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Perceptions of threat are associated with transcriptomic profiles

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Perceptions of threat are associated with transcriptomic profiles

Objective: Perceived threat is hypothesized to activate the fight-or-flight response which, in turn, eventuates in up-regulation of proinflammatory genes and down-regulation of antibody production and interferon response genes. We report the first test of this hypothesized threat-gene expression link in a non-clinical sample.

Methods: A subset of participants in the Durham Child Health and Development Study provided data describing threat ambiguity (assessed with reactions to CAUSE videos) and threat vigilance (assessed with an affect misattribution procedure). A venous blood draw was also performed, and leukocyte mRNA expression was quanitfied.

Results: A heightened vigilance for threat predicted mRNA abundance of genes associated with interferon response and antibody production, and a tendency to view ambiguous situations as threatening predicted mRNA abundance of genes associated with antibody production.

Conclusions: Two dimensions of threat perception—vigilance and ambiguity—were independently associated with key transcriptomic elements of the stress response.

KEYWORDS: Threat, Stress, Transcriptome, CTRA, Antibody, Interferon

ACRONYMS: CTRA = conserved transcriptional response to adversity; CAUSE = Cognitive Appraisal and Understanding of Social Events; AMP = affect misattribution procedure; CREB = cAMP response element-binding protein; NF-Y = nuclear factor Y; SES = socioeconomic status; IL-5 = interleukin 5; mRNA = messenger ribonucleic acid; PBMC = peripheral blood mononuclear cells; cRNA = complementary deoxyribonucleic acid; CNS = central nervous system, TFBM = transcription factor binding motif

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Individual differences in the perception of threat are widely considered to be responsible for CNS-mediated chronic inflammation (Kemeny, 2009; Nusslock & Miller, 2016; O'Donovan, Slavich, Epel, & Neylan, 2013). Expanding on this view, a social genomic model posits that perceptions of threat can activate transcription factors that are associated with up-regulation of pro-inflammatory genes and down-regulation of Type I interferon response and antibody production genes (i.e., the conserved transcriptional response to adversity [CTRA]; (Cole, 2014)).

Evidence for the specific hypothesis that individual differences in threat perception are indeed related to the CTRA is surprisingly limited but suggestive. In a small sample of asthmatic youth, a tendency to view ambiguous situations as threatening (assessed with the CAUSE videos) mediated the associations between SES and CREB and SES and NF-kB transcriptional signaling activity (from bioinformatic analyses) in T lymphocytes (Chen et al., 2009). A larger body of research examines associations between threat and biomarkers of inflammation, although the relevance of this work hinges on reliable associations between such markers and the CTRA, for which the evidence is mixed. Among asthmatic youth, CAUSE scores were associated with the stimulated production of IL-5 and eosinophil counts, which reflect asthma-related inflammatory processes (Chen et al., 2006). And using an affect misattribution procedure (AMP) involving weapons, Hostinar and colleagues found that threat vigilance was associated with metabolic syndrome, which reflects, in part, inflammatory mechanisms (Hostinar, Nusslock, & Miller, 2018).

These findings reveal that, despite the central role of perceived threat in conceptual models of stress and its physiological consequences, evidence for an association between threat and stress-related, transcriptional patterns is limited to one asthmatic sample. The present study examines individual differences in threat perception—vigilance and tendency to view ambiguous situations as threatening (hereafter, "threat vigilance" and "threat ambiguity")—and the CTRA as indicated by mRNA abundance in human peripheral blood leukocytes in a community sample. As in previous research, we use the CAUSE and AMP measurement strategies. Results reveal that both threat vigilance and threat ambiguity are independently associated with regulation of genes associated with antibody response and the former is also associated with the regulation of genes associated with interferon response and the CTRA composite.

METHODS

Sample. Data come from a subsample of the Durham Child Health and Development Study, which used purposive sampling in a mid-sized, diverse American city to recruit white and black families of differing socioeconomic status backgrounds and with a newborn child. Data collection began in 2002 when the child was approximately 3 months of age. When the children were about 12 years-old, a small sample was recruited for the purpose of this study. Thirty-one subjects reported to the data collection site, a dedicated lab space at an EPA facility associated with a major research university. Of the 31 subjects, 25 had valid values on other variables (9 black, 1 mixed race; 11 females). The income-to-needs ratio (income/number of household members) indicated considerable diversity, ranging from $1535.00 to almost $40,000.00.

Individual differences in threat perception. The CAUSE videos were used to assess the extent to which subjects interpreted circumstances that have ambiguity in them as threatening. Subjects watched three brief films depicting such situations. The subject then provided open-ended responses to prompts that elicited his/her interpretation of what would happen next and why. Responses were transcribed and trained raters coded them on a scale ranging rom -2 (very benign) to +2 (very threatening). (See SI for further detail). The AMP procedure was used to assess unintended, unconscious, efficient, and uncontrollable activation by potentially threatening stimuli (Payne, 2001) and has been used to assess threat vigilance (Hostinar et al., 2017). The AMP was based on images that may involve a physical threat (see SI for further detail) and the analyses focus on overall accuracy of classification, with higher values indicating greater accuracy in classifying images as threatening or not.

Blood draw. Blood was drawn from study subjects by standard venipuncture technique and collected into PAXgene RNA tubes obtained from PreAnalytiX (Franklin Lakes NJ). RNA was extracted from the whole blood using the Qiagen RNA extraction protocol. Total RNA integrity was established using the Agilent Bioanalyzer. (see S1 for further details).

Conserved transcriptional response to adversity. Cole (2014) has proposed a composite of leukocyte transcriptional shifts that represent fight-or-flight signaling pathways (the CTRA) comprising the up-regulation of pro-inflammatory genes and the downregulation of antibody production and Type I interferon genes. We therefore consider the relation between threat perception and the CTRA *en masse* and the three

CTRA subcomponents: inflammatory, antibody and Type I interferon. He also proposed that distinct leukocytes subpopulations (particularly monocytes, dendritic cells, B cells, and T cells) mediate these changes, and so we examine cell subpopulations bioinformatically. Finally, for genes that are over- and under-expressed as a function of individual differences in threat perceptions, we can examine the prevalence of transcription factor binding motifs (TFBMs) that are believed to cause the transcriptional shifts associated with CTRA. (See S1 for details)

Body mass index. Because body mass index is associated with inflammation, we also assessed height and weight with a medical-grade scale and wall-mounted height rod. Calculated BMIs ([pounds/inches*inches)*703]) ranged from 12.64 to 19.71 with a mean of 15.23.

Data analysis. Three procedures are used to examine whether individual differences in threat are associated with leukocyte transcriptional activity. First, we examine whether the CTRA and its subcomponents are associated with these individual differences. Because the number of parameters associated with the CTRA candidates (19 proinflammatory +31 interferon +3 antibody = 53) exceeds the number of subjects, standard regression models are underdetermined. We therefore consider a generalization of the F-test, the so-called G-test or global test (Goeman & Bühlmann, 2007), which examines the same null hypothesis as the F-test, namely that H_0:β=0, with $\beta = (\beta_1,,,, \beta_p)$, where each β_i represents the association between one gene and our phenotype of interest, i.e. threat perception. In separate analyses, for the different

CTRA subsets, p variously equals the 19 proinflammatory genes, the 31 interferon genes and 3 antibody genes. The G-test exploits an empirical Bayes framework, testing whether the mean and variance of the hyperparameters of the set of coefficients (β_i), considered as random effects, equal zero (i.e., H_0:β=0). In other words the hyperparameters of the empirical Bayes model asks whether the distribution of coefficients – each of which relates one gene to the phenotype (e.g., threat vigilance) - has a mean and variance of 0.

Second, we examine whether individual differences in threat are associated with compositions of leukocyte subpopulations. We used compositional regression (Aichison 1982) as implemented in R by Tolosana-Delgado (2006). A compositional vector of 6 genes was regressed on threat vigilance and ambiguity. Each gene was a proxy for one of the corresponding 6 principle cell types: T cells (CD3D, CD3E), CD4+ subset of T cells (CD4), CD8+ subset of T cells (CD8A), B cells (CD19), NK cells (FCGR3A), and monocytes (CD14).

Third, we examine whether individual differences in threat are associated with the prevalence of specific transcription factor binding motifs (TFBMs) among the genes that are differentially expressed by threat perceptions. The specific TFBMs were identified *a priori* based on findings from previous research and include pro-inflammatory factors (NF-κB, AP-1), interferon response (ISRE), SNS response factors (CREB), and the GR. The DNA binding motif data refer to the set of loci that are upstream of the start site and in open chromatin which host any motif targeted by at least one known whole-blood factor. After identifying differentially expressed genes based on threat perceptions (k genes) from the set of all genes (n_g), we look at the

prevalence of these *a priori* specified TFBMs in *k*, compared to the prevalence of these same TFBMs in a random subset of *k* genes from the entire set of all n_g genes. This contrast (for each TFBM) suggests the extent to which differences in gene expression based on threat perceptions are being driven to some degree by the TFBMs over-represented in *k.*

FINDINGS

The models include sex, race, and BMI as covariates, and we examine threat vigilance and threat ambiguity separately (Models 1 and 2, respectively) and together (Model 3). Figure 1 reports results in terms of p-values for models of the CTRA composite and the antibody, inflammatory, and interferon sub-components. Our central result for threat vigilance (labeled "VIGILANCE") is based on Model 3, and shows that threat vigilance is associated with antibody production (G=19.10, see S1, Table 1); interferon response (G=27.9) and the entire CTRA (G=22.4). Thus, accurate, unconsciously-processed classifications of threats, as indicated by the AMP scores, are clearly associated with CTRA.

Our central result for threat ambiguity (labeled "CAUSE;" Model 3) indicates that a tendency to view ambiguous situations as threatening is significantly associated with antibody production mRNA (G=15, see S1 Table 2 for more statistical detail on the results of the global test). Thus, youth who described ambiguous situations with an increasing sense of threat also exhibited more different expression levels of the antibody gene set Together these findings reveal independent, additive effects of these two dimensions of threat.

Except for a relation between BMI and interferon, none of the controls predict transcriptomic signatures, likely reflecting a lack of power.

One advantage of our multivariate global test - over analyses based on summary composite scores - is that it does not assume that all elements of a gene set (e.g., the three genes in the antibody set) have the same relation to the phenotype (e.g., vigilance). Indeed, underlying the significant omnibus global tests outlined above we observed heterogeneity in the size and sign of these relationships. An inspection of the histogram of partial regression estimates indicates that antibody genes have a negative partial association with threat vigilance, as expected. Only one antibody gene (of three), however, was negatively associated with threat ambiguity. Overall our results clarify that threat vigilance is negatively, significantly associated with genes associated with antibody production, although the pattern is mixed for threat ambiguity.

We also examined whether threat perceptions were associated with compositions of leukocyte subpopulations. Neither threat vigilance nor threat ambiguity was associated with cell type composition at a significance level of $p = 0.05$. Among genes that are differentially expressed as a function of threat perceptions, are TFBMs associated with stress response observed more often than expected by chance? The results show that TFBMs do not appear to vary by threat perceptions.

DISCUSSION

The present study observed associations between individual differences in threat perceptions and the CTRA in a non-clinical sample. Despite a restricted sample size, both threat vigilance and threat ambiguity had independent effects on the expression

levels of genes associated with antibody production and, in the case of the former, interferon response and the CTRA composite. Interestingly, no association was observed between threat perceptions and inflammatory genes, although some evidence links threat with biomarkers of inflammation.

The results suggest caution in the study of the CTRA or any mRNA signature: univariate composite scores (e.g., the CTRA or the antibody gene set) may indicate a significant positive or negative association, but such a conclusion may not be warranted in the presence of heterogeneity at the level of specific genes. That is, discussing complex multivariate relations in terms of "the" up or down-regulation of a gene set risks overgeneralization, and may be an artifact of the univariate composite summary approach but not the underlying biology.

Three limitations should be noted. First, the data do not allow us to examine the role of anxiety in the associations between threat and interferon gene expression patterns. Anxiety is associated with tendency to detect threats and to regard ambiguous situations as threatening, and these patterns support the hypothesis that anxiety causes these perceptual tendencies or that anxiety and threat perceptions are cyclically related (O'Donovan et al., 2013). Second, the study is undoubtedly unpowered. In this context, the rejections of the null hypotheses in Model 3 are noteworthy, but failure to reject other hypotheses – a possible connection between threat perceptions and inflammation, and differences in cell types and TFBMs – could reflect false negatives and thus are highly provisional findings. And third, we examine only two aspects of threat perception, although the social psychological mechanisms that could activate the CTRA are undoubtedly more extensive and complex.

Future research could profitably collect longitudinal data from more people and including more dimensions and measures of threat (and anxiety). A larger sample would also allow for tests of hypotheses suggested by research on biomarkers of inflammation: that perceptions of threat themselves reflect circumstances in the household (especially socioeconomic status and parenting), and indeed such perceptions may trace back to pre- or perinatal experiences precipitated by maternal stress. In any event, coupled with the research of Chen and her colleagues, the present study supports the role of threat perceptions in the CTRA, although further research is clearly needed. As the social psychology of the CTRA is more fully understood, more precise interventions and treatments may be possible.

Supplementary On-Line Material

CAUSE Videos. For example, “Billy” shows a high school student who did well on a math test, although the teacher informs the class that many students cheated on the test, and that such students will be punished. As the class ends, the teacher asks Billy to talk to him in private. Children were shown these videos depicting potentially threatening social situations. Next, they were asked open-ended questions about their interpretation. Their answers were taped and later rated by judges on a scale of –2 (very benign interpretation) to +2 (very threatening interpretation). Subjects might predict, for example, that the teacher is adamant that Billy himself has cheated (=2, most threatened) or that the teacher will compliment Billy for his examination result (=-2, least threatened), with many shades of meaning between these extremes. In order to facilitate accurate data collection, these open-ended question and answer sessions were audiotaped and later transcribed and coded by two research graduate assistants.

Films included “Billy,” “Caught in the Act,” and “Shopping;” a fourth film, “Going Hungry,” lacked coding guidelines). Two raters, graduate students in sociology, scored the responses and discussed several cases of discrepancy to clarify coding rules. The scorers then revised their codings without discussion or compromise. The results led to decreased reliability in the Billy codings and it was agreed that reliable codings of Billy would not attained. The resulting score thus reflects “Caught in the Act” and “Shopping.”

Past research reports reliability as the percent of ratings within 1 point of each other, with prior research reporting 89-94% such agreement; the present study had 97.7% agreement across the three videos. However, other measures of reliability—kappa (.17)

and Krippendorf (.60)—suggest that this instrument may have considerable measurement error in this set of observations.

Affect Misattribution Procedure. For each trial, children saw a sequence of two photographs on a computer screen. They were instructed to do nothing with the first photo (the prime), but to categorize the second photo (the target) as depicting something “dangerous” or “not dangerous” by pressing one of two keys. Half of the pictures (for both the first and second photo) depict objects or situations that could be physically threatening, whereas the other half depict non-threatening objects or situations. Seventeen images depicting physical threat were identified from the IAPS bank, along with three images deemed appropriate from other sources and nutral images. All items were validated in a pretest by ratings of pleasantness and arousal (Data available on request). In the final implementation, four threat items were replaced as they were deemed potentially alarming to participants. Children were instructed to respond quickly (in under one second) for each response. If they responded too slowly they were given feedback asking them to respond faster. Responses were scored for (1) how accurately children classify the second images and (2) the extent to which the first (to-be-ignored) image biased responses.

Blood draw. We used a globin reduction protocol prior to expression analysis (Fry et al., 2007)(Fry et al 2007). 10 µg of total RNA was then extracted from 107 PBMC’s (Qiagen RNEasy) using PCR-clean and RNAse-free techniques, and frozen at -80 C until the

end of the project. The assays began with RNA quality/quantity checks using an Agilent NanoDrop BioAnalyzer (Wilmington, DE), followed by the synthesis of fluorescently-labeled cRNA, which was then hybridized to an Affymetrix Human Gene Focus Microarray (Affymetrix; Santa Clara, CA). The microarray used a Affymetrix® HT WT Terminal Labeling and Controls Kit, and the GeneChip® Human Gene 1.1 ST Array Plate and Ambion WT Expression Kit. Low-level measures of differential gene expression were calculated using Robust Multiarray Averaging, followed by independent sample t-test (with control of False Discovery Rate at 10%). To confirm gene expression changes Real Time polymerase chain reaction was performed using the RT2 Real-Time SYBR Green PCR Master Mix (both from SA Biosciences) on a Roche LightCycler 480 (the array includes house-keeping genes for data normalization, RT and PCR controls). Signal intensities of the .cel files were then normalized using RMA processing (Bolstad et al., 2003; Irizarry et al., 2003).

CTRA genes. 19 proinflammatory genes which are upregulated in CTRA on average; (b) 31 genes involved in type I IFN responses down-regulated in the CTRA (c) 3 genes involved in antibody synthesis down-regulated in the CTRA. These molecules have been historically designated by their HGNC names (HUGO gene nomenclature committee). IL1A, IL1B, IL6, IL8, TNF, PTGS1, PTGS2, FOS, FOSB, FOSL1, FOSL2, JUN, JUNB, JUND, NFKB1, NFKB2, REL, RELA, RELB, GBP1, IFI16, IFI27, IFI27L1, IFI27L2, IFI30, IFI35, IFI44, IFI44L, IFI6, IFIH1, IFIT1, IFIT2, IFIT3, IFIT5, IFIT1L, IFITM1, IFITM2, IFITM3, IFITM4P, IFITM5, IFNB1, IRF2, IRF7, IRF8, MX1, MX2, OAS1, OAS2, OAS3, OASL, IGJ, IGLL1, IGLL3. In the present case, 50 of the 53 CTRA

were on GeneChip® Human Gene 1.1 ST Array Plate of the Affymetrix Human Gene Focus Microarray: IL1A, IL1B, IL6, CXCL8, TNF, PTGS1, PTGS2, FOS, FOSB, FOSL1, FOSL2, JUN, JUNB, JUND, NFKB1, NFKB2, REL, RELA, RELB; Interferon type-I: IFI16, IFI27, IFI27L1, IFI27L2, IFI30, IFI35, IFI44, IFI44L, IFI6, IFIH1, IFIT1, IFIT2, IFIT3, IFIT5, IFIT1B, IFITM1, IFITM2, IFITM3, IFITM4P, IFITM5, IFNB1, IRF2, IRF7, IRF8, MX1, OAS1, OAS2, OAS3, OASL. Antibody: JCHAIN, IGLL1. Note that 4 of the original 53 CTRA have been renamed: IL8, IFIT1L, IGJ, IGLL3 are now CXCL8, IFIT1B, JCHAIN, IGLL3P.

Leukocyte subpopulations. We used compositional regression (Aichison 1982) as implemented in R by Van Der Boogaart & Tolosana-Delgado (2006).

Transcript analysis. The TFBM analysis was done in the framework of TeLiS (Cole et al 2005), with the most up-to-date transcription factor count data available from the R suite, Biomart. (In Biomart nomenclature, “NF-kB” is identified with NFKB1 or NFKB2. AP-1 is called JUN. ISRE is identified with the set of motifs including IRF2, IRF3, IRF4, 5, 7, 8, 9. CREB is identified with CREB3 or CREB3L1. GR is called NR3C1. This leaves us with 13 regulators plus one complex CEBPG::CREB3L1 (CEBPG_CREB3L1), as follows: “CEBPG_CREB3L1”, “CREB3”, “CREB3L1”, “IRF2”, “IRF3”, “IRF4”, “IRF5”, “IRF7”, “IRF8”, “IRF9”, “JUN”, “NFKB1”, “NFKB2”, “NR3C1”.) We conducted promoter-based bioinformatics analyses of TF-binding motif (TFBM) prevalence for a pre-specified set of TFs involved in inflammation (NF-κB and AP-1), IFN response

(interferon-stimulated response elements; ISRE), SNS activity (CREB, which mediates SNS-induced β-adrenergic signaling), and glucocorticoid signaling (glucocorticoid receptor; GR), using TeLiS (Cole, 2004).

Table 1: Vigilance on each gene set (M3)

gene_set	p-value	Statistic	Expected	Std.dev	#Cov
Inflammatory	0.29	6.35	5.56	5.81	19
Interferon	0.028	27.90	5.54	7.69	29
Antibody	0.049	19.10	5.53	6.40	2
All	0.045	22.40	5.54	7.27	50

Table 2: Ambiguity on each gene set (M3)

gene_set	p-value	Statistic	Expected	Std.dev	#Cov
Inflammatory	0.82	1.21	4.18	4.20	19
Interferon	0.27	5.29	4.17	5.44	29
Antibody	0.045	15.00	4.17	4.94	2
All	0.28	5.01	4.17	5.26	50

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Figure 1. Prediction of mRNA Signatures by Threat Perceptions