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Ventromedial prefrontal cortex activity differentiates sick from healthy faces: Associations with inflammatory responses and disease avoidance motivation

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ABSTRACT

Background: Humans are able to discern the health status of others using olfactory and visual cues, and subsequently shift behavior to make infection less likely. However, little is known about how this process occurs. The present study examined the neural regions involved in differentiating healthy from sick individuals using visual cues.

Methods: While undergoing a functional magnetic resonance imaging scan, participants ($N = 42$) viewed facial photos of 30 individuals (targets) who had been injected with an inflammatory challenge–low-dose endotoxin (i.e., sick) or placebo (i.e., healthy), and rated how much they liked each face. We examined regions implicated in processing either threat (amygdala, anterior insula) or cues that signal safety (ventromedial prefrontal cortex [VMPFC]), and how this activity related to their liking of targets and cytokine levels (interleukin-6, tumor necrosis factor-α) exhibited by the targets.

Results: Photos of sick faces were rated as less likeable compared to healthy faces, and the least liked faces were those individuals with the greatest inflammatory response. While threat-related regions were not significantly active in response to viewing sick faces, the VMPFC was more active in response to viewing healthy (vs. sick) faces. Follow-up analyses revealed that participants tended to have lower VMPFC activity when viewing the least liked faces and the faces of those with the greatest inflammatory response.

Conclusions: This work builds on prior work implicating the VMPFC in signaling the presence of safe, non-threatening visual stimuli, and suggests the VMPFC may be sensitive to cues signaling relative safety in the context of pathogen threats.

1. Introduction

Infectious disease continues to be a major cause of death worldwide, especially in low-income countries (World Health Organization, 2020). Although the global mortality rate for infectious diseases has decreased, the number of infectious disease outbreaks has steadily increased over the past several decades (Christiansen, 2018). The spread of communicable diseases such as the common cold, influenza, or the more recent coronavirus (COVID-19) is facilitated through person-to-person contact, including direct physical contact or transmission through the air of respiratory droplets when someone nearby talks, coughs, or sneezes (Liu et al., 2020; Wat, 2004; Killingley and Nguyen-Van-Tam, 2013). As a result, identifying and maintaining distance from infected individuals becomes critical in avoiding pathogen exposure and subsequent infection. Thus, the capacity to detect disease cues within other individuals is an adaptive skill crucial for survival, as it reduces the risk of communicable disease transmission. A growing body of work has shown that both animals and humans are able to detect sickness in others. Non-human animals detect and avoid infected conspecifics (i.e., individuals of the same species) via detecting sensory (e.g., olfactory, visual) cues

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that signal infection (Kavaliers and Colwell, 1995; Kavaliers and Colwell, 1995; Kavaliers and Colwell, 2010; Kavaliers et al., 2005; Behringer et al., 2006). Likewise, humans are able to identify sick individuals based on briefly viewing facial photos, relying on subtle sickness cues such as a more swollen face or more negative affective expressions (Axelsson et al., 2018; Tskhay et al., 2016; Sarolidou et al., 2019).

Following the detection of sickness cues in another individual, a person may engage in certain behaviors (e.g., reduced affiliation with others), or experience certain emotions (e.g., disgust), that serve to reduce the likelihood of social contact, thereby reducing the likelihood of pathogen exposure (Schaller, 2006). In support of this pattern of responses, past work has found that healthy individuals rate sick others as less likable compared to healthy others (Sarolidou et al., 2020; Regenbogen et al., 2017), and tend to rate the odors of sick individuals as more aversive than odors of healthy individuals (Regenbogen et al., 2017; Moshkin et al., 2012; Olsson et al., 2014; Gordon et al., 2018). Such negative evaluations likely serve to motivate behavioral avoidance of sick others, reducing risk of pathogen exposure. Interestingly, the most aversive body odors of sick individuals tend to be from those experiencing the greatest inflammatory responses (indexed by interleukin-6 [IL-6] and tumor necrosis factor-α [TNF-alpha]) (Olsson et al., 2014). However, past work has not examined whether inflammatory cytokine levels in a sick individual also are related to the detection of sickness through visual cues observed by others.

Further, existing work has tended to examine whether healthy individuals are able to detect sickness cues in a within-subjects context (e.g., repeated exposure to the same subject in a healthy and sick state) (Axelsson et al., 2018; Sarolidou et al., 2019; Sarolidou et al., 2020; Regenbogen et al., 2017; Moshkin et al., 2012; Olsson et al., 2014). While a within-subjects approach may be more relevant to identifying sickness in familiar individuals where a healthy baseline is known, many sources of pathogen exposure are likely to be from unfamiliar individuals in the surrounding environment. Thus, a between-subjects context (e.g., single exposure to individuals who are either sick or healthy) more closely approximates everyday pathogen threats. One prior study employing a randomized between-subjects design has shown that olfactory cues of sickness can be detected in this context (Gordon et al., 2018). However, most prior work employing between-subject stimuli has utilized photos of individuals with or without existing communicable diseases, allowing observers to utilize cues such as socioeconomic status as a proxy for likely disease status (Tskhay et al., 2016). The present study aimed to extend this past work by examining whether naïve observers visually differentiate between healthy and sick individuals in a between-subjects context (e.g., based on a single exposure), and where health status has been randomly assigned, meaning many proxy cues for disease status (e.g., socioeconomic status, poorer hygiene) would not aid in accurate sickness detection.

In addition, the present study explored neural mechanisms associated with threat and safety that may underlie sickness detection and/or avoidance. To date, only one study has examined the neural mechanisms of disease detection in humans (Regenbogen et al., 2017). This study found that neural regions typically involved in face processing were active while viewing faces of sick individuals (compared to viewing the same individuals in a healthy state) (Regenbogen et al., 2017), fitting with past work suggesting that perceiving certain facial cues (e.g., swollen face, negative affective expressions) may aid sickness detection (Axelsson et al., 2018). In the present study, we focused on three a priori regions implicated in the processing of threatening cues (amygdala, anterior insula [AI]) or processing visual cues signaling safety (ventromedial prefrontal cortex [VMPFC]) to examine whether they play a role in pathogen detection and avoidance.

The amygdala has been implicated in processing negatively-valanced or threatening stimuli (Adolphs, 2008; Phelps and LeDoux, 2005), such as viewing facial expressions of fear (Whalen et al., 1998) or other negative emotions (Whalen et al., 2001; Blair et al., 1999). In addition, the amygdala is implicated more broadly in responding to visual social stimuli (e.g., tracking eye gaze) (Adolphs, 2003; Kawashima et al., 1999). Given that sick individuals tend to look more fatigued with droopier corners of the mouth (Axelsson et al., 2018) and show more negative facial expressions (Sarolidou et al., 2019), naïve observers may perceive sick individuals as more threatening, hence eliciting greater amygdala activity.

The AI, another region implicated in threat-processing (Mobbs et al., 2009), may be particularly relevant in the context of pathogen detection given its purported role in processing disgust. The emotional experience of disgust in response to sickness cues is thought to motivate the avoidance of potential pathogens (Oaten et al., 2009). In addition, the AI is involved in processing facial expressions of disgust in others (Phillips et al., 1997; Sambataro et al., 2006; Stark et al., 2007), as well as the first-hand experience of disgust (Wicker et al., 2003; Heining et al., 2003). Finally, the AI has been shown to react to contamination and other pathogen threats (Wright et al., 2004). Thus, to the extent that visual sickness cues may elicit pathogen threat via feelings of disgust, the AI may be responsive to viewing sick faces.

Finally, past research has identified the VMPFC as being involved in tracking the safety value of stimuli (Eisenberger et al., 2011; Phelps et al., 2004; Harrison et al., 2017). For example, heightened VMPFC activity has been associated with relearning a cue as safe following fear conditioning (Phelps et al., 2004), as well as responding to the safety value of an attachment figure 35. In addition, the VMPFC has been shown to have an inhibitory effect on threat-related amygdala activity (Motzkin et al., 2015). Thus, the VMPFC may also be responsive to the safety value of healthy faces, relative to the pathogen threat represented by visual sickness cues.

In order to examine the neural mechanisms that differentiate sick from healthy faces, participants in the present study underwent a functional magnetic resonance imaging (fMRI) scan while viewing photos of 30 individuals who had either received low-dose endotoxin that experimentally induces a systemic inflammatory response (i.e., sick) or placebo (i.e., healthy). After viewing each photo, participants rated how much they liked the observed individual. Here, we examined: 1) differences in liking ratings of healthy and sick individuals; 2) how inflammatory responses (IL-6, TNF-alpha) of those in the photos was associated with their average liking ratings; and 3) whether threat- and safety-related neural regions responded differentially to sick vs. healthy photos, as well as whether neural activity was modulated by liking ratings or the inflammatory responses of those in the photos.

2. Methods and materials

2.1. Participants

Participants ($N = 42$) were recruited from the University of California Los Angeles (UCLA) campus and surrounding area via flyers. (See Table S1 for demographic information.) All participants met the following inclusion criteria: (i) fluent in English, (ii) free of any serious mental or physical health problems, (iii) not taking any prescription mental health-related medications, (iv) right-handed, and (v) had no conditions that prevented scanning (e.g., pregnant, claustrophobia, nonremovable metallic implants). The UCLA Institutional Review Board approved all study procedures. Participants provided written informed consent.

2.2. Procedure overview

Participants completed a task in which they viewed photos of 15 healthy faces and 15 sick faces while undergoing an fMRI scan. Photo stimuli were collected in a prior study (Moieni et al., 2015) in which participants were either injected with low-dose endotoxin (causing a transient inflammatory response) or placebo. Inflammatory data was collected hourly for 6 h from those in the endotoxin study. In the present

study, participants provided a liking rating for each photo they viewed while in the scanner. Participants were naïve to the purpose of the study and the source of the photos. Participants were compensated $50.

2.3. fMRI task design and image acquisition

While undergoing an fMRI scan, participants completed a task in which they viewed photos of 15 healthy faces and 15 sick faces in a pseudo-randomized order. Each face was presented once (2000 ms), with the same face type (healthy or sick) not presented more than three times in a row. Each face was followed by a brief inter-stimulus interval (250–750 ms). Then, a rating screen was presented (4000 ms) where participants responded to the question, "How much do you like this person?", by providing a rating on a 7-point scale (1 = *not at all*, 7 = *very much*). Participants moved the rating slider using two buttons, and pressed a third button to submit their final rating. Following each rating, there was an inter-trial interval fixation (1000–2000 ms) before the next face was presented. The task was based on past work examining neural responses to viewing sick and healthy faces (Regenbogen et al., 2017), and was adapted to present 30 distinct individuals in the photos (rather than the same individuals repeated in both a healthy and sick state).

2.4. Photo stimuli

Photos of faces were collected in a prior study (Moieni et al., 2015) in which participants ($N = 115$) were either injected with a low dose of endotoxin (derived from *Escherichia coli*; 0.8 ng/kg of body weight), a bacterial agent which induces an inflammatory response (sick faces), or with placebo (healthy faces). Photos were taken of a subset of participants who provided consent ($n = 52$) and 30 of these photos (15 sick, 15 healthy) were selected for use in the current task. Participants were instructed to produce a relaxed, neutral facial expression for the photo. The same digital camera was used for all pictures. The experimenter stood at the foot of the hospital bed and took one picture of the participant making a neutral facial expression while the subject was sitting up against their hospital bed in their hospital room. If the subject closed their eyes during the picture, another picture was taken. No lighting adjustments were made. Photos were taken by the experimenter at baseline (prior to endotoxin administration) and at approximately 2 h post-injection, corresponding to the peak inflammatory response as indexed by plasma levels of IL-6 and TNF-alpha (reported elsewhere: (Moieni et al., 2015). In the current study, participants were only shown the photos taken from the post-injection period. This was done in order to simulate the real-world situation in which one encounters healthy or sick individuals, with whom one has had no prior interaction and thus has no immediate comparison to determine if they are sick or healthy.[1] Photos were then cropped to a standard size, and the background was removed so that only the head was visible.

Photos with participants who wore glasses in the photo, had baseline depression, or were missing baseline cytokine data were excluded from the selection procedure. To be included in the healthy faces condition, placebo participants could not have reported more than one physical symptom at the time of the photograph. To be included in the sick faces condition, endotoxin participants must have reported at least two physical symptoms at the time of the photograph. (The number of available photos meeting each of these criteria for inclusion is detailed in Figure S2.)

Although the individuals within the healthy and sick faces did not statistically differ from each other in terms of age, sex, race, or BMI (*ps* > 0.05), group differences in sex and BMI were trending. Specifically, the sick faces condition had more females (87%) than the healthy faces (53%) condition ($p = .11$), and the sick faces condition had marginally higher body mass index (BMI) compared to the healthy faces condition ($p = .10$). In order to account for these differences, and due to known effects of BMI on cytokines, we controlled for sex and BMI in reported analyses. (See Table S2 for characteristics of the photo stimuli set.)

2.5. Inflammatory assessment of photographed individuals

Participants in the prior study, whose photos were included in the present task, provided blood samples hourly over six hours for assessment of inflammatory response, including a baseline, pre-injection blood draw. We focus on levels of IL-6 and TNF-alpha, as participants in the full prior study sample displayed elevated levels of these two cytokines in response to endotoxin (reported previously, (Moieni et al., 2015). To assess the total cytokine response for each cytokine during the study period, we calculated the area under the time-concentration curve (AUC). AUC values were natural log-transformed for analyses to correct for non-normality. As expected, photo participants in the sick condition ($n = 15$) showed higher total levels of IL-6 and TNF-alpha across the study compared to those in the healthy condition ($n = 15$; *ps* < 0.001). (See Figure S1 for hourly cytokine trajectory by condition for the photo participants.) Information related to the processing of the inflammatory assays is included in Supplemental Material.

2.6. fMRI data acquisition and analysis

Neuroimaging data were acquired on a Siemens Prisma 3.0 Tesla MRI scanner at the UCLA Brain Mapping Center. Head movements were restrained with foam padding. For each participant, a T1-weighted magnetization prepared rapid gradient echo (MPRAGE) anatomical image (slice thickness = 0.9 mm, 192 slices, TR = 2300 ms, TE = 2.32 ms, flip angle = 8°, matrix = 256 × 256, FOV = 240 mm) was acquired coplanar with the functional scan. One functional scan was acquired (echo-planar T2*-weighted gradient-echo, 129 volumes, slice thickness = 3 mm, gap = 1 mm, TR = 2000 ms, TE = 24 ms, flip angle = 90°, matrix = 64 × 64, FOV = 200 mm).

Neuroimaging data were analyzed using Statistical Parametric Mapping (SPM12; Wellcome Department of Cognitive Neurology, Institute of Neurology, London). Images were realigned to correct for head motion, normalized to Montreal Neurological Institute (MNI) space using diffeomorphic anatomical registration through exponentiated lie algorithms (resampled at 3 × 3 × 3 mm), and spatially smoothed using a 5 mm Gaussian kernel, full width at half maximum, to increase signal-to-noise ratio. General linear models (GLMs) were constructed for each participant, and linear contrasts of interest were computed. The time series was high-pass filtered (128 Hz) and serial autocorrelation was modeled as an autoregressive AR(1) process.

Anatomical ROIs were created for the amygdala and the AI (anterior portion divided at $y = 0$, (Bonthius et al., 2005; Öngür et al., 2003) using the Automated Anatomical Labeling atlas (Tzourio-Mazoyer et al., 2002). The VMPFC ROI was created by centering a 15 mm sphere on the peak coordinates of a cluster (MNI coordinates: $x = 0$, $y = 42$, $z = -12$) reported to be more active in response to cues signaling safety (Phelps et al., 2004). Mean parameter estimates were extracted for each ROI and entered into standard statistical software (SPSS 25) for further analysis. All ROI analyses were conducted as one-tailed tests, given convention in neuroimaging research.

2.7. Analytic overview

2.7.1. Behavioral analyses

First, multiple linear regression was used to examine whether average liking ratings differed as a function of the health status of the target faces (sick vs. healthy), controlling for BMI and sex of the faces.

[1] Follow-up analyses of these baseline photos with a separate group of raters (n = 61) demonstrated that there were no differences in the likeability ("how much do you like this person?" on a 1–7 scale) of the faces that would later receive placebo ("healthy:" M = 3.64, SD = 0.88) vs. endotoxin ("sick:" M = 3.64, SD = 0.94) (t(60) = 0.07, $p = .93$).

Next, we examined whether the extent of the inflammatory sickness response of individuals in the photos was associated with liking, controlling for BMI and sex of target faces. An average liking rating across all subject ratings was computed for each face. For each cytokine (IL-6, TNF-alpha), partial correlations were used to examine whether total cytokine responses were significantly associated with liking ratings.

2.7.2. Neuroimaging main effect analyses

In order to compare neural activity in response to viewing healthy vs. sick photos, a GLM was constructed in which the presentation of face stimuli was modeled in a single regressor.[2] Parametric modulators for covariates (BMI, sex of target face) were included, followed by a third modulator differentiating healthy vs. sick faces. One-sample t-tests were conducted on the parameter estimates from a contrast weighted on the modulator of interest (face condition) for each ROI (amygdala, AI, VMPFC) to examine whether activity in each area responded differentially to healthy vs. sick faces.

2.7.3. Neuroimaging follow-up analyses

For any a priori regions that were significantly active in the main effect analysis, follow-up analyses were conducted to further explore the extent of the region's involvement in pathogen detection and avoidance. We first examined whether neural activity was associated with liking ratings, on a trial-by-trial basis. A GLM was constructed for each subject in which the presentation of face stimuli was modeled in a single regressor. Parametric modulators for covariates were included, followed by a third modulator for the liking rating of each face.[3] One-sample t-tests were conducted on resulting parameter estimates to examine associations between liking ratings and neural activity in each ROI that responded differentially to sick vs. healthy faces.

Next, to investigate whether neural activity in these regions was associated with the degree of cytokine response in the photo participants, GLMs were constructed for each cytokine (IL-6, TNF-alpha) in which the presentation of face stimuli was modeled in a single regressor. Parametric modulators for covariates were included, followed by a third modulator for the AUC cytokine response. One-sample t-tests were conducted on resulting parameter estimates to examine associations between the inflammatory response and neural activity in each ROI that significantly responded to viewing sick vs. healthy faces.

3. Results

3.1. Behavioral analyses

We first examined whether liking ratings differed between the two face conditions (healthy vs. sick) to determine if liking would be lower for sick faces, as expected. As predicted, there was a main effect of face condition such that sick faces were less liked than healthy faces, $F(1,26) = 5.32$, $p = .03$, partial $\eta^2 = 0.17$. Ratings for healthy faces were not significantly different from the neutral point (a rating of 4) of the liking scale ($M = 4.1$, $SE = 0.2$, 95% CI:[3.7,4.4]), while average ratings for sick faces were below the neutral point ($M = 3.4$, $SE = 0.2$, 95% CI: [3.0, 3.9]) (Fig. 1).

Next, we examined whether the magnitude of the inflammatory response of the individuals in the photos was associated with liking. The average liking ratings of faces was significantly related to IL-6 responses, $r(26) = –0.37$, $p < .05$. In addition, the average liking ratings of faces was significantly related to TNF-alpha responses, $r(26) = –0.41$, $p = .03$. Thus, the faces of those who displayed the greatest inflammatory responses tended to be the least liked by naïve raters (Fig. 2).[4]

3.2. Neuroimaging main effect analyses

Next, we examined whether regions typically associated with threat, such as the amygdala and the AI, were more active in response to viewing sick (vs. healthy) faces. However, there was neither significant amygdala (left: $t(41) = –0.28$, $p = .39$, $d = –0.04$; right: $t(41) = –0.23$, $p = .41$, $d = –0.04$) nor AI (left: $t(41) = –0.16$, $p = .44$, $d = –0.03$; right: $t(41) = –0.01$, $p = .50$, $d = –0.001$) activity in response to viewing sick (vs. healthy) faces (Fig. 3).

We then examined whether the VMPFC, a region implicated in safety signaling, would be more active when viewing healthy (vs. sick) faces. In line with hypotheses, the VMPFC was significantly more active in response to viewing photos of healthy faces relative to sick faces, $t(41) = 1.82$, $p = .04$, $d = 0.28$.

3.3. Neuroimaging follow-up analyses

To further examine the role of the VMPFC in the avoidance of pathogen threats, we examined whether VMPFC activity during face viewing was modulated by trial-by-trial liking ratings. In line with predictions, VMPFC activity significantly correlated with liking ratings ($t(40) = 2.53$, $p = .008$, $d = 0.40$), such that greater self-reports of liking a face were associated with greater activity in the VMPFC while viewing that face.

Finally, we explored whether the degree of inflammatory response exhibited by the individual whose face was viewed was associated with VMPFC activity while viewing the photos. VMPFC activity while viewing the photos was modulated by differences in overall IL-6 levels, $t(41) = –1.95$, $p = .03$, $d = –0.30$. In addition, VMPFC activity while viewing photos was also similarly modulated by differences in overall TNF-alpha levels, $t(41) = –2.97$, $p = .003$, $d = –0.46$. Thus, VMPFC activity was modulated on a trial-by-trial basis such that neural activity decreased in response to viewing faces of individuals who experienced a greater inflammatory response, potentially presenting the most salient threat.

4. Discussion

Sick faces were rated as less liked than healthy faces, suggesting that visual sickness cues impacted participants' initial liking of individuals, an indicator of their motivation to avoid such individuals. Additionally, participant's liking ratings of individuals negatively correlated with the degree of inflammatory response displayed by the targets in the photos, suggesting that reduced liking of faces may be partially due to a more salient pathogen threat. The VMPFC responded more strongly to viewing healthy (vs. sick) faces. Lending support for a safety-signaling role of the VMPFC, greater activity in this region was associated with reduced liking for faces on a trial-by-trial level. Further, reduced activity in the VMPFC also tracked greater proinflammatory cytokine responses of those in the photos. In sum, activity in this region appears to be

[2] For all neuroimaging GLMs, rating periods for healthy and sick faces were modeled in separate regressors of non-interest, while inter-trial fixations were included as the implicit baseline.

[3] For any faces for which a liking rating was missing, the face onset for that photo was modeled in a separate regressor (as it did not have a value for a modulation analysis). One participant was excluded from this analysis due to no variability in their liking ratings. Most participants (83.33%) provided liking ratings for all 30 faces. Six participants failed to provide liking ratings for a single face during their scan, and one additional participant failed to provide liking ratings for two faces. Rates of missed liking ratings were evenly distributed across the conditions.

[4] To further explore this data, and given significant mean differences between IL and 6 and TNF-alpha as a function of condition, we also provide more in-depth analyses looking at the relationship between liking and inflammation across time and by group (sick, healthy) to supplement this analysis (in Supplemental Information and Figures S3-S4). However, these should be interpreted with caution given the small sample size within-group of $n = 15$.

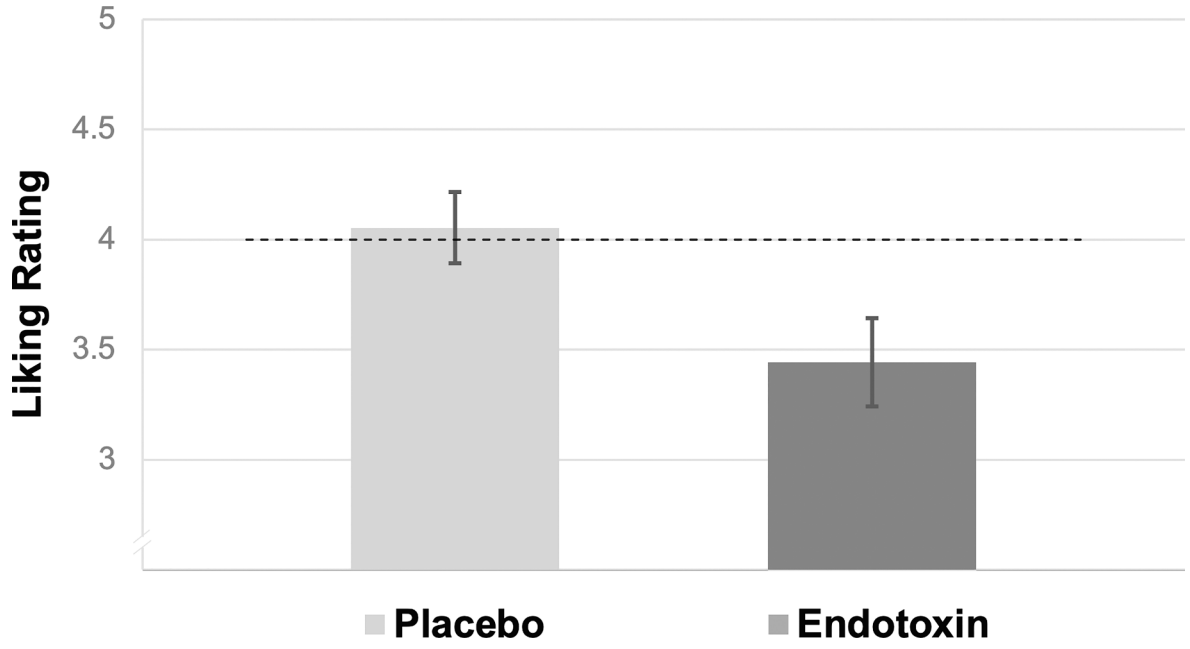

Fig. 1. Liking as a Function of Health Status. Sick faces (targets who were injected with endotoxin) were consistently rated as less liked than healthy faces (who were injected with placebo) ($p = .03$). Analyses controlled for BMI and sex of the target face. Estimated marginal means and standard errors are plotted. The dotted line represents the neutral point on the 7-point rating scale.

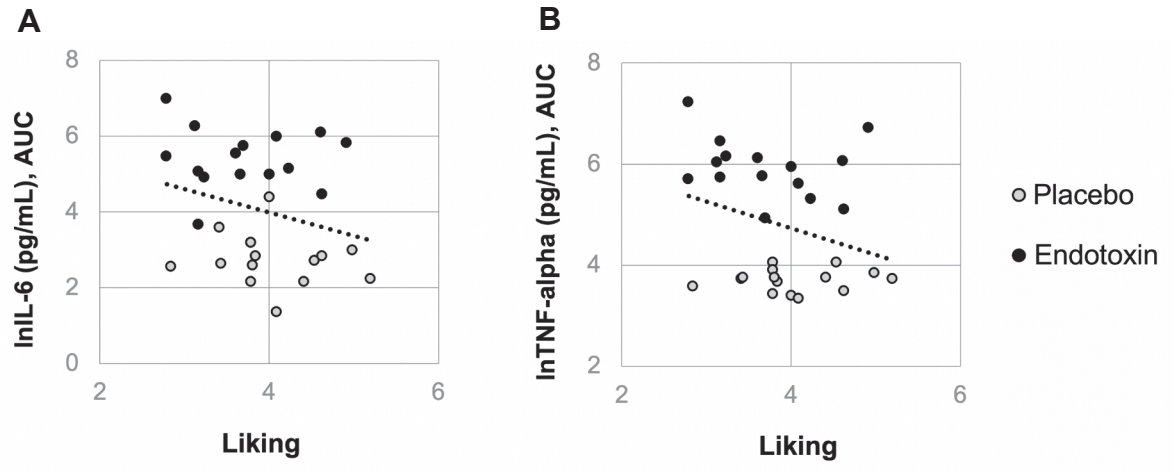

Fig. 2. Association between Liking by Raters and Inflammatory Responses in Photo Targets. Liking ratings of faces by naïve raters were negatively associated with total plasma levels of interleukin-6 (IL-6; $p < .05$, panel A) and tumor necrosis factor-α (TNF-alpha; $p < .05$, panel B) of the target individuals, as measured by area under the time-concentration curve (AUC). Natural log transformed values are plotted for cytokines. Analyses controlled for body mass index and sex, given trending group differences on these variables. Raw (uncontrolled) correlations are plotted for ease of interpretation. Trendlines reflect trends for the full sample.

involved in tracking the safety value of individuals in the context of potential pathogen threats.

Several of the present findings fit with and extend prior literature. First, we extend past work in which healthy individuals rate sick others as less likeable compared to when the same individuals are healthy (Sarolidou et al., 2020; Regenbogen et al., 2017). We provide the first evidence that the same distinction by health status can be found in randomized between-subjects paradigms, which more closely parallel pathogen threats within everyday life. Interestingly, we found that participants' liking ratings of individuals negatively correlated with the overall magnitude of inflammatory response (IL-6, TNF-alpha) displayed by the individuals in the photo. This finding extends prior work showing that cytokine levels may mediate olfactory sickness detection (Olsson et al., 2014), by suggesting similar cytokine effects may underlie detection of visual sickness cues as well.

In the present study, we examined the AI because of its role in processing disgust and its theorized role in motivating pathogen avoidance. Indeed, prior work has found that perceiving overt signs of sickness (e.g., coughing) tends to elicit feelings of disgust (Hedman et al., 2016). However, it is likely that we did not observe disgust-related activation in the AI because the subtle cues available in posed facial photos (e.g., fatigue) would not be strong enough to elicit overt feelings of disgust, particularly for observers naïve to the presence of any disease-relevant stimuli. Thus, a mechanism other than disgust may support avoidance behaviors during earlier phases of illness prior to the manifestation of overt symptoms. The observed VMPFC activation, and its correlation with liking and cytokines responses, suggest that this region may play a critical role in underlying the early detection of sickness cues.

It is important to note that VMPFC activity may have tracked sick faces in this study because participants were asked to focus on the extent

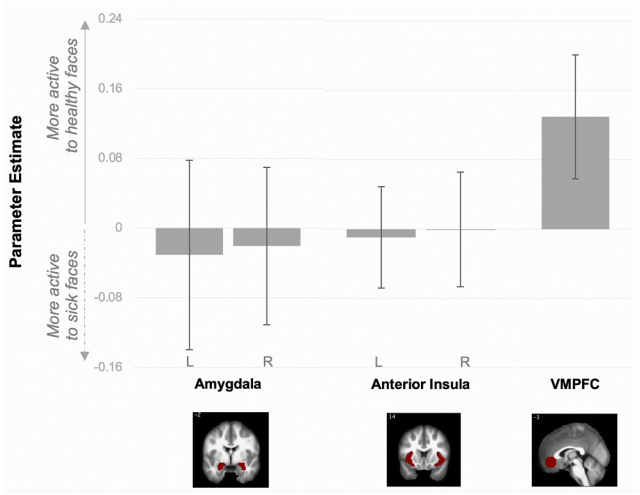

Fig. 3. Neural Activity in Response to Healthy (vs. Sick) Faces. The amygdala and anterior insula were not differentially responsive to healthy or sick faces ($ps > 0.39$). In contrast, the ventromedial prefrontal-cortex (VMPFC) was significantly more active in response to viewing photos of healthy faces (as compared to sick faces) ($p = .04$). Analyses controlled for body mass index and sex of the target face. Error bars represent standard error of the means. L = left; R = right.

to which they liked the person in each photograph. Thus, the observed VMPFC activity may be partially driven by participants' engagement in the valuation of information, a process which recruits the VMPFC (Lim et al., 2011; Lebreton et al., 2009). Similarly, the VMPFC is active during impression formation of new faces (Cloutier and Gyurovski, 2014; Dang et al., 2019). In the present study, the fact that VMPFC activity was additionally negatively correlated with inflammatory responses suggests that sickness cues may play a role in informing these first impressions. The explicit likability ratings that participants made while viewing the photos may have contributed to the lack of neural activity in regions traditionally implicated in threat processing (amygdala, AI). Had participants engaged in passive observation of the faces or made assessments relating to a less positive dimension than likability, these regions may have been active in response to viewing sick vs. healthy faces.

Most prior work has utilized stimuli showing within-subject variations in health status. A major focus of the present study was advancing prior work by utilizing photo stimuli showing between-subject variations in health status, which more closely approximates the nature of pathogen threats from unfamiliar others. Despite the increased ecological validity of this approach, certain limitations should be noted. For example, it can be difficult to match the sick and healthy face conditions on demographic variables when using a between-subjects sickness manipulation. Due to potential group differences within the present stimuli set, we had to statistically control for sex and BMI in the present analyses. Critically, prior work has shown that facial adiposity and facial cues to body fat inform perceptions of health status (Henderson et al., 2016). In addition, we know from prior research that facial adiposity is related to perceptions of attractiveness (de Jager et al., 2018), another key variable that may play a role in perceptions of health status (Kalick et al., 1998). Moreover, other factors, like skin color, have been shown to change as a function of sickness (Henderson et al., 2017) and to be associated with perceptions of attractiveness (Fink et al., 2001) and health (Stephen et al., 2011). Thus, future work using this between-subject approach should aim to directly examine the effect that demographic factors, as well as other facial traits such as adiposity, attractiveness and skin color, have on sickness detection or avoidance. This will require the collection and use of larger stimuli sets of sick and healthy faces so that such explorations are well-powered.

In summary, the present findings highlight that naïve observers can detect subtle visual sickness cues which inform less positive first impressions, capturing observers' motivation to avoid potential pathogen exposure. In addition, this work builds on prior work implicating the VMPFC in signaling the presence of safe, non-threatening visual stimuli, and suggests the VMPFC may be sensitive to cues signaling relative safety in the context of pathogen threats. Critically, the VMPFC appears to track visual social cues that correspond with the inflammatory response, suggesting that humans can flexibly respond to pathogen threats along a continuum, such that those exhibiting the greatest pathogen threat are more likely to be avoided.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2021.11.011.

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