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Full-length Article

Typhoid vaccine does not impact feelings of social connection or social behavior in a randomized crossover trial among middle-aged female breast cancer survivors

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ABSTRACT

Background: Inflammation can have social consequences, which may be relevant to inflammation's link with depression. The current study tests whether a typhoid vaccine increases feelings of social disconnection and avoidance behavior.

Method: In two full-day visits at least three weeks apart, 172 postmenopausal breast cancer survivors (Stage I-IIIA) each received a typhoid capsular polysaccharide vaccination and a saline placebo injection in a random sequence. Blood was drawn prior to the injection, as well as every 90 min thereafter for 8 h to assess the inflammatory response (interleukin-6, IL-6; interleukin-1 receptor antagonist, IL-1Ra). At both visits, women completed the Social Connection Scale at 0 and 8.5 h post-vaccination as well as implicit and explicit social avoidance tasks at 7 h post-vaccination.

Results: The typhoid vaccine triggered rises in both inflammatory markers ($ps < 0.01$), but it did not impact feelings of social connection ($p = .32$), or performance on the implicit ($p = .34$) or explicit tasks ($p = .37$). Inflammatory rises did not predict feelings of social connection ($ps > 0.64$) or performance on explicit ($ps > 0.73$) or implicit ($ps > 0.88$) social avoidance tasks.

Conclusion: Milder inflammatory stimuli may not affect social processes. Higher levels of inflammation or, relatedly, more sickness symptoms may be necessary to recapitulate prior findings of social avoidance.

Abbreviations: LPS, lipopolysaccharide; IL-6, interleukin-6; IL-1Ra, interleukin-1 receptor antagonist.

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1. Introduction

Inflammation can have social consequences. In fact, social avoidance is considered a primary "sickness behavior," or symptom following an inflammatory stimulus – a well-established model for the inflammatory subtype of depression (Kelley et al., 2003; Lasselin et al., 2020). One of the first relevant and well-replicated observations was that adult male rats injected with lipopolysaccharide (LPS; endotoxin), a strong inflammatory stimulus, avoid juvenile males (Bluthe et al., 1994, 1992; Marvel et al., 2004). These findings prompted the notion that inflammation may partially explain the altered social behavior commonly observed in depression, yet the corresponding translational research was slow to emerge. Until recently, human studies measured subjective social experience rather than social behavior, showing that people injected with LPS felt less socially connected (Eisenberger et al., 2010; Moieni et al., 2015) and had less social motivation (Hannestad et al., 2011). However, studies with milder inflammatory stimuli (i.e., the influenza vaccine) did not replicate these results (Jolink et al., 2022; Kuhlman et al., 2018). Observed LPS-induced changes in self-reported feelings of social connection corresponded with neuroimaging findings (Eisenberger et al., 2009; Inagaki et al., 2012; Muscatell et al., 2016). For example, those exposed to endotoxin had greater amygdala responses to socially threatening images (Inagaki et al., 2012). Even so, these studies did not measure social behavior and therefore did not directly correspond with the above murine findings.

Only two known studies to date have measured human social behavior following an inflammatory stimulus – a gap in the literature (Muscatell and Inagaki, 2021a). Among 31 healthy young people, those who had greater interleukin-6 (IL-6) responses to an influenza vaccine were faster to approach a support figure and more accurate in avoiding vaguely familiar celebrities (Jolink et al., 2022). Indeed, when it comes to milder inflammatory stimuli, only people with strong inflammatory responses may have altered social behavior, whereas more powerful inflammatory stimuli may have a universal impact on social behavior. A randomized crossover trial with 22 healthy adult participants found that participants were more likely to moan and complain when communicating with a healthcare provider after receiving a relatively high dose of LPS (2.0 ng/kg of body weight), compared to saline placebo (Lasselin et al., 2018b). Notably, the latter study's LPS injection boosted IL-6 levels to nearly 1,000 pg/ml (Lasselin et al., 2018b), while IL-6 levels remained below 3 pg/ml following the influenza vaccine in the former study (Jolink et al., 2022).

A recent review highlighted the importance of considering the strength of an inflammatory stimulus when examining its impact on social behavior. The authors, who themselves have used the LPS injection paradigm to examine changes in social experience, state that changes in social behavior following LPS may simply model acute sickness, while vaccination models may be more relevant to stress and depression (Muscatell and Inagaki, 2021a). An article in this journal underscored the novelty of Jolink et al.'s findings regarding social behavior following the influenza vaccine, and highlighted the need for extension with a typhoid vaccine paradigm, given that it provokes inflammatory increases "approximating levels of systemic inflammation associated with depression" (Lindsay, 2022). Further, the author suggests that as the inflammatory response increases, so might neural sensitivity and subconscious social processes; only the strongest inflammatory stimuli might affect social motivation via mood degradation (Lindsay, 2022). The current study examines both feelings of social connection and social behavior following and the typhoid vaccine and saline placebo injection in a large sample. Even though the typhoid vaccine triggers a smaller inflammatory response (IL-6 levels lower than 2 pg/mL after three hours and below 7 pg/mL after six to eight hours), it nonetheless can affect mood without inducing fever or notable discomfort, except mild injection site pain (Chia et al., 2003; Hingorani et al., 2000; Kharbanda et al., 2002; Lacourt et al., 2015; Wright et al., 2005). Therefore, this model may afford the opportunity to tease out the effect of inflammation on social processes when participants are not as sick as they would be in the LPS paradigm.

This study assessed self-reported feelings of social connection and performance on implicit and explicit social avoidance tasks featuring unfamiliar others following a typhoid vaccine and saline injection. We tested the effect of the vaccine and the vaccine-induced inflammatory response on self-reported social connection as well as implicit and explicit social avoidance task performance. The Jolink et al. (2022) findings are the most relevant to our study, as they used also used a mild inflammatory stimulus, which altered implicit social behavior but not self-reported motivation. To explain this pattern of results, Lindsay et al. (2022) reasoned that small inflammatory rises may only impact subtle, subconscious, or implicit social behaviors, but larger increases may be necessary to impact self-reported social feelings. Therefore, we hypothesized that the typhoid vaccine and related inflammatory rise would not be associated with feelings of social connection or performance on the explicit task but would predict avoidance on the implicit task. In particular, because there is variability in the inflammatory response to the typhoid vaccine, we expected that greater inflammatory response magnitude would be an especially potent predictor of implicit avoidance. Although the implicit and explicit task featured faces with differing emotions (described in detail below), we made no specific hypotheses about social behavior toward each emotion type due to a lack of prior relevant literature.

2. Methods

2.1. Participants

For the parent study that analysed the inflammatory responses to the typhoid vaccine (Kiecolt-Glaser et al., 2022), we recruited 172 postmenopausal breast cancer survivors (Stage I-IIIA) one to nine years after primary treatment completion – except for longer-term hormonal therapies (tamoxifen, aromatase inhibitors) (Table 1, Fig. 1). Participants were primarily recruited from the James Cancer Hospital breast cancer clinics or the Army of Women website. Women were excluded if they had a prior history of any other cancer besides basal or squamous cell skin cancer, stroke, diabetes, anemia, liver disease, autoimmune disease, current heart disease or unmanaged hypertension, alcohol or drug

Table 1
Sample Demographic Information.

	N	Mean (SD) or N (%)	Range
Age	172	56.6 (8.4)	36–78
Race	172		
White		159 (92.4 %)	
Black		10 (5.8 %)	
Asian		1 (0.6%)	
Multiracial		2 (1.2 %)	
Years since treatment	172	3.5 (2.3)	0.8–9.9
Chemotherapy treatment	172	116 (67.4 %)	
Radiation treatment	172	105 (61.0 %)	
Current hormone therapy	172	138 (80.2 %)	
Cancer stage	172		
Stage I		81 (47.1 %)	
Stage II		82 (47.7 %)	
Stage III		9 (5.2 %)	
Any comorbidities	171	22 (12.9 %)	
UCLA Loneliness (Screen)	172	34.9 (9.5)	20–68
Social Connection Scale*			
Visit 1	172	5.1 (0.9)	1.3–6.0
Visit 2	163	5.1 (0.9)	2.3–6.0
Fasting IL-6, pg/mL			
Visit 1	171	2.8 (6.1)	0.4–78.4
Visit 2	163	2.8 (6.4)	0.4–80.6
Fasting IL-1Ra, pg/mL			
Visit 1	171	542 (394)	121–1991
Visit 2	163	526 (369)	143–2389

*Baseline Administration of Social Connection Scale.

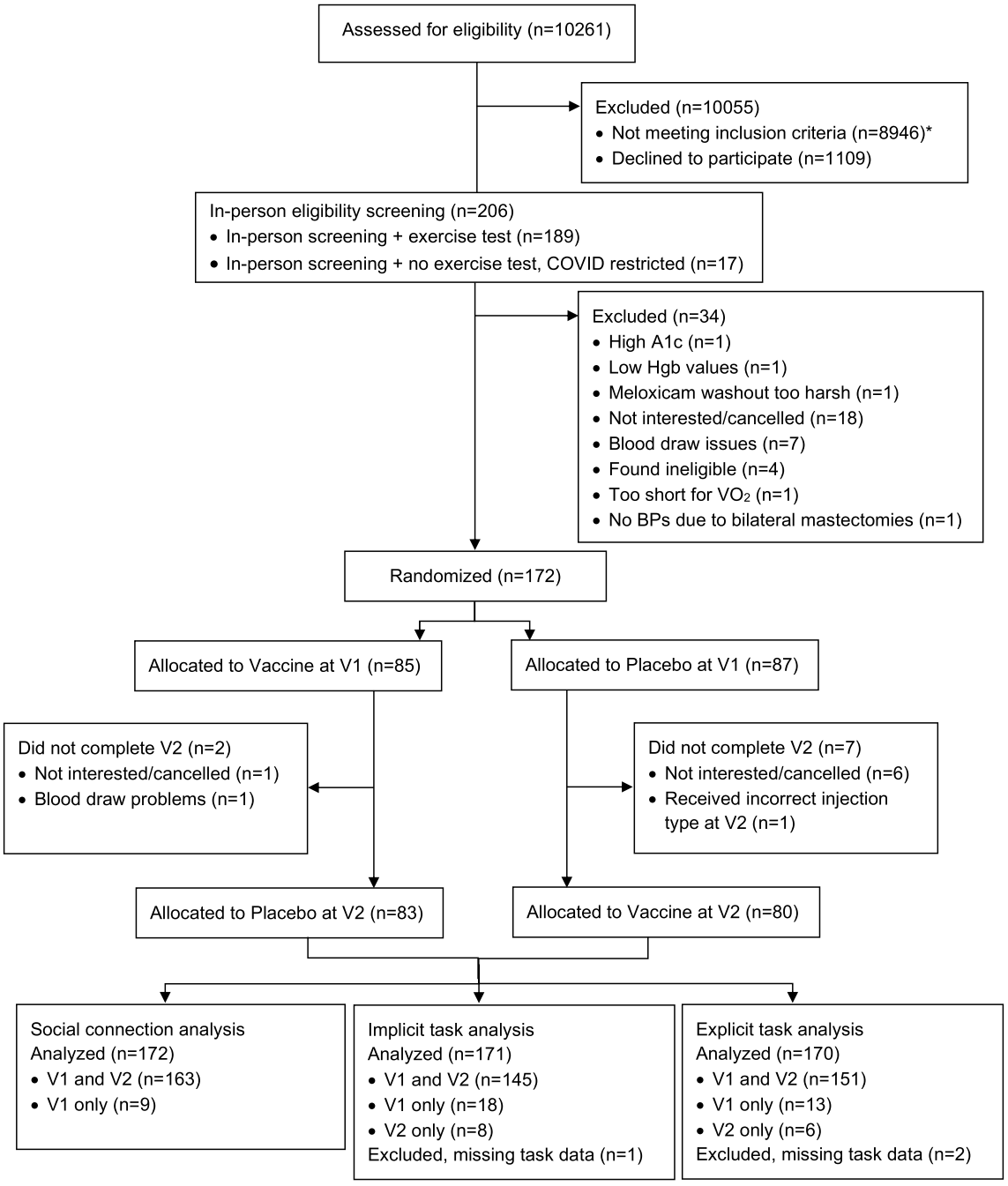

Fig. 1. Flow of Participants through the Study.

abuse, smoking, or other medical conditions that would have affected participation, such as cognitive impairment. Women were not allowed to participate if they had a prior typhoid vaccination or any other vaccine within the past month. The Ohio State University Institutional Review Board approved this study, and each participant provided informed consent.

2.2. Study procedures and design

In this double-blind crossover trial, women were randomized to either the vaccine/placebo or the placebo/vaccine sequence at the first of two full-day study visits after completing an initial screening visit to determine eligibility. The Data Manager created the randomization sequence but had no contact with participants. The research assistants were blind to condition assignments. The nurse who collected blood throughout the day was not the same nurse who administered the injections.

We asked women to eat their last meal before 7:30 pm the night before each visit and to avoid alcohol and vigorous physical activity two days prior to each visit. We also asked them to discontinue aspirin and nonsteroidal anti-inflammatory drugs one week prior to each visit. The two 9.5-hour sessions were scheduled 26–420 days apart (M = 46.87, SD = 47.48); the initial goal was 10–30 days apart, but the COVID-19 pandemic substantially delayed some of the second visits.

On the morning of each study visit, a nurse took participants' oral temperature, which served as the baseline measurement. Then the nurse inserted an intravenous catheter and drew a baseline blood sample following a 20–30 min adaptation period. After the baseline blood draw and around 8:30 am, the nurse injected saline (the placebo) or Typhoid capsular polysaccharide vaccine (Typhim-Vi, Sanofi Pasteur) into the

non-dominant deltoid muscle. Within five minutes after the injection, participants completed the Social Connection Scale and then ate a standardized breakfast. For the next 8 h, blood draws occurred every 90 min. Participants also rated their mood and sickness symptoms after each blood draw. A trained experimenter took an oral temperature every 0.5 h following the injection. Participants completed other tasks relevant to the parent study (e.g., questionnaires, 20-minute resting metabolic measurements) throughout the day. Seven hours after the injection, participants completed the 15-minute implicit and five-minute explicit social avoidance tasks, respectively. Ninety minutes later (approximately 8.5 h post-injection), participants completed the Social Connection Scale a second time. The timing of these measures corresponds with the typhoid vaccine's peak inflammatory response (6–8 h post-vaccine; Paine et al., 2013).

2.3. Questionnaires

Twice at each visit, participants were asked to rate on a seven-point Likert scale (0, strongly disagree; 6, strongly agree) the extent of their agreement or disagreement with various statements regarding their current feelings of social connection (e.g., "Right now I feel appreciated") on the eight-item Social Connection Scale (αs = 0.83 - 0.87 at all administrations) (Jaremka et al., 2017). To account for loneliness as a covariate, participants completed the University of California, Los Angeles (UCLA) Loneliness Scale at the screening visit (Russell, 1996; Cronbach's $\alpha = 0.91$).

2.4. Mood and sickness symptoms

As reported in the first publication from this trial (Kiecolt-Glaser et al., 2022), women rated the intensity of their physical symptoms (pain, muscle aches, headache, feverishness, focus, memory, and hunger) on a 10-point Likert scale after each blood draw. At this time, they also completed the Self-Assessment Manikin (SAM) scale, which pictorially represents 4 × 9-point continuums of symptoms ranging from (1) sadness to happiness, (2) relaxed to anxious, (3) submissive to dominant, and (4) cheerful to angry (Bradley and Lang, 1994).

2.5. Implicit approach/avoidance task

Participants were presented with pictures of faces with a variety of expressions (happy, sad, anger, fear, neutral) from a validated set that healthy controls can accurately recognize (Gur et al., 2002). They were instructed to push a joystick away for faces that were surrounded by a yellow box and pull towards them for faces surrounded by a blue box. This task was performed on the DirectRT software with a joystick that was confined to forward and backward movement. Reaction time, the outcome of interest, was defined as the time from stimulus presentation to the lever's maximal extension. Response times that were less than 200 ms or greater than 4000 ms – two percent of all trials – were eliminated. Previous studies demonstrate that pulling is faster for appetitive stimuli (indicating approach tendencies) and pushing is faster for aversive stimuli (indicating avoidance tendencies) (Marsh et al., 2005). Participants committed an error when they pulled the joystick when a yellow box was presented or pushed the joystick when a blue box was presented.

There were 120 total trials (60 approach trials, 60 avoidance trials; 60 female trials, 60 male trials). There were 24 stimuli for each of the four emotional categories and 24 neutral stimuli. Stimuli were randomized, and each stimulus was presented once as an avoidance trial and once as an approach trial. Each distinct individual's face expressed only one emotion; the same face was never displayed with a different emotion. Participants engaged in a practice block in which they were presented with 20 faces. Then they began the main task, which lasted approximately 15 min.

2.6. Explicit ratings task

This task was also completed on DirectRT software and immediately followed the implicit task. The explicit ratings task lasted approximately five minutes and was composed of 50 trials with no practice – 10 per emotion and 10 neutral faces. In this task, each stimulus was presented only one time and facial stimuli from the implicit trial were represented. The instructions were: "You will see a picture of a face on the screen; imagine standing face-to-face with the person shown in the picture and rate your tendency to approach or avoid this person using the scale provided on the screen (-4 to + 4). A "0" means that you would not approach or avoid; each other number corresponds to the number of steps participants would make towards (+) or away (-) from the person. Do not base your rating on the attractiveness or trustworthiness of the person, but only on the emotional expression."

2.7. Cytokines

Prior research showed that serum IL-6 and interleukin-1 receptor antagonist (IL-1Ra) increase in response to the typhoid vaccine (Hingorani et al., 2000; Kharbanda et al., 2002) and track with social processes (Eisenberger et al., 2017). IL-1Ra is often measured instead of interleukin-1 (IL-1) because IL-1 levels in circulation are very low – even in septic shock patients (Cannon et al., 1990; Granowitz et al., 1991). Also, compared to IL-1, IL-1Ra is a stronger correlate of disease severity across a wide variety of conditions (Fischer et al., 1992; Granowitz et al., 1991; Pereira et al., 1994). We assayed serum IL-1Ra using an electrochemiluminescence method with Meso Scale Discovery kits and serum IL-6 with the Quantikine HS ELISA kit (R & D Systems, Minneapolis, MN). Sensitivity for IL-1Ra was 6.3 pg/mL, the intra-assay coefficient of variation was 4.1 %, and the inter-assay coefficient of variation was 8.6 %. Corresponding values for IL-6 were 0.03 pg/mL, 4.1 %, and 6.5 %.

2.8. Analytic method

As a manipulation check, we first examined whether the typhoid vaccine provoked increases in the cytokines of interest, compared to the placebo injection. For this analysis, we used linear mixed effects models with fixed effects of injection type, time (across the day, categorical), and their interaction. Of primary interest was the interaction between injection type and time. These models used natural log-transformed IL-6 and IL-1Ra to better approximate normality of residuals. The random effects were a random intercept per subject per visit, with random intercepts on the same subject (i.e., for each of the two visits) allowed to be correlated to capture within-subject correlations within- and between-visits, and random assay plate effects to reduce error variance due to between-plate variability. In these and the primary models (below), we excluded data from the final blood draw because: (1) it occurred after the outcomes of interest, and (2) preliminary analyses showed that there was only a placebo-related increase between 6.5 and 8 h post-injection ($ps < 0.001$) but not a vaccine-related increase ($ps > 0.09$), suggesting that the peak inflammatory response to the typhoid vaccine occurred at 6.5 h. Additionally, after discovering in a subsample that IL-1Ra did not change at the earlier time points, we elected to measure it once at baseline and then not again until 6.5 h later to capture its peak – primarily due to the cost of the assay. As a result, there were five repeated measurements of IL-6 (baseline, 1.5, 3, 5, 6.5 h post-injection) but only two of IL-1Ra (baseline, 6.5 h post-injection). We also used a similar modeling strategy (i.e., linear mixed models with fixed effects of injection type, time, and their interaction and a subject-specific visit random effect) to test whether injection type influenced body temperature. Next, we used generalized estimating equations with robust standard error estimates (Ballinger, 2004; Zeger and Liang, 1986) to test whether injection type influenced mood ratings. GEE models are appropriate for these repeated measures analyses with a discrete, categorical outcome because they model participants' average responses, thereby providing

efficient, unbiased estimates of how much the average response changes for every one-unit increase in a predictor variable (Ballinger, 2004; Zeger and Liang, 1986).

For the primary analyses, we were interested in the effects of (1) the vaccine/placebo injection, and (2) change in inflammation throughout the day on (1) self-reported Social Connection Scale scores, (2) implicit social behavior task performance (response times), and (3) explicit social behavior task performance (ratings). Due to the multiple visits per participant and repeated measurements within a visit, all analyses used linear mixed effects models, and each predictor/outcome combination was examined in separate models.

In models assessing the effect of vaccine versus placebo, the predictor of interest was injection type. In models assessing the effect of change in IL-6, the area under the curve with respect to increase (AUCi) was the predictor of interest, with baseline (pre-injection) IL-6 additionally included to guard against regression to the mean (raw, untransformed values used for both). In models assessing the effect of change in IL-1Ra, the predictor of interest was the change from baseline to 6.5 h post-injection, with baseline IL-1Ra additionally included. In order to better approximate normality of residuals, the change score for IL-1Ra was calculated on the natural-log scale and baseline IL-1Ra was log-transformed for inclusion in the models.

For models with Social Connection as the outcome, the measurement at 8.5 h post-injection was used as the outcome and the baseline measurement was included as a covariate. The main effect of either injection type or cytokine change measure was of primary interest. Random subject-specific intercepts were used to capture the within-subject correlation due to the two visits. For models with implicit task performance as the outcome, of primary interest was the three-way interaction of the predictor of interest (injection type, IL-6 AUCi, ln(IL-1Ra) change score) with stimulus emotion and trial type (approach/avoidance) to account for the task design. For models with explicit ratings as the outcome, of primary interest was the two-way interaction of the predictor of interest with stimulus emotion, which reflects the task design, as there were not specific approach/avoidance trials. All lower-order interactions and main effects were also included. Both the implicit and explicit tasks involved repeated measurements per visit, thus random subject-specific visit effects were included to allow the within-subject correlations to vary across visits. All models controlled for age, loneliness, visit (first vs second), and season (fall, winter, spring, summer) via fixed effects.

The analytic sample size differed across models due to intermittently missing outcome values and/or cytokine values. All 172 study participants contributed data to the models of IL-6 and IL-1Ra over time. When using injection type as a predictor, models for both Social Connection and implicit task performance had n = 171; the model for explicit ratings had n = 170. When using change in cytokines as a predictor, models for Social Connection had n = 167 while implicit and explicit task models had n = 164.

Blinding indices are reported in the parent publication (Kiecolt-Glaser et al., 2022). All analyses were conducted in SAS version 9.4 (Cary, North Carolina) except the blinding index, which was calculated using R. Alpha levels were set at 0.05.

3. Results

3.1. Manipulation check

As expected, compared to the placebo injection, the typhoid vaccine triggered rises in both cytokines (IL-6: ($F(4, 1247) = 82.38$, $p < .0001$); IL-1Ra ($F(1, 302) = 73.76$, $p < .0001$). These inflammatory markers were significantly elevated for vaccine compared to placebo at all post-baseline measurement timepoints (Fig. 2).

3.2. Mood and fever

As reported in the initial publication from this trial, women reported more pain, aches, and headache after they received the vaccine compared to placebo, but the vaccine did not affect self-reported focus, memory, hunger, nor feverishness (Kiecolt-Glaser et al., 2022). Here we additionally report that the vaccine did not affect oral temperature compared to the placebo ($p = .44$) nor ratings of sadness, anger, anxiety, and submissiveness ($ps > 0.21$) (Supplemental Fig. 1A-B).

3.3. Self-reported social connection

Supplemental tables show the full results from the regression models that are relevant to the primary hypotheses. There was no vaccine effect on the second administration of the self-report social connection scale when controlling for the first administration of this scale ($p = .32$). Neither change in IL-6 nor change in IL-1Ra across the day predicted scores on the second administration of the Social Connection Scale ($ps > 0.64$).

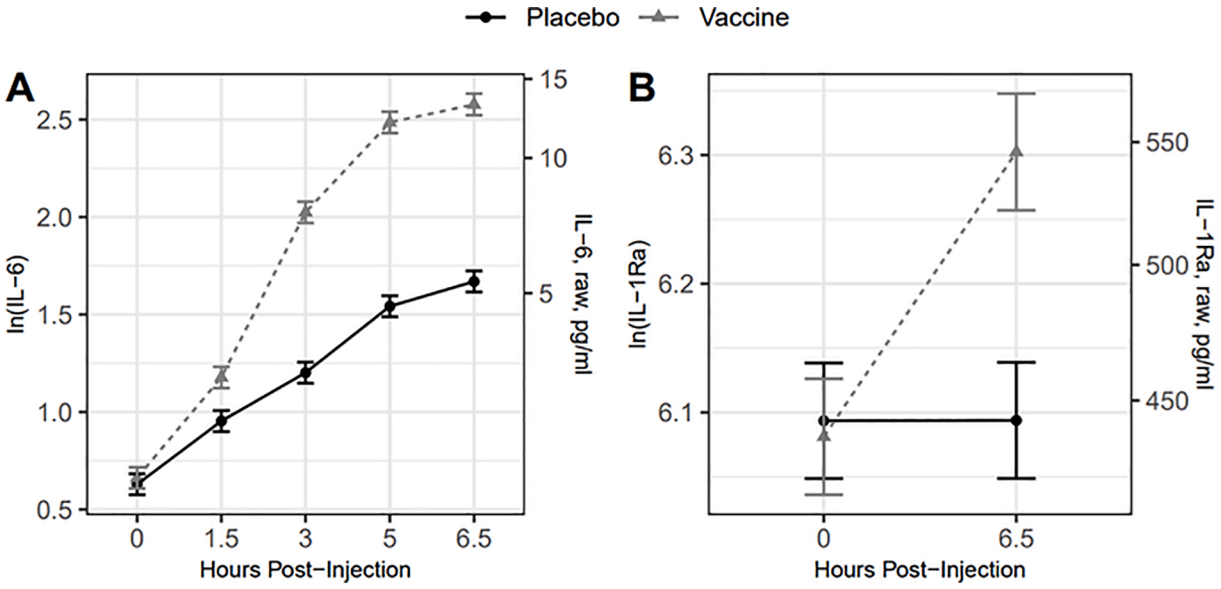

Fig. 2. Trajectories of Cytokines. Compared to the placebo, the typhoid vaccine provoked a rise in IL-6 and IL-1Ra ($ps < 0.0001$). These inflammatory markers were significantly elevated for vaccine compared to placebo at all post-baseline measurement timepoints.

3.4. Implicit task performance

Task errors did not differ between the vaccine ($M = 1.65$, $SE = 0.14$) and placebo ($M = 1.65$, $SE = 0.14$) visits ($t(127) = -0.03$, $p = .98$). People responded faster to pull trials than push trials ($B = -6.33$, $SE = 2.57$, $t(2818) = -2.46$, $p = 0.014$), but there was no difference in task errors for push ($M = 1.60$, $SE = 0.13$) and pull ($M = 1.70$, $SE = 0.13$) trials ($t(314) = 0.87$, $p = 0.39$).

Neither the typhoid vaccine ($p > .40$) nor either inflammatory cytokine increase ($ps > 0.14$) interacted with emotion and/or trial type to predict implicit task performance. Moreover, neither the typhoid vaccine ($p = .34$) nor either inflammatory cytokine increase ($ps > 0.89$) independently predicted response times on the implicit task.

3.5. Explicit task performance

The vaccine did not interact with stimulus emotion ($p = .98$) nor did it independently predict explicit task performance ($p = .37$). Inflammatory marker changes throughout the day did not predict explicit ratings – either independently ($ps > 0.74$) nor in interaction with stimulus emotion ($ps > 0.31$). Null results depicting means for the vaccine and placebo for the primary outcome variables are depicted in Supplemental Fig. 2-4.

4. Discussion

In the first randomized, placebo-controlled trial examining the effects of a mild inflammatory stimulus on social avoidance behavior toward unfamiliar others with a variety of emotional expressions, our hypothesis that the typhoid vaccine or the resulting inflammatory response would be related to more implicit social avoidance was unsupported. Notably, these null findings emerged even though the inflammatory response peaked shortly before the implicit task. However, as expected, there was no relationship between this mild inflammatory stimulus or the resulting inflammatory response and ratings of social connectedness or explicit social behavior.

4.1. Social disconnection

As hypothesized, our null results with respect to social disconnection align most closely with two recent single-arm trials among healthy adults who received another relatively mild inflammatory stimulus – the influenza vaccine. In those studies, participants did not have any post-vaccination changes in feelings of social disconnection (Kuhlman et al., 2018) nor self-reported motivation to socially engage with close others or with vaguely recognizable celebrities (Jolink et al., 2022). Similarly, we found that the typhoid vaccine did not change self-reported feelings of social connection, compared to placebo. Our results, when combined with these prior findings, suggest that the acute inflammatory response to vaccination does not impact either social motivation or feelings of social disconnection. In contrast, LPS, a stronger inflammatory stimulus, promotes feelings of social disconnection (Eisenberger et al., 2010; Moieni et al., 2015).

These conflicting results demonstrate that the inflammatory stimulus's strength is paramount in determining whether social experience is affected. In trials that captured a signal for social disconnection, even the relatively low dose of LPS (0.8 ng/kg of body weight) caused IL-6 to rise to well over 100 pg/ml two to three hours post-vaccination, at the same time that sickness symptoms, depressed mood, and feelings of social disconnection also reached their peak (Eisenberger et al., 2010; Moieni et al., 2015). In stark contrast, in the trials with null results for social motivation and disconnection, the influenza vaccine caused mean IL-6 levels to increase by only 1.2 pg/ml (Jolink et al., 2022) and 0.3 pg/ml (Kuhlman et al., 2018) 24 h after the vaccine – the established timeframe for the peak inflammatory response (Radin et al., 2021). We had similar null results in our trial, in which the peak IL-6 response to the typhoid vaccine was below 15 pg/ml. It is reasonable to expect divergent social outcomes given these vast differences in inflammatory stimuli, yet the question remains as to which type of stimulus is more informative from a clinical perspective, discussed in greater detail below.

4.2. Social avoidance behavior

With respect to social avoidance behavior, we did not find a relationship between rises in IL-6 following the vaccine and socially avoidant behavior toward emotional facial stimuli featuring unfamiliar others, in contrast to the Jolink et al. study (Jolink et al., 2022), which found that healthy people with stronger inflammatory responses to an influenza vaccine were more accurate in avoiding vaguely familiar celebrities. Unlike the facial stimuli used in that study, ours were emotionally valenced, completely unfamiliar strangers, and were not situated among stimuli featuring close others. Presenting unfamiliar others in quick succession of close others may help to draw out approach/avoidance tendencies (Inagaki et al., 2015). These methodological differences may explain the divergent results and therefore are notable for future work. Another prior study that revealed an inflammatory stimulus's effect on social behavior utilized a relatively high dose of endotoxin (Lasselin et al., 2018b), and the magnitude of the inflammatory response likely explains these discordant results.

The above mixed results reflect the fact that social avoidance is a nebulous term that refers to a variety of underlying mechanisms, processes, and behaviors, and its relationship with inflammation depends upon several factors – including context as well as the individual's sex and degree of familiarity with the target (Eisenberger et al., 2017; Inagaki et al., 2015; Irwin and Eisenberger, 2017; Lasselin, 2021; Lasselin et al., 2018a). In terms of context, sickness behaviors reflect a reorganization of priorities in line with conserving energy to fight the pathogen, but sickness is inherently a motivational state that contextual factors (e.g., an unsafe environment) can override (Cohn and de Sá-Rocha, 2006; Lasselin, 2021; Lopes, 2014). The current study's within-subjects crossover design largely negates this concern because each woman's performance after the vaccine was compared to her performance after receiving the placebo injection, and women completed the social avoidance tasks after completing the same full-day uniform protocol at each visit.

4.3. Clinical relevance of inflammatory stimuli

Compared to endotoxin, mild inflammatory stimuli (e.g., typhoid and influenza vaccines) may be more realistic approximates of the proinflammatory cytokine levels present in the inflammatory subtype of depression (Lindsay, 2022). Meta-analytic evidence suggest that the weighted mean difference in IL-6 levels for depressed versus non-depressed people is 1.78 pg/mL with raw IL-6 means in depressed people never exceeding 16 pg/ml in individual studies (Dowlati et al., 2010) – on par with the inflammatory response magnitude in the current study. Granted, the inflammation underlying inflammation-associated depression is more chronic than the short-lived inflammatory responses to vaccination: IL-6 peaks two hours after an endotoxin injection, between six and eight hours post-typhoid vaccine, and 24 h post-influenza vaccine, and returns to baseline in the same order – four to six hours following endotoxin, within 24 h following typhoid vaccination, and days to weeks for the influenza vaccine (Copeland et al., 2005; Paine et al., 2013; Radin et al., 2021). Although the inflammation is shorter-lived in vaccination models than in depression, the current study demonstrates that at a comparable *magnitude* of inflammation, changes in social behavior may not be expected.

Another important consideration when considering paradigms to model the inflammatory subtype of depression: Steep endotoxin-induced inflammatory responses correspond with many sickness symptoms (e.g., malaise, nausea) that are not usually present in depression.

Therefore, such paradigms may simply demonstrate the intuitive connection between the sickness symptoms and social withdrawal (Muscatell and Inagaki, 2021b). Although endotoxin is most commonly used in the sickness behavior model of depression, milder inflammatory stimuli that do not provoke as many intense sickness symptoms may provide a more accurate model. Even so, it is important to note that unlike other trials utilizing the typhoid vaccine as an inflammatory stimulus (e.g., Wright et al., 2005), we did not find vaccine-related mood alterations, compared to placebo, perhaps limiting its relevance to the inflammatory subtype of depression. The length of our protocol (9.5 continuous hours) may provoke a lower mood across all participants (Supplemental Fig. 1), thereby possibly masking a vaccine-related effect; another trial which found vaccine-related mood degradations across the day allowed participants to go about their daily activities as usual, rather than being confined to a hospital research unit (Wright et al., 2005). Nonetheless, it is entirely possible that only inflammatory stimuli that dampen mood in turn affect social processes.

4.4. Strengths and limitations

This is the first large, randomized, placebo-controlled crossover trial to examine the effect of an inflammatory stimulus on feelings of social disconnection and social approach/avoidance behavior. The within-subjects crossover design and use of mixed linear models to account for repeated measurements are strengths of this study. Also, the sample size is much larger than previous trials that detected a signal (Eisenberger et al., 2010; Jolink et al., 2022), suggesting that our null results are not due to a lack of statistical power. Even so, it is important to consider that participants were female breast cancer survivors. It is unclear whether these findings generalize to a non-cancer or male population; yet prior research suggests that if the typhoid vaccination impacts feelings of social connection or social avoidance, it would be most evident in a female sample like ours (Lasselin et al., 2018a). Along the same line, participants were mostly White people, and further study among a more diverse population is needed. Further, it is unclear how well our computerized social avoidance behavior tasks translate to in-person social interactions, and we did not include stimuli featuring close others. An additional consideration is that inflammation may impact psychomotor speed, calling into question the use of reaction time to index implicit social avoidance; yet, we did not observe slower reaction times across visits and even stronger inflammatory stimuli may not promote psychomotor slowing (Handke et al., 2020).

4.5. Conclusions

In this randomized, crossover trial, neither the typhoid vaccination nor the resulting inflammatory rise predicted social avoidance behavior toward unfamiliar others or feelings of social disconnection. Although stronger inflammatory stimuli like LPS may affect social processes, our findings suggest that lower levels of inflammation may not shape social experience or avoidance behavior toward strangers.

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.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2022.09.021.

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