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Alterations in Resting-State Functional Connectivity Link Mindfulness Meditation With Reduced Interleukin-6: A Randomized Controlled Trial

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ABSTRACT

BACKGROUND: Mindfulness meditation training interventions have been shown to improve markers of health, but the underlying neurobiological mechanisms are not known. Building on initial cross-sectional research showing that mindfulness meditation may increase default mode network (DMN) resting-state functional connectivity (rsFC) with regions important in top-down executive control (dorsolateral prefrontal cortex [dlPFC]), here we test whether mindfulness meditation training increases DMN-dlPFC rsFC and whether these rsFC alterations prospectively explain improvements in interleukin (IL)-6 in a randomized controlled trial.

METHODS: Stressed job-seeking unemployed community adults ($n = 35$) were randomized to either a 3-day intensive residential mindfulness meditation or relaxation training program. Participants completed a 5-minute resting-state scan before and after the intervention program. Participants also provided blood samples at preintervention and at 4-month follow-up, which were assayed for circulating IL-6, a biomarker of systemic inflammation.

RESULTS: We tested for alterations in DMN rsFC using a posterior cingulate cortex seed-based analysis and found that mindfulness meditation training, and not relaxation training, increased posterior cingulate cortex rsFC with left dlPFC ($p < .05$, corrected). These pretraining to posttraining alterations in posterior cingulate cortex-dlPFC rsFC statistically mediated mindfulness meditation training improvements in IL-6 at 4-month follow-up. Specifically, these alterations in rsFC statistically explained 30% of the overall mindfulness meditation training effects on IL-6 at follow-up.

CONCLUSIONS: These findings provide the first evidence that mindfulness meditation training functionally couples the DMN with a region known to be important in top-down executive control at rest (left dlPFC), which, in turn, is associated with improvements in a marker of inflammatory disease risk.

Keywords: fMRI, Functional connectivity, IL-6, Mindfulness meditation, Stress, Unemployment

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Mindfulness meditation training programs, which train receptive attention and awareness to one's present moment experience, have been shown to improve a broad range of stress-related psychiatric and physical health outcomes in initial randomized controlled trials (e.g., depression relapse, anxiety, human immunodeficiency virus progression) (1–7). For example, recent well-controlled studies indicate that mindfulness meditation training may reduce markers of inflammation (C reactive protein, interleukin [IL]-6, neurogenic inflammation) in stressed individuals (8–11). However, little is known about the neural mechanisms underlying the effects of mindfulness training on health among these individuals (12,13).

One possibility is that mindfulness meditation training alters resting-state functional connectivity (rsFC) of brain networks implicated in mind wandering (the default mode network [DMN]) and executive control (the executive control [EC] network), which, in turn, improves emotion regulation, stress resilience, and stress-related health outcomes in at-risk patient populations (1,14–16). Two lines of research support this hypothesis. First, a cross-sectional study ($n = 25$) showed that advanced mindfulness meditation practitioners had increased functional connectivity of a key hub in the default mode network (i.e., posterior cingulate cortex [PCC]) with regions considered to be important in top-down executive control (dorsolateral prefrontal cortex [dlPFC], dorsal anterior cingulate cortex [dACC]), both at rest and during a guided mindfulness meditation practice (17). This coupling of one's DMN at rest with regions of the EC network may be important

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for emotion regulation and stress resilience effects, as greater activation and functional connectivity of EC regions, such as the dlPFC, is associated with reduced pain, negative affect, and stress (18–21). A second line of research demonstrates initial links between alterations in DMN rsFC and psychiatric (e.g., Alzheimer's disease, schizophrenia) (22,23) and physical (e.g., obesity, diabetes) (24,25) health risks; for example, there is reduced rsFC of the posterior cingulate cortex and the dlPFC observed in schizophrenia patients relative to matched control subjects (26).

Here, we provide the first experimental test of whether an intensive 3-day mindfulness meditation training intervention (relative to a relaxation training intervention) alters DMN connectivity and circulating IL-6 in a high-stress unemployed job-seeking community sample. IL-6 is an established clinical health biomarker that is elevated in high-stress populations (27,28) and is associated with elevated cardiovascular disease and mortality risk (29–31). Moreover, unemployment is a well-known chronic stressor that can foster a loss of control, helplessness, and financial setbacks (32), and unemployment is associated with elevated inflammation (33). Building on initial cross-sectional evidence (17), we hypothesized that mindfulness meditation training would increase rsFC between the DMN and regions implicated in attention and executive control (dlPFC and dACC). Moreover, we tested whether mindfulness meditation training (relative to relaxation training) decreased circulating IL-6 at 4-month follow-up and whether preintervention-postintervention increases in DMN-dlPFC rsFC mediated IL-6 improvements at 4-month follow-up.

METHODS AND MATERIALS

Participants

Thirty-five right-handed unemployed job-seeking community adults participated (see Supplemental Table S1 for inclusion/exclusion study criteria) (see Supplemental Figure S1 for CONSORT flowchart). Participants had moderate to high levels of job-seeking stress over the past month, scoring >5 on an adapted 4-item Perceived Stress Scale (34) (mean [M] = 9.60, SD = 2.35; for job-seeking stress measure see Supplemental Table S1). After complete description of the study to the participants, written informed consent was obtained.

Procedure

Beginning 4 weeks before the 3-day training intervention, participants completed a baseline neuroimaging session, which included a 5-minute resting-state scan where they passively viewed a fixation cross. After neuroimaging, participants were invited to a nearby residential retreat center where they provided a blood sample (for measurement of circulating IL-6) and were then randomized (via a random number generator by the study principal investigator) to either a 3-day intensive mindfulness meditation training (n = 18) or a matched 3-day relaxation residential retreat intervention (n = 17). Posttreatment study personnel were blinded to participant study condition (including personnel running the posttreatment magnetic resonance imaging session and magnetic resonance imaging data preprocessing). Participants returned for a neuroimaging assessment within 2 weeks of completing the 3-day intervention and completed an identical scanning procedure as at baseline (participants verbally confirmed that they did not engage in meditation or relaxation activities during the resting-state scan at both time points). At 4-month follow-up, participants were invited back to the retreat center, at which time they provided a blood sample and completed a measurement battery. The measures described in this report are a subset of measures collected in this trial (Supplement).

Interventions

We developed a 3-day residential mindfulness meditation retreat format from activities used in the Mindfulness-Based Stress Reduction program (35), called Health Enhancement through Mindfulness (HEM). Delivery of the HEM program in a structured residential retreat format improves compliance with training and reduces treatment attrition; greater experimental control is also afforded by offering a parallel matched relaxation training retreat (in a separate wing of the retreat center). Briefly, the HEM program consists of mindfulness training through body scan awareness exercises, sitting and walking meditations, mindful eating, mindful stretching, and discussion. We developed a structurally matched Health Enhancement through Relaxation (HER) program that included similar behavioral training activities (e.g., walking, stretching, and didactics) as HEM, but emphasized participation in these activities in a restful rather than a mindful way. The use of a structurally matched active comparison group was designed to control for nonmindfulness specific factors such as positive treatment expectancies, group support, teacher attention, physical activity, and mental engagement. An hour-by-hour outline of interventions is provided in Supplemental Table S4.

Neuroimaging Measures

Image Acquisition and Preprocessing. Structural and functional images were acquired on a Siemens Verio 3T scanner (Erlangen, Germany) using a 32-channel head coil. High-resolution T1-weighted gradient-echo images were acquired (repetition time [TR] = 1800 ms, echo time = 2.22 ms, flip angle = 9°, matrix size = 256 × 256, number of slices = 256, field of view = 205 mm, 0.8 mm slices, generalized autocalibrating partially parallel acquisitions acceleration factor phase encoding = 2, voxel size = 0.8 × 0.8 × 0.8 mm). Next, four functional echo-planar imaging runs were acquired, including a 300-second resting-state scan (TR = 2000 ms, echo time = 30 ms, flip angle = 79°, matrix size = 64 × 64, number of slices = 36, field of view = 205 mm, 3.2-mm-thick slices echo-planar imaging with rate 2 generalized autocalibrating partially parallel acquisitions, voxel size = 3.2 mm × 3.2 mm × 3.2 mm).

Three participants were excluded from functional magnetic resonance imaging (fMRI) data analyses due to neuroimaging session problems (at the baseline appointment: one participant reported sleeping, one had poor coverage, and one did not understand directions; at the posttreatment appointment: one participant reported sleeping, one did not understand directions, and one had poor coverage). Functional blood oxygen level–dependent (BOLD) data were processed using

SPM8 (Wellcome Department of Cognitive Neurology, London, United Kingdom; implemented by MATLAB, The MathWorks, Inc., Natick, MA). Functional images were first realigned to the mean image of the first run and then smoothed with a 4-mm full-width at half maximum Gaussian kernel. Data were then submitted to motion correction using the Art Repair utility (36), an interpolation-based motion correction program. The functional data were then normalized to the standard Montreal Neurological Institute (MNI) T1 template. Finally, the images were smoothed with a 7-mm full width at half maximum kernel, resulting in an overall full width at half maximum smoothing of 8 mm (36).

IL-6 and Psychosocial Measures

IL-6. Blood samples were collected (between the hours of 10:00 AM and noon) and processed (then frozen) in batch at baseline and at 4-month follow-up by technicians blinded to treatment conditions. IL-6 levels were determined from plasma in duplicate by high-sensitivity quantitative sandwich enzyme immunoassay kit (R& D Systems, Minneapolis, MN) run according to manufacturer's directions. Two participants had insufficient samples for IL-6 determination and were excluded.

Psychosocial Measures. To evaluate whether the relaxation retreat program produced equivalent positive beliefs about its value (a placebo control) compared with the mindfulness retreat program, participants completed a six-item measure of perceived positive treatment benefits at the conclusion of the 3-day retreat program using an adapted version of the Credibility/Expectancy Questionnaire (37) (study $\alpha = .87$, sample item: "At this point, how much do you really feel that this therapy will help you reduce your stress symptoms?" 1 = not at all to 9 = very much). Reemployment status was assessed at 4-month follow-up; participants indicated whether they were unemployed, defined as having no job for more than 20 hours per week in the 4-month posttreatment period.

Data Analysis

Resting-State Functional Connectivity Analysis. PCC-seeded resting-state BOLD fMRI images were generated in the CONN toolbox, following the recommended CONN analysis procedures (35). Specifically, CONN implements several additional processing and first-level analysis routines before rsFC analysis: CONN estimates an orthogonal time series using principal component analysis of the BOLD time series in each noise region of interest (ROI) (subject-specific white matter and cerebrospinal fluid masks). Structural magnetization prepared rapid acquisition gradient-echo images were segmented to define gray matter, white matter, and cerebrospinal fluid areas. BOLD signal from the subject-specific white matter and cerebrospinal fluid masks, motion parameters (six dimensions), and the effect of rest (an average across the session) were used as regressors to account for further temporal confounding factors. CONN uses a component-based noise reduction (CompCor) that avoids regression of the global signal. A covariate for each subject's head motion was entered at the first level. A band-pass filter of 0.008 Hz to 0.09 Hz was used. A hemodynamic response function was used to down-weight the initial scans within each resting-state block to minimize potential ramping effects.

The PCC seed was anatomically defined using the Talairach Daemon database in the Wake Forest University (Winston-Salem, NC) PickAtlas (38) centered on MNI: −4, −50, 40. Seeded first-level maps in CONN were then submitted to a second-level full factorial analysis in SPM8 with two factors specified, time and group. To test study predictions in the brain, we specified a time by group spreading interaction contrast that tested for baseline to postintervention increases in rsFC in the HEM program relative to no change in the HER program from baseline to postintervention using contrast weights: [−1 (pre, HEM), −1 (pre, HER), 3 (post, HEM), −1 (post, HER)]. This *t* contrast models the specific hypothesized differential group change from baseline to posttreatment. The strength of this approach (relative to testing for significant voxels using the more standard overall *F* contrast or just comparing the two groups at posttreatment only) is that it tests the specific prediction that the mindfulness meditation program increases rsFC from baseline to posttreatment compared with no change in the relaxation group (as opposed to other types of interaction patterns that might be significant with an *F* contrast analysis). Note that this approach compares the mindfulness group at posttreatment with the average of the other cells in this 2 × 2 design, testing the spreading interaction prediction (and not other interaction patterns, e.g., crossover interactions). Furthermore, we then plotted the parameter estimates from this spreading interaction contrast to visually confirm the specific interaction pattern (Figure 1B, C).

For purposes of testing rsFC with the PCC in this study, two ROI masks were created using the Wake Forest University PickAtlas Anatomical Automatic Labeling atlas (each with dilation of 1 mm): the first mask consisted of the left and right anterior cingulate and the second ROI mask consisted of the left and right middle frontal cortex [based on (17)]. Cluster-level correction for multiple comparisons was obtained using a Monte Carlo simulation in AlphaSim (National Institute of Mental Health, Bethesda, MD). AlphaSim was first run on the anterior cingulate Anatomical Automatic Labeling-defined mask, with significant clusters ($p < .05$, corrected) defined as those involving $k > 49$ contiguous voxels, each at $p < .005$. AlphaSim was then run on the middle frontal cortex Anatomical Automatic Labeling-defined ROI mask, with significant clusters ($p < .05$, corrected) defined as those involving $k > 82$ contiguous voxels, each at $p < .005$.

IL-6 and Head Motion Analysis. IL-6 values at baseline and 4-month follow-up were log transformed. Analyses adhered to intent-to-treat principles using mixed effect linear models (MLMs) conducted in SPSS 21.0 (IBM Corp., Armonk, NY). All variables were modeled as fixed effects in models fit with a compound symmetric variance-covariance structure, using maximum likelihood estimation. These models included a condition factor (HEM vs. HER program), a time factor (baseline, 4-month follow-up), and their interaction, with *F*-statistics used to evaluate significant effects. All IL-6 MLMs included participant age as a covariate given the significant range in participant age in this sample (22–54 years old), as

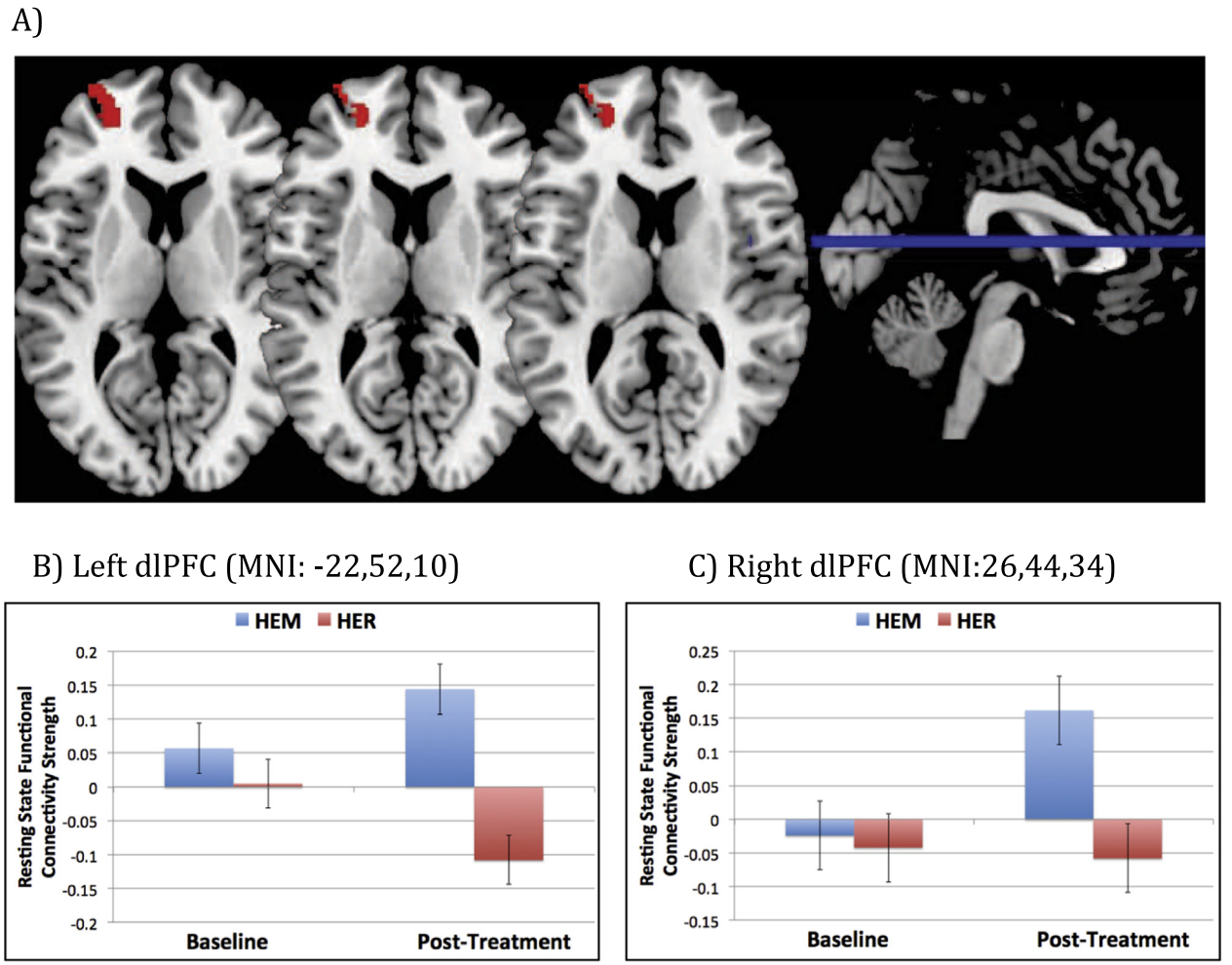

Figure 1. Baseline to posttreatment resting-state functional connectivity in the mindfulness and relaxation training groups. **(A)** Left dorsolateral prefrontal cortex (dlPFC) cluster (Montreal Neurological Institute [MNI]: −22, 52, 10; $k = 111$, $p < .05$ corrected) that showed increased resting-state functional connectivity with posterior cingulate cortex from before to after mindfulness meditation training (Health Enhancement through Mindfulness [HEM]) relative to relaxation training (Health Enhancement through Relaxation [HER]). Specifically, a time × condition spreading interaction analysis revealed a significant cluster in left dlPFC **(A, B).** A cluster in right dlPFC (MNI: 26, 42, 38; $k = 24$) showed the same pattern of effects as the left dlPFC cluster, but it did not survive correction, thus the **(C)** right dlPFC resting-state functional connectivity results should be interpreted with caution. The spreading interaction effects that mediate interleukin-6 effects are depicted for left **(B)** and right **(C)** dlPFC. Specifically, mean connectivity strength is shown for the mindfulness (HEM) and relaxation (HER) training groups at baseline and posttreatment. Error bars depict ± 1 standard error.

age is robustly associated with increasing IL-6 (27,39). To test whether there were condition (and time × condition) differences in fast head motion (defined as TR-TR head motion greater than 0.25 mm or 0.25 degrees in any plane), a hypothesis and condition-blind coder summed total high head motion TRs for each participant's baseline and posttreatment resting-state scan, which was then tested in a MLM with total head motion as the outcome.

PCC Resting-State Functional Connectivity Mediation Analysis. Statistical mediation analyses were conducted following recommended procedures for testing intervening variable effects using MLMs (40,41). Specifically, rsFC cluster-level parameter estimates were extracted from the SPM8 group-level analyses and tested as mediating variables in a series of MLMs. The MLMs consisted of (MLM #1) testing a time × condition interaction effect on change in PCC rsFC (path a, see Figure 2); (MLM #2) testing for a significant effect of PCC rsFC on change in IL-6 (path b), when the PCC rsFC variable (baseline, posttraining) was entered simultaneously along with the condition, time, and time × condition interaction variables; and (MLM #3) whether the original time × condition interactive effect on IL-6 (path c) was no longer statistically significant when the PCC rsFC effect was entered as a simultaneous predictor variable (path c′). Statistical mediation was defined as present when 1) there were significant intervening variable paths (paths a and b) and 2) when the original time × condition interaction on IL-6 (path c′) was no longer significant when the change in rsFC variable was entered as a simultaneous predictor variable.

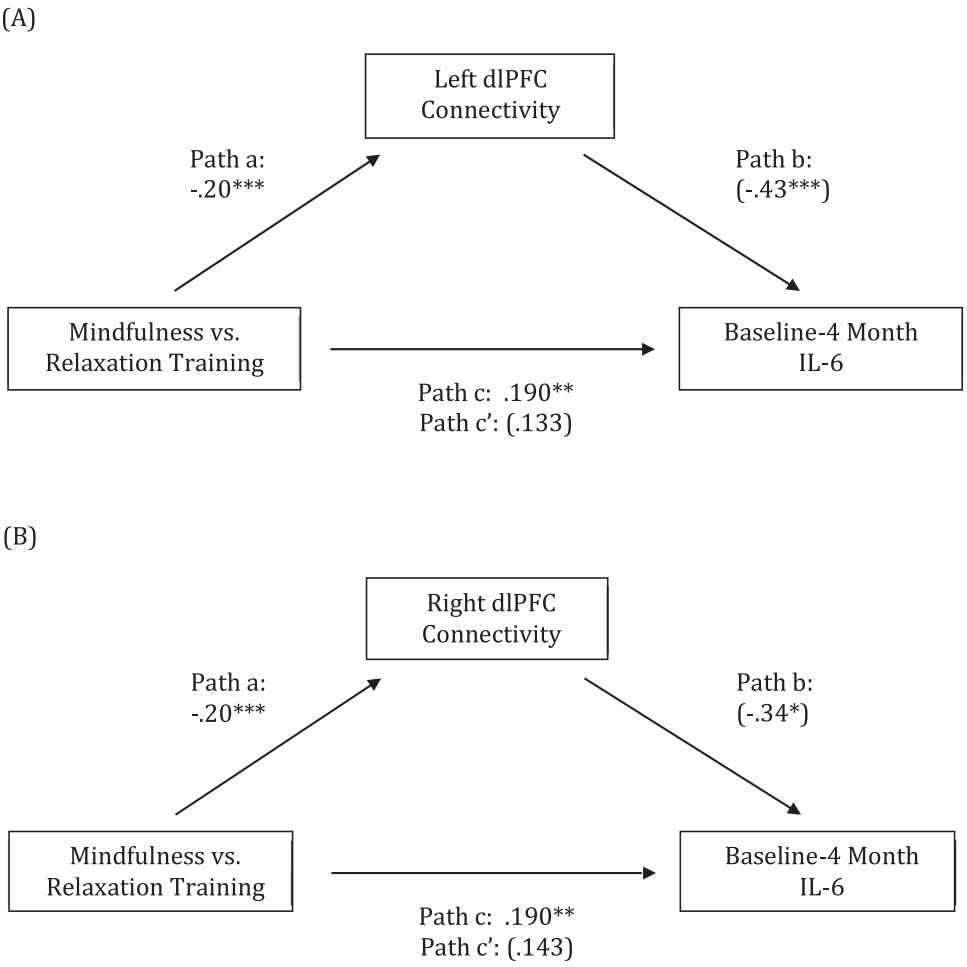

Figure 2. Interleukin (IL)-6 mediation analyses. Increases in left dorsolateral prefrontal cortex (dlPFC) connectivity (Montreal Neurological Institute: −22, 52, 10) significantly mediate **(A)** the time × treatment interaction on circulating (log transformed) IL-6. Increases in right dlPFC (Montreal Neurological Institute: 26, 42, 38) marginally significantly mediate **(B)** IL-6 effects. Numbers represent *b* coefficients from mixed-effect linear models, with parentheses representing *b* coefficients when the main effect and time × treatment condition interaction terms and dlPFC connectivity parameter estimates are entered in a mixed-effect linear model simultaneously. *$p = .06$; **$p = .05$; ***$p < .05$.

RESULTS

Preliminary Analyses—Success of Randomization and Treatment Program Measures

There were no significant differences between the mindfulness (HEM) and relaxation (HER) groups on study baseline characteristics, indicating successful randomization (Table 1). There were no significant group differences in fast head motion during the resting-state scan period ($F_{1,35} = 0.002$, $p = .97$) and no differential fast head motion changes from baseline to the posttreatment fMRI resting-state scan between groups (time × condition, $F_{1,34} = 0.001$, $p = .98$). The relaxation training program was an effective placebo control; there were no significant group differences in perceived treatment benefits at the conclusion of the 3-day retreat (independent samples, $t_{31} = 1.06$, $p = .30$; HEM M = 38.76, SE = 2.19, HER M = 42.03, SE = 2.13).

Ninety-seven percent of randomized participants completed the 3-day training programs and 97% were retained at the 4-month follow-up assessment (see Supplemental Figure S1 for CONSORT flowchart). These high retention rates may have reflected the high (and equivalent) treatment satisfaction reported by participants in both the mindfulness (HEM M = 4.15, SE = 0.18) and relaxation (HER M = 4.12, SE = 0.19) programs at follow-up (single item: "Would you recommend this program to other people you know?": 1 = not at all to 5 = a great deal; independent samples, $t_{32} = 0.11$, $p = .91$). Despite our efforts to encourage home practice after the 3-day retreats (along with sending participants home with customized compact discs containing guided condition-specific mindfulness and relaxation exercises), participants did not complete much formal practice in the 4-month follow-up period. HEM participants reported using their home practice CD an average of 1.24 times per week (SD = 1.28) over the last month (at the 4-month follow-up assessment), while HER participants reported using their home practice CD .38 times per week (SD = .86) over the last month ($t_{31} = 2.27$, $p = .03$). (We also did not collect measures of home practice in the days following the retreat program leading up to the posttreatment fMRI session, a study limitation.)

Primary Analyses—Alterations in DMN Resting-State Functional Connectivity

Consistent with predictions, there was a significant preintervention-postintervention increase in rsFC between the PCC (the DMN seed region) and left dlPFC (MNI: −22, 52,10; $k = 111$; $Z = 3.44$, $p < .05$, corrected) in the mindfulness

Table 1. Baseline Characteristics of Randomized Participants

Characteristic	HEM	HER	Difference Statistic
Age, Years (SD)	37.94 (10.96)	41.00 (9.55)	$t_{33} = -0.48$, $p = .64$
Gender			$\chi^2_1 = 0.24$, $p = .63$
Male	11	9	
Female	7	8	
Ethnicity			$\chi^2_5 = 6.37$, $p = .27$
Caucasian	10	13	
African American	6	2	
Asian American	1	0	
Latino	0	1	
Native American	0	0	
Biracial	1	0	
Other	0	1	
Months Unemployed	8.17 (12.48)	10.58 (20.31)	$t_{33} = -0.43$, $p = .67$
Education			$\chi^2_8 = 8.43$, $p = .39$
No high school degree	1	0	
GED	1	0	
High school degree	1	2	
Technical training	3	2	
Some college	4	3	
Associate degree	2	0	
Bachelor's degree	2	7	
Master's degree	3	3	
MD/PhD/JD/PharmD	1	0	
Body Mass Index	27.15 (4.30)	26.44 (5.50)	$t_{32} = 0.423$, $p = .68$
Cognitive Impairment (MMSE)	29.39 (0.70)	28.88 (1.15)	$t_{32} = 1.60$, $p = .12$
IL-6 pg/mL	1.81 (2.03)	1.21 (0.76)	$t_{28} = 1.03$, $p = .31$
Post-fMRI Days Elapsed	5.06 (3.29)	5.29 (4.52)	$t_{32} = -0.17$, $p = .86$

Values are presented as mean (SD) or *n*. Participants indicated their ethnicity/gender via self-report.

fMRI, functional magnetic resonance imaging; GED, general educational development; HEM, 3-day Health Enhancement through Mindfulness group; HER, 3-day Health Enhancement through Relaxation group; IL-6, interleukin-6; MMSE, Mini-Mental State Examination; Post-fMRI Days Elapsed, number of days elapsed at post magnetic resonance imaging session from the conclusion of the retreat program.

meditation training group relative to the relaxation training control group. As shown in Figure 1, there was no coupling of PCC with the left dlPFC (Figure 1B) at baseline in both the mindfulness and relaxation groups, but at posttreatment, the mindfulness (but not relaxation) group showed positive coupling of PCC and left dlPFC. Although the clusters did not survive multiple comparison correction, a homologue region of right dlPFC showed a similar effect (MNI: 26, 44, 34; $k = 24$; $Z = 3.22$; Figure 1C), along with another cluster in left dlPFC (MNI: −30, 42, 38; $k = 77$; $Z = 3.11$). Contrary to predictions, mindfulness meditation training did not significantly alter rsFC of the PCC with dACC. We also observed no alterations in intra-DMN rsFC; specifically, mindfulness meditation training did not decouple the PCC with other DMN nodes (e.g., PCC with ventromedial prefrontal cortex) (42). We provide exploratory time × condition interaction results for PCC rsFC across the whole brain in Supplemental Tables S2 and S3 (thresholded at uncorrected $p < .005$, $k > 50$ voxels).

Circulating IL-6

There were no significant baseline differences in log-transformed IL-6 (or raw IL-6; Table 1) between the mindfulness meditation and relaxation training groups ($t_{28} = 1.04$, $p = .31$). However, mindfulness meditation training, relative to relaxation training, reduced circulating levels of IL-6 at 4-month follow-up. A mixed-effect linear model (controlling for participant age) indicated that mindfulness meditation training significantly reduced circulating log-transformed IL-6 from baseline (M = 0.13, SE = 0.07) to 4-month follow-up (M = 0.08, SE = 0.08) compared with increases in the relaxation training group (baseline M = −0.06, SE = 0.08; 4-month follow-up M = 0.08, SE = 0.07) (time × condition interaction, $F_{1,29} = 4.14$, $p = .05$, $d = .71$) (Table 2). Changes in reemployment during the 4-month follow-up period could have explained these changes in IL-6, but this was not the case; there were equal rates of reemployment in the two groups at the 4-month follow-up assessment (48% of participants in both groups were reemployed at 4-month follow-up).

Alterations in DMN rsFC Mediate Mindfulness Meditation Training Improvements in IL-6

As shown in Figure 2, increases in preintervention-postintervention PCC-left dlPFC rsFC statistically mediated changes in circulating IL-6 from baseline to 4-month follow-up.

Table 2. Circulating IL-6 by Treatment Group and Time

	Mean HEM	SE	Mean HER	SE
Log-Transformed IL-6				
Baseline	0.13	0.07	−0.06	0.08
4-month follow-up	0.08	0.08	0.08	0.07
Raw IL-6				
Baseline	1.87	0.31	1.17	0.32
4-month follow-up	1.45	0.32	1.41	0.30

Means and standard errors (SE) from IL-6 mixed-effect linear models, with baseline age as a covariate. All IL-6 values are in pg/mL.

IL-6, circulating interleukin-6; HEM, Health Enhancement through Mindfulness program; HER, Health Enhancement through Relaxation program.

Specifically, a mediation model indicated that the strength of the time × condition interaction effect on IL-6 (path c) was no longer significant when the change in the left dlPFC cluster (MNI: −22, 52, 10; $k = 111$) connectivity predictor variable was entered simultaneously in a mixed-effect linear model (path c′). Although the right dlPFC cluster did not survive multiple comparison correction (MNI: 26, 44, 34; $k = 24$), it also marginally significantly mediated the IL-6 effects (also shown in Figure 2). Mindfulness meditation training alterations in rsFC observed in the second left dlPFC cluster (MNI: −30, 42, 38) did not mediate IL-6 effects. These findings in Figure 2 indicate that change in PCC-dlPFC connectivity accounts for 30% (left dlPFC MNI: −22, 52, 10) and 25% (right dlPFC MNI: 26, 44, 34) of the overall mindfulness meditation (vs. relaxation training) effect on reductions in IL-6 at 4-month follow-up.

DISCUSSION

There has been considerable recent interest in characterizing resting-state neural networks, such as the DMN, and understanding their role in health and disease (23). We report that mindfulness meditation training (compared with relaxation training without a mindfulness component) increased rsFC between the DMN (PCC) and left dlPFC. These findings corroborate and experimentally extend cross-sectional findings in advanced mindfulness meditation practitioners (17), suggesting that mindfulness meditation training may couple one's resting-state DMN with regions implicated in executive control (dlPFC). These findings were specific to coupling of the PCC with dlPFC and not with dACC [cf. (17)]. Notably, some work has shown that dlPFC and dACC are involved with dissociable executive control and salience processing networks, respectively (43); furthermore, whereas increased dACC connectivity is associated with anxiety, increased dlPFC connectivity is associated with behavioral improvements in executive task performance (e.g., attention task switching) (43).

Although studies commonly report that the DMN is anticorrelated with regions in the executive control network (44,45), some studies indicate positive PCC-dlPFC coupling during self-focused and process-oriented mental simulations (45–47) and during guided mindfulness meditation practice (17). These findings suggest that a process-oriented focus on the self during meditation may shift intranetwork DMN rsFC toward internetwork connectivity, coupling DMN with regions in the executive control network. Notably, the present results show that these alterations in DMN-EC rsFC can be maintained in the weeks following brief, intensive mindfulness meditation training.

We also provide an initial indication in this randomized controlled trial sample ($n = 35$) that mindfulness meditation training reduces IL-6 from baseline to 4-month follow-up compared with increases in IL-6 in the relaxation training control group. This finding suggests that mindfulness meditation training may decrease biomarkers of inflammatory disease risk in populations whom have elevated inflammation (such as stressed unemployed adults), although we note this was a small sample and this IL-6 finding should be treated with some caution. But like this study finding, several studies show that mindfulness meditation training interventions reduce markers of inflammation in stressed populations (8–11). Moreover, the present work shows that pretraining-posttraining changes in PCC-dlPFC rsFC mediate these effects on IL-6. Currently, very little is known about top-down neural modulation of peripheral inflammation in humans, despite a large number of epidemiologic studies linking peripheral circulating proinflammatory cytokines with cardiovascular morbidity and all-cause mortality (29–31,48). Some initial studies in humans highlight reciprocal brain-peripheral inflammation links (49,50), although the present study offers a novel top-down regulatory pathway for the modulation of circulating IL-6 in humans. Notably, studies have implicated activation and functional connectivity of dlPFC with improved executive control resources and reduced pain, negative affect, and stress (18–21,43). We speculate that mindfulness meditation training coupled the brain's DMN with regulatory areas of prefrontal cortex (dlPFC), which facilitated more effective emotion regulation and stress resilience in this high-stress unemployed job-seeking sample, reduces circulating IL-6. Specifically, efferent projections from dlPFC might modulate medial prefrontal cortex and subcortical cell groups known to trigger peripheral stress and inflammatory response cascades (51,52). A small experimental literature provides initial support for this neural stress-buffering account of mindfulness meditation training (14), although more research is needed to evaluate these stress reduction mediated pathways.

An important question going forward will be to evaluate whether these observed PCC-dlPFC rsFC changes reflect neuroplasticity in white matter connectivity after mindfulness meditation training. Several lines of evidence provide suggestive support: anatomic studies in primate models have shown that measures of rsFC have high correspondence to underlying white matter architecture (53), and there are established white matter tracts linking PCC with dlPFC (54). Notably, an initial experimental study demonstrates that even brief meditation training (~11 hours) fosters neuroplasticity by increasing white matter connectivity (as measured by fractional anisotropy) of the anterior corona radiata (55).

Unlike a previous study (17), mindfulness meditation training did not increase PCC-dACC rsFC in this sample. We speculate that one possibility for these divergent effects may be due to differences in study methodology. The previous study measured rsFC using 2-minute resting-state periods at the beginning of each run, followed by 4.5-minute meditation practice periods (17). By contrast, the present study collected rsFC at the beginning of the fMRI session (immediately

following the collection of the structural scans). Some neuroimaging evidence indicates that acute meditation practice activates the anterior cingulate cortex (56,57), and it may be that anterior cingulate cortex activity during these 4.5-minute meditation practice periods had some residual carryover into the rsFC scans in the previous study. Indeed, there is research showing that cognitive tasks have carryover effects on PCC-anterior cingulate cortex functional connectivity during subsequent rsFC periods (58). The present study design is less susceptible to potential task-based carryover effects and provides an initial indication for nontask-based PCC-dlPFC rsFC effects in the weeks following a 3-day mindfulness meditation training intervention.

Conclusions

Little is known about how behavioral interventions, such as mindfulness meditation training, can impact the brain and physical health. We provide the first well-controlled evidence that 3 days of mindfulness meditation training increases rsFC of the DMN with neural regions important in executive control (left dlPFC) and that these rsFC changes statistically mediate improvements in circulating levels of IL-6 at follow-up.

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