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Low-Grade Inflammation Decreases Emotion Recognition – Evidence from the Vaccination Model of Inflammation

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Keywords: Inflammation, social cognition, sickness behavior, typhoid vaccination, cytokines, reading the mind in the eyes, theory of mind, psychoneuroimmunology

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ABSTRACT

The ability to adequately interpret the mental state of another person is key to complex human social interaction. Recent evidence suggests that this ability, considered a hallmark of 'theory of mind' (ToM), becomes impaired by inflammation. However, extant supportive empirical evidence is based on experiments that induce not only inflammation but also induce discomfort and sickness, factors that could also account for temporary social impairment. Hence, an experimental inflammation manipulation was applied that avoided this confound, isolating effects of inflammation and social interaction.

Forty healthy male participants (mean age = 25, *SD* = 5 years) participated in this double-blind placebo-controlled crossover trial. Inflammation was induced using Salmonella Typhi vaccination (0.025 mg; Typhim Vi, Sanofi Pasteur, UK); saline-injection was used as a control. About 6h30m after injection in each condition, participants completed the Reading the Mind in the Eyes Test (RMET), a validated test for assessing how well the mental states of others can be inferred through observation of the eyes region of the face.

Vaccination induced systemic inflammation, elevating IL-6 by +419% ($p < .001$), without fever, sickness symptoms (e.g., nausea, light-headedness), or mood changes (all *p's* > .21). Importantly, compared to placebo, vaccination significantly reduced RMET accuracy ($p < .05$). RMET stimuli selected on valence (positive, negative, neutral) provided no evidence of a selective impact of treatment.

By utilizing an inflammation-induction procedure that avoided concurrent sicknesses or symptoms in a double-blinded design, the present study provides further support for the hypothesis that immune activation impairs ToM. Such impairment may provide a mechanistic link explaining social-cognitive deficits in psychopathologies that exhibit low-grade inflammation, such as major depression.

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1. INTRODUCTION

Human and animal studies have identified inflammation as a powerful regulator of social behavior (Eisenberger, Moieni, Inagaki, Muscatell, & Irwin, 2017). Initial animal studies identified social withdrawal as a core-feature of sickness behavior, i.e., the depression-like constellation of symptoms that also includes anhedonia, fatigue, and depressed mood (Ashwood et al., 2011; Dantzer & Kelley, 2007; Dowlati et al., 2010; Miller, Buckley, Seabolt, Mellor, & Kirkpatrick, 2011). Subsequent neurocognitive analyses in humans expanded this understanding beyond mere social withdrawal, and showed, for example, that inflammation induces heightened feelings of social disconnection and alters sensitivity to social threats and rewards (Eisenberger et al., 2010; Inagaki, Muscatell, Irwin, Cole, & Eisenberger, 2012; Moieni, Irwin, Jevtic, Breen, & Eisenberger, 2015; Muscatell et al., 2016; Wright, Strike, Brydon, & Steptoe, 2005).

More recently, experimental human research revealed that inflammation-induction reduces the ability to infer the affect and mental states of other people on the basis of facial expressions (Moieni et al., 2015), which is considered indicative of impaired Theory of Mind (ToM). The concept of ToM, sometimes called mentalizing (Frith & Frith, 2006), was developed in the context of research on autism-spectrum disorders and refers to the ability to interpret someone else's desires, beliefs, and intentions, all of which are essential to human social interaction (Premack & Woodruff, 1978). However, impairments of ToM more broadly characterize a number of mental health disorders, most notably depression (Bora & Berk, 2016). Impaired ToM is thought to explain why depressed individuals tend to withdraw from social contacts, report less enjoyment in social interactions, and have fewer social contacts than non-depressed individuals (Hirschfeld et al., 2000), whereby mood and social interactions may operate in a bidirectional manner (Bora & Berk, 2016). Meta-analyses have consistently established that depression is associated with a state of low-grade inflammation (although there is marked heterogeneity between studies) (Dowlati et al., 2010; Leighton et al., 2017). The observation that inflammation impairs ToM might thus provide a mechanistic link connecting inflammation with the interpersonal difficulties that characterize depression.

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Two studies have provided direct human experimental evidence for a link between inflammation and ToM (Kullmann et al., 2013; Moieni et al., 2015). Both induced acute inflammation through the administration of bacterial endotoxin (i.e., lipopolysaccharide, LPS) and assessed ToM using the Reading the Mind in the Eyes Test (RMET), a validated test for assessing ToM (Baron-Cohen, Wheelwright, Hill, Raste, & Plumb, 2001). In this test, participants are presented photographs showing only the eye regions of emotional facial expressions, and are asked to select an emotion word that best describes what the person in the photograph might think or feel. Although Moieni et al. (2015) reported that inflammation impaired RMET performance. Kullmann et al. (2013) found no such impairment, but observed an enhanced response to RMET images in brain regions relevant to ToM (i.e., superior temporal gyrus, temporo-parietal junction), which might suggest that more effort was required to produce impairment-free performance. While potentially important, the interpretation of the above data is somewhat complicated by the fact that endotoxin administration, besides inflammation, also provokes fever and flu-like symptoms including nausea, headache and fatigue in a dose-dependent manner (e.g., Eisenberger et al., 2010; Kullmann et al., 2013; Lasselin et al., 2016; Moieni et al., 2015). Considering that physical discomfort alone may produce neuropsychological performance decrements (Keogh, Moore, Duggan, Payne, & Eccleston, 2013; Smith, 2016), the above observations would benefit from further experimental validation using a model of inflammation that minimizes such secondary illness effects. Such alternative is provided by inflammation-induction through vaccination against *Salmonella typhi* (the causal agent of typhoid fever). Vaccination likewise reliably initiates an acute systemic inflammatory response, lasting up to 12 hours, but without generating flu-like symptoms (Brydon et al., 2009; Harrison et al., 2015; Lacourt et al., 2015; Paine, Ring, Bosch, Drayson, & Veldhuijzen van Zanten, 2013 but also Harrison et al., 2009).

The present study tested whether inflammation would lead to a decrement in the ability to accurately identify mental states in others and whether interpretation of positive versus negative facial expressions would be equally affected. The latter consideration stems from previous research showing that inflammation increased sensitivity to both positive and negative social feedback (Muscatell et al., 2016).

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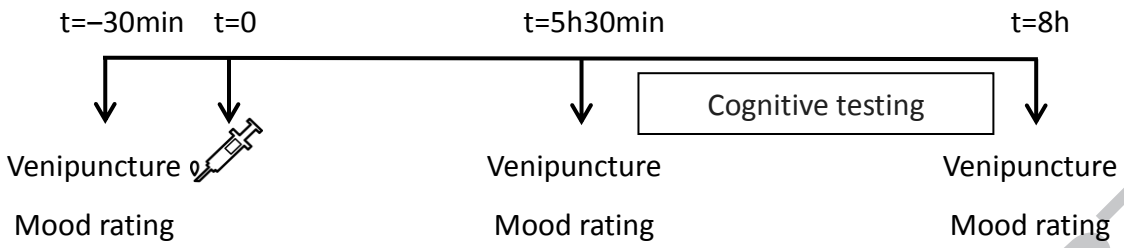

Figure 1. Study timeline. Participants were tested on two occasions, separated by a 7-day wash out, using an identical protocol. Cognitive testing was performed between 5.5h and 8h after application of vaccine or placebo (t=0). Before participation, participants visited the laboratory for a single 70 minutes familiarization session.

2. METHOD

2.1 Participants

Forty healthy young male participants from the University of Birmingham were enrolled a result of recruitment via online advertisement (*M* age = 24.7, SD = 5.2 years). Mean body mass index (BMI) was 23.7 (SD = 3.2 kg/m^2, 16.6-29.2 kg/m^2). Individuals were excluded if they report a history of or suspected vaccine-related allergy, food allergy/intolerance, inflammatory, cardiovascular, neurological, mental health or immune-related disorders, smokers, visual impairments (unless corrected to normal), and those on any medication 7 days prior to the test days.

Participants received research credits or were paid a minimum of £40 to reimburse travel and expenses. Participants could win an additional £12 depending on task performance during one of the cognitive tasks (reward learning task; data not reported here). The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the local Research Ethics Committee of the National Health Service (NHS).

2.2 Procedures

Participants visited the behavioral immunology laboratory on three separate occasions: the first visit was intended as a familiarization session, followed by two separate test days (i.e.,

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vaccination or control) planned at least one week apart. During the familiarization session, which took about 70 minutes, written informed consent was obtained, inclusion and exclusion criteria were verified, an initial set of questionnaires were completed, and participants performed abbreviated versions of the computer tasks for the purpose of familiarization. Figure 1 presents the time line of the subsequent experimental sessions. On each of the two the test days, participants arrived at the laboratory between 8:00 and 9:00 am after an overnight fast. Participants were instructed to refrain from strenuous physical activity and alcohol intake for at least 24 hours, and were asked to avoid high fat and high sugar products for at least 12 hours prior to the test days. Before vaccination or placebo, participants verified absence of acute illness, and mood and sickness symptoms were assessed (as described below), and tympanic body temperature was measured. Subsequently the first blood sample was taken. After vaccination/placebo, participants received a standardized breakfast (granola with skimmed milk, approx. 430 kcal), and after a 5-hour break participants again received a standardized meal consisting of cheese sandwiches (approx. 328 kcal). For a subset of participants, EEG data was also collected from about 5.5 hours to 6.5 hours post-injection (N = 20, not reported here); and these participants arrived back at the laboratory 1 hour earlier to allow EEG preparations. During the 5-hour break participants were instructed to refrain from eating, drinking (except for water and the meals provided), or strenuous physical activity. Fifteen minutes post-lunch a second blood sample was taken and tympanic body temperature was measured; then, a set of cognitive tests that included the computerized RMET were started. Other tasks, not reported here, included measures of memory, attention, learning and response inhibition. Mood was also assessed at several intervals during the test day; before injection and 5h30m and 8 hours post-injection. The final blood sample was taken about 8 hours post-injection. Test timings were identical across visits, as were the procedures, except for the type of injection (vaccine or saline-placebo)

2.3 Randomization

Participants were randomly assigned to receive vaccine or placebo (control) on the first day of formal testing. Randomization was performed by supporting staff who had no contact

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with the study participants. Both participants and researchers were blind to condition order, and only the nurse administering the injections was aware of the order whereby a sealed envelope containing information about the condition was handed to the nurse before administering the injection. The nurse followed identical procedures for placebo and vaccine injection ensuring participant's blindness to the condition. The researchers where not present when the injection was administered.

3. MATERIALS

3.1 Reading the Mind in the Eyes test (RMET)

The RMET is considered a test of theory of mind, a cognitive process sometimes referred to as "mind-reading" and "social intelligence" (Baron-Cohen et al., 2001). The RMET was developed to measure social sensitivity in typical adults with normal intelligence (Baron-Cohen et al., 2001).

Stimuli

A gray-scale digital image (subtending 9 x° X 3.6 y° of visual angle) of the eye region of a face (including eyes and eyebrows) was presented in the middle of a grey field on a computer monitor. Four words describing mental states accompanied each test stimulus, presented in black Arial font (subtending 2.6 x° X 0.7 x° of visual angle).

Procedure

The test display comprised a test eye image and four words placed in the centre of the screen. The participant was instructed to select the word that best described what the person in the test image was thinking or feeling by pressing one of four computer keys (Q, P, A, L) that spatially corresponded to the position of each word. The correct (target) word had the same emotional valence as the accompanying three foil words. For example, the target word, 'panicked', was accompanied by 'arrogant', 'jealous' and 'hateful'. Target words were equally likely to appear in one of the four word locations on the screen. Each test display remained visible until a key response was made; the next test display was immediately presented

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thereafter. One block comprised of 18 test displays was completed on each day, with a different set of 18 test displays used on the second test day; the order of the display sets used was counterbalanced across participants. The two sets were comparable with regards to the sex of the faces (50% female) and number of items depicting positive, neutral and negative emotional expressions (fully crossed with sex, making three test displays for each sex X expression condition within each block). In line with previous studies, a glossary containing a definition of each word was available to the participant. Accuracy and response time were calculated as the percentage of correct responses and time to complete the task, respectively. Additional accuracy scores were calculated to assess the effect of emotional valence of the mental state discrimination (positive, neutral, negative expressions; see Maurage et al. 2011).

3.2 Typhoid vaccination

Participants received 0.5 mL *Salmonella typhi* capsular polysaccharide vaccine (0.025 mg in 0.5 mL, Typhim Vi, Sanofi Pasteur, UK) and a saline placebo (0.5 mL) via intra-muscular injection in the deltoid muscle of the non-dominant arm by a certified nurse. Typhoid vaccine was selected as a low-grade inflammatory stimulus, since this vaccine is known to induce increases in circulating pro-inflammatory cytokine levels with no significant effect on body temperature (Paine et al., 2013).

3.3 Mood and sickness symptoms

Current mood and presence of sickness symptoms was assessed using a modified version of the Profile of Mood States – Short Form (POMS-SF; Curran, Andrykowski, and Studts 1995). The POMS-SF consists of 32 items asking 'How are you feeling right now:' followed by the item. Items were rated on a five-point Likert scale (0 = not at all, 1 = a little, 2 = moderately, 3 = quite a bit, to 4 = extremely). Scores for POMS subscales (tension-anxiety, anger-hostility, fatigue-inertia, vigour-activity, confusion-bewilderment, depression-dejection) were computed by summing ratings on individual items. Six items were added to assess physical sickness (light-headed, nausea, faint, disgusted) and behavioral sickness (withdrawn, sociable).

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3.4 Blood sampling

Blood was collected into one 6 ml vacutainer containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant (Becton Dickinson Diagnostics, Oxford, United Kingdom). Samples were immediately centrifuged at 1500*g* for 10 min at 4 °C and plasma was aliquoted and stored at -80 °C for later cytokine assessment of plasma interleukin-6 (IL-6). Plasma IL-6 was measured in duplicate using high-sensitivity enzyme-linked immunosorbent assay (ELISA) (Quantikine HS Human IL-6 ELISA, R&D Systems, UK) in accordance with the manufacturer's instructions. The limits of detection of this assay was .11 pg/mL, with an intra-assay coefficient of variation (CVs) of 4.2%. All samples from the same participants were assayed in the same run.

3.5 Anthropomorphic Measures

Participants were asked to remove footwear and coats and empty their pockets before a body composition measurement was taken using a TANITA BC-545N body composition analyser (Tanita Europe, Amsterdam, The Netherlands), a device that uses bioelectrical impedance analysis. A stadiometer was used to measure height.

4. STATISTICAL ANALYSIS

All data were analyzed using SPSS v.24.0 (IBM-SPSS Inc., Chicago, IL, USA). Individuals with accuracy scores > 2.5 SD from the mean were excluded from analysis (N = 1). ANOVAs were performed to compare treatment (placebo vs vaccine). For POMS subscales, linear mixed models were used. Model simplicity and likelihood ratio tests were used to select appropriate covariance structures. Treatment and time were fixed and repeated factors; subject was entered as a random factor; and baseline scores were entered as a time-varying covariate. The effects of interest were interactions between time and treatment and main effects of treatment. Tests of simple main effects were performed on the linearly independent pairwise comparisons between the estimated marginal means for all analyses.

For IL-6 analyses, log10 transformation was applied because of the skewed distribution of raw IL-6 values. Three participants were excluded because of high baseline values indicative of a possible infection. Injection order (p = .21) and stimulus set order (p = .80) were added as

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between-subjects factors but showed no effect in any of the analyses and were therefore omitted from the final analyses. To correct for mood and sickness (POMS), difference scores were calculated for each subscale for the vaccine condition from baseline to peak IL-6 level (either 5h30m or 8h post-injection) and added as covariate if of interest. Correlational analysis with IL-6 was conducted on the peak IL-6 difference scores during the vaccine condition.

5. RESULTS

5.1 Responses to Typhoid vaccination

5.1.1 Physiological responses

As shown in Table 1, after typhoid vaccination, participants showed a significantly greater increase in plasma IL-6 at 5h30m (+348%, mean difference 4.1, *SE* = 0.3) as compared to placebo (-14%, mean difference -0.1, *SE* = 0.0), $t(32) = -15.14$, $p < .001$. IL-6 remained elevated until at least 8 hours post injection (+227%, mean difference = 2.7, SE = 0.2), $t(33) = -15.41$, $p = <.001$; treatment (placebo, vaccine) x time (0h, 5h30, 8h) interaction, $F(2, 50) = 58.04$, $p < .001$, $\eta_p^2 = .84$). The peak IL-6 response occurred at 5h30m post-injection for most participants (average peak increase was 419%). Core body temperature showed no effect of treatment, $F(2, 54) = 1.32$, $p = .28$, $\eta_p^2 = .05$).

	0 hours	5h30m	8 hours
Placebo	1.11 (0.58)	0.95 (0.65)	0.97 (0.69)
Vaccine	1.18 (0.62)	5.29 (1.72)	3.86 (1.26)

Table 1. Mean (SD) IL-6 in pg/mL for the placebo and vaccine condition. Column labels represent time since vaccination

5.1.2 Affective responses, physical symptoms, and expectancy

No significant time by condition interactions were evident for any of the POMS subscales or total mood score (all *F*'s < 1). POMS data are summarized in Table 2. Results also indicated that participants were blind to their condition at the first visit: on test day 1, 55.6% of

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the participants reported the condition correctly at the end of the test day, which is at chance level, $\chi^2(1) = .44$, $p = .51$. At the end of test day 2, 83.8% correctly guessed the condition they were in, $\chi^2(1) = 16.89$, $p < .001$. To rule out partial expectancy effects, sensitivity analyses were run including only test day 1 (using between-subject comparisons), which yielded essentially identical results (results presented below).

	0 hours		5h30m		8 hours	
	Placebo	Vaccine	Placebo	Vaccine	Placebo	Vaccine
Anger	1.6 (2.2)	1.3 (2.1)	0.7 (1.4)	1 (2.4)	1.4 (2.6)	1.4 (2.1)
Confusion	4 (2.3)	3.5 (2.3)	3.2 (2.2)	3.4 (2.0)	4.5 (2.2)	4.3 (2.2)
Depression	1.3 (2)	1.3 (2.2)	0.6 (1.2)	0.8 (1.9)	1 (2.1)	1.4 (2.3)
Fatigue	3.9 (4.1)	3.8 (3.8)	2.8 (4.7)	3 (4.5)	5.3 (3.7)	6 (3.9)
Tension	1.6 (2.2)	1.2 (1.1)	1 (1.9)	1.5 (2.2)	1.7 (2.9)	1.3 (2)
Vigour	7.2 (4.2)	7.3 (3.3)	7.0 (3.6)	7.2 (3.4)	4.7 (3.7)	4.3 (3.3)
Behavioral Sickness	2.5 (1.5)	2.2 (1.3)	2.3 (1.3)	2.1 (1.3)	2.6 (1.3)	2.4 (1.3)
Physical Sickness	0.8 (1.6)	0.8 (1.2)	0.8 (1.9)	0.8 (1.4)	1.1 (1.8)	0.8 (1.3)

Table 2. Mean POMS subscales (SD) (mood and physical and behavioral symptoms) separated by condition. No interaction effects between treatment and time were evident (all F's < 1). Column labels represent time since vaccination.

5.2 Reading the eyes in the mind test

As can be seen in Figure 1a, vaccination (vs. placebo) led to a significant decrease in performance on the RMET, $F(1, 38) = 4.78$, $p = .035$, $\eta_p^2 = .11$. These results remained significant after adjusting for mood, behavioral or physical symptoms as well as for vaccination order. Sensitivity analyses including only test day 1 (using a between-group comparison), yielded virtually identical results, $F(1, 37) = 9.08$, $p = .005$, $\eta_p^2 = .20$). Similar responses times were observed for vaccine (M = 8.1 sec, SD = 4.4 sec) and placebo (M = 8.2 sec, SD = 4.5 sec), $F(1, 38)$

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= .03, p = .867, η_p^2 = .00. Separate analyses of RMET stimuli selected on valence (positive, negative, neutral) provided no evidence of a selective impact of treatment (see Figure 1b), F(2, 76) = .08, p = .921, η_p^2 = .00. Finally, the decreased RMET performance was not correlated with changes in IL-6 in the vaccine condition, r(36) = .16, p = .367.

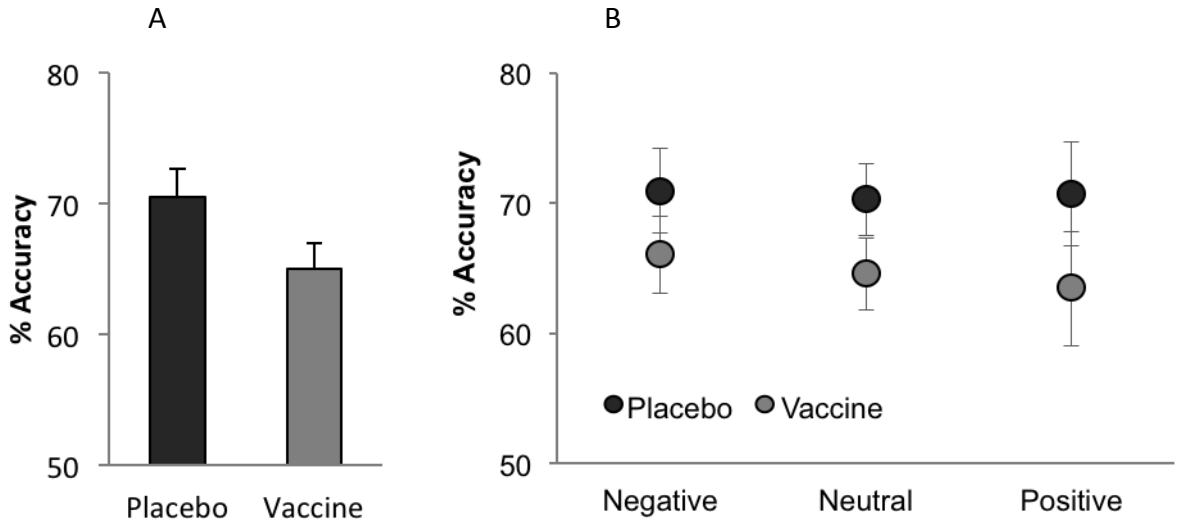

Figure 2. Mean accuracy scores for RMET total score (A), and for each emotional valence (B) for the placebo and vaccine condition. Errors bars indicated SEM. Please note that the y-axes start at 50% accuracy.

6. DISCUSSION

The present study tested whether inflammation impairs the ability to infer other's mental states and affect, which is considered a key aspect of Theory of Mind (Frith & Happe, 1999). Inflammation, as measured by IL-6, was effectively induced using a typhoid vaccination without causing sickness symptoms (e.g., fever, light-headedness, nausea, faint, withdrawn) or deterioration of mood. Moreover, analyses indicated that participants were blind to their condition at the first visit, which largely excluded confounding by expectancy effects. In line with the hypothesis, vaccine resulted in poorer performance on the RMET compared to the placebo condition. The current findings provide further experimental support to the idea that inflammation may drive social-cognitive deficits in psychopathologies that exhibit enhanced

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inflammation, such as major depression (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; Eisenberger et al., 2017).

A possible alternative interpretation of the current results is that the lower performance during inflammation reflects reduced motivation. Motivational changes, which are considered a characteristic effect of inflammation, could thus account for the decreased RMET performance (e.g., Draper et al., 2017; Harrison et al., 2015; Lasselin et al., 2016). If this were the case, participants should have performed more poorly and taken less time to carefully inspect the stimuli. However, the results did not seem consistent with this interpretation; the time participants took to complete the task was identical across conditions while performance accuracy was not. Although the current study was not specifically designed to assess social-related motivational changes, the observed pattern of results would not seem consistent with weakened motivation explaining the RMET performance deficits shown here.

The results further suggested that the inflammatory stimulus applied in the current study impaired ToM skills independent of emotional valence. I.e., the ability to infer positive mental states was affected to a similar degree as inferences on negative and neutral mental states. This observation is consisted with that of Muscatell et al. (2016), who showed that endotoxin-induced inflammation enhanced neural responsivity in threat-related (e.g., bilateral amygdala) and reward-related (e.g., ventral striatum) brain regions, as well as in a region involved in inferring mental states of others (dorsomedial prefrontal cortex (DMPFC)) to the same degree for negative versus positive social feedback. Muscatell et al. (2016) and Kullmann et al. (2013) further showed endotoxin-induced heightened neural sensitivity in the core region implicated in mentalizing. The fact that Kullmann et al. (2013) failed to find a behavioral effect, may perhaps be taken to suggest that individuals with inflammation may find the RMET task harder and so require more brain resources to achieve the same level of performance.

Clinically, although still somewhat speculatively at this point, the current findings suggest that individuals with inflammation find social interactions more complex, which could possibly contribute to social withdrawal and further amplification of depressive symptoms (Cacioppo, Hughes, Waite, Hawkley, & Thisted, 2006; Heinrich & Gullone, 2006). Likewise, impairments in social emotion recognition may hinder optimal support seeking, whereby

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patients might be less in tune with their social environment, e.g., less sensitive in picking up social cues that would otherwise guide symptom reporting and help-seeking behavior. The inflammatory component of depressive disorders may similarly contribute to social impairments and withdrawal that characterize these disorders (Miller & Raison, 2016).

A notable feature of the current study is that the typhoid vaccination used here elicited a smaller inflammatory response, raising IL-6 levels 4-fold, compared to the endotoxin manipulations used previously, which raised IL-6 levels between approximately 100-fold with a lower endotoxin dose (0.4 ng/kg body weight) up to roughly 1000-fold with a high dose (2 ng/kg body weight) (Draper et al., 2017; Eisenberger, Inagaki, Rameson, Mashal, & Irwin, 2009; Grigoleit et al., 2011; Kullmann et al., 2013; Lasselin et al., 2016; Moieni et al., 2015; Muscatell et al., 2016). Although it could be argued that the modest elevation of IL-6 observed here simply reflects diurnal variation (Agorastos et al., 2014; Nilsonne, Lekander, Åkerstedt, Axelsson, & Ingre, 2016), this account is unlikely as IL-6 changes were specific to the vaccine condition and not found in the placebo condition, even though measurements were obtained at the same time of the day in both conditions. Producing subtle increases inflammation can be considered an advantage for research aimed at uncovering cognitive consequences of inflammation. For example, in terms of generalizability, the level of immune activation seen here is more akin to the low-grade inflammatory levels seen in depressed individuals, as well as medical conditions such as diabetes and atherosclerosis that have been linked to increased depression risk. Moreover, as argued earlier, the manipulation minimizes potentially confounding side effects such as sickness symptoms or significant mood deterioration (e.g., Dowlati et al., 2010; Maes et al., 1995; O'Brien, Scully, Fitzgerald, Scott, & Dinan, 2007).

Typhoid vaccination is used to induce inflammation and subsequent neuropsychological effects subsumed under sickness behaviour, but typically without physical malaise that more typically denotes sickness (e.g., fever, nausea). This similarly applies to other human data in which low-grade elevated inflammatory activity is present without overt sickness (e.g., such as in depression), but still showing neuropsychological phenomena like fatigue, anhedonia and motor slowing (e.g., Goldsmith et al., 2016; Treadway, Bossaller, Shelton, & Zald, 2012). Hence, there seems reasonable ground to further discuss whether the term sickness behaviour

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remains appropriate or whether we should consider new terminology (e.g., inflammation-associated cognitive changes. Some studies using typhoid vaccination have reported modest elevations in fatigue after vaccination, although this has not been uniformly observed (Brydon et al., 2008; Harrison et al., 2015; Harrison et al., 2009; but also Paine et al., 2013). Inspection of the data suggests that studies which measured fatigue 2-4 hours post vaccination observed elevated fatigue, whereas those using later time-points did not. However, further studies are needed to establish if timing is indeed a factor. The performance decrement of 5.6% on the RMET we observed in the current study is comparable to the study of Moieni et al. (2015) (about -5%). Interestingly, such differences are also observed in individuals with major depression as compared to controls (Kettle, O'Brien-Simpson, & Allen, 2008; Lee, Harkness, Sabbagh, & Jacobson, 2005; Szanto et al., 2012). However, perhaps the more significant point to be taken from these data goes beyond the exact magnitude of the observed effects, but is the experimental demonstration that mild inflammation affects social cognition. A further strength of the study is the relatively large sample size, which is the largest typhoid vaccination study to date, although it remains possible that some more subtle effects may have been missed: i.e., analyses established that at a power of .80, and assuming an alpha of .05, the current samples size was sufficient to detect small to moderate effect sizes. Limitations are that the current model induces an acute inflammatory state, and generalization to chronic inflammation remains to be determined and only healthy young males were assessed. Even though Moieni et al. (2015) reported no sex differences in acute inflammation or its consequent cognitive effects, further studies are clearly needed to confirm the generalizability of the current effects to females and to younger and older individuals. Indeed, inflammation is a hallmark of ageing and replication of results to such a relevant group is advised. Moreover, even though Lacourt et al., (2015) showed that vaccination does not affect pain tolerance or pain threshold and no side effects were reported in the current study, pain could have influenced performance and lack of assessing pain is a limitation of the study. Similarly, even though modified versions of the Profile of Mood Scales-Short Form (POMS-SF) have been used extensively in typhoid vaccination studies (e.g., Brydon et al., 2009; Harrison et al., 2015), the use of a modified POMS-SF to measure mood and sickness can be considered as a limitation of

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the current study. Finally, similar to the study of Moieni et al. (2015), we found that the magnitude of the vaccine-induced inflammatory response was not related to behavioral performance. They speculated that the RMET might not be sensitive enough to capture incremental changes in inflammation. However, the current study showed that also a modest inflammatory stimulus impairs RMET performance. An alternative explanation might be that IL-6 is not a causal factor, and perhaps more proximal inflammatory biomarkers could be explored (Leighton et al., 2017).

In summary, typhoid vaccination elicited a transient low-grade inflammatory response in healthy young men and decreased performance on the Reading the Mind in the Eyes Test, tested in a double-blinded placebo-controlled crossover design. Hereby the current study provided direct empirical evidence for a link between heightened inflammation and lower ability to infer mental states of others. This finding, together with related recent reports, warrants a more comprehensive program of research linking inflammation and social cognition.

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Highlights

1. Typhoid vaccination elicited a transient low-grade inflammatory response without physical sickness or mood changes.
2. Inflammation resulted in lower ability to interpret the mental state of someone else, suggestive of transient impairment of Theory of Mind.
3. Inflammation may be a contributing factor to social-cognitive deficits in psychopathologies that exhibit enhanced inflammation, such as major depression.

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