Brain, Behavior, and Immunity 95 (2021) 444–453

Contents lists available at ScienceDirect

Brain Behavior and Immunity

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

Exploring neural mechanisms of the health benefits of gratitude in women: A randomized controlled trial

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ARTICLE INFO

Keywords:
Gratitude
Intervention
Inflammation
Health
fMRI

ABSTRACT

Background: Gratitude has received growing interest as an emotion that can bring greater happiness and health. However, little is known about the effects of gratitude on objective measures of physical health or the neural mechanisms that underlie these effects. Given strong links between gratitude and giving behavior, and giving and health, it is possible that gratitude may benefit health through the same mechanisms as giving to others. Thus, this study investigated whether gratitude activates a neural 'caregiving system' (e.g., ventral striatum (VS), septal area (SA)), which can downregulate threat responding (e.g., amygdala) and possibly cellular inflammatory responses linked to health.

Methods: A parallel group randomized controlled trial examined the effect of a six-week online gratitude (n = 31) vs. control (n = 30) writing intervention on neural activity and inflammatory outcomes. Pre- and post-intervention, healthy female participants (ages 35–50) reported on support-giving behavior and provided blood samples to assess circulating plasma levels and stimulated monocytic production of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6)). Post-intervention, participants completed a gratitude task and a threat reactivity task in an fMRI scanner.

Results: There were no significant group differences (gratitude vs. control intervention) in neural responses (VS, SA, or amygdala) to the gratitude or threat tasks. However, across the entire sample, those who showed larger pre- to- post-intervention increases in self-reported support-giving showed larger reductions in amygdala reactivity following the gratitude task (vs. control task). Additionally, those who showed larger reductions in amygdala reactivity following the gratitude task showed larger pre-to-post reductions in the stimulated production of TNF-α and IL-6. Importantly, gratitude-related reductions in amygdala reactivity statistically mediated the relationship between increases in support-giving and decreases in stimulated TNF-α production.

Conclusion: The observed relationships suggest that gratitude may benefit health (reducing inflammatory responses) through the threat-reducing effects of support-giving.

1. Introduction

Gratitude is a positive emotion that can arise when one receives kindness, or something of value, from another person (Algoe et al., 2016). Although fleeting, this emotional response to another person's kindness plays an important role in forming and maintaining social relationships (Algoe et al., 2008), and is a powerful driver of prosocial behavior (Bartlett & DeSteno, 2006). Over the past few decades, gratitude has also received growing interest as an emotion that can bring greater happiness and health. Gratitude has been linked to improvements in mental health and well-being (Wood et al., 2010) as well as self-reported markers of health (e.g. decreases in perceived stress (Wood et al., 2008), and better sleep quality (Wood et al., 2009)). However, we know relatively little about the effects of gratitude on health-relevant

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https://doi.org/10.1016/j.bbi.2021.04.019
Received 15 January 2021; Received in revised form 5 April 2021; Accepted 26 April 2021
Available online 28 April 2021

physiology, or the neural mechanisms that underlie those effects (Boggiss et al., 2020). The current study aimed to address these gaps in the literature by examining whether gratitude, through its ability to inspire prosocial behavior, activates a caregiving neural system, which in turn reduces cellular inflammatory responses linked to health (S. L. Brown & Brown, 2015; Inagaki, 2018).

1.1. Gratitude and prosocial behavior

A growing body of work suggests that gratitude functions to strengthen supportive social relationships (Algoe, 2012) and supports the theory that gratitude evolved to facilitate prosocial behavior, or behavior intended to help others (McCullough et al., 2008). For example, experimentally induced gratitude has been found to reliably increase helping behavior in laboratory settings (Tsang, 2006), even when it comes at a cost to the self (Bartlett & DeSteno, 2006). Outside of the laboratory, gratitude has been shown to increase effortful acts of kindness (Layous et al., 2017) and the provision of emotional support to others (Emmons & McCullough, 2003) in daily life. Additionally, the gratitude intervention in the current study led to increases in support-giving over the course of the six-week intervention (Moieni et al., 2019).

1.2. Prosocial behavior and health

A robust literature supports a link between prosocial behavior and a wide range of positive health outcomes (Konrath & Brown, 2013). Cross-sectional as well as large-scale longitudinal studies have shown that giving support to others is associated with markers of physical health, including lower systolic and diastolic blood pressure (Piferi & Lawler, 2006), morbidity (Brown et al., 2005), and reduced risk of mortality (Brown et al., 2003). Moreover, evidence from randomized controlled trials suggests that interventions that increase prosocial behavior can lead to improvements in inflammatory outcomes associated with health (Moieni et al., 2019; Nelson-Coffey et al., 2017; Schreier et al., 2013). Thus, to the extent that gratitude triggers support-giving behavior, gratitude should affect inflammatory processes through the same neural mechanisms involved in giving to others.

1.3. Caregiving system as a neural mediator of the link between gratitude and health

In light of the strong connections between gratitude and prosocial behavior, and between prosocial behavior and health, it is possible that gratitude may benefit health through the activation of a neural caregiving system, which promotes prosocial behavior and reduces threat responding to facilitate adaptive caregiving during threat. Animal work has shown that caregiving behavior relies on reward-related neural regions, including the ventral striatum (VS) and septal area (SA), such that lesions to either the VS or SA critically disrupt maternal behavior (Hansen, 1994; Slotnick & Nigrosh, 1975). In humans, VS activity has been associated with responsive maternal behavior (Strathearn et al., 2009), and giving support to a loved one was found to increase neural activity in both the VS and SA (Inagaki & Eisenberger, 2012). Interestingly, the SA is also involved in reducing fear and threat responding (Thomas, 1988), and has direct inhibitory connections to the amygdala (Adolphs et al., 1995; Melia et al., 1992), a neural region involved in threat responding.

Indeed, this dampening of amygdala reactivity by caregiving-related neural regions may help explain the link between support-giving and health (Eisenberger, 2013; Inagaki, 2018). In support of this idea, increased SA activity during support-giving was found to relate to decreased amygdala activity (Inagaki & Eisenberger, 2012), and greater self-reported support-giving was associated with reduced amygdala reactivity to a stress task (Inagaki et al., 2016). As part of a neural 'alarm system,' the amygdala plays a role in triggering threat-related physiology, such as inflammation (Eisenberger & Cole, 2012; Irwin & Cole, 2011), which can promote disease over time (Furman et al., 2019). Thus, reducing amygdala reactivity should, at least in part, reduce threat-related physiology and health outcomes. Taken together, this evidence suggests that the threat-reducing capacity of the neural caregiving system may be a key mechanism underlying the health benefits of support-giving. To the extent that gratitude also activates this system, gratitude may affect downstream threat-related physiology (i.e. inflammation) through this same pathway.

1.4. The present study

A six-week gratitude intervention was conducted in healthy, middle-aged women in order to investigate whether gratitude could improve inflammatory outcomes through the activation of a neural caregiving system, and a corresponding reduction in threat-related neural responding. Previously published analyses of the behavioral and inflammatory outcomes of this intervention showed that, while the gratitude intervention led to significant increases in support giving, it did not have a significant effect on inflammatory outcomes. However, when collapsing across the entire sample, greater increases in support-giving were associated with greater decreases in the percentage of stimulated measures of inflammation (Moieni et al., 2019). The present study examined the neuroimaging component of this intervention in order to: 1) assess whether there were between-group differences in neural activity as a function of the intervention, and 2) explore the neural regions underlying the previously reported link between support-giving behavior and reduced inflammation across the full sample.

Thus, the current research examined: 1) whether the gratitude intervention led to enhanced activity in caregiving-related neural regions (VS, SA), 2) whether increases in caregiving-related neural activity (VS, SA) or self-reported support-giving over the course of the intervention across the full sample predicted reduced threat-related neural responding (e.g., amygdala activity to negative stimuli), and 3) whether differences in levels of threat-related neural activity mediated the previously reported relationship (Moieni et al., 2019) between increases in support-giving and decreases in cellular inflammatory responses. The first aim was made a priori (i.e. before data collection began, as part of the original aims of the gratitude intervention), and the latter two were made following the results reported in Moieni et al., 2019, but a priori to analyzing the neuroimaging data in the current study.

2. Materials and methods

2.1. Participants

The study sample size was determined by the sample size needed to produce significant gratitude-related neural activations in our two regions of interest, the VS and SA. Based on our pilot study examining a separate task that induced gratitude, we calculated (using fMRIpower; http://www.fmripower.org/) that with 25 subjects per group we would have at least 80% power to detect an effect size of Cohens' $d = 0.88$ in the VS and SA. Thus, a target sample size of 40 participants per group was defined in order to obtain usable neural activation data for at least 25 participants per group. After exclusions, no between-group analyses involved <28 participants per group, and no whole-sample analysis involved <58 participants across both groups.

Participants were recruited from UCLA and the greater Los Angeles community, and were screened via a structured phone interview. To be eligible, participants had to meet the following inclusionary criteria: (a) be a healthy woman between 35 and 50 years of age, (b) be fluent in English, and (c) have access to a computer and the Internet to complete the weekly online assessments. This study was conducted in women only for several reasons. Because this study is the first to explore the neural correlates of gratitude felt towards close others, and to investigate neural-immune regulation in the context of gratitude, we elected to reduce noise by selecting a more homogenous sample. Additionally,

there is evidence to suggest that women show a greater pro-inflammatory response to acute stress as well as greater behavioral consequences of inflammation (e.g., depressed mood and social disconnection) than men (Bekhbat and Neigh, 2018; Moieni et al., 2015). Exclusionary criteria relevant to the neuroimaging component included claustrophobia, left-handedness, and metallic implants that would jeopardize safety in the MRI scanner. Other exclusionary criteria included chronic physical or mental health conditions that might influence study outcomes (e.g. autoimmune disorders, major depression), regular use of certain prescription medications (e.g. anti-inflammatory medications, psychotropic medications, steroids, opioids), body mass index (BMI) >30, regular smoking, excessive use of caffeine (>600 mg/day), and recent nightshift work or time zone shifts (>3 h).

Our final study sample consisted of 76 middle-aged women (mean age = 42.6 ± 4.8 years) who were randomized into either a gratitude condition or control condition for 6 weeks. Of these participants, eight (four in each condition) did not complete the study. Of the 68 participants who completed the study, two did not complete the neuroimaging component (one due to claustrophobia, and one due to a metallic implanted device not reported at enrollment), leaving a final sample of 66. All participants provided written consent before participating in this study, and all procedures were approved by the UCLA Human Subjects Protection Committee. A CONSORT flow diagram illustrates the flow of participants through each phase of the trial (Fig. 1), and a complete CONSORT checklist is included in the Supplementary Material.

2.2. Procedure

The study design is a parallel group randomized controlled trial, following the protocol previously reported in Moieni et al., 2019.

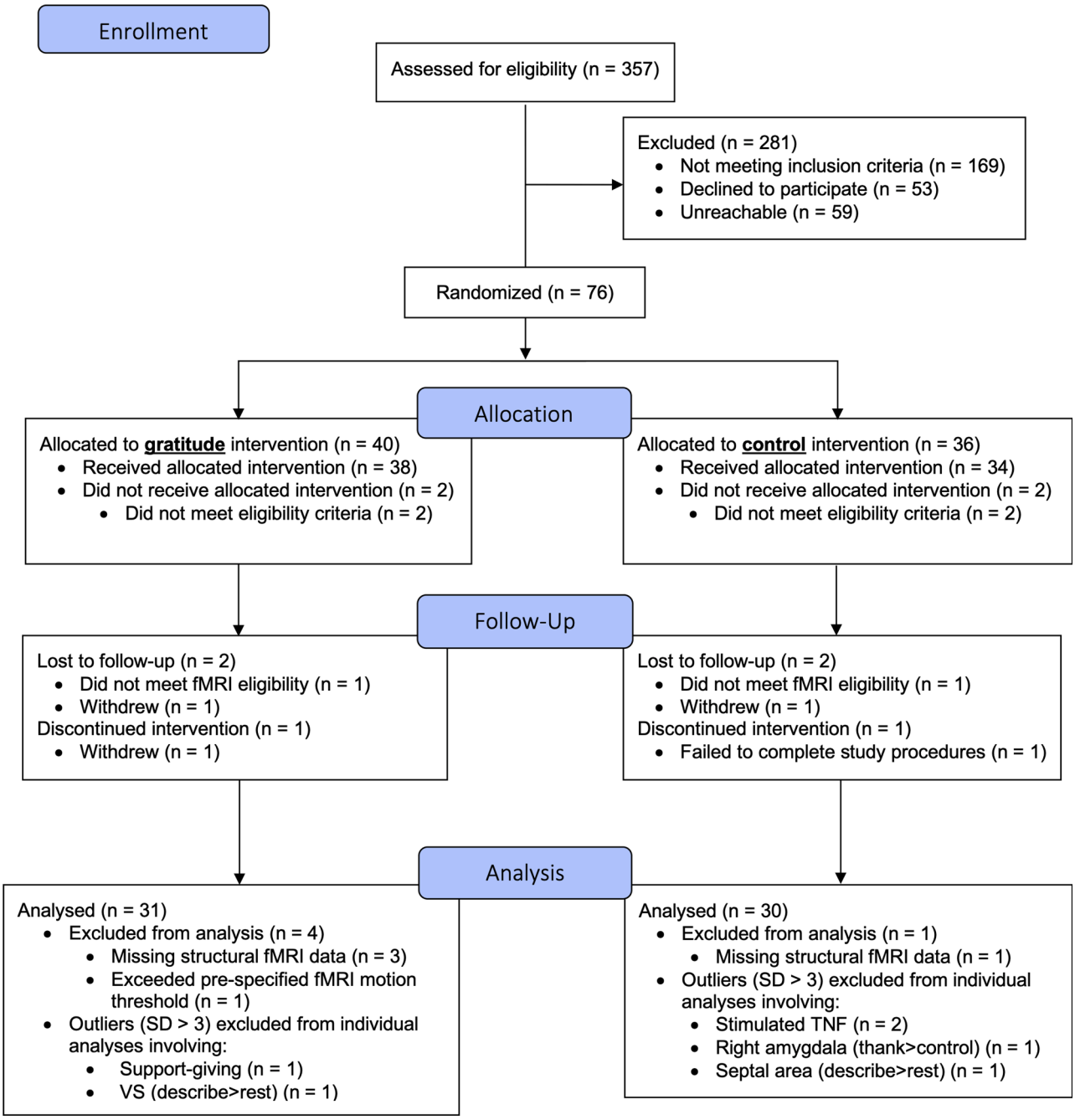

Fig. 1. CONSORT flow chart of participants retained at each phase of the trial.

Subjects were screened, provided informed consent, and completed a pre-intervention session, 6 weeks of gratitude or control writing sessions, and a post-intervention session. Blood samples to assess inflammatory measures were obtained and self-report questionnaires were completed at both the pre- and post-intervention sessions.

The pre-intervention session took place at the UCLA Clinical and Translational Research Center (CTRC), where a nurse blind to condition drew blood for assessment of inflammatory measures. The timing of pre- and post- intervention blood draws was kept consistent (all samples were scheduled for 9am), and if a participant was feeling sick, their session was rescheduled for another day. Participants then completed self-report questionnaires (including a measure of support-giving), and were given instructions for the intervention. After completing the pre-intervention session, participants were randomized in blocks of 10 using a random assignment generator into either a gratitude or control condition. The study coordinator generated the random allocation sequence, enrolled participants (along with graduate students and trained research assistants), and assigned subjects to interventions. Researchers involved in randomization and enrollment were not blinded after assignment to interventions; however, they did not interact with participants during the intervention period outside of scheduling the follow-up visit, and were not involved in data analysis. Participants were blind to their condition and the purpose of the intervention throughout the six-week intervention.

Once per week for six weeks (on Sundays), participants in both conditions were emailed a link to complete an online writing assignment. Given that we were investigating support giving as a key mechanism, all prompts in the gratitude condition were intentionally designed to focus participants on their feelings of gratitude towards other people, rather than objects or good circumstances in general. Participants in the gratitude condition received a prompt focusing participants on their feelings of gratitude for people in their life (e.g., "Think of someone in your life who you feel like you have never fully or properly thanked for something meaningful or important that they did for you…"), and participants in the control condition received a prompt intended to be a descriptive, neutral writing prompt (e.g., "Think about the longest distance that you walked today…"). Based on prior research suggesting that variety can increase the efficacy of positive psychological interventions (Sheldon et al., 2013), participants received slightly different prompts each week. They were asked to complete the writing session at a time they could sit alone without distractions, and to spend at least 5–10 min writing, although they were welcome to spend longer if they wished. They were also told not to worry about grammar, and encouraged to immerse themselves in the writing experience. Participants also engaged in a 'booster,' in which they reviewed their writing later on in the week (on Wednesday evenings). This was meant to reinforce feelings of gratitude for participants in the gratitude condition. All six writing prompts from the gratitude and control interventions are included in the Supplementary Material.

After the six-week intervention, participants returned for the post-intervention blood draw, questionnaires, and then completed an fMRI scan at the Ahmanson-Lovelace Brain Mapping Center. To examine the neural correlates of gratitude and its possible role in reducing threat-related neural activity, the fMRI scan involved a task designed to elicit feelings of gratitude intermixed with a threat reactivity task widely used to elicit amygdala activation (Tottenham et al., 2009).

2.3. Measures

2.3.1. fMRI task and image acquisition

To examine the neural correlates of gratitude and the effect of gratitude on threat-related neural activity, participants underwent an fMRI scan while they completed a novel gratitude task designed for this study intermixed with a standard threat-reactivity task. For the gratitude task in the scanner, participants were asked prior to beginning the 6-week study to provide the names of the eight people they feel most grateful to have in their life. The experimenters then selected six of these (excluding young children, pets, and deceased close others) to include in the gratitude task in the scanner.

The gratitude task consisted of presenting participants with two conditions: a "thank" condition in which participants were shown the name of one of the close others, and instructed to think about why they are grateful to have this person in their life and a "describe" (control) condition in which participants were shown the same name, but instead asked to mentally describe the physical appearance of this person. Each of these conditions was displayed once per close other, for a total of six times each. Each block began with an instructions slide presented for 4 s, then a "thank" or "describe" condition which lasted 20 s, followed by 2 s of rest (a fixation crosshair) before the next block. Following each of these conditions, participants saw a threat-related block for 16 s. These blocks consisted of viewing 8 threatening facial expressions (from the NimStim database (Tottenham et al., 2009), a widely used threat-reactivity task), for 2 s each. Given our hypotheses of the effect of gratitude on threat responding, threat-related blocks all contained items from the same set of stimuli but were divided into three separate conditions: threat immediately following the "thank" condition, threat immediately following the "describe" condition, and threat which did not immediately follow either. The scan consisted of two runs, each presenting the thank-related blocks and describe-related blocks in a different order. The order of runs was also counterbalanced between participants. Finally, to get a sense of the variability in gratitude within and across participants during the gratitude task, participants were asked after completing the scan to write down what came to mind during the "thank" and "describe" conditions for each of their six close others.

Imaging data were acquired using a Siemens Trio 3.0 Tesla MRI scanner at the UCLA Ahmanson-Lovelace Brain Mapping Center. A T1-weighted MPRAGE anatomical image was acquired for functional image registration and normalization, as well as 404 functional T2-weighted EPI volumes (slice thickness = 3 mm, TR = 2000 ms, TE = 25 ms, flip angle = 90°). The dependent variables of interest are parameter estimates extracted from each region of interest.

2.3.2. Inflammatory assessments

Inflammatory outcomes were assessed as previously described (Moieni et al., 2019), using two complementary measures: (a) assessment of production of inflammatory cytokines by monocytes by flow cytometry following *in vitro* TLR-4 stimulation by lipopolysaccharide (LPS), and (b) circulating (plasma) levels of inflammatory cytokines. Stimulated monocyte production of cytokines was assessed using flow cytometry, following procedures previously described in (Irwin et al., 2006). In short, heparin-treated whole blood was mixed with 100 pg/mL LPS (Sigma, St. Louis, MO) and 10 μg/mL brefeldin A (Sigma) or brefeldin A only. Red blood cells were lysed and remaining cells were fixed with fluorescence-activated cell-sorting Lysis Buffer (BD Biosciences, San Diego, CA), then stored at –80 °C. When ready to process, samples were thawed and stained for intracellular cytokines. Our dependent variables of interest were the percentage of monocytes producing interleukin-6 (IL-6), the percentage of monocytes producing tumor necrosis factor-α (TNF-α), and the percentage of monocytes coproducing IL-6 and TNF-α. In vivo circulating levels of cytokines were assessed using the following ELISA kits from R&D Systems (Minneapolis, MN): Human IL-6 Quantikine High Sensitivity ELISA (HS600B/SS600B, limit of detection 0.2 pg/mL), with an inter-assay CV of 6.7% and mean intra-assay CV < 1%, and Human TNF-alpha Quantikine High Sensitivity ELISA (first generation; limit of detection 0.5 pg/mL), with an inter-assay CV of 8.1% and mean intra-assay CV of 5.2%.

2.3.3. Support-giving

Giving social support to others was measured with the 2-Way Social Support Scale (2-Way SSS) (Shakespeare-Finch & Obst, 2011) pre- and post-intervention. This scale measures giving and receiving emotional and instrumental support, asking participants how much they agree with

statements such as "I give others a sense of comfort in times of need," rated on a 6-point Likert scale ranging from 0 (*not at all*) to 5 (*always*). The measure used in this study combined the giving instrumental support and giving emotional support subscales into one overall measure of giving social support by averaging items across the two subscales at pre- and post-intervention. One item from the giving emotional support subscale ("People close to me tell me their fears and worries") was missing due to technical issues; however, the reliability of the giving support measure was high ($\alpha = 0.84$).

2.4. Data analyses

Imaging data were pre-processed in SPM12 (The Wellcome Centre for Human Neuroimaging, 2014) using the DARTEL procedure. This procedure included image realignment to correct for head motion, normalization into Montreal Neurological Institute (MNI) space, and spatial smoothing using a 8 mm Gaussian kernel full-width at half-maximum to increase signal-to-noise ratio (code template and details on specific algorithmic steps are provided on github). Following pre-processing, a general linear model was constructed for each participant, in which activation during each active task block was convolved with a canonical hemodynamic response function. Regressors of interest coded for type of block ("thank," "describe," threat following "thank," threat following "describe," and control threat), and six motion parameters and their derivatives were included as covariates. To control for time points with significant levels of noise or motion, acquisitions that exceeded 3 SDs of global signal change or 1.5 mm of motion in any direction were included as additional nuisance regressors. This approach has been found to decrease variance in parameter estimates, reduce residual error in GLM estimation, and improve the quality of task fMRI data across a range of parameter spaces (Siegel et al., 2014). Following estimation, linear contrasts were computed for each participant according to pre-specified comparisons ("thank" vs. implicit baseline, "describe" vs. implicit baseline, "thank" vs. "describe," threat following "thank" vs. threat following "describe," and threat following "thank" vs. control threat). Contrast images for each participant were then entered into random effects analyses at the group level for statistical inference.

Given our hypotheses concerning caregiving-related and threat-related neural activity, *a priori* regions-of-interest (ROI) analyses were performed focusing on the ventral striatum (VS), septal area (SA), and amygdala. VS and SA ROIs were structurally defined based on previous work on the neural correlates of giving support (Inagaki & Eisenberger, 2012); the VS was made by combining the caudate and putamen from the AAL and constraining at $-24 < x < 24$, $4 < y < 18$, and $-12 < z < 0$, and the SA ROI was created by dilating a sphere with a 5 mm radius centered at (0, 2, −4) using MarsBaR (Brett et al., 2016). Amygdala ROIs were defined anatomically based on the automated anatomical labeling atlas (AAL) (left amygdala: $-30 < x < -12$, $-8 < y < 4$, $-28 < z < -12$; right amygdala: $18 < x < 36$, $-8 < y < 8$, $-30 < z < -12$). When examining differences between the "thank" and "describe" conditions, one-tailed t-tests were conducted to test specific directional hypotheses.

Due to the known effects of BMI on markers of inflammation, we controlled for BMI in all analyses involving inflammatory markers (regardless of significance). Circulating cytokine values were not normally distributed at any time point, and as a result were natural log-transformed for analyses. Finally, participants whose scores were over 3 *SD*s on variables of interest were removed from the respective analyses to improve accuracy and robustness of the results to replication (Osborne & Overbay, 2004). In sum, there were six participants who scored above 3 SDs on a variable of interest (see Fig. 1 for more details), however no more than three participants total were removed from any single analysis, and their removal did not affect significance.

All analyses were conducted using *statsmodels*, a standard library for statistical analysis in Python (Seabold & Perktold, 2010), and Pingouin, a statistical package written in Python (Vallat, 2018). When testing between-group effects on post-intervention measures, controlling for pre-intervention values, analyses of covariance (ANCOVA) were conducted. ANCOVA (vs. change from baseline) is the recommended, more powerful strategy to detect longitudinal changes in randomized studies (Van Breukelen, 2006). When collapsing across intervention groups and testing the effect of a continuous variable on post-intervention values while controlling for pre-intervention values, linear regression was performed.

For mediation analyses, PyProcessMacro, a Python implementation of the PROCESS macro for SPSS (Hayes, 2012) was used to estimate 95% confidence intervals using bootstrapping (10,000 samples). It should be noted that we are not examining a 'time-lagged' mediational model, in which gratitude-related processes at time 1 alter neural sensitivity at time 2 which then alter inflammatory outcomes at time 3, but rather a 'levels of analysis' mediation, in which we are exploring whether neural activity to a gratitude task underlies the relationship between gratitude-related processes (in this case, post-intervention support-giving) and inflammatory outcomes. Although mediational analyses can have the connotation of being time-lagged, our tests of statistical mediation fit with the standard definition of mediation, in which one variable (in this case, neural responses) carries the influence of an independent variable (post-intervention support giving) on a dependent variable (post-intervention inflammatory activity).

3. Results

3.1. Participant characteristics

Recruitment and data collection took place between May 2013 and November 2014. Of the 66 participants who completed the intervention and neuroimaging session, four were missing structural fMRI MPRAGE acquisition data, and were excluded from preprocessing and analyses. One participant was excluded due to her fMRI data surpassing a pre-specified motion threshold (over 50% of total acquisitions exceeding 0.5 mm framewise displacement). This left a total sample of 61 participants with usable neuroimaging data that were analyzed (mean age = 43.1 ± 4.8 years). Of these participants, 31 had been randomized into the intervention condition, and 30 had been randomized into the control condition. The intervention and control group did not significantly differ at pre-intervention on average age, BMI, support-giving, or inflammatory measures (*ps* > 0.1, Table 1). However, the groups were significantly different on racial/ethnicity distribution ($p < 0.01$). As a result, race/ethnicity was included as a covariate in all between-groups analyses.

3.2. Did the gratitude intervention increase activity in caregiving-related neural regions (VS, SA)?

We first examined whether the 6-week gratitude intervention increased caregiving-related neural activity during the gratitude task in the scanner by testing differences between the two study groups in VS and SA activity to the "thank" vs. "describe" conditions. Contrary to our hypotheses, the gratitude intervention (vs. control) did not lead to increases in caregiving-related neural activity to "thank" vs. "describe," controlling for race/ethnicity (left VS: $F(1, 56) = 0.11$, $p = .74$, $\eta^2 = 0.02$, right VS: $F(1, 57) = 0.01$, $p = .92$, $\eta^2 < 0.01$; SA: $F(1, 57) = 0.25$, $p = .62$, $\eta^2 < 0.01$). Therefore, subsequent analyses collapsed across both groups (for results separated by the intervention conditions, see Table 2).

Participants across the whole sample did not show significantly greater activity in the left or right VS during the "thank" (gratitude) condition compared to the "describe" (control) condition of the gratitude task (left VS: $t(59) = 1.46$, $p = 0.08$; right VS: $t(59) = 0.75$, $p = 0.23$). There was also no significant difference in activity between these conditions in the SA ($t(59) = 0.64$, $p = .26$). However, activity in each of these regions during the "thank" condition (vs. implicit baseline) was significant (left VS: $t(60) = 2.98$, $p < 0.001$, $d = 0.38$, 95% CI [0.03,

Table 1
Baseline (pre-intervention) characteristics of participants with usable neuroimaging data in the Gratitude and Control intervention groups.*

	Condition	
	Gratitude (N = 31)	Control (N = 30)
Age (years)	43.9 (5.0)	41.8 (4.3)
Body mass index (kg/m^2)	23.0 (3.0)	23.9 (3.4)
Race/Ethnicity		
Asian	4	6
African American	0	7
Latina/Hispanic	9	4
Caucasian	26	16
Other	1	1
Employed	27 (87.1%)	24 (80%)
Education		
High school degree or equivalent	1 (3.2%)	0 (0.0%)
Some college but no degree	2 (6.5%)	2 (6.7%)
College/post-graduate degree	28 (90.3%)	28 (93.3%)
Married	15 (48.4%)	11 (36.7%)
Has children	15 (48.4%)	19 (63.3%)
Support-giving (2-way SSS scores)	4.2 (0.7)	4.2 (0.6)
Inflammatory markers		
Plasma IL-6, pg/mL	1.4 (2.6)	1.2 (1.8)
Plasma TNF-α, pg/mL	0.8 (0.4)	0.9 (0.4)
Stimulated monocyte production of IL-6, % of monocytes	23.3 (11.2)	26.5 (16.4)
Stimulated monocyte production of TNF-α, % of monocytes	20.2 (8.1)	22.0 (14.0)
Stimulated monocyte coproduction of IL-6 and TNF-α, % of monocytes	11.8 (6.1)	14.1 (11.4)

*Data are mean (SD) or number (%).

Table 2
Neural activity (parameter estimates) for pre-determined contrasts (n = 61) in the Gratitude and Control intervention groups.*

	Condition	
	Gratitude (N = 31)	Control (N = 30)
Left ventral striatum (thank > describe)	0.08 (0.21)	0.006 (0.2)
Right ventral striatum (thank > describe)	0.032 (0.18)	0.006 (0.21)
Septal area (thank > describe)	0.01 (0.39)	0.066 (0.29)
Left amygdala (faces after thank > control)	0.005 (0.35)	0.015 (0.47)
Right amygdala (faces after thank > control)	0.011 (0.31)	−0.012 (0.34)

*Data are mean (SD). No differences between groups are significant.

0.16]; right VS: $t(59) = 2.5$, $p < 0.001$, $d = 0.32$, 95% CI [0.02, 0.14]; SA: $t(59) = 1.72$, $p < 0.05$, $d = 0.22$, 95% CI [−0.01, 0.17]). Activity in the left and right VS during the "describe" condition (vs. implicit baseline) was also significant (left VS: $t(59) = 2.21$, $p = 0.02$, $d = 0.28$, 95% CI [0.01, 0.13]; right VS: $t(59) = 1.96$, $p = 0.03$, $d = 0.25$, 95% CI [0, 0.11]), although activity in the SA was not ($t(59) = 1.3$, $p = 0.1$) (Fig. 2) (for results separated by the intervention conditions, see Table 2).

To further explore why caregiving-related neural activity was not greater during the "thank" condition relative to the "describe" condition, we conducted exploratory analyses testing the possibility that the "describe" instructions inadvertently primed caregiving-related psychological responses. We instructed 5 independent raters to rate each participant's post-scan comments as to how grateful, caring, and neutral the participants' responses seemed on a Likert scale from 1 (*not at all*) to 7 (*very much so*) (α = 0.79). The post-scan comments for the "describe" condition were rated on average as significantly more grateful (M = 2.77) and caring ($M = 3.49$) than the lowest point on the scale (1, *not at all*); they were also rated as significantly less neutral ($M = 4.62$) than the highest point on the neutral scale (7, *very much so*) ($ps < 0.001$). This suggests that the "describe" condition may not have been an effective control. As a result, in subsequent analyses we focused primarily on the

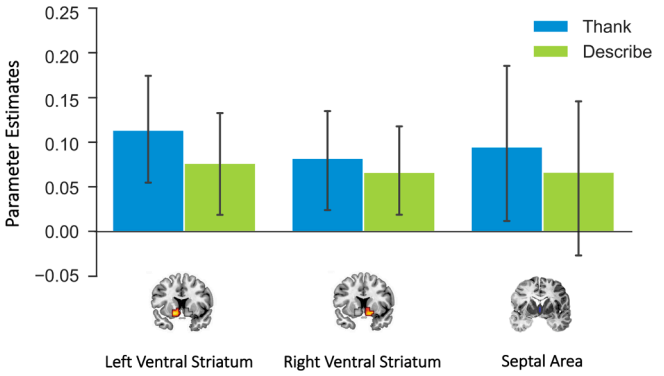

Fig. 2. Caregiving-related Neural Activity During a Gratitude Task. Mean parameter estimates from left ventral striatum (VS) and septal area (SA) regions of interest (ROIs) during the "thank" (gratitude) and "describe" (control) conditions, when compared to activity at rest (implicit baseline). Error bars reflect standard errors. Mean data shown are from across the entire sample (n = 61, collapsed across gratitude and intervention groups), from the fMRI scan and tasks performed at the post-intervention visit. Brain images illustrate the VS and SA regions of interest.

threat responses following the "thank" condition compared to the control threat condition that did not follow either "thank" or "describe," rather than the threat condition that followed the "describe" condition.

3.3. Did increases in support-giving or caregiving-related neural activity (VS, SA) predict reductions in threat-related neural activity (amygdala)?

Across the entire sample, greater increases in support-giving post-intervention, controlling for pre-intervention support-giving, predicted lower left amygdala activity during the threat condition that followed the "thank" condition vs. control ($b = -0.33$, $p = .03$, $R^2 = 0.09$, 95% CI [-0.61, −0.04]) (Fig. 3), but not right amygdala activity during the same condition ($b = 0.02$, $p = .88$). Contrary to our hypotheses, neither VS nor

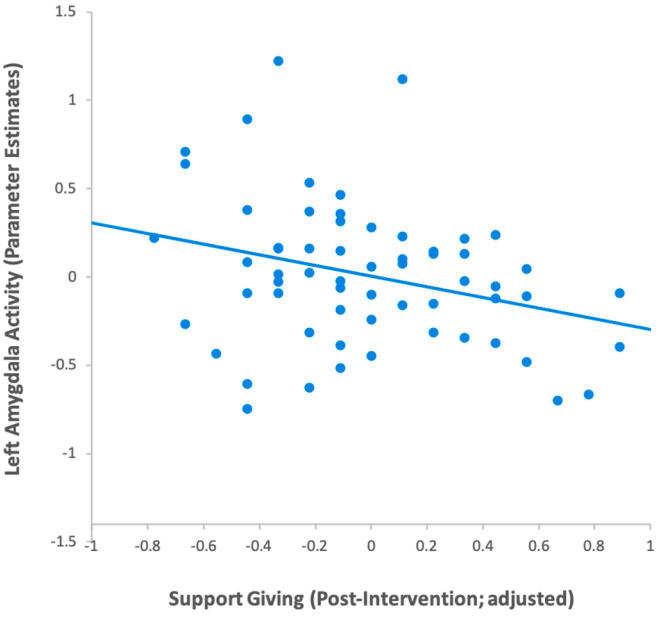

Fig. 3. Correlation Between Support-Giving and Gratitude-related Left Amygdala Activity During Threat. Partial correlation across the entire sample (n = 61) between giving support to others (post-intervention Social Support Scale score, controlling for pre-intervention scores), and left amygdala activity (parameter estimates) during the threat condition that immediately followed the Gratitude ("thank") condition (vs. control) at the post-intervention fMRI scan (r = -0.29, p = .02).

SA activity during "thank" (vs. implicit baseline) predicted left or right amygdala activity to the threat condition that followed the "thank" condition (vs. control) (*ps* > 0.05; results reported fully in Supplementary Table 1).

3.4. Did amygdala activity predict post-intervention inflammatory outcomes?

Greater reductions in left amygdala activity during the threat task that followed the thank condition (vs. control) predicted a greater reduction in the *in vitro* stimulated monocytic production of TNF-α (b = 9.52, $p < 0.001$, $R^2 = 0.35$, 95% CI [3.14–15.9]) (Fig. 4a) and a greater reduction in stimulated monocytes co-expressing TNF-α and IL-6 at post-intervention, (b = 5.01, $p < 0.05$, $R^2 = 0.29$, 95% CI [0.11–9.9]) (Fig. 4b), but not for monocytic production of IL-6 (b = 6.06, $p = 0.15$, $R^2 = 0.26$, 95% CI [−2.25–14.36]). This same relationship was not observed for the right amygdala (*ps* > 0.1). Moreover, neither left nor right amygdala activity predicted post-intervention circulating markers of inflammation (*ps* > 0.1). All analyses controlled for pre-intervention values and BMI, and all analyses are reported fully in Supplementary Table 2.

3.5. Did reduced amygdala activity mediate the relationship between increased post-intervention support-giving and reduced post-intervention inflammatory responses?

To examine whether reduced amygdala activity mediated the relationship between support-giving and reduced inflammatory responses, we first confirmed the relationship between support-giving and inflammatory activity within the current subset of 61 women with usable neuroimaging data (previously published analyses were conducted on 68 subjects; Moieni et al., 2019). Within the neuroimaging sample, increases in support-giving were associated with decreases in stimulated monocytes, adjusting for pre-intervention values and BMI (TNF-α: b = -9.93, $p = .007$, $R^2 = 0.38$; IL-6: b = -10.89, $p = .017$, $R^2 = 0.35$; TNF-α and IL-6: b = -7.04, $p = .008$, $R^2 = 0.41$). Next, we tested whether threat-related neural activity after the intervention might statistically mediate the effect of support-giving on these stimulated inflammatory measures. Left amygdala activity (during the threat condition that followed the "thank" condition vs. control) mediated the relationship between increased support-giving and decreased stimulated monocytic production of TNF-α (effect = −2.39, 95% CI [−7.06, −0.15]) Fig. 5. No mediation effect was seen for stimulated monocyte production of IL-6, nor for coproduction of TNF-α and IL-6. To provide evidence for the directionality of these relationships, the reverse pathway was also tested: whether post-intervention inflammatory activity predicted post-intervention support-giving, mediated by amygdala reactivity. This pathway was not significant (effect = −0.01, 95% CI [−0.01, 0.001]). All analyses controlled for BMI and pre-intervention levels of inflammatory measures and support-giving.

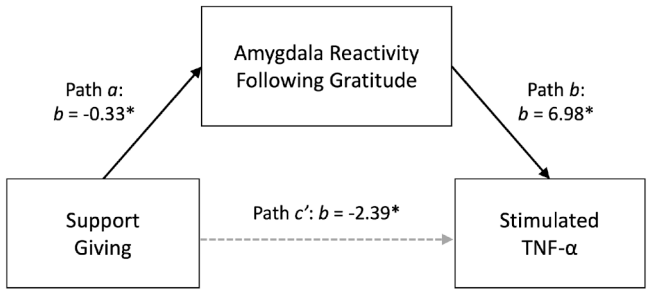

Fig. 5. Amygdala Reactivity as a Mediator of Psychological Support-Giving and Immunological Inflammatory Responses. Model showing the effect of post-intervention support-giving on post-intervention percentage of monocytes producing TNF-α following stimulation (controlling for pre-intervention values and BMI), as mediated by amygdala activity during the threat condition that immediately followed the Gratitude ("thank") condition (vs. control) during the post-intervention fMRI scan. The effect of support-giving on amygdala reactivity (path a) and the effect of amygdala reactivity on stimulated TNF-α controlling for support-giving (path b) comprise the indirect effect of support-giving on stimulated TNF-α through reductions in amygdala reactivity (path c').

3.6. Summary of key results

While the gratitude intervention did not appear to alter neural responses to the gratitude or threat reactivity tasks, several interesting relationships emerged across the whole sample. First, those who showed larger pre- to post-intervention increases in support giving also showed less left amygdala reactivity following the gratitude task. In other words, the more support participants gave over the course of the intervention, the more the gratitude task appeared to reduce amygdala-related threat responding. Second, those who showed greater reductions in amygdala reactivity following experiences of gratitude in the scanner also showed greater reductions in measures of stimulated TNF-α from pre- to post-intervention. Finally, we found evidence for a full mediation pathway linking these two effects, in which gratitude-related reductions in amygdala reactivity statistically mediated the previously observed relationship between support-giving and reductions in a stimulated measure of inflammation (TNF-α) from pre- to post-intervention.

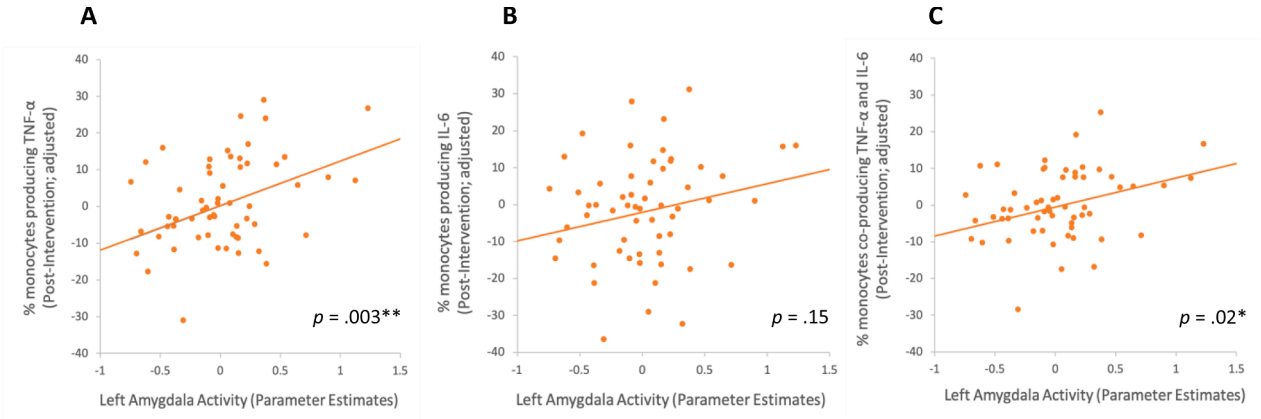

Fig. 4. Correlation Between Gratitude-related Left Amygdala Activity During Threat and Stimulated Inflammatory Responses. Partial correlations across the entire sample (n = 61) between left amygdala activity during the threat condition that immediately followed the Gratitude ("thank") condition (vs. control) at the post-intervention fMRI scan, and percent of stimulated monocytes producing TNF-α (r = 0.38, p = .003) (Panel A), producing IL-6 (r = 0.20, p > .1) (Panel B), and coproducing TNF-α and IL-6 (r = 0.29, p = .02) (Panel C) at post-intervention, adjusted for pre-intervention values and BMI.

4. Discussion

Although gratitude has been linked to numerous health benefits, we know relatively little about the neural mechanisms that underlie these effects. To address this gap in the literature, the current study explored possible neural-inflammatory regulation in the context of a gratitude intervention. Given links between gratitude and giving behavior, and between giving behavior and inflammation, we hypothesized that gratitude would activate a neural caregiving system, which would in turn reduce neural threat responding and predict decreases in cellular inflammatory responses.

We began by utilizing a novel, personalized fMRI gratitude task that elicited feelings of gratitude for loved ones, completed post-intervention, to evaluate the effects of the six-week intervention on neural caregiving systems (VS, SA activity) during experiences of gratitude. This task was immediately followed by a threat task to evaluate whether the gratitude task moderated amygdala reactivity. Contrary to our hypotheses, we did not find an effect of the gratitude intervention on neural activity in any of the neural regions examined here. One possible explanation for this lack of between-group differences may come from the fact that several participants in the control condition spontaneously reported that they believed they were in the gratitude condition, likely because they were asked before beginning the intervention to provide the names of eight people for whom they were grateful. This may also help explain the prior observation that the gratitude intervention did not have a total effect on inflammatory outcomes.

We then examined the entire sample as a single group, and investigated whether the "thank" condition of the gratitude fMRI task (in which participants mentally thanked loved ones they previously identified as being grateful for) elicited greater caregiving-related neural activity (VS, SA) than the "describe" condition (in which participants mentally described the same loved ones' physical appearance). Contrary to our hypotheses, this was not the case. However, both conditions led to greater activity in the VS relative to implicit baseline (i.e. rest), and the "thank" condition additionally led to greater SA activity relative to implicit baseline. Upon further investigation, we discovered that the "describe" condition, although intended to be a neutral control condition, generated feelings that were somewhat grateful and caring, and clearly not neutral. Although the VS and SA are known to be involved in various forms of rewarding experience (Gottfried, 2012), they are also known to be important to maternal caregiving behavior (Hansen, 1994; Slotnick & Nigrosh, 1975) and to activate in response to giving support to close others (Inagaki & Eisenberger, 2012). Hence, these results are consistent with the hypothesis that gratitude activates neural regions involved in caregiving behavior, although they do not speak to the effect of feeling grateful over and above merely bringing a person to mind for whom one is grateful.

We next explored whether increases in support-giving over the course of the intervention, or greater caregiving-related neural activity (VS, SA) during the gratitude task in the scanner, related to subsequent decreases in amygdala reactivity during a threat task that immediately followed the "thank" condition of the gratitude task (relative to control). As hypothesized, pre- to post-intervention increases in support-giving across the sample predicted greater gratitude task-related reductions in left amygdala reactivity. While prior work has established a link between support-giving and reduced amygdala-related threat-responding (Eisenberger, 2013; Inagaki et al., 2016), this is the first study to provide evidence for a link between support-giving and a specific immediate effect of gratitude on amygdala reactivity, supporting our hypothesis that gratitude may activate the same threat-reducing pathways involved in support-giving.

Importantly, we found that this gratitude-related dampening of left amygdala reactivity significantly predicted post-intervention stimulated monocytic production of TNF-α alone, as well as coproduction of TNF-α and IL-6, controlling for pre-intervention levels and BMI. This suggests that the greater the immediate threat-reducing effect of the gratitude task, the greater the reduction in cellular production of inflammation. The percentage of monocytes that produce inflammatory cytokines such as TNF-α and IL-6 following *in vitro* stimulation is viewed as an indicator of the sensitivity of an individual's innate immune system to an inflammatory trigger, and higher percentages are often viewed as an increased likelihood to mount an exaggerated and therefore potentially harmful immune response. Thus, in the current analyses, we extend results from this intervention to the neural level to show that reduced left amygdala responses to threat immediately following a gratitude task were significantly associated with post-intervention decreases in monocytes producing TNF-α alone, and to a lesser degree, those coproducing TNF-α and IL-6—another beneficial result in the context of gratitude.

Finally, we found that gratitude-related reductions in left amygdala reactivity statistically mediated the relationship between increases in support-giving and decreases in stimulated production of TNF-α over the course of the gratitude intervention. Moreover, this relationship appeared to be directional, as reductions in amygdala activity did not mediate the reverse relationship between decreases in inflammation and increases in support-giving. This adds to work linking positive social experiences more broadly (e.g., social support) with reduced amygdala reactivity and inflammatory processes (Muscatell et al., 2016), and supports the idea that, to the extent that gratitude motivates support-giving, reductions in amygdala reactivity may be one mechanism through which gratitude can affect inflammatory outcomes. Furthermore, these results suggest that over time, prosocial behavior may build the capacity of prosocial emotions, like gratitude, to reduce neural and physiological threat responding. This research contributes to a growing literature on the reinforcing relationships between positive emotions, prosocial behavior, and physiological processes related to physical health (Fredrickson & Joiner, 2018; Kok et al., 2013; Kok & Fredrickson, 2010; Layous et al., 2017), and further suggests that the amygdala may be an important neural structure involved in these processes.

Contrary to our hypotheses, caregiving-related neural activity (VS or SA) during experiences of gratitude did not predict reductions in amygdala reactivity or inflammatory outcomes. Although these results do not shed light on the role of caregiving-related neural activity in the health benefits of gratitude, they raise several possibilities. One is that the caregiving response, and its subsequent capacity to reduce stress, may primarily be a regulatory response to distress. Indeed, previous literature has focused on caregiving responses triggered by cues of distress or perceived need in another (Brown & Brown, 2015; Taylor et al., 2000). More specifically, previous research on the effect of support-giving on amygdala reactivity has relied on paradigms in which participants underwent a stressful task in the fMRI scanner (Inagaki et al., 2016), or gave support by holding the arm of a loved one undergoing electrical shock (Inagaki & Eisenberger, 2012). In the current study, participants were not given a stressful task or the opportunity to help loved ones in need, but were simply asked to bring to mind feelings of gratitude for their loved ones. This suggests a promising avenue for future research may be testing whether perceived need or distress is a precondition for the neural caregiving response and its subsequent stress-reducing capacity.

Another possibility is that gratitude increases prosocial motivation, which in turn reduces threat responding, but these effects take place over time rather than simultaneously. Like any emotion, feeling grateful is fleeting, and recent work suggests that intermediary emotional states (e.g., elation (Layous et al., 2017), trust (Dunn & Schweitzer, 2005), and felt closeness (Algoe et al., 2008)), perceptions (e.g., perceived responsiveness and communal norms (Algoe et al., 2008)), and behavior (e.g., expressions of gratitude, and cycles of support within relationships (Algoe et al., 2013)), may play roles in the translation of gratitude into prosocial behavior. Moreover, the *broaden-and-build* theory of positive emotions posits that the real benefits of gratitude lie in the lasting personal resources it cultivates, including close, supportive relationships (Emmons & McCullough, 2004). Given the design of the current study,

we were only able to capture the moment gratitude was felt, rather than the psychological states and actions that feeling grateful might inspire within participants' relationships afterwards. Therefore, it may be that the caregiving response plays a greater role when examined closer to when prosocial behavior takes place, rather than when gratitude is felt. Candidate mechanisms to explore in future work during experiences of gratitude might be the mu-opioid and oxytocin systems. Both of these systems are involved in social bonding and prosocial behavior, are known immune-modulators (Brown & Brown, 2015), and have been proposed as key to the experience and benefits of gratitude (Algoe & Way, 2014; Henning et al., 2017).

These findings should be interpreted in light of several important limitations. First, this study was conducted within women only, and therefore this study does not allow us to explore possible sex differences. Second, this study was conducted in a healthy sample and therefore these findings are not generalizable to clinical populations. Finally, results were not corrected for the multiple hypothesis tests conducted across multiple neural regions and left and right lateralization, and therefore should be interpreted with caution.

While more work is needed to differentiate the neural mechanisms of gratitude from other positive social emotions, this is the first study to our knowledge that has examined the neural correlates of gratitude towards close others (prior work has instead focused on gratitude towards unknown benefactors: (Fox et al., 2015; Kini et al., 2016; Liu et al., 2020; Yu et al., 2017, 2018). According to the *find, remind, and bind* theory of gratitude, gratitude serves the evolutionary function of promoting intimacy, closeness, and mutual support within social relationships (Algoe, 2012). Therefore, studying the neurobiology of gratitude in the context of close relationships is critical to understanding gratitude as experienced in daily life, and the use of ecologically valid, personalized stimuli is a strength of this study. Thus, the present study extends previous literature on the neuroscience of gratitude by providing preliminary evidence that feelings of gratitude towards close others may activate the mammalian caregiving system.

5. Conclusions

Although these findings do not provide evidence for a direct effect of gratitude on health and should be interpreted with caution (given their correlational nature and lack of clinical health outcomes), they provide novel insight into the brain-body pathways that may link gratitude and physical health. These results provide support for the idea that, to the extent that gratitude motivates support-giving behavior, gratitude may exert its benefits through the same threat-reducing neural pathways as giving support to others. More broadly, this work adds to the growing body of scholarship showing us how deeply the emotions and actions that nurture social relationships affect our physiology (Eisenberger & Cole, 2012; Slavich, 2020), and the importance of giving support to others for health and well-being (Brown et al., 2003; Inagaki, 2018). That support-giving seems to be a primary driver of the neural and inflammatory mechanisms of gratitude adds to the growing evidence that prosocial activities more generally (such as volunteering or performing acts of kindness for others) are beneficial for health. Future work is needed to clarify the immediate and long-term effects of gratitude on threat responding. However, the present study provides evidence supporting that intentionally cultivating gratitude towards loved ones may be an effective way to motivate prosocial behavior and potentially improve health.

Funding

This research was supported by a grant from the Greater Good Science Center at the University of California, Berkeley, CA.

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.

Acknowledgements

The authors wish to thank Kristine Chua, Lauren Labac, Steven Lam, Ann Kim, Vy Nguyen, and Joby Joseph for their help with participant recruitment and data collection and entry.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2021.04.019.

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