoxin (Carobrez et al., 2002). Although the sample in the current study was not being directly exposed to a social threat or having their social relationships experimentally altered, individuals with a greater sensitivity to social disconnection may have increased thoughts, feelings, or worries related to these types of negative social experiences even when not directly confronted with such experiences (Cacioppo and Hawkley, 2009). Thus, our results in a human sample extend these animal findings by showing that greater sensitivity to social disconnection may lead to greater proinflammatory activity to endotoxin.

Interestingly, it has been proposed that this increased inflammatory activity in response to social disconnection may have been evolutionarily adaptive in ancestral times (Cole, 2013, 2014; Eisenberger and Cole, 2012; Irwin and Cole, 2011). Because humans are social beings and viral infections are typically transmitted through social contact, it would be adaptive for humans to exhibit a strong anti-viral bias to fight such infections. However, when individuals risk being excluded or ostracized from the social group and thus suffer increased risk of wounding, bacterial infections also become more likely. As such, it would become adaptive to temporarily up-regulate pro-inflammatory gene expression (even if that comes at the expense of anti-viral gene expression). Indeed, the human immune system appears to have developed a conserved transcriptional response to adversity (CTRA) which involves up-regulating pro-inflammatory gene expression (e.g., cytokines, chemokines) while conversely down-regulating anti-viral gene expression (e.g., interferon response genes) in response to threatening environmental conditions, such as subjective social isolation (Cole, 2013).

Results from the bioinformatics analyses in the present study support this hypothesis that sensitivity to social disconnection activates a CTRA-like pattern involving enhanced activation of pro-inflammatory transcription control pathways and reduced activation of antiviral transcription factors. This CTRA profile may contribute to the elevated inflammatory responsiveness of individuals highly sensitive to social disconnection. Furthermore, these findings are consistent with the broader CTRA literature on other types of social adversity (Cole, 2013; Irwin and Cole, 2011). People facing various types of chronic social threat, such as social isolation (Cole et al., 2007), low socio-economic status (Chen et al., 2011; Miller et al., 2009) or caring for a mortally ill spouse (Miller et al., 2008) also exhibit a CTRA profile. The results from the present study combined with this prior literature suggest that social disconnection may prime the body for enhanced responses to immunologic insults (e.g., microbes, injury, etc.) and thereby contribute to a variety of chronic illnesses associated with social isolation in the epidemiologic literature. Thus, while once adaptive, this pro-inflammatory CTRA profile may be promoting inflammatory-related chronic disease in modern society's complex social systems.

As noted in the Results section and as displayed in Figure 1, the placebo group did not show larger inflammatory responses as a function of sensitivity to social disconnection. That is, those with high social disconnection sensitivity did not show elevations in pro-inflammatory cytokines under control conditions; they only showed hyper-responsiveness when exposed to endotoxin. This stands in slight contrast to the aforementioned work showing higher levels of inflammatory activity in relation to social disconnection or chronic social threat under baseline conditions. Healthy young individuals, as examined in this study (average age = 24), typically have lower levels of pro-inflammatory cytokines as compared to older adult samples (O'Connor et al., 2009). As such, social disconnection-related differences in basal inflammation levels that have been observed in some other older samples may not yet be evident in this young, healthy cohort.

Relatedly, it is important to also address the limitations of this study. First, while this study may ultimately help us understand the associations between social isolation and poor health outcomes, the present study is limited in that it was conducted in a young and healthy sample. Future studies should be done in older and/or clinical samples in order to better flesh out the findings. Furthermore, although the experimental nature of the endotoxin administration allowed us to investigate the impact of social disconnection sensitivity on a well-controlled, uniform inflammatory challenge, we did not experimentally manipulate sensitivity to social disconnection. As such, we cannot conclusively determine that sensitivity to social disconnection causally led to enhanced pro-inflammatory responses. While we cannot disentangle these complicated pathways using this single study, the present findings generally support the notion that there is a relationship between social disconnection and inflammation that warrants future research.

5. Conclusions

In summary, we found that in response to endotoxin (vs. placebo), individuals who are more sensitive to social disconnection exhibited heightened pro-inflammatory activity, including increased TNF-α and IL-6 responses in plasma, as well as up-regulation of multiple genes related to inflammation. Furthermore, bioinformatics analyses revealed that those in the endotoxin group who were more sensitive to social disconnection exhibited a CTRA regulatory profile, involving up-regulation of pro-inflammatory transcription control pathways and down-regulation of antiviral transcription factors in response to endotoxin. This work suggests that those more sensitive to threats to their social relationships may be primed to exhibit heightened inflammatory responses to immunological insults, which may ultimately lead to chronic inflammatory-related illnesses. Thus, these findings may have implications for understanding the links between social isolation, inflammation, and health.

Conflict of interest

None declared.

Funding

This research was funded by an R01 from NIMH to NIE (5R01MH091352). The authors also acknowledge the additional support provided by R01AG034588; R01AG026364; R01CA160245-01; R01CA119159; R01HL095799; R01DA032922-01; P30AG028748 to MRI; R01AG043404; R01AG033590; P30AG017265 to SWC; and National Center for Advancing Translational Sciences UCLA CTSI Grant UL1TR000124, and the Cousins Center for Psychoneuroimmunology. Additionally, the first author was supported by a pre-doctoral NRSA individual fellowship from NIA (1F31AG048668) and a pre-doctoral NRSA training fellowship from NIGMS (5T32GM084903). The aforementioned funders provided financial support for the study, but they were not involved in the conduct of the study in any other capacity (e.g., design, data collection, manuscript preparation, etc.).

Contributors

N.I.E. and M.R.I. were responsible for the study's concept and design. M.M. and S.W.C. were responsible for statistical analyses. I.J. was the study coordinator for the study and was responsible for running the experimental sessions. E.C.B was responsible for the performance, review, and quality control of all cytokine assays. H.J.C. provided funding to analyze the gene expression data. J.M.G.A. and J.M. were responsible for running assays for the gene expression data. The report was drafted by M.M., S.W.C., and N.I.E. All authors approved the final article.

Acknowledgements

We would like to thank the staff and support of the UCLA Clinical and Translational Research Center, as well as Anthony Suffredini, M.D. at the National Institutes of Health, Warren Grant Magnuson Clinical Center, for providing the standard reference endotoxin, as well as Spencer Bujarski, M.A. for providing statistical consulting; neither Dr. Suffredini nor Mr. Bujarski were compensated.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2015.08.020.

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