Prefrontal Regulatory Mechanisms of Mindfulness and Stress Reduction and Links to Markers of Health

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PREFRONTAL REGULATORY MECHANISMS OF MINDFULNESS AND STRESS REDUCTION AND LINKS TO MARKERS OF HEALTH

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Mindfulness is, at its core, an open or receptive attention to present-moment experience. In recent years, interest in understanding the underlying neurobiology of mindfulness training has grown exponentially as more and more studies show the psychological and physiological health benefits of mindfulness practice, particularly in stressed populations. A primary goal of this emerging field has been to identify the neural mechanisms by which mindfulness training interventions may be producing such beneficial effects, which include decreased stress-responding as well as increased attentional focus, enhanced cognitive flexibility, and greater capacity for emotion regulation, cognitive processes that can be broadly classified as "executive control". Here, across three studies, I focus on the intrinsic neural circuitry underlying stress-responding and executive control. Using functional MRI data, I investigate changes in functional neural connectivity after a randomized controlled trial of a mindfulness training intervention (relative to a relaxation control intervention) in a high-stress, unemployed community adult population. In Chapter 2, I identify stress-related increased resting state functional connectivity in an amygdala-subgenual anterior cingulate pathway that is decoupled by mindfulness training. In Chapter 3, I characterize mindfulness training-associated changes in the functional connectivity of dorsolateral prefrontal cortex to other attention and executive control-associated brain regions. Extending these region-specific functional connectivity findings, in Chapter 4 I show network-level changes in information processing within attention and salience-responding networks after mindfulness training. Collectively, these results demonstrate that mindfulness training may decrease baseline functional coupling between regions implicated in stress-responding and increase connectivity between regions implicated in executive control, and enhance the efficiency of information transfer between distributed neural circuitry for attentional monitoring.

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PREFACE

My professional development over the past five years has been heavily shaped by my advisors and colleagues at both the University of Pittsburgh and Carnegie Mellon University. First, I would like to acknowledge that the studies presented in this dissertation have been collaborative efforts. Specifically, I would like to thank Peter Gianaros and Leijyuan Sheu for access to data from the Pittsburgh Imaging Project, as well as their insights on numerous analyses. I am equally indebted to Michael Hallquist and Scott Marek for their assistance with resting state functional connectivity network metric analyses, and for helping shape my understanding of graph theory and its application to cognitive neuroscience. Finally, I also want thank my former mentor, Scott Huettel, whose training allowed me to “hit the ground running” in graduate school, and colleagues from the Huettel lab, particularly David V. Smith and McKell Carter, for their continued support throughout graduate school and teaching me that the computer is always right.

The Creswell lab has been my academic home for the past 4 years, and I would like to thank David J. Creswell for being a great mentor and academic advisor. He has played a major role in my personal and intellectual growth, and his guidance has allowed me to think about my work in broader terms that will heavily inform my future research and medical career. Finally I want to thank my dissertation committee – Michael Tarr, Peter Gianaros, Beatriz Luna, Michael Hallquist, and Kirk Erickson – for their input and guidance throughout my dissertation work.

1.0 INTRODUCTION

Psychological stress is a serious health problem that contributes to the pathogenesis of a broad spectrum of chronic diseases, including cardiovascular, metabolic, gastrointestinal, psychiatric, and immunologic disorders (1,2). At its core, mindfulness is a psychological process involving attention, non-judgmental acceptance, and receptivity to what is happening in one's moment-by-moment experience (3–5). Mindful traits as well as mindfulness-based interventions have been associated with increased measures of well-being and decreased incidence of depression, anxiety, mood disorders, and psychopathology (4,6). Additionally, mindfulness has been linked to better outcomes in a number of stressed patient populations, including those with chronic pain, HIV, cancer, cardiovascular disease, and fibromyalgia (7,8). Given the expanding clinical literature documenting the positive physical and mental outcomes associated with mindfulness meditation, considerable interest has developed in investigating the neurological basis for these changes.

Mindfulness has been studied using both self-report measures of trait mindfulness such as the Mindful Attention and Awareness Scale (MAAS) (9) and Five-Facet Mindfulness Questionnaire (FFMQ) (10), and structured mindfulness training programs, such as the 8-week Mindfulness Based Stress Reduction program (3). There has been some recent debate as to whether self-report mindfulness questionnaires and mindfulness meditation training measure the same underlying construct (7,11,12). Recent studies have shown some convergence between self-report trait mindfulness and trained mindfulness, such that mindfulness meditation training interventions increase self-reported mindfulness (10,11,13). Studies of trait mindfulness are largely cross-sectional correlational and have associated increased trait mindfulness with a variety of psychological and health outcomes, including positive associations with conscientiousness, agreeableness, positive affect, life satisfaction, and feelings of autonomy (9,14–16) and negative associations with neuroticism, depression, rumination, social anxiety, and poor affect regulation (9,15,17–21). More recently, trait mindfulness has also been linked to brain function; greater trait mindfulness has been associated with decreased limbic and increased prefrontal brain activity during an affect labeling task (22) and decreased activity during the resting state in regions responsible for self-referential processing (e.g. medial prefrontal cortex, parietal cortex) (23). Additional cross-correlational studies have examined the relationship between mindfulness meditation experience and similar psychological, behavioral, and neural outcomes; compared to non-meditators, meditation practitioners report greater psychological well-being, self-compassion, and trait mindfulness, and decreased levels of rumination, emotion suppression, and psychological symptoms (24,25); better attentional performance and cognitive flexibility (26,27); increased cortical thickness (28) and gray matter concentration (29) in brain regions involved in interoceptive awareness and attention and that are active during meditation (e.g. right anterior insula, prefrontal cortex, hippocampus); and increased activity in brain regions associated with attention and response inhibition on a distractor task (30). Cumulatively, these psychological and brain findings are consistent with the enhanced attention, self-referential processing, interoception, and affect regulation associated with mindful personality and mindfulness practice.

As such cross-correlation work limits the claims that can be made for a causal role of mindfulness in driving these psychological and neural outcomes, mindfulness has also been studied using randomized controlled trials of training interventions, including MBSR and Mindfulness-Based Cognitive Therapy (MBCT). Consistent with trait mindfulness associations, MBSR has been shown to reduce depression, anxiety, perceived stress and rumination (31–35), and increase positive affect, life satisfaction, empathy, self-compassion, and general emotional well-being (33,36,37). Recent fMRI and EEG studies of 8-week MBSR training programs have shown increased activation in right lateralized prefrontal cortex, insula, and inferior parietal lobule and decreased recruitment of medial prefrontal cortex, postulated to relate to changes in self-referential processing (38); increased left-lateralized prefrontal cortex electrical activity (an EEG finding associated with positive affect) (39); and greater right-lateralized recruitment of visceral and somatosensory processing areas in response to sadness provocation relative to waitlisted controls (40). This body of work thus provides initial indications that mindfulness meditation training interventions produce longitudinal neural changes commensurate with the psychological processes they train.

An understanding of the neurobiological changes associated with mindfulness will lend further empirical validity to the use of MBSR and other mindfulness training programs in clinical settings. This work, although rooted in basic science, has translational implications for clinicians seeking new treatments for patients with stress-sensitive illnesses, particularly since mindfulness training may be most beneficial to stressed populations. In particular, intervention studies in high-stress subject samples have thus far indicated that MBSR participation reduces state and trait anxiety and psychological distress and increases empathy in stressed medical students (35), that Mindfulness-Based Mind Fitness Training decreases perceived stress in a high-stress pre-deployment military cohort (41), and that MBSR decreases depression, anxiety, and psychological distress in patients with chronic somatic diseases (42). Mindfulness-Based Stress Reduction (MBSR) is thus a novel clinical treatment for improving outcomes in stress-related illnesses.

This series of studies uses a condensed version of the standardized MBSR intervention to test the neurobiological effects of mindfulness training in stressed community adults in an innovative randomized controlled trial. Thirty-five unemployed job-seeking community adults reporting high perceived stress levels were recruited for a rigorous single-blind RCT of 3-day intensive mindfulness meditation or relaxation training intervention. Four weeks before the 3-day training intervention, participants came to the Scientific Imaging and Brain Research (SIBR) center at Carnegie Mellon for a baseline neuroimaging session, which included a 5-minute resting state functional MRI scan. Participants then were randomized to either a 3-day intensive mindfulness meditation training or a structurally matched 3-day relaxation residential retreat program that included similar activities but emphasized participation in a restful rather than mindful manner. Participants returned to SIBR within two weeks of completing the 3-day intervention and completed the same resting state fMRI scanning procedure as at baseline. Participants also completed a battery of psychosocial measures and provided a blood draw at baseline and at 4-month follow-up. As high-stress populations are also hard to reach and retain, and often drop out of 8-week training programs (4), our novel approach has several advantages. By delivering both interventions at the same time in a 3-day residential retreat format, we increase experimental control over treatment delivery, improve treatment compliance, and decrease subject attrition, thus improving our study’s internal validity.

There has been much recent interest in understanding how mindfulness training changes the brain. Despite an increasing number of studies reporting positive associations between mindfulness and physical and mental well-being, the neurobiological processes that underlie the health benefits of increased mindfulness are not well-understood. To date, there has been work investigating both structural neural changes (28,43–46) and task-based functional brain activity associated with mindfulness (5,22,38,40,47–49). From these studies, it has been proposed that both "top-down" regulatory pathways and "bottom-up" stress reactivity pathways are influenced by individual differences in trait mindfulness as well as mindfulness training; more mindful individuals show increased prefrontal cortical activity (suggestive of increased top-down regulation of limbic system stress responding, and enhanced executive control) as well as decreased limbic system activity (consistent with decreased bottom-up stress responding) (22,38). Given the emerging evidence for structural and functional neural changes associated with dispositional and trained mindfulness, the goal of this series of studies is to further elucidate the neural mechanisms underlying the connections between mindfulness, decreased stress responding, and enhanced executive function using resting state functional MRI. There are several advantages to using resting state data in evaluating functional connectivity. First, it is task-independent, and thus avoids certain confounding factors in the interpretation of task-based studies (e.g. practice effects, task adaptation, effort, task strategy) (50). Second, consistent networks are identified from spontaneous fluctuations in the BOLD signal during resting conditions regardless of methodological variation (e.g. eyes open, eyes closed, fixation on a cross-hair) that reflect known functional topography (51). These spatial correlation patterns of spontaneous activity have been shown to predict task-based activity (52) as well as task performance (53,54). Additionally, ongoing spontaneous neuronal activity consumes the majority of the brain's energy, whereas task-associated increases in neuronal metabolic activity are relatively small; thus, it has been suggested that alterations in resting state neural activity may be a particularly valuable indicator of altered neural processing in the global scheme of brain metabolism (50). Finally, the signal-to-noise ratio may be higher in resting state fMRI than task-based studies, which require significant averaging over large numbers of trials to obtain a robust signal (55). Indeed, much of the “noise” that must be dealt with in task-based studies represents the ongoing spontaneous BOLD fluctuations that comprise the resting state signal (50,55). Resting state functional connectivity analyses are thus an ideal tool for investigating fundamental neural changes that may occur with a mindfulness training intervention in a high-stress community adult population.

This program of research seeks to answer several major open questions about the neural mechanisms for mindfulness training effects. Is the decreased stress-responding associated with greater mindfulness reflected in baseline functional connectivity changes between stress-responding regions? Does mindfulness training enhance executive function by strengthening the connectivity of prefrontal circuits for executive control and attentional processes? How do attentional, executive control, and salience network properties change in response to a mindfulness training program? To examine these questions, I describe three sets of analyses from a 3-day mindfulness training intervention (versus an active, well-matched relaxation control) with pre- and post-intervention resting state functional MRI scans. In Study 1 (Chapter 2), I identify stress-related resting state functional connectivity changes reduced by mindfulness training. In Study 2 (Chapter 3), I investigate mindfulness-associated changes in the functional connectivity of dorsolateral prefrontal cortex to inform our understanding of how mindfulness may enhance executive control. In Study 3 (Chapter 4), I expand upon these region-specific functional connectivity results to look at network-level changes in information processing within attention and stimulus-responding networks after mindfulness training.

2.0 STRESS-RELATED RESTING STATE FUNCTIONAL CONNECTIVITY CHANGES AFTER A MINDFULNESS TRAINING INTERVENTION

2.1 INTRODUCTION

There has been significant recent interest in identifying interventions and pathways for stress resilience in at-risk individuals (56). Recent studies suggest that mindfulness meditation interventions, which train the capacity to be more open and aware of present-moment experience, may increase stress resilience (57,58). For example, RCTs show that mindfulness meditation training interventions reduce reactivity to acute stressors (59,60) and improve health outcomes in stress-related disorders and diseases (e.g., depression, PTSD, HIV-infection: 61,62,63). Moreover, cumulative evidence has linked individual differences in stress reactivity and the experience of stress to a broad range of physiological health outcomes (1,64–67). While such studies are promising, the neural mechanisms for mindfulness training and stress resilience are unknown.

Resting state functional connectivity (rsFC) methods provide one approach for interrogating how mindfulness alters neural dynamics among stress-responsive brain regions. RsFC metrics of inter-regional dynamics specifically afford the advantage of being task-independent, providing reliable estimates of neural circuit functionality corresponding to structural topography (51,68). Here, we tested the extent to which mindfulness alters stress-related amygdala rsFC for three reasons: (1) The amygdala is a cell complex that is centrally involved in processing psychological stressors and coordinating physiological stress responses (69,70). (2) Recent studies show that greater reported mindfulness is associated with reduced amygdala volumes and task-based amygdala activation (71,44,23,43,72,73). (3) Anatomical studies in animal models indicate robust amygdala connectivity to other brain regions considered to be integral for processing stressors and orchestrating stress reactions (e.g. anterior cingulate cortex [ACC] and medial prefrontal cortex, hypothalamus, periaqueductal gray, and pontine/medullary autonomic control regions) (67,74). Moreover, recent human studies have shown that amygdala functional connectivity with the ACC is associated with greater stressor-evoked physiological reactivity (75) and amygdala-ACC functional connectivity is enhanced after acute stress exposures (76,77).

Accordingly, to test whether mindfulness reduces stress-related amygdala-ACC rsFC, we conducted two studies: (1) an initial discovery study evaluated whether self-reported perceived stress was associated with greater amygdala-ACC rsFC in a large sample of community volunteers (N=130). Although some human studies implicate increased stress-related amygdala rsFC with spatially adjacent areas of the ACC (e.g., 75), the discovery study tested for associations between perceived stress with amygdala rsFC across the whole brain. (2) We then conducted a randomized controlled trial (RCT) of mindfulness meditation training (compared to a matched relaxation training intervention without a mindfulness component) in a high-stress unemployed community sample to test whether mindfulness training prospectively reduces stress-related amygdala-ACC rsFC.

2.2 METHODS

2.2.1 Discovery Study

Participants, Procedure, and Analysis

133 healthy adults were recruited from the community by mass mailings to residents of Allegheny County, PA (3 participants were removed due to poor co-registration during preprocessing, resulting in a final sample of N=130). This discovery study was drawn from a larger ongoing NIH-funded parent study focused on understanding the neurobiological, psychosocial, and behavioral correlates of health among community adults (the Pittsburgh Imaging Project). Demographic characteristics of this study sample are provided in Table 1. All participants gave written informed consent as part of protocols approved by the Institutional Review Boards of the University of Pittsburgh and Carnegie Mellon University. Inclusion criteria included no history of (1) cardiovascular disease (including treatment for or diagnoses of hypertension, stroke, myocardial infarction, congestive heart failure, and atrial or ventricular arrhythmias); (2) prior neurosurgery or neurological disorder; (3) current treatment for or self-reported psychiatric disorder; (4) typical consumption of greater than 15 alcoholic beverages per week; (5) daily use of corticosteroid inhaler; (6) current use of psychotropic, lipid lowering, or any cardiovascular medication, including any medication to control blood pressure; (7) metal implants or exposure; (8) colorblindness; and (9) claustrophobia. All participants were right-handed, as assessed by the Edinburgh Handedness Inventory (78). Women were excluded if pregnant (verified by urine test). Participants completed a psychosocial survey battery, which included the Perceived Stress Scale (PSS). The PSS is a 10-item questionnaire measuring the frequency of stressful feelings and thoughts within the last month (0=Never, 4=Very Often). Four of the items are reverse-scored and an overall composite measure is computed, with higher values indicating a greater perceived stress (79).

Table 1 Discovery Study Subject Characteristics (n = 130)

Variable	**Mean**	**St. Dev.**
Age	40.15	6.14
Gender	71 male, 59 female	-
Household Income	$39,199	$17,713
Years of School	17.31	3.21
PSS-10: Perceived Stress	1.33 (Range 0.1 – 3.2)	0.61

Notes: PSS = Perceived Stress Scale

All participants completed a separate neuroimaging session, which included a 5-minute resting state functional connectivity scan where participants were asked to rest quietly with their eyes open. Images were acquired on a 3 Tesla Trio TIM whole-body scanner (Siemens, Erlangen, Germany), equipped with a 12-channel phased-array head coil. Three-dimensional magnetization prepared rapid gradient echo (MPRAGE) high-resolution T1-weighted neuroanatomical images were acquired for each subject over 7 minutes 17 seconds by these parameters: field of view = 256×208 mm, matrix size = 256×208 mm, time to repetition = 2100 ms, time-to-inversion = 1100 ms, time to echo = 3.29 ms, and flip angle = 8° (192 slices, 1mm thick, no gap). For each subject, a single 300 second run of resting state BOLD data was acquired with a repetition time of 2 seconds. Preprocessing of images was conducted in SPM8 (Welcome Department of Cognitive Neurology, London, UK; run on MATLAB, MathWorks, Inc., Natick, MA, USA) and rsFC analysis was conducted using the CONN toolbox (80). BOLD images for each participant were realigned to the first image of the series, corrected for distortion due to movement, and spatial normalized to the Montreal Neurological Institute 152 template. Normalization was conducted using the structural gray matter image segmented from the T1-weighted MPRAGE image; the structural gray matter image was co-registered with the mean realigned BOLD image, and registered to the MNI template by non-linear affine transformation. The normalized BOLD images were smoothed with a 6-mm full-width at half-maximum isotropic Gaussian kernel for statistical analysis. In the CONN toolbox, structural MPRAGE images were segmented to define gray matter, white matter, and cerebrospinal fluid areas. A time series was extracted from each region of interest using functional BOLD fMRI resting state data. Additional preprocessing was carried out in the CONN toolbox to account for further temporal confounding factors, including BOLD signal from the subject-specific white matter and CSF masks, motion parameters (six dimensions), and the effect of rest (an average across the session) to account for global signal. A covariate for each subject's head motion was entered at the first level. A band-pass filter of 0.008-0.09 Hz was used. The CONN toolbox estimates orthogonal time series using principal component analysis of the BOLD signal in each noise ROI.

At the single subject level, functional connectivity was measured by calculating the average BOLD time series across all voxels in each seed region and calculating a bivariate correlation between each seed region of interest and every other voxel. A hemodynamic response function was used to down weight the initial scans within each resting state block to minimize potential ramping effects. Left and right amygdala seeds were anatomically defined using the Wake Forest University (WFU) pickatlas (81) for generating whole-brain rsFC maps in CONN, which were then related to PSS scores as a covariate of interest in separate group-level GLM analyses. Whole-brain analyses relating PSS to left and right amygdala rsFC were conducted using a discovery threshold of uncorrected $p<.001$ and $k>30$.

2.2.2 Randomized Controlled Trial

2.2.2.1 Participants

Thirty-five stressed unemployed job-seeking community adults (who indicated moderate to high levels of perceived job-seeking stress over the past month, scoring >9 on an adapted 4-item PSS for job-seeking stress; $\alpha = 0.55$) participated in a single-blind RCT of 3-day intensive mindfulness meditation or relaxation training intervention (see Table 2 for participant characteristics). Participants were recruited via newspaper advertisements and through employment agencies in Pittsburgh, PA. Participants were English-speaking, had no pre-existing health conditions, were willing and available to participate in all study assessments, and were willing to be randomly assigned to one of two study conditions. Callers who met these qualifications were invited to come to Carnegie Mellon University for an in-person screening interview and baseline assessment. A more in-depth screening interview followed, including assessments of basic cognitive ability, right- or left-handedness and internal metal content (for fMRI eligibility), employment background (to probe for unemployment-related stress), medical history, and health behavior. Subjects taking psychotropic medications were excluded. Figure 1 depicts the flow of participants through the RCT. This study was approved by the Carnegie Mellon University Internal Review Board and all participants provided informed consent.

Table 2 Baseline Characteristics of Randomized Controlled Trial Participants

Characteristic	Mindfulness Group	Relaxation Group	Difference Statistic
Age [mean years (SD)]	37.94	41.00	$t(33)= -.48$, $p= 0.64$
	(10.96)	(9.55)	
Gender			$\chi^2(1)=.24$, $p= 0.63$
Male	11	9	

Female	7	8	
Ethnicity			$\chi^2(3)= 4.36$, *p*= 0.23
Caucasian	10	13	
African American	6	2	
Asian American	1	0	
Latino(a)	0	0	
Native American	0	0	
Other	0	1	
Years Unemployed	8.17 (12.48)	10.58 (20.31)	*t*(33)= -.43, *p*= 0.67
Education			$\chi^2(8)= 8.43$, *p*= 0.39
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	

Notes: Standard deviation values are provided in parentheses. Mindfulness group refers to the 3-Day Health Enhancement thru Mindfulness (HEM) intervention. Relaxation group refers to the 3-Day Health Enhancement thru Relaxation (HER) intervention.

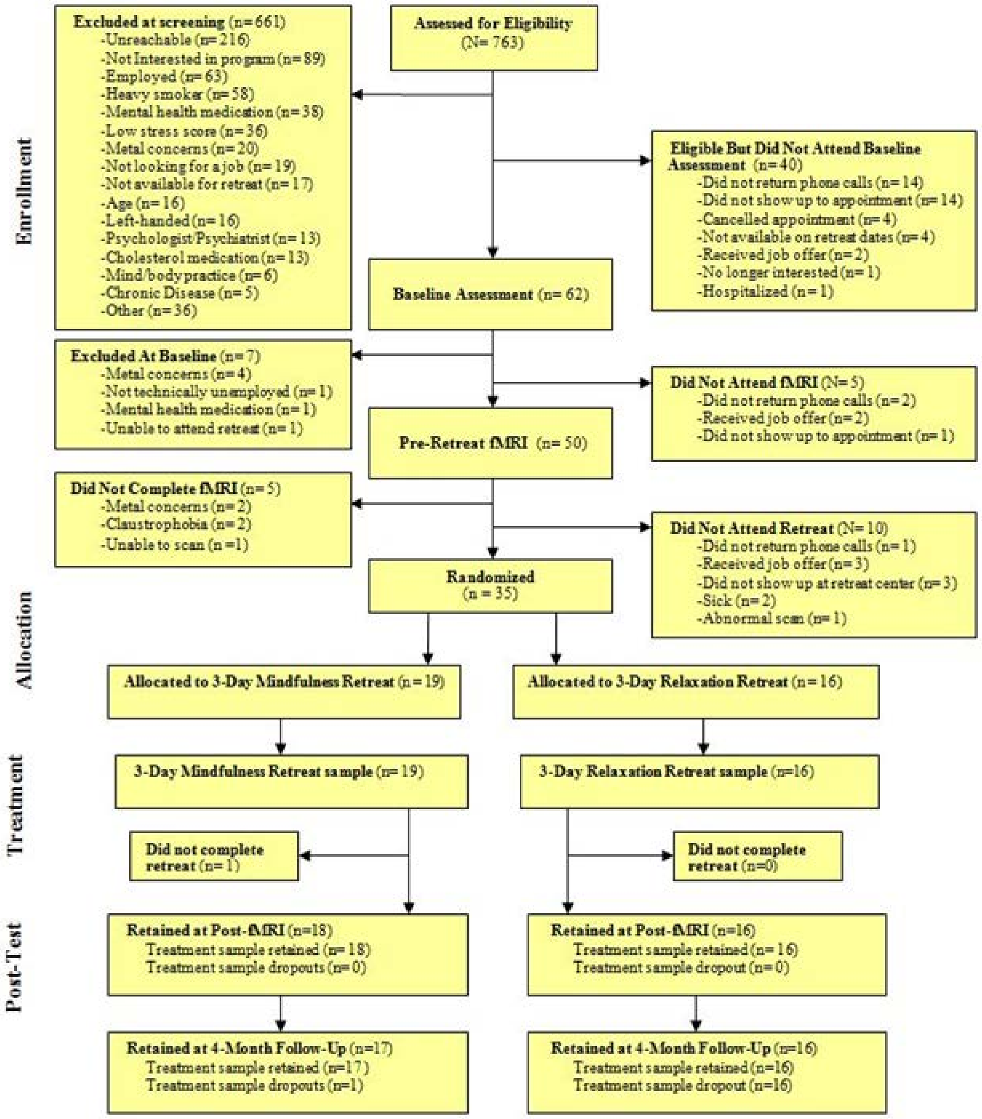


Figure 1. CONSORT flowchart of participants retained at each stage of the Mindfulness Meditation Training RCT.

2.2.2.2 Procedure

We conducted this RCT between December 2010 and October 2011. Beginning four weeks before the 3-day training intervention, participants completed a baseline neuroimaging session. All participants began with a 5-minute resting state scan (where they passively viewed a fixation cross), followed by three functional tasks in counterbalanced order (Multi-Source Interference Task, Affect Labeling, and a Personalized Stress Task), and a 7-minute perfusion MRI scan with a guided awareness of breathing task (the results of these functional tasks are not reported here). After neuroimaging, participants were invited to a nearby residential retreat center where they were randomized to either a 3-day intensive mindfulness meditation training (N=19) or matched 3-day relaxation residential retreat intervention (N=16). Only the participant, project manager, treatment program staff members, and the treatment program instructor were aware of the participant's study condition. All other study personnel responsible for collecting study assessments remained blinded. Participants returned for a neuroimaging assessment within two weeks of completing the 3-day intervention and completed an identical scanning procedure as at baseline, including the same 5-minute resting state scan. At both neuroimaging sessions, participants were instructed to passively view a fixation cross during the resting state scan period and not to sleep or engage in any meditation or relaxation practices (which was verbally confirmed in all participants at the conclusion of the neuroimaging session). 97% of randomized participants were retained at the post-intervention neuroimaging assessment (3% study attrition, see Figure 1). As part of the larger study, participants completed a comprehensive battery of psychosocial measures and provided a blood draw at baseline and at 4-month follow-up; the present report focuses on testing how mindfulness meditation training changes rsFC patterns using the 5-minute resting state BOLD scan at baseline and in the two weeks following the 3-day intensive training period (post-intervention). In order to measure cumulative hypothalamic-pituitary-adrenal (HPA) axis activation over the 4-month follow-up period, participants were invited to provide a hair sample at the 4-month follow-up appointment (82,83).

2.2.2.3 Interventions

We adapted the standardized and manualized 8-week Mindfulness-Based Stress Reduction (MBSR) program (which includes a day-long retreat) (3,84) into a condensed 3-day residential retreat format, entitled Health Enhancement through Mindfulness (HEM). Delivery of the HEM program in a structured residential retreat format improves compliance with training, reduces treatment attrition, and greater experimental control is afforded by offering a parallel matched relaxation training retreat (in a separate wing of the retreat center). The HEM instructor was a doctoral level psychologist with 7 years of MBSR teaching experience. Subjects were not informed that the mindfulness intervention was called HEM, so as to avoid any non-specific demand characteristics. Briefly, the HEM program consists of mindfulness training through body scan awareness exercises, sitting and walking meditations, mindful eating, and mindful movement (gentle hatha yoga postures). After each formal meditation period, participants engaged in discussion of their observations about themselves and the practices. The instructor modeled and encouraged attitudes to foster mindfulness such as letting go of judgment and expectations, cultivating self-care, patience, and friendly curiosity toward present moment experience. On the third day, formal meditation practices were extended to discussions about how participants could use mindful awareness for their unemployment and job-seeking stress.

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 all trainings emphasized participation in these activities in a restful way rather than a mindful way. The HER program instructor was a licensed social worker with over 2 decades of clinical experience in stress management. The use of a structurally-matched active comparison group was designed to control for non-mindfulness specific factors such as positive treatment expectancies, group support, teacher attention, physical activity, and mental engagement.

2.2.2.4 Image Acquisition

Structural and functional images were acquired on a Siemens Verio 3T scanner using a 32-channel head coil. High-resolution T1-weighted gradient-echo images were acquired at the start of the scanning session, with a slice orientation of AC-PC aligned, temporal lobes up (TR=1800ms, TE=2.22ms, flip angle= 9º, matrix size= 256x256, number of slices= 256, FOV= (205mm, 0.8mm thick slices), GRAPPA accel. factor PE= 2, voxel size= 0.8x0.8x0.8mm). Four functional echo-planar imaging runs were acquired, including a 300 second resting state scan (TR=2000ms, TE=30ms, flip angle=79º, matrix size=64x64, number of slices=36, FOV= 205mm, 3.2mm thick slices EPI with rate 2 GRAPPA, voxel size=3.2mmx3.2mmx3.2mm).

2.2.2.5 Image Preprocessing

Functional BOLD data were processed using SPM8 (Welcome Department of Cognitive Neurology, London, UK; implemented by MATLAB, MathWorks, Inc., Natick, MA, USA). First, the data were realigned to the mean image of the first run and then smoothed with a 4mm FWHM Gaussian kernel to be in the preferred format for the motion correction program, ArtRepair. Data were then submitted to motion correction using the ArtRepair utility (85,86), an interpolation-based motion correction utility program. Motion correction in ArtRepair follows a two-step process. In the first step, an algorithm is applied to each run of data to suppress interpolation errors due to large motion. The algorithm applies a larger correction to edge-wise voxels than to central voxels, since the effects of motion on BOLD signal are most pronounced in these areas. In the second step, TRs with large amounts of fast motion or large global signal variation are flagged for repair. A default motion threshold of 1mm was used, so that TRs with motion greater than 1mm were flagged for repair. Repair of the data was done through linear interpolation, so that volumes flagged for repair were filled in with the average signal value from the two nearest unrepaired TRs. After motion correction the functional data was normalized to the standard Montreal Neuroimaging Template (MNI) T1 template using indirect normalization, in which the functionals are first coregistered to the MPRAGE, and then the MPRAGE is normalized to the T1 template. Finally, the images were smoothed a second time with a 7mm FWHM kernel, resulting in an overall FWHM smoothing of 8mm (85).

2.2.2.6 Connectivity and Data Analyses

Left and right amygdala seeded resting state BOLD fMRI images were generated in the CONN toolbox (80) (using the same procedures as the discovery study), which were then applied in a group-level flexible factorial analysis in SPM8 with two factors specified, time (pre- and post-intervention) and group (HEM vs HER groups). We generated a time-by-group spreading interaction contrast that tested for baseline to post-intervention decreases in rsFC in the HEM program (relative to the HER program, in which we did not expect to see this rsFC decrease) using contrast weights: [1(pre,HEM), 1(pre,HER), -3(post, HEM), 1(post,HER)]. In a model with two independent variables, a spreading (or ordinal) interaction exists when an effect exists at one level of a second independent variable but is weaker or does not exist at another level of the independent variable. The discovery study indicated stress-related bilateral amygdala-sgACC rsFC, thus we pursued amygdala rsFC analyses with a bilateral anatomical (AAL atlas) ACC ROI (defined by the WFU Pickatlas (81)) in the mindfulness meditation training RCT. Cluster-level correction for multiple comparisons was obtained using a Monte Carlo simulation implemented by AlphaSim

(National Institute of Mental Health, Bethesda, MD). AlphaSim was implemented with a bilateral ACC mask generated from the AAL atlas using an 8mm smoothing kernel and 10000 iterations. Significant clusters ($p < 0.05$, corrected) were defined as those involving $k > 27$ contiguous voxels, each at $p < .005$.

Measuring Stress in the Mindfulness Meditation Training RCT: Hair Sampling

In order to evaluate whether changes in amygdala-ACC rsFC prospectively predict stress-related biomarkers after mindfulness meditation training, we conducted an exploratory analysis testing whether pre-post intervention changes in rsFC were associated with hair-derived cumulative measures of HPA-axis activation during the 4-month follow-up period (83). Hair samples have been used to measure cumulative HPA-axis activation, with approximately 1cm of hair length corresponding to one month of HPA-axis activity. Recent studies indicate higher HPA-axis activation among the unemployed relative to employed adults in hair samples (82). Hair was acquired and assayed using standard procedures described by Kirschbaum, Dettenborn and colleagues (82,87). Briefly, about 40 hairs were cut with scissors as close to the back of the scalp as possible from a posterior vertex position. The follicle end of each hair sample was labeled and clipped to a piece of aluminum foil and then sent to a specialized laboratory in Dresden, Germany for assay. Each sample was washed, dried, and spun for steroid extraction (see 82). Cortisone and cortisol determination was then assessed using a commercially available immunoassay (with chemiluminescence detection, CLIA-IBL-Hamburg, Germany). The lab reports intra-assay and inter-assay coefficients of variance as below 8%.

Data on demographic and lifestyle factors that consistently affect hair glucocorticoid levels is limited, but previous studies suggest that age, gender, and exercise volume (as vigorous exercise is associated with HPA axis activation) may be associated with hair cortisol (83,88–90). Thus, participant age, gender, and frequency of vigorous exercise were included as covariates in hair analyses in order to reduce noise attributable to these potential confounding factors. We analyzed both hair cortisone and cortisol; both are glucocorticoids released by the adrenal glands, with similar physiologic functions. Although cortisol is the more active form; previous research reports that hair cortisone may be higher than hair cortisol (as opposed to in plasma, where cortisol concentrations are higher than cortisone) due to increased activity of the enzyme responsible for converting cortisol to cortisone in the hair bulb (88,91) (Tiganescu et al., 2011). We first conducted a one-way ANCOVA comparing the mindfulness to relaxation groups on hair-derived measures of cortisone and cortisol at the 4-month follow-up appointment. Parameter estimates were then extracted in SPM8 using cluster analysis centered on the peak voxel in subgenual ACC at baseline and post-training. Exploratory analyses then tested whether greater pre-post (change score) training-related changes in amygdala-ACC rsFC co-varied with lower levels of these hair-derived markers of cumulative HPA-axis activation at 4-month follow-up. Specifically, associations between hair cortisone, cortisol and post-pre training change in functional connectivity across groups were examined using bivariate Pearson correlation analyses. N=4 participants were unable or unwilling to provide hair samples at the 4-month follow-up time point, and were excluded from these analyses. Statistical analyses were carried out in SPSS with a significance threshold of $p<0.05$.

2.3 RESULTS

2.3.1 Discovery Study Results

A whole-brain analysis indicated a positive association between self-reported chronic stress and greater amygdala coupling with the sgACC. Specifically, higher levels of stress were associated with increased rsFC between right amygdala and subgenual ACC (sgACC) (MNI: 0,16,-13, k=120 voxels), the only region of significant correlation with the right amygdala in a whole-brain analysis ($p<0.001$, uncorrected) (Figure 2a, Table 3). Although smaller in spatial extent, greater self-reported stress was also associated with increased left amygdala-sgACC (MNI: 1,22,-4, k=24 voxels) rsFC ($p<0.001$, uncorrected) (Figure 2b). Higher levels of self-reported perceived stress were also associated with greater left amygdala rsFC with perigenual right anterior cingulate cortex, parahippocampal gyrus, left insula, and temporal cortex in the discovery study (Table 4).

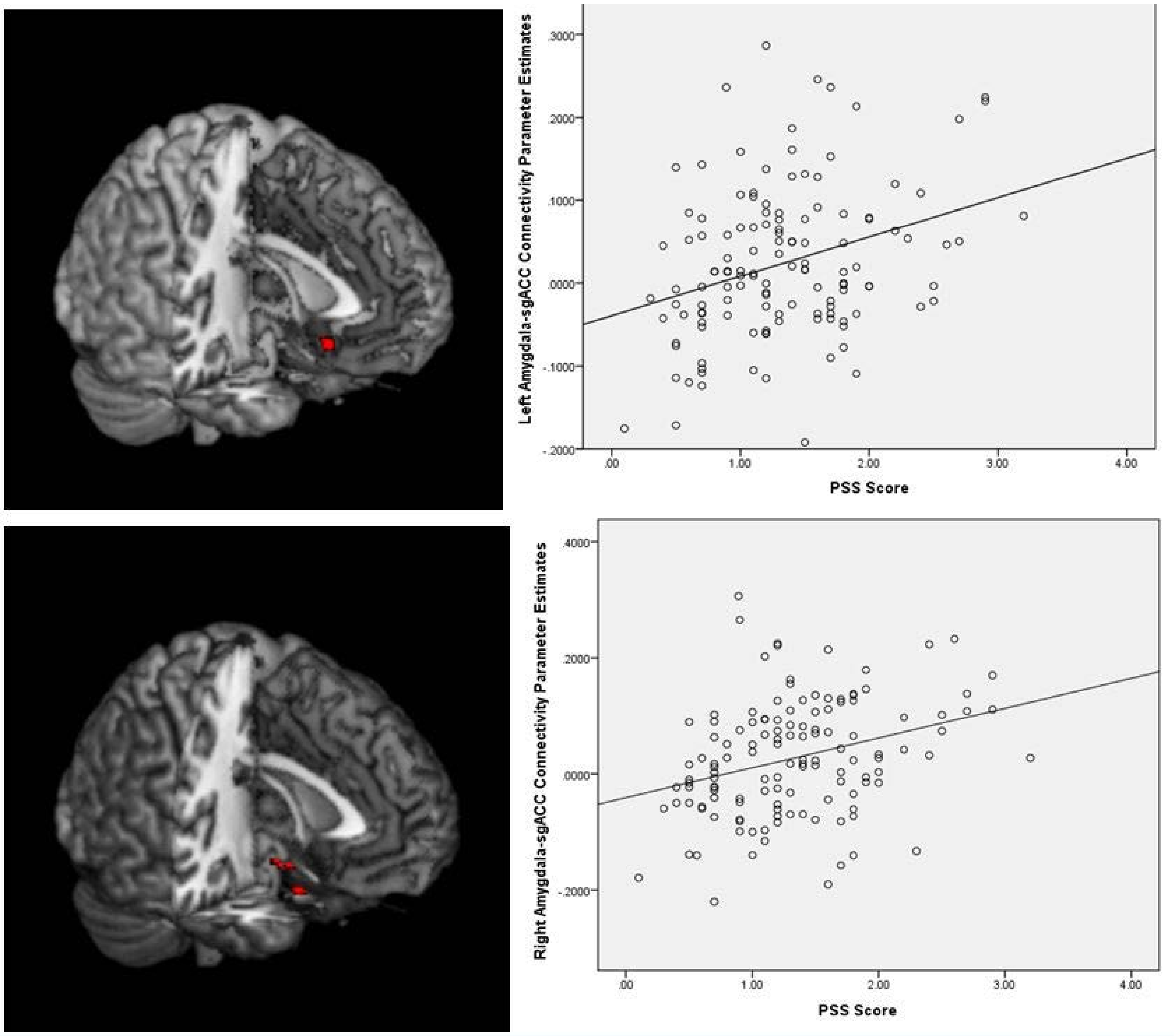


Figure 2. Relationship between perceived stress and amygdala-sgACC rsFC.

Discovery Study: **(a, top left panel)** Greater self-reported perceived stress on the PSS (item average) is associated with greater resting state functional connectivity between right amygdala and subgenual ACC ($p < 0.001$). **(a, top right panel)** Scatterplot between perceived stress and right amygdala-sgACC rsFC parameter estimates ($n = 130$, $R = -0.15$). **(b, bottom left panel)** Greater self-reported perceived stress on the PSS (item average) is associated with greater resting state functional connectivity between left amygdala and ACC ($p < 0.001$). **(b, bottom right panel)**

Scatterplot between perceived stress and left amygdala-sgACC rsFC parameter estimates (n = 130, R = -0.101).

Table 3 Subgenual ACC clusters identified in amygdala rsFC analyses.

Analysis	**Subgenual ACC MNI Peak Coordinates**	***k***	***p***	***T***	***Z***
Study 1, PSS, Right Amygdala	(0, 15.5, -13)	120	<0.001, uncorrected	3.71	3.61
Study 1, PSS, Left Amygdala	(1, 21.5, -4)	24	<0.001, uncorrected	3.35	3.28
Study 2, Spreading Interaction HEM < HER at Post-Intervention relative to Baseline	(0, 18, -12)	28	<0.05, corrected	2.93	2.82

Table 4 Discovery Study. PSS Left Amygdala Seed Connectivity, Whole-Brain Analysis

Increased Coupling associated with higher PSS score, *p* < 0.001, *k* > 30	Region (aal)	Peak MNI coordinate	Cluster Size	T	*Z*
	Parahippocampal Gyrus	-13 -11 -22	152	3.69	3.59
	Right Superior Temporal	56 -7 3	80	3.77	3.66
	Left Insula	-39 -13 11	112	3.92	3.80

	Right Perigenual Anterior Cingulum	11 30 14	236	4.48	4.31

2.3.2 Mindfulness Meditation Training RCT Results

Building on the discovery study findings showing that stress was associated with greater amygdala-sgACC rsFC, it was predicted that mindfulness meditation training (relative to a well-matched relaxation training program without a mindfulness component), would decrease right amygdala-sgACC rsFC in stressed, unemployed community adults. Consistent with this prediction, a flexible factorial random effects analysis showed that a 3-day intensive mindfulness meditation training program reduced right amygdala-sgACC (MNI: 0,18, -12) rsFC, compared to relaxation training without a mindfulness component (time × treatment interaction $p<0.05$, $k=28$, corrected for multiple comparisons) (Table 3, Figure 3). As shown in Figure 3, we observed positive right amygdala-sgACC rsFC pre-intervention in this high stress unemployed sample, which is consistent with the discovery study findings suggesting that stress is associated with elevated amygdala-sgACC rsFC (there were no significant difference in right amygdala-sgACC connectivity between the HEM and HER groups at baseline, $p=0.877$). But notably this was a spreading interaction driven by mindfulness meditation training reductions in right amygdala rsFC at post-treatment (Figure 3). A parallel flexible factorial random effects analysis also tested for training related changes in left amygdala-ACC rsFC, but there was no significant differential decreases in left amygdala-ACC rsFC pre-post training (no significant time × treatment interaction). Although the primary intent was to test for stress-related amygdala-ACC rsFC in an ACC ROI analysis, exploratory uncorrected whole-brain results are provided in Appendix A.

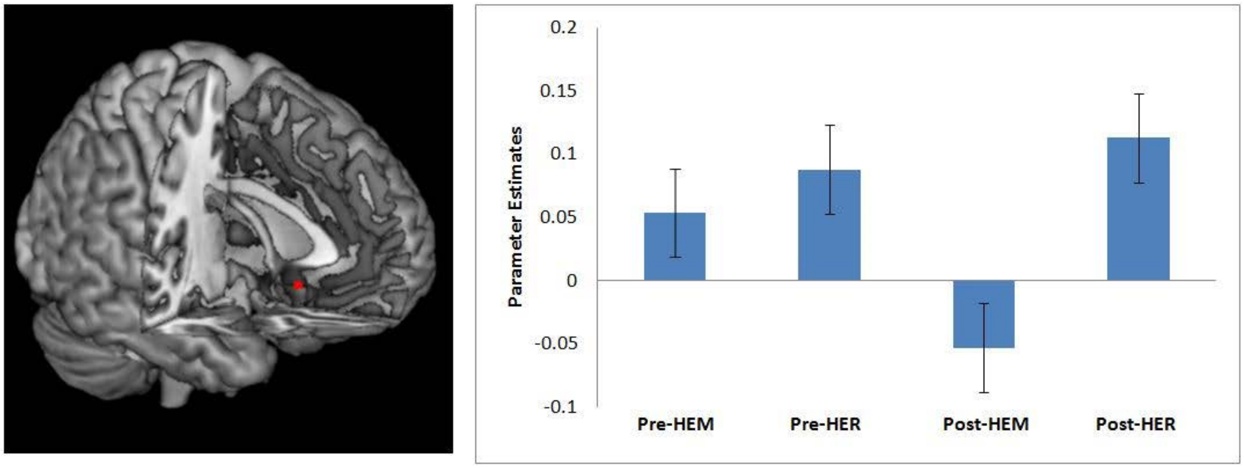


Figure 3. Mindfulness Meditation Training RCT right amygdala rsFC.

Mindfulness Meditation Training RCT. The left panel depicts the region of sgACC that showed decreased resting state functional connectivity with right amygdala from before to after mindfulness meditation training (HEM) relative to relaxation training (HER) ($p<0.05$, corrected for multiple comparisons). The right panel depicts the mean percent signal change for subgenual ACC cluster for the mindfulness (HEM) and relaxation (HER) training groups at each of the two time points (pre-intervention and post-intervention). Error bars depict +/- 1 standard error. Parameter estimates were extracted in SPM8 and plotted in a random effects mixed model conducted in SPSS.

Change in Amygdala rsFC Co-Vary with Biomarkers of Stress

An exploratory analysis evaluated whether hair-derived markers of cumulative HPA-axis activation were associated with brain activation during the 4-month follow-up period. There were no statistically significant differences between the mindfulness meditation and relaxation groups in cortisone (one-way ANCOVA $F(1,25)= 0.08$, $p=0.779$; mindfulness group $M=54.99$, $SE=43.63$; relaxation group $M=72.12$, $SE=40.75$)) or cortisol (one-way ANCOVA $F(1,25)= 0.783$, $p=0.385$; mindfulness group $M=52.80$, $SE=12.31$; relaxation group $M=37.72$, $SE=11.50$) at 4-month follow-up. However, pre-post intervention decreases in amygdala-sgACC rsFC were associated with less cumulative HPA-axis activation in hair across all subjects. Specifically, training-related decreases for right amygdala-sgACC rsFC were inversely associated with hair cortisone ($r(24)$= -0.39, p=0.049) and showed a trending association with hair cortisol ($r(24)$= -0.31, p=0.102).

Despite recommendations to continue home practice after the 3-day retreat with customized compact discs, the high stress participants in the HEM and HER programs did not complete much formal guided practice in the 4-month follow-up period. HEM group participants reported using their home practice CD an average of 1.24 times per week (SD = 1.28) over the last month (at 4-month follow-up), while HER group participants reported using their home practice CD an average of 0.382 times per week (SD = 0.86) over the past month (t_{31} =2.272, p = 0.03).

2.4 DISCUSSION

We provide an initial indication that alterations in amygdala-ACC rsFC track with perceived stress and can be altered by a mindfulness meditation intervention. Specifically, self-reported perceived stress was found to be associated with greater amygdala-sgACC rsFC. Moreover, mindfulness meditation training decreased amygdala-sgACC functional coupling relative to a well-matched comparison relaxation treatment without a mindfulness component. These findings agree with a neural circuitry-based account of previous studies suggesting that mindfulness alters amygdala structure and function (22,23,43,44,71), identifying a candidate amygdala-sgACC pathway that may link mindfulness training with reduced stress and stress-related health outcomes (57,58). While brain regions other than the amygdala are involved in stress reactivity, our approach was to first identify a candidate stress processing region that is central for gating stress responding (i.e., the amygdala) (67,70,74) and test how trained mindfulness alters stress-related rsFC.

The present discovery study indicates a positive association between perceived stress and amygdala–sgACC coupling. This finding is in accord with several studies, which link the right amygdala and ACC to stress. Specifically, the amygdala has well-known anatomical connectivity with regions of ACC (92–95), including sgACC (93,95)—and neurobiological accounts highlight the importance of amygdala and ACC/medial PFC networks in driving central fight-or-flight stress response cascades via activation of the HPA and sympathetic-adrenal-medullary axes (70). ACC regions spatially similar to regions identified in the present study have been implicated in stress responding; stressor-evoked physiological reactivity has been positively associated with right amygdala-perigenual ACC connectivity (75). One important question for the present work is whether mindfulness training related changes in amygdala-sgACC rsFC could be prospectively driving changes in stress. We provide some initial suggestive evidence that pre-post training related decreases in right amygdala-sgACC are associated with lower cumulative markers of HPA-axis activation over the 4-month follow-up period in the mindfulness meditation RCT study. However, this exploratory analysis should be viewed with some caution (and tested in new studies) given the lack of any robust training differences between the mindfulness meditation and relaxation conditions in these cumulative HPA-axis activation biomarkers, and the small subsample who provided hair samples at 4-month follow-up in the RCT study (N=30).

One limitation of the present research is that there were no stress-related disease outcomes in the RCT study with high stress community adults, and thus no way to evaluate whether mindfulness training changes in amygdala rsFC might drive potential improvements in stress-related disease. Nonetheless, this work offers a novel candidate pathway for explaining how mindfulness interventions benefit stress sensitive psychiatric populations. Previous studies have implicated disrupted rsFC in stress-related psychiatric disorders (e.g., PTSD, generalized anxiety disorder, major depressive disorder) (96–99), and recent RCTs indicate robust effects of mindfulness training on improving psychiatric outcomes in these same patient groups (63,100–103). Moreover, higher levels of self-reported perceived stress have been linked to poorer mental and physical health outcomes (104). The present research offers a testable new prediction for future studies; namely, mindfulness interventions may reduce amygdala-sgACC rsFC, which serves as a neurobiological mechanism for improvements in emotion regulation, stress reactivity, and improved stress-related health and disease outcomes.

There has been a recent interest in how mindfulness meditation alters structural and functional activity in the ACC. For example, integrative mind-body meditation training has been shown to increase network efficiency and connectivity of the ACC (46) and recent work has shown that this form of meditation training increases white matter integrity in the corona radiata – the major tract projecting through ACC (45). Notably, sgACC is thought to be an important hub in networks for negative affect and mood disorders (96,105) and anatomical studies have shown direct connections between sgACC and the amygdala, hypothalamus, nucleus accumbens, and orbitofrontal cortex (whereas pregenual ACC is more strongly connected to medial prefrontal and mid-cingulate cortex, sgACC, and subcortical regions)(106). In combination, these studies and our work here suggest potential dissociable effects of mindfulness meditation training on spatially distinct regions of ACC; such as mindfulness meditation training increasing rsFC on more dorsal and pregenual ACC tracts associated with enhanced self-regulation (45,cf. 107) and pain modulation (48), while also decreasing stress-related functional connectivity of the sgACC with the amygdala. Together, this work suggests the possibility that mindfulness meditation training fosters dorsal/pregenual ACC connectivity for attention monitoring while decoupling stress-related sgACC connectivity for stress resilience.

An important limitation of the present seed-based rsFC analytic approach is that it precludes inferences about the directionality of amygdala-sgACC connectivity; in the future, effective connectivity analyses (e.g., with dynamic causal modeling) offer opportunities to test causal interactions between these stress-sensitive nodes. Moreover, we utilized 5 minute resting state scans; longer scans periods as well as cardiorespiratory data can be used to reduce measurement error in future studies (108,109). An additional limitation is the lack of inclusion of a usual care group, which would provide an additional level of comparison to the HER and HEM intervention groups, allowing examination of the effects of attending a 3-day relaxation retreat and the potential stress-buffering gained from this (above and beyond no intervention). Given prohibitive cost and subject burden concerns, hair samples were not collected at time 1, only at 4-month follow-up; thus, our analyses are necessarily limited to relating change in amygdala-ACC resting state functional connectivity to cortisone and cortisol levels at follow-up. Finally, this work more broadly suggests a new amygdala rsFC pathway for stress resilience; future work should evaluate whether similar changes in amygdala rsFC can be achieved with other clinically impactful psychological interventions (e.g., cognitive behavioral therapy) or anxiolytic pharmacological treatments in at-risk stressed populations.

Conclusions

The present findings significantly advance our understanding of resting state functional connectivity in stress and mindfulness training interventions, and implicate decoupling of the amygdala and sgACC as a potential neurobiological mechanism underlying mindfulness-based stress reduction effects.

2.5 BRIDGE

Here, I have implicated altered rsFC of the right amygdala as a potential neural mechanism for the stress-buffering effects of a mindfulness training intervention. Using an anatomical seed-based approach for functional connectivity analysis, I demonstrated that mindfulness training decoupled the right amygdala from subgenual ACC, while perceived stress was associated with increased coupling between these two regions. This study provides initial evidence that mindfulness moderates baseline connectivity between two known stress-processing regions, providing a neural mechanism for the reduced stress-responding associated with increased mindfulness and an underlying explanation for the reduced limbic system activity that has been observed in previous task-based fMRI studies (22,72); however, it leaves open the question of whether similar functional changes could explain the concomitantly observed enhanced executive function in more mindful individuals. This could potentially be experimentally observed by demonstrating a parallel increase in executive control-associated resting state functional connectivity in this subject sample. One hypothesis is that there is a two-pronged mechanism driving the positive psychological outcomes and improvements in cognitive function fostered by mindfulness training – a simultaneous decoupling of stress-related brain regions and enhanced coupling of executive control-associated brain regions.

It has been previously established that mindfulness interventions in healthy adults increase a broad range of executive functions, including attention and working memory (110,111), self-regulation (111), and perceptual discrimination (110). A recent study specifically examining the effects of mindfulness training on cognitive-affective neural changes found improved cognitive control and executive processing; specifically, brief mindfulness training (relative to an active control group) was associated with decreased conflict (better performance) on an affective Stroop task and increased dorsolateral prefrontal cortex (dlPFC) activity during the task (47). This suggests a pathway by which mindfulness may encourage executive control, as dlPFC - a key region in the central executive network - is implicated in top-down attention regulation and working memory (112). Thus, I next investigate resting state functional connectivity of the dlPFC after a mindfulness training intervention versus a relaxation control program in our sample of 35 high-stress community adults.

3.0 EXECUTIVE CONTROL REGION RESTING STATE FUNCTIONAL CONNECTIVITY AFTER A RANDOMIZED CONTROLLED TRIAL OF MINDFULNESS TRAINING

3.1 INTRODUCTION

Mindfulness meditation interventions, which train the capacity to both focus attention and to be more open to present-moment experience, produce many positive physical and psychological health effects, including increased stress resilience and greater executive control (3,113,114). However, the specific aspects of mindfulness training that increase executive control– and their neural mechanisms - have yet to be fully elucidated.

Dorsolateral prefrontal cortex (dlPFC) - a key region in the central executive network - is broadly implicated in the regulation of attention, decision making, working memory, and cognitive control (112), and is the key hub of a dorsal neural pathway for the control of behavior (115). Moreover, a growing body of literature shows that dlPFC is active during meditative states, including focused attention meditation practices (30,116), open-monitoring mindfulness meditation practices (38), and in response to affective stimuli (sadness) in trained meditators (40). This suggests a dlPFC-specific pathway by which mindfulness may encourage executive control, which is further supported by behavioral evidence that mindfulness increases performance on various cognitive tasks, including attention and working memory (110,111), self-regulation (111), and perceptual discrimination (110). As mindfulness trains the capacity for focused attention as well as open monitoring – cognitive processes that recruit dlPFC as well as dorsal and ventral regions for cognitive control, e.g. parietal cortex, superior temporal cortex, ventrolateral prefrontal cortex – I posit that increased functional coupling between dlPFC and these regions may be a neural mechanism underlying the enhanced cognitive control observed with mindfulness training.

A hypothesis not previously explored in the literature is that mindfulness fosters greater executive control (e.g. attention, working memory, emotion regulation, cognitive control) by strengthening the intrinsic functional connections between dlPFC and the dorsal and ventral frontoparietal control regions – specifically, intraparietal sulcus, frontal and supplementary eye fields, posterior parietal cortex, temporoparietal junction, ventrolateral frontal cortex, and inferior frontal gyrus - that coordinate executive control.

Dorsolateral PFC has functional and anatomic connections to dorsal (e.g., bilateral intraparietal sulcus, frontal and supplementary eye fields, and superior and posterior parietal cortex; involved in top-down directing of attention to specific inputs) and ventral- (e.g., right-lateralized temporoparietal junction, ventral frontal cortex, superior temporal gyrus, and inferior frontal gyrus; thought to be responsible for monitoring and reorienting attention in response to salient stimuli) networked regions for attention and cognitive control (112,117,118). Anatomical tracing studies in primates demonstrate that dlPFC (primate brain areas 9 and 46) is densely connected to these regions, with axonal tracts projecting to cingulate cortex, lateral prefrontal cortex, superior, middle and inferior frontal gyri, premotor and supplementary motor areas, orbitofrontal cortex, and insular cortex (119). Yet, no research has evaluated how mindfulness training might modulate dlPFC resting state functional connectivity to these key ventral and dorsal frontoparietal control network regions.

Resting State Functional Connectivity of dlPFC and Mindfulness

Resting state functional connectivity (rsFC) has proven to be a robust method of evaluating inter-regional dynamics; it has the advantage of being task-independent, reliable, and shows consistent correlations with known functional and structural topography (51,68). It is thus an ideal tool for investigating dlPFC functional connections in the context of mindfulness training, allowing us to build a functional network-based account of mindfulness effects for cognitive control. Previous studies provide evidence that dlPFC resting state functional connectivity changes with mindfulness; specifically, increased coupling is observed between dlPFC and default mode network regions (e.g. dorsal anterior cingulate, posterior cingulate cortex), consistent with decreased mind-wandering in experienced meditators (120). A similarly increased resting state functional coupling between posterior cingulate cortex and dlPFC has been reported after 3 days of intensive mindfulness training, relative to a relaxation control intervention; this study focused on mindfulness-related default mode network rsFC by using a posterior cingulate seed (Creswell et al, in submission).

In addition to these studies of mindfulness-associated dlPFC functional connectivity changes, clinical studies have shown that dlPFC resting state functional connectivity is altered by neuropsychiatric conditions; in schizophrenia, rsFC is reduced between dlPFC and parietal cortex, posterior cingulate, thalamus, and striatum, and increased between dlPFC and paralimbic structures as well as left temporal lobe (121). In euthymic bipolar disorder patients, right dlPFC-medial PFC rsFC is increased relative to controls (122). In patients with chronic hallucinations, reduced rsFC was observed between right dlPFC and right IFG (123). In all these conditions, altered dlPFC rsFC is thought to underlie the cognitive changes associated with these disorders, including working memory deficits, emotion regulation, and somatosensory processing. Although no studies have directly tested for dlPFC alterations after mindfulness training (cf. Creswell et al., 2015), there are studies showing that these executive functions are enhanced by mindfulness training (47,110,111). Moreover, there is evidence that dlPFC functional connectivity changes may relate to behavioral measures of executive control; during a 2-back working memory task, decreased FC between right dlPFC and left inferior parietal cortex and increased FC between left dlPFC and the right inferior temporal lobe was observed in autistic subjects relative to a control group (124). Finally, dlPFC activity has been shown to be stress-sensitive; acute psychological stress decreases dlPFC activity during working memory tasks, indicating a shift of neural resources away from executive control network regions (125). Chronic psychosocial stress disrupts functional connectivity between dlPFC and other frontoparietal network regions associated with attentional shifts; significantly, this disrupted connectivity was shown to be reversed after 1 month of decreased stress, indicating that stress-related changes in dlPFC connectivity are highly plastic (126). Therefore, it is plausible that a brief mindfulness training intervention in a high-stress sample could produce similar changes in dlPFC resting state functional connectivity.

The Present Study

We developed an innovative well-controlled training format for rigorously evaluating mindfulness meditation training effects on the brain in a high-stress community sample by adapting 8-week mindfulness meditation and relaxation training programs (3,84,127) to a 3-day residential retreat format. We recruited high stress unemployed job-seeking adults and randomized them to either a 3-day mindfulness meditation training program or a matched 3-day relaxation training lacking a mindfulness training component, allowing us to test for effects specific to mindfulness training and not general relaxation. This approach improves study internal validity by increasing experimental control of treatment delivery (as both the meditation and relaxation programs were delivered at the same time in the same relaxing retreat setting) and fosters improved treatment compliance and reduced participant attrition in hard-to-reach-and-retain high-stress patient populations. In the present study, we utilized resting state fMRI to probe the dlPFC functional connectivity to specific *a priori* defined brain regions of interest in intraparietal sulcus, frontal eye fields, posterior parietal cortex, temporoparietal junction, ventrolateral frontal cortex, middle and inferior frontal gyrus. To investigate how mindfulness training may modulate resting state functional connectivity of the dlPFC, we tested the hypothesis that this high-stress unemployed sample of community adults would show increased connectivity between dlPFC and regions that comprise the dorsal and ventral regulatory pathways (intraparietal sulcus, frontal and supplementary eye fields, posterior parietal cortex, temporoparietal junction, ventrolateral frontal cortex, and inferior frontal gyrus) after a mindfulness training intervention, relative to a well-matched relaxation control program.

3.2 METHODS

Randomized Controlled Trial of Mindfulness Meditation Training

Participants

Thirty-five stressed unemployed job-seeking community adults (who indicated moderate to high levels of perceived job-seeking stress over the past month, scoring >9 on an adapted 4-item PSS for job-seeking stress; i.e., "In the last month, how often have you felt confident about your ability to handle your job-related problems?" α=.6) participated in a single-blind RCT of 3-day intensive mindfulness meditation or relaxation training (see Table 1 for participant characteristics). Participants were recruited via newspaper advertisements and through employment agencies in Pittsburgh, PA. Participants were also English-speaking, had no pre-existing health conditions, were willing and available to participate in all study assessments, and were willing to be randomly assigned to one of two study conditions. Callers who met these qualifications were invited to come to Carnegie Mellon University for an in-person screening interview and baseline assessment, where the full study procedures were explained. Interested participants provided informed consent. A more in-depth screening interview followed, including assessments of basic cognitive ability, right- or left-handedness and internal metal content (for fMRI eligibility), employment background (to probe for unemployment-related stress), medical history, and health behavior. Subjects taking psychotropic medications were excluded. Demographic information was collected as well, including age, race, education, income, and marital and family status. Qualified participants completed a baseline psychosocial assessment on their own, described below. Participants were compensated $20 for this assessment. This study was approved by the Carnegie Mellon University Internal Review Board and all participants provided informed consent.

Procedure

We conducted this RCT between December 2010 and October 2011. Beginning four weeks before the 3-day training intervention, participants completed a baseline neuroimaging session. All participants began with a 5-minute resting state scan (where they passively viewed a fixation cross), followed by several functional tasks in counterbalanced order and an 8-minute perfusion MRI scan (the results of these tasks will be reported in separate papers). After neuroimaging, participants were invited to a nearby residential retreat center where they were randomized to either a 3-day intensive mindfulness meditation training (N=18) or matched 3-day relaxation residential retreat intervention (N=17) (described in Interventions below). Only the participant, project manager, treatment program staff members, and the treatment program instructor were aware of the participant's study condition. Participants returned for a neuroimaging assessment within two weeks of completing the 3-day intervention and completed an identical scanning procedure as at baseline, including the same 5-minute resting state scan. At both neuroimaging sessions, participants were instructed to passively view a fixation cross during the resting state scan period and not to sleep or engage in any meditation or relaxation practices (which was verbally confirmed in all participants at the conclusion of the neuroimaging session). 97% of randomized participants were retained at the post-intervention neuroimaging assessment (3% study attrition). As part of the larger study, participants completed a comprehensive battery of psychosocial measures and provided a blood draw at baseline and at 4-month follow-up; the present report focuses on testing how mindfulness meditation training changes rsFC patterns using the 5-minute resting state BOLD scan at baseline and in the two weeks following the 3-day intensive training period (post-intervention).

Interventions

We adapted the standardized and manualized 8-week Mindfulness-Based Stress Reduction (MBSR) program (which includes a day-long retreat) (3,84) into a condensed 3-day residential retreat format, entitled Health Enhancement through Mindfulness (HEM). Delivery of the HEM program in a structured residential retreat format improves compliance with training, reduces treatment attrition, and greater experimental control is afforded by offering a parallel matched relaxation training retreat (in a separate wing of the retreat center). The HEM instructor was a doctoral level psychologist with 7 years of MBSR teaching experience. Briefly, the HEM program consists of mindfulness training through body scan awareness exercises, sitting and walking meditations, mindful eating, and mindful movement (gentle hatha yoga postures). After each formal meditation period, participants engaged in discussion of their observations about themselves and the practices. The instructor modeled and encouraged attitudes to foster mindfulness such as letting go of judgment and expectations, cultivating self-care, patience, and friendly curiosity toward present moment experience. On the third day, formal meditation practices were extended to discussions about how participants could use mindful awareness for their unemployment and job-seeking stress.

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 all trainings emphasized participation in these activities in a restful way rather than a mindful way. The HER program instructor was a licensed social worker with over 2 decades of clinical experience in stress management. The use of a structurally-matched active comparison group was designed to control for non-mindfulness specific factors such as positive treatment expectancies, group support, teacher attention, physical activity, and mental engagement.

Image Acquisition

Structural and functional images were acquired on a Siemens Verio 3T scanner using a 32-channel head coil. High-resolution T1-weighted gradient-echo images were acquired at the start of the scanning session, with a slice orientation of AC-PC aligned, temporal lobes up (TR=1800ms, TE=2.22ms, flip angle= 9º, matrix size= 256x256, number of slices= 256, FOV= (205mm, 0.8mm thick slices), GRAPPA accel. factor PE= 2, voxel size= 0.8x0.8x0.8mm). Four functional echo-planar imaging runs were acquired, including a 300 second resting state scan (TR=2000ms, TE=30ms, flip angle=79º, matrix size=64x64, number of slices=36, FOV= 205mm, 3.2mm thick slices EPI with rate 2 GRAPPA, voxel size=3.2mmx3.2mmx3.2mm).

Image Preprocessing

Functional BOLD data were processed using SPM8 (Welcome Department of Cognitive Neurology, London, UK; implemented by MATLAB, MathWorks, Inc., Natick, MA, USA). First, the data were realigned to the mean image of the first run and then smoothed with a 4mm FWHM Gaussian kernel to be in the preferred format for the motion correction program, ArtRepair. Data were then submitted to motion correction using the ArtRepair utility (85,86), an interpolation-based motion correction utility program. Motion correction in ArtRepair follows a two-step process. In the first step, an algorithm is applied to each run of data to suppress interpolation errors due to large motion. The algorithm applies a larger correction to edge-wise voxels than to central voxels, since the effects of motion on BOLD signal are most pronounced in these areas. In the second step, TRs with large amounts of fast motion or large global signal variation are flagged for repair. A default motion threshold of 1mm was used, so that TRs with motion greater than 1mm were flagged for repair. Repair of the data was done through linear interpolation, so that volumes flagged for repair were filled in with the average signal value from the two nearest unrepaired TRs. After motion correction the functional data was normalized to the standard Montreal Neuroimaging Template (MNI) T1 template using indirect normalization, in which the functionals are first coregistered to the MPRAGE, and then the MPRAGE is normalized to the T1 template. Finally, the images were smoothed a second time with a 7mm FWHM kernel, resulting in an overall FWHM smoothing of 8mm (85).

Connectivity and Data Analyses

Preprocessing of images was conducted in SPM8 (Welcome Department of Cognitive Neurology, London, UK; run on MATLAB, MathWorks, Inc., Natick, MA, USA) and rsFC analysis was conducted using the CONN toolbox (80). The CONN toolbox estimates orthogonal time series using principal component analysis of the BOLD signal in each noise ROI. At the single subject level, functional connectivity was measured by calculating the average BOLD time series across all voxels in each seed region and calculating a bivariate correlation between each seed region of interest and every other voxel. A hemodynamic response function was used to weight down the initial scans within each resting state block to minimize potential ramping effects. Seed regions were defined by creating 8mm spheres around peak coordinates of four dlPFC clusters. We defined bilateral ROIs based on a previous study of resting state functional connectivity that showed increased dlPFC connectivity in meditators versus controls, to investigate dlPFC-associated rsFC that may be mindfulness-specific (MNI = 42, 21, 14; -48, 36, 15) (120). In order to also investigate dlPFC regions classically associated with executive control, we identified two additional ROIs by searching the features "attention" and “executive control” in the Neurosynth database (MNI = 32 50 12; -28 0 54) (128–130). Using Neurosynth to identify ROIs adds the value of an automated meta-analysis to identify neural regions that have been associated with features of interest (i.e. “attention”). See Table 5 for a complete list of ROIs and MNI coordinates.

Table 5. ROIs generated for seed-based analyses.

Region of Interest	MNI coordinates	Radius
Left dlPFC	-48, 36, 15	8mm
Left dlPFC	-28, 0, 54	8mm
Right dlPFC	42, 21, 14	8mm
Right dlPFC	32, 50, 12	8mm

Seeded resting state BOLD fMRI images were then applied in a group-level flexible factorial analysis in SPM8 with two factors specified, time (pre- and post-intervention) and group (HEM vs HER groups). We generated a time-by-group spreading interaction contrast that tested for baseline to post-intervention decreases in rsFC in the HEM program (relative to the HER program) using contrast weights: [1(pre,HEM), 1(pre,HER), -3(post, HEM), 1(post,HER)]. In a model with two independent variables, a spreading (or ordinal) interaction exists when an effect exists at one level of a second independent variable but is weaker or does not exist at another level of the independent variable. Cluster-level correction for multiple comparisons was obtained using a Monte Carlo simulation implemented by AlphaSim (National Institute of Mental Health, Bethesda, Maryland). AlphaSim was implemented with an anatomical ROI mask (generated using the Wake Forest Univeresity pick atlas, that covered middle frontal cortex, inferior frontal cortex, superior and posterior parietal lobule, and middle temporal cortex) using an 8mm smoothing kernel and 10000 iterations. Significant clusters ($P < 0.05$, corrected) were defined as those involving $k > 22$ contiguous voxels, each at $P < .005$.

3.3 RESULTS

Given that the dlPFC is implicated in executive control and modulated by mindfulness, it was predicted that mindfulness meditation training (relative to a well-matched relaxation training program without a mindfulness component) would increase rsFC between dlPFC and ventral and dorsal system regions (intraparietal sulcus, supplementary and frontal eye fields, posterior parietal cortex, temporoparietal junction, ventrolateral prefrontal cortex) in stressed, unemployed community adults. Consistent with this prediction, left dlPFC showed increased connectivity to the right inferior frontal gyrus (or vlPFC, a key ventral attention control region) ($p<0.05$, $k=28$, corrected for multiple comparisons; Table 6, Figure 4), and to the right middle frontal gyrus ($k=34$), right supplementary eye field ($k = 38$) and right superior/posterior parietal cortex ($k = 23$) (dorsal attention network regions) ($p<0.05$, corrected for multiple comparisons; Table 6, Figure 5), and to the left middle temporal gyrus ($p<0.05$, $k=52$, corrected for multiple comparisons; Table

6, Figure 5) following mindfulness training relative to the relaxation control. This pattern of mindfulness-associated increased rsFC supports the idea that mindfulness may increase top-down regulation by modulating task-independent functional connectivity between executive and attentional brain regions.

Right dlPFC (MNI = 32, 50, 12) showed increased connectivity to right middle frontal gyrus ($p < 0.05$, $k = 30$, corrected for multiple comparisons; Table 6, Figure 6). Right dlPFC cluster (MNI = 42, 21, 24) showed no significant time by group differences in resting state functional connectivity. For all dlPFC seed regions, no significantly reduced pre-post rsFC with other brain regions was observed in the mindfulness training group relative to the relaxation control group ($p < 0.05$, $k > 22$, corrected for multiple comparisons).

Table 6. Clusters with significantly increased rsFC to dlPFC seed regions after mindfulness training relative to a relaxation control intervention (p < 0.05, corrected for multiple comparisons). Notes: ***IFG = inferior frontal gyrus, SFG = superior frontal gyrus, SEF = supplementary eye fields, MFG = middle frontal gyrus.***

Seed ROI		**MNI**	***k***	**T**
Left dlPFC (-48, 36, 15)	Right IFG	54, 16, 14	28	3.74
Left dlPFC (-28, 0, 54)	Right SEF (BA 6)	22, 12, 58	38	4.29
	Right MFG	34, 2, 58	34	3.98
	Left Superior parietal lobule (BA 7)	-10, -78, 36	23	4.44
	Left Middle Temporal/Angular Gyrus	-42, -58, 10	52	3.97
Right dlPFC	Right MFG	46, 20, 40	30	4.97

(32, 50, 12)				

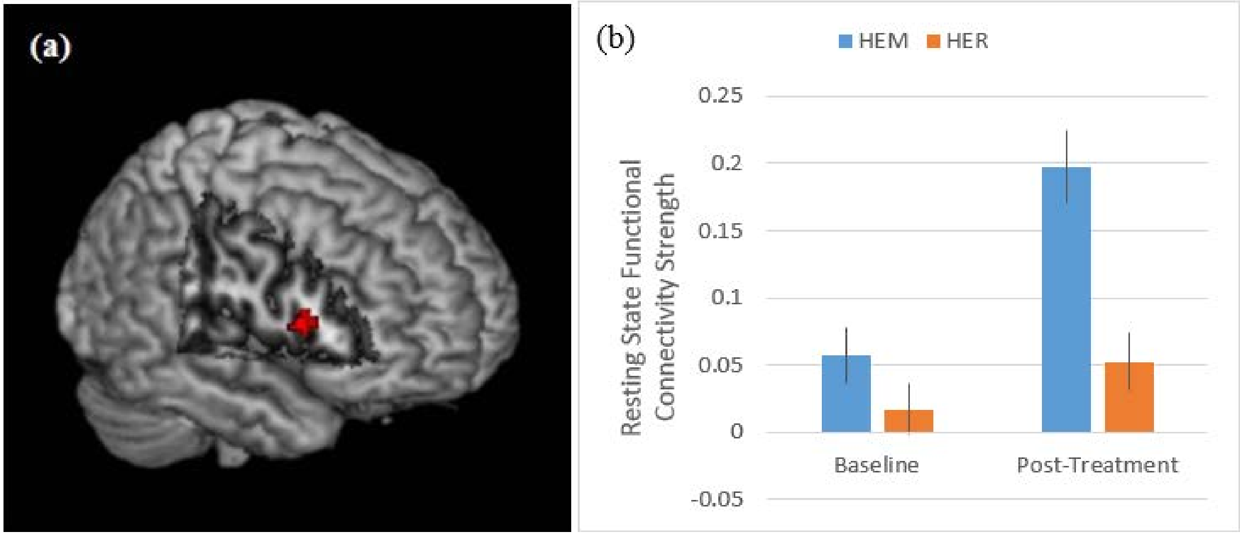
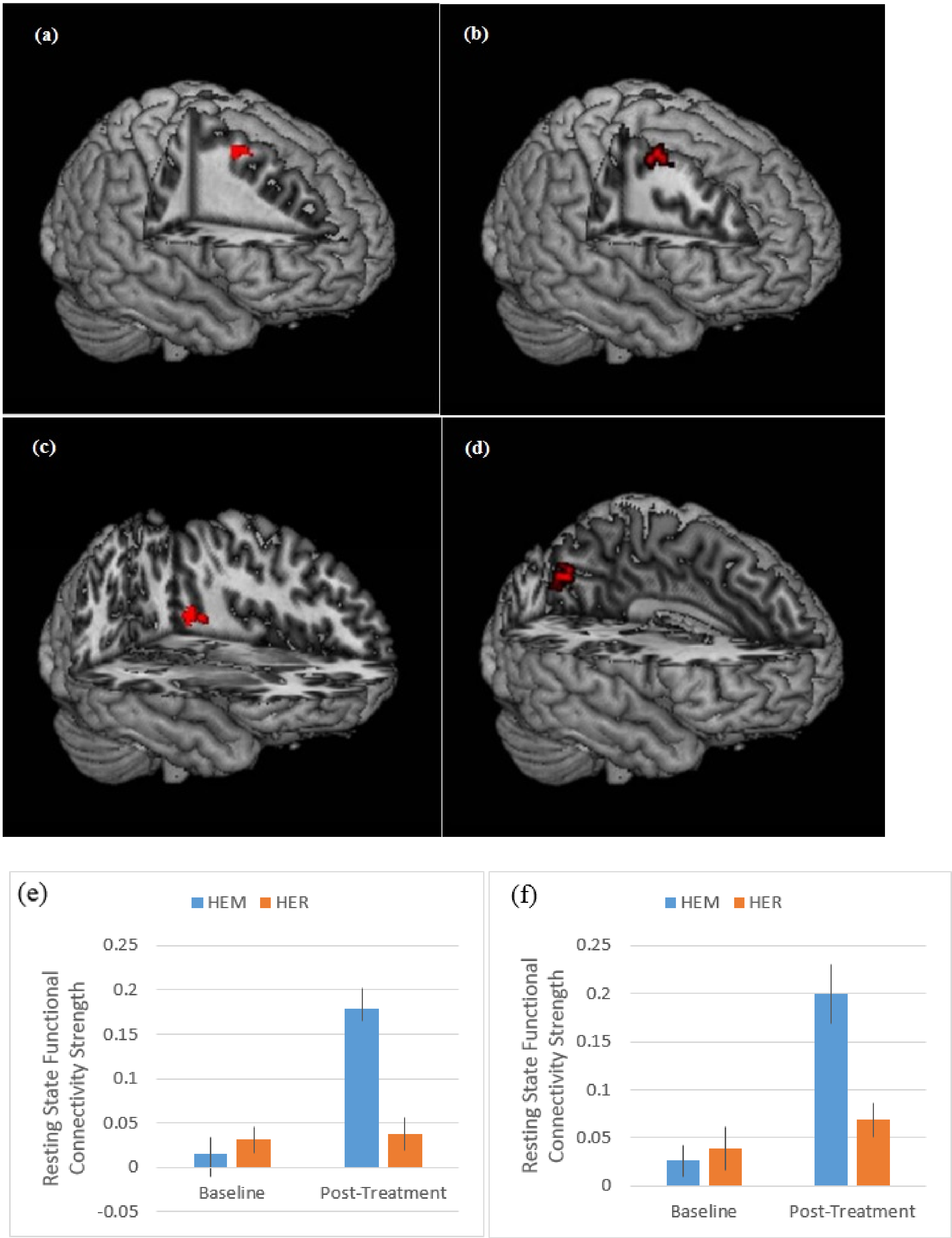


Figure 4. Increased left dlPFC-right IFG rsFC after mindfulness training.

(a) Regions that showed increased resting state functional connectivity with left dlPFC (-28, 0, 54) from before to after mindfulness meditation training (HEM) relative to relaxation training (HER) ($p<0.05$, corrected for multiple comparisons, cluster-thresholded $k>21$). Specifically, a condition by time spreading interaction analysis revealed a significant cluster in right inferior frontal gyrus ($k = 28$, peak MNI coordinates (54, 16, 14), $T = 3.74$). **(b)** Mean connectivity strength signal change for right IFG for the mindfulness (HEM) and relaxation (HER) training groups at each of the two time points (pre-intervention and post-intervention). Error bars depict +/- 1 standard error. Parameter estimates were extracted in SPM8.


(a)
(b)
(c)
(d)
(e)
HEM
HER
Resting State Functional Connectivity Strength
0.25
0.2
0.15
0.1
0.05
0
-0.05
Baseline
Post-Treatment
(f)
HEM
HER
Resting State Functional Connectivity Strength
0.25
0.2
0.15
0.1
0.05
0
Baseline
Post-Treatment

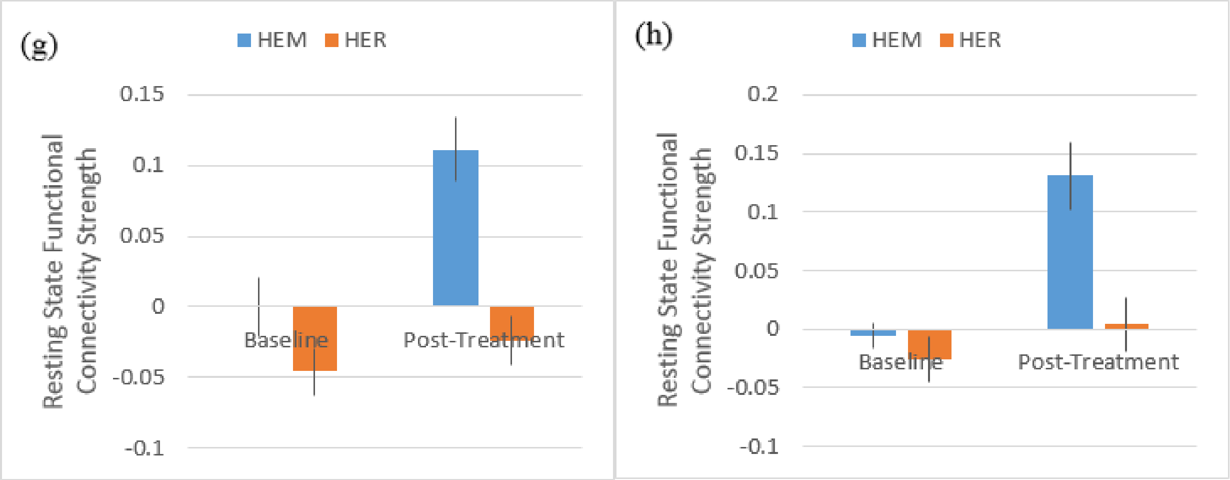


Figure 5. Regions of increased rsFC with left dlPFC after mindfulness training.

Regions that showed increased resting state functional connectivity with left dlPFC (-48, 36, 15) from before to after mindfulness meditation training (HEM) relative to relaxation training (HER) ($p<0.05$, corrected for multiple comparisons, cluster-thresholded $k>21$). Specifically, a condition by time spreading interaction analysis revealed significant clusters in (a, e) right SEF (k = 38, peak MNI coordinates (22, 12, 58), $T = 4.29$), (b, f) right middle frontal gyrus (k = 34, peak MNI coordinates (34, 2, 58), $T = 3.98$), (c, g) left middle temporal/angular gyrus (k =52, peak MNI coordinates (-42, -58, 10), $T = 3.97$), and (d, h) left posterior parietal cortex (k = 23, peak MNI coordinates (-10, -78, 36), $T = 4.44$). **(e – h)** Mean connectivity strength signal change for the mindfulness (HEM) and relaxation (HER) training groups at each of the two time points (pre-intervention and post-intervention). Error bars depict +/- 1 standard error. Parameter estimates were extracted in SPM8.

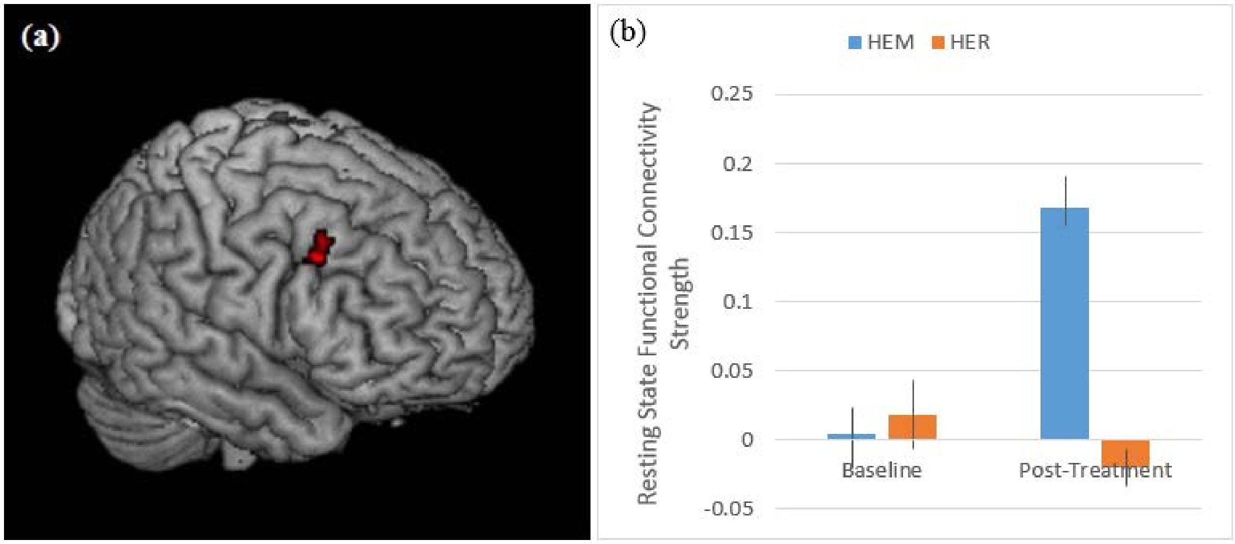


Figure 6. Increased right dlPFC-right MFG rsFC after mindfulness training.

(a) Right MFG region that showed increased resting state functional connectivity with right dlPFC (32, 50, 12) from before to after mindfulness meditation training (HEM) relative to relaxation training (HER) ($p<0.05$, corrected for multiple comparisons, cluster-thresholded $k>21$). Specifically, a condition by time spreading interaction analysis revealed a significant cluster in right middle frontal gyrus ($k = 30$, peak MNI coordinates (46, 20, 40), $T = 4.97$). **(b)** Mean connectivity strength signal change for right MFG for the mindfulness (HEM) and relaxation (HER) training groups at each of the two time points (pre-intervention and post-intervention). Error bars depict +/- 1 standard error. Parameter estimates were extracted in SPM8.

3.4 DISCUSSION

Here we report that mindfulness training, relative to a well-matched relaxation control intervention, increases resting state functional connectivity between dlPFC and dorsal network (superior parietal lobule, supplementary eye field, MFG) and ventral network (right IFG, middle temporal/angular gyrus)-associated regions. Consistent with our hypotheses, these findings broadly suggest that brief mindfulness training increases functional connectivity between a hub in the executive control network (the dlPFC) and dorsal and ventral corticolimbic circuits involved in cognitive control. These findings build upon previous work showing that functional connectivity amongst broadly distributed brain regions associated with attention, interoception, and emotional processing increases during active meditation (131), and that dlPFC connectivity is strengthened following stress-reduction interventions (125), by identifying specific neural circuits in which resting state functional connectivity is enhanced by a mindfulness training intervention in a high-stress subject sample.

The superior parietal cortex, supplementary eye fields, and MFG are key regions functionally connected to left dlPFC and associated with a dorsal circuit for goal-directed, sustained control of behavior and attention allocation (115). Strong coactivation is particularly reported between dlPFC and parietal cortex; posterior and superior parietal regions play a role in spatial orientation and focused visuospatial attention, and the dlPFC-posterior parietal pathway is thought to be engaged when extra cognitive control is required to process incoming stimuli and select behavioral outputs (132). One of the primary skills trained by mindfulness is focused attention, and focused attention meditation has been previously associated with increased dlPFC activity (116). Increased resting state functional connectivity between dorsal stream regions and dlPFC suggests that the focused attention trained by mindfulness may be enhancing the ability of dlPFC to exert executive control for attention and action selection via strengthening of this dorsal neural circuit.

The SEF has direct anatomical projections to dlPFC (133), and plays a functional role in planning saccadic eye movements, updating and error monitoring for movement plans, and mapping stimulus-response associations for cognitive-behavioral learning (134,135). Our finding of greater rsFC between the SEF and dlPFC may indicate greater executive control over action output (in particular, modifying behavior based on visual stimuli) among mindfulness-trained subjects. As previously discussed, SEF is considered part of a dorsal frontoparietal attention network that also includes dlPFC and posterior parietal cortex, including the superior parietal lobule (136). SEF activity is observed during both attention shifting (requiring a saccade) and peripheral attention tasks that do not require a saccadic eye movement (135), indicating a broader role for SEF in attentional processes, such as the focused attention and open monitoring trained by mindfulness. Of note, previous studies have shown that the SEF shows increased functional connectivity specifically to the left superior parietal lobule during active allocation of attention (136). Here, we similarly observe left-lateralized increased dlPFC rsFC with SEF and superior parietal lobule, supporting a lateralized, functionally connected dorsal attention system enhanced by mindfulness training.

Recently, it has been recognized that ventral corticolimbic circuitry also plays a distinct role in top-down regulation; in contrast to the dorsal control pathway, ventral circuitry is thought to link salience processing to immediate behavioral control (115). The right IFG is a key hub in this pathway, where it is responsible for active maintenance of stimulus information and integrating salient, interoceptive, and sensory inputs, creating a top-down biasing effect that leads to immediate action selection by posterior cortical regions (132). Importantly, right IFG is thought to have an orienting function in switching between internally and externally oriented control modes in response to salient stimuli (137) and coordinate further processing of salient stimuli (138). The role of IFG in salience processing and responding is corroborated by studies showing that cognitive control-related ventrolateral prefrontal cortex activity (including IFG and anterior insula) inhibits processing of emotional stimuli (139,140); such top-down control has been posited to be an important mechanism for emotion regulation and coping – that engaging right IFG suppresses retrieval of emotional memories (141) and predicts self-reported pain symptom improvements after administration of a placebo (142). Moreover, dispositional mindfulness is associated with more successful cognitive reappraisal of negative emotions (143) and increased activation in vlPFC during affect labeling (22). Together, these studies suggest that more mindful individuals may be better able to use this ventral pathway, including right IFG, for top-down regulation of emotion. Our finding of increased dlPFC-right IFG coupling supports the theory that mindfulness training strengthens a ventral control pathway for salience processing and emotion regulation. Furthermore, we postulate that at the behavioral level, mindfulness causes this effect by training open monitoring skills, which promote active awareness and maintenance of internal and external stimuli as they arise – functions attributed to a right-lateralized ventral frontoparietal network.

We also report increased rsFC after mindfulness training from left dlPFC to left middle temporal gyrus extending to the angular gyrus, another ventral control-associated region that plays a role in attention allocation to salient stimuli (144), lending further support to the theory that mindfulness strengthens the functional connections between executive control and salience-responding ventral attentional regions. Additionally, this finding accords with previous imaging studies showing changes in the left temporal lobe with meditation practice, including increased grey matter concentration (145,146) and volume (147). Moreover, dlPFC and middle temporal regions are structurally connected by the temporal component of the superior longitudinal fasciculus (tSLF), and enhanced connectivity of the left tSLF has been previously observed in long-term meditators (148). Our finding of increased rsFC between dlPFC and left middle temporal gyrus extends these findings and suggests that functional connectivity changes from brief mindfulness training may precede these structural changes associated with long-term meditation practice.

Contrary to hypotheses, we observed no mindfulness-associated rsFC changes between dlPFC and the frontal eye fields or intraparietal sulcus. This may be due to topographical differences in frontal-posterior parietal cortical functional connectivity. For example, in a previous study of spatial attention, whereas SEF showed increased functional connectivity specifically to the superior parietal lobule (SPL) (regions in which we do observe increased rsFC to dlPFC here), the frontal eye fields showed greater functional connectivity to intraparietal sulcus (IPS) (136). In the same study, robust structural connections between FEF-IPS and between SEF-SPL were also shown, consistent with the observed attention-associated functional connectivity patterns (136). It has been suggested that there are thus distinct FEF-IPS and SEF-SPL pathways for spatial attention (136). Although all of these regions have anatomical connections to dlPFC, our pattern of results suggests that mindfulness training may specifically enhance left dlPFC rsFC to the SEF/SPL pathway, but not the FEF/IPS pathway. As there are also different behavioral correlates for these pathways (SEF and SPL play greater roles in task-switching (149), condition-action associations (134), and object- and gaze-centered attentional representations (136), whereas FEF and IPS respond to viewer-centered representations (136) and are thought to contain a salience map of the visual environment for focused spatial attention (150)), our positive findings with one pathway and not the other could also be due to the particular aspects of attention that were trained within our brief mindfulness intervention.

We also saw no changes in dlPFC rsFC with temporoparietal junction (TPJ) rsFC, a region implicated in responding to salient stimuli (151), theory of mind (152), and attentional orienting (151) and associated with mindfulness meditation (with greater TPJ activation observed during focused breathing and greater TPJ cortical thickness observed after an 8 week MBSR program) (146,153). Previous studies investigating the functional connectivity of TPJ have produced variable results; greater positive functional connectivity has been observed between TPJ and ventral PFC during the resting state (154) and between TPJ and anteromedial PFC on a social emotion task (155), while both positive and negative TPJ rsFC has been reported with dlPFC (156). Recent work has suggested that this may be due to topographical differences in structural and functional connectivity of TPJ subregions to prefrontal cortex (157); one explanation for our negative finding may be that our particular dlPFC seed regions do not have robust connections to TPJ.

The lateralization in dlPFC functional connectivity changes we report here (e.g. left dlPFC to right SEF, IFG, and MFG and left parietal lobule and temporal/angular gyrus; right dlPFC to right MFG) may be a product of hemispheric differences in dlPFC function. Furthermore, human and primate studies have demonstrated that lateral frontal cortex is organized axially into functionally distinct areas with different axonal projections (119), suggesting that functional connectivity will be highly seed region-dependent. Left dlPFC activation has been associated with response choice, rapid attention adjustment, neutrally valenced reasoning, and higher-level motor planning (158–161), and greater left dlPFC and posterior parietal co-activation is thought to reflect increased task-positive attention allocation and executive control (162); this emphasis on the function of left dlPFC in neutral higher cognitive functions and action output is consistent with our account of increased left dlPFC to SEF, middle frontal, and parietal connectivity. In contrast, right dlPFC is implicated in working memory for emotional stimuli (163), attentional conflict (160), and planning performance (164). Right dlPFC activations spatially similar to our seed region have been reported in association with increased neuroticism-associated functional connectivity during viewing of angry and fearful facial expressions (165), in perceptual tasks as a function of task difficulty (166), during attention shifting (135), response inhibition tasks (167), and encoding and retrieval of valenced words (168). We report stronger right dlPFC rsFC to middle frontal gyrus, a region frequently coactivated with right dlPFC on emotional and attention tasks (135,168), and previous mindfulness studies suggest that mindfulness training enhances the ability to regulate emotion (40,71,169,170). Increased right-lateralized dlPFC-MFG functional connectivity may potentially underlie mindfulness-associated improvements in top-down control of emotion regulation.

While we investigated resting state functional connectivity changes in the present study, in future studies, it will be important to probe these same functional connections in task-based cognitive control tasks, particularly given the previous literature relating meditation to greater dlPFC activity during cognitive tasks (22,48). Moreover, while we posit that the focused attention and open monitoring aspects of mindfulness training underlie these neural changes, behavioral experiments can directly test this theory.

3.5 BRIDGE

The results of the present study suggest that mindfulness training alters point-to-point rsFC between specific brain regions that ultimately function as part of larger neural networks in the regulation of executive function and stimulus responding. However, such seed-based analyses do not answer questions about how mindfulness affects interactions between multiple networked brain areas. Previous studies of mindfulness meditation have attempted to answer network-level functional connectivity questions by using independents components analysis to identify intrinsic connectivity networks (171) or by running large seed-based analyses (131). These studies provide an initial indication that mindfulness practice is associated with functional connectivity changes in auditory, salience, attention, and visual networks, consistent with increased attentional, sensory, and self-referential processing (131,171). This accords with my findings in the present chapter; namely, increased resting state functional connectivity between dlPFC and regions belonging to dorsal (posterior parietal cortex, supplementary eye fields, middle frontal cortex) and ventral (inferior frontal gyrus, angular gyrus) frontoparietal control networks (also referred to in the literature as the dorsal and ventral attention networks). The seed-based analyses here (e.g. right inferior frontal gyrus, dlPFC) and in Chapter 1 (e.g. amygdala, ACC) also suggest rsFC changes in regions associated with the salience network (53,115).

Based on the seed-based analyses presented thus far, I next investigate how mindfulness training alters the behavior of these dorsal and ventral control and stimulus-responding networks. Using novel graph theoretical analyses, I test the hypothesis that mindfulness-trained participants will show enhanced function within the salience, dorsal attention, and ventral attention networks relative to a relaxation control group.

4.0 MINDFULNESS TRAINING CHANGES ATTENTIONAL AND SALIENCE RESTING STATE NETWORKS

4.1 INTRODUCTION

Initial neuroimaging studies of mindfulness meditation have evaluated whether mindfulness training alters functional connectivity between specific brain regions (38,107,120,172,173). Such studies indicate that mindfulness changes both resting state and task-based functional connectivity among stress-responding regions and prefrontal regulatory regions, suggesting that interactions among networked brain areas for salience responding, attention, and executive control may be altered on a larger scale with mindfulness practice (107,120,148,173). Multiple studies show that mindfulness meditation training improves behavioral measures of alerting, attentional orienting and responding, and executive attention, hallmark features of the salience network, ventral attention network (VAN), and dorsal attention networks (DAN), respectively (111,154,174–178). Additionally, neuroimaging studies highlight increased activity in individual regions belonging to these networks in meditators (29,47,179) and during the meditative state (153,180) during tasks requiring increased attention and executive control, including the anterior cingulate cortex (29,153,179,180), dorsolateral prefrontal cortex (38,47), fronto-parietal regions (153), and medial prefrontal cortex (47). To date, how the flow of information within and between these regions and networks changes after mindfulness meditation training is a largely uninvestigated area. This is the first study to use graph theoretical analyses to investigate resting state neural network properties (specifically, within the salience network, DAN, and VAN) in the context of mindfulness training.

Complex neural networks can be modeled by graphs, in which nodes (representing specific brain regions) are linked by edges, indicating an interaction between them. Graph theory offers several metrics that can be generated to further describe properties of individual and collections of nodes (communities, typically comprising different functional networks), including measures of functional integration, segregation, and small-worldness, which can be compared within and between networks. First, one can calculate the clustering coefficient (*C*) for a network – the proportion of connections that exist between a node and its neighbors (out of total connections) (181). In highly clustered networks, a given node and its neighboring nodes are highly likely to be interconnected. A related measure is a network's *local efficiency,* the average of the individual nodes' clustering coefficients (182) or average inverse shortest path length. Together, high local efficiency and clustering describe a network that efficiently transfers information between nodes connected by short paths. Such networks are referred to as *small world,* as they are both functionally segregated (high clustering and local efficiency) and functionally integrated (short path lengths) (183). Network connectivity can also be described by the *participation coefficient* and *connectivity degree*. Participation coefficient is a measure of the degree to which nodes within a community connect to nodes outside of their community (versus connections to other intra-community nodes), with a greater value indicative of more interactions with other communities (greater functional integration with other networks), or decreased functional segregation from other networks – thought to reflect a greater ability to quickly combine information from regions across the brain (181). Connectivity degree, calculated as the total number of connections per node, gives an indication of the relative density of connections in a network. Here, we apply these measures to resting state functional MRI data after a randomized controlled trial of a brief mindfulness intervention versus a relaxation control in a high-stress community adult population.

Resting state fMRI has the advantage of being task-independent, reliable, and shows consistent correlations with known functional and structural topography (51,68). Spontaneous brain activity has been repeatedly shown to be organized into sets of distributed brain regions that exhibit correlated patterns of activity; these resting state networks topographically overlap with functional networks identified during cognitive tasks (51,184–186). Resting state functional networks are thus an ideal means for examining information transmission and processing across functionally and structurally connected brain regions.

Although graph theoretical methodologies have become an important tool in neuroimaging over the last decade (181,183,187), there is currently very little known about how graph theoretical network metrics change with cognitive training programs in randomized controlled trials. Previous cross-sectional studies have used such graph theoretical metrics to show altered functional network components in some clinical conditions, including path length, global efficiency, and nodal centrality in depression (188), clustering and integration in schizophrenia (189,190), clustering, nodal centrality, and path length in Alzheimer's disease (191–193), and clustering and path length in autism (194), suggesting that disrupted network interactions may underlie certain neuropsychiatric conditions. Moreover, there is evidence that network metrics can be altered by training over relatively short time courses; particularly, the degree and modularity of the hippocampus and ACC is changed by exercise training in older adults and corresponds to changes in regional cerebral blood flow (195), and 5 days of motor pattern learning increased clustering coefficients and number of network connections, and shortened path lengths across five networks defined based on fMRI task-related activations (196).

There are currently no published graph theory-based mindfulness training studies (to our knowledge), despite significant calls for clarifying the neural network dynamics underlying mindfulness training (5,197). Some initial studies have attempted to describe network-level interactions after mindfulness training using independent components analysis (ICA) (171) and inter-region connectivity matrices (173). These studies have shown differences in auditory, salience, dorsal attention and visual networks, consistent with increased attentional and self-referential processing, key components of mindfulness (171,173). Specifically, after 8 weeks of Mindfulness-Based Stress Reduction (MBSR) training (3), subjects showed increased right dorsomedial prefrontal cortex, left parietal operculum, and left posterior insula rsFC with an auditory/salience network identified by ICA, which included regions of dlPFC, anterior cingulate, superior temporal gyrus, primary auditory cortex, and posterior insula (171). The authors suggest that this increased positive connectivity between auditory, salience, and attentional regions may indicate greater top-down control and less mindless processing after MBSR training (171). In a similar study of experienced mindfulness meditators, calculating time series correlations between nodes within the dorsal attention network (DAN) showed increased rsFC between right anterior intraparietal sulcus (IPS) and left frontal eye field (FEF), right MT and left FEF, right posterior IPS and left anterior IPS, and right posterior IPS and left MT in mindfulness meditation practitioners relative to non-meditators (173). Additionally, functional connectivity between DAN and default mode network (DMN) nodes, as well as between DAN nodes and a right PFC node in the salience network, was greater during the meditative state relative to the resting state amongst mindfulness meditation practitioners (173). Finally, years of meditation experience predicted stronger rsFC between left posterior IPS (DAN) and medial and right anterior prefrontal cortex regions associated with the executive control and salience networks, suggesting that meditation strengthens rsFC between networked regions responsible for attention, emotion regulation, and self-referential processing (173). Thus, studies of both MBSR training and long-term mindfulness practice provide converging evidence for increased rsFC in attentional and salience networks, which may be related to greater top-down control and allocation of attention toward internal and external sensory experience.

We and others have shown that mindfulness training interventions produce functional connectivity changes between specific executive control regions (e.g. dorsolateral prefrontal cortex) and regions belonging to dorsal (supplementary eye fields, frontal gyrus, superior parietal lobule) and ventral (right inferior frontal gyrus, left middle temporal cortex) attention circuits (Chapter 2), supporting the hypothesis that mindfulness training-induced enhancements in top-down control may strengthen interactions on a larger scale in the dorsal and ventral attention and salience networks. Here, we build upon these findings (and those of previous studies demonstrating increased mindfulness-associated within-network rsFC, (171,173)) to examine network-level resting state brain changes after a mindfulness training intervention in a high-stress community adult population relative to a well-matched active control intervention; this allows us to investigate network-level interactions that our previous seed-based analyses could not answer. Given the evidence that mindfulness improves executive attention, open monitoring, and orienting and altering to stimuli (110,111,174), it was predicted that mindfulness-trained subjects will show changes in the dorsal attention, ventral attention, and salience networks reflective of more efficient information transfer; namely, increased small-world properties (e.g. clustering and efficiency) and decreased segregation (e.g. participation). Such changes would support a neural network-level account for enhanced top-down control of attention and salience responding associated with mindfulness meditation training, thereby identifying the neural mechanism driving a broad range of outcomes observed in the literature with mindfulness training and meditation experience –

including greater attentional stability, task switching, working memory, and affective stimulus responding, functions attributed to healthy salience and attentional networks.

4.2 METHODS

Participants

Thirty-five stressed unemployed job-seeking community adults (who indicated moderate to high levels of perceived job-seeking stress over the past month, scoring >9 on an adapted 4-item PSS for job-seeking stress) participated in a single-blind RCT of 3-day intensive mindfulness meditation or relaxation training intervention (see Table 1 for participant characteristics). Participants were recruited via newspaper advertisements and through employment agencies in Pittsburgh, PA. Participants were English-speaking, had no pre-existing health conditions, were willing and available to participate in all study assessments, and were willing to be randomly assigned to one of two study conditions. Callers who met these qualifications were invited to come to Carnegie Mellon University for an in-person screening interview and baseline assessment. A more in-depth screening interview followed, including assessments of basic cognitive ability, right- or left-handedness and internal metal content (for fMRI eligibility), employment background (to probe for unemployment-related stress), medical history, and health behavior. Subjects taking psychotropic medications were excluded. Supplementary Figure 1 depicts the flow of participants through the RCT. All analyses were based on a final sample size of 29 participants at pre-intervention (6 were excluded during data analysis due to poor quality structural images) and 26 participants at post-intervention (8 were excluded during data analysis due to poor quality structural images). This study was approved by the Carnegie Mellon University Internal Review Board and all participants provided informed consent.

Intervention

We conducted this RCT between December 2010 and October 2011. Beginning four weeks before the 3-day training intervention, participants completed a baseline neuroimaging session. All participants began with a 5-minute resting state scan (where they passively viewed a fixation cross), followed by three functional tasks in counterbalanced order, and a 7-minute perfusion MRI scan with a guided awareness of breathing task (the results of these functional tasks are not reported here). After neuroimaging, participants were invited to a nearby residential retreat center where they were randomized to either a 3-day intensive mindfulness meditation training (N=19) or matched 3-day relaxation residential retreat intervention (N=16). Only the participant, project manager, treatment program staff members, and the treatment program instructor were aware of the participant's study condition. All other study personnel responsible for collecting study assessments remained blinded. Participants returned for a neuroimaging assessment within two weeks of completing the 3-day intervention and completed an identical scanning procedure as at baseline, including the same 5-minute resting state scan. At both neuroimaging sessions, participants were instructed to passively view a fixation cross during the resting state scan period and not to sleep or engage in any meditation or relaxation practices (which was verbally confirmed in all participants at the conclusion of the neuroimaging session). 97% of randomized participants were retained at the post-intervention neuroimaging assessment (3% study attrition, see Figure 1). As part of the larger study, participants completed a comprehensive battery of psychosocial measures and provided a blood draw at baseline and at 4-month follow-up; the present report focuses on testing how mindfulness meditation training changes rsFC patterns using the 5-minute resting state BOLD scan at baseline and in the two weeks following the 3-day intensive training period (post-intervention).

We adapted the standardized and manualized 8-week Mindfulness-Based Stress Reduction (MBSR) program (which includes a day-long retreat) (3,84) into a condensed 3-day residential retreat format, entitled Health Enhancement through Mindfulness (HEM). Delivery of the HEM program in a structured residential retreat format improves compliance with training, reduces treatment attrition, and greater experimental control is afforded by offering a parallel matched relaxation training retreat (in a separate wing of the retreat center). The HEM instructor was a doctoral level psychologist with 7 years of MBSR teaching experience. Subjects were not informed that the mindfulness intervention was called HEM, so as to avoid any non-specific demand characteristics. Briefly, the HEM program consists of mindfulness training through body scan awareness exercises, sitting and walking meditations, mindful eating, and mindful movement (gentle hatha yoga postures). After each formal meditation period, participants engaged in discussion of their observations about themselves and the practices. The instructor modeled and encouraged attitudes to foster mindfulness such as letting go of judgment and expectations, cultivating self-care, patience, and friendly curiosity toward present moment experience. On the third day, formal meditation practices were extended to discussions about how participants could use mindful awareness for their unemployment and job-seeking stress.

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 all trainings emphasized participation in these activities in a restful way rather than a mindful way. The HER program instructor was a licensed social worker with over 2 decades of clinical experience in stress management. The use of a structurally-matched active comparison group was designed to control for non-mindfulness 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 Supplementary Materials.

Image Acquisition

Structural and functional images were acquired on a Siemens Verio 3T scanner using a 32-channel head coil. High-resolution T1-weighted gradient-echo images were acquired at the start of the scanning session, with a slice orientation of AC-PC aligned, temporal lobes up (TR=1800ms, TE=2.22ms, flip angle= 9º, matrix size= 256x256, number of slices= 256, FOV= (205mm, 0.8mm thick slices), GRAPPA accel. factor PE= 2, voxel size= 0.8x0.8x0.8mm). Four functional echo-planar imaging runs were acquired, including a 300 second resting state scan (TR=2000ms, TE=30ms, flip angle=79º, matrix size=64x64, number of slices=36, FOV= 205mm, 3.2mm thick slices EPI with rate 2 GRAPPA, voxel size=3.2mmx3.2mmx3.2mm).

Connectivity Analysis Pipeline

Structural images were preprocessed in a pipeline that first performed bias field correction to decrease spatial variation in intensity. Next, brain extraction from the T1 image was performed. The structural image was then warped to standard stereotactic space using a linear affine transformation with FLIRT, and warped to stereotactic space using nonlinear transformation with FNIRT using affine coefficients from the linear warping as starting values. A brain mask was generated based on the inverse warp from MNI template. Finally, tissue-type segmentation was carried out.

Functional images were preprocessed using tools from FSL 5.0, AFNI, and Python 2.7. Images were first reoriented to match LPI/RPI orientation. Next, slice timing and motion correction were applied using a four-dimensional algorithm implemented in the NiPy toolbox (Roche, 2011, *IEEE Transactions on Medical Imaging*). Each subject's mean functional image was warped to the structural image, using a white matter boundary-based registration approach to register the EPI to T1 image, followed by nonlinear warping into MNI space using the warp information from the structural-to-MNI warp mentioned above. Functional images were then skull-stripped and intensity thresholding was performed using FSL. Frequency-dependent nonstationary events associated with intensity spikes in the BOLD signal (often due to rapid head movement) were removed from voxelwise time series using wavelet despiking (198). Union and intersection files were created using motion censoring criteria of a dvars threshold of 20 and fd threshold of 0.2. Functional images were smoothed with a 6mm FWHM kernel. Nuisance regression and bandpass filtering (.009 – .08 Hz) were applied simultaneously using 3DBandpass in AFNI (199). Nuisance signals included six motion parameters, average white matter and cerebrospinal fluid time series, and their derivatives (199).

Graph Theory Metrics

We utilized a graph theoretical approach to examine global and local properties brain network topology, represented by nodes (regions of interest) and edges (functional connectivity between regions). For each participant, a 264 x 264 correlation matrix was created by computing temporal correlations among regions of interest (ROIs) using the preprocessed resting state functional time series. A mask was created containing 264 5mm radius spheres, based on the 264 ROIs referenced in Power et al. 2011 that represent a reasonably comprehensive set regions involved in resting-state networks and cognitive tasks. An MNI brain mask was applied to ensure that no voxels outside of the brain were considered. Prior to ROI correlation, voxels within each ROI were combined by extracting the first eigenvector, which represented maximum shared variance among voxels. Correlations among ROI time series were computed using a high breakdown correlation estimator (Fast MCD) in order to mitigate the effects of potential outliers or high leverage data points on correlation estimates. In addition, volumes in which there was both high movement (i.e., FD > 0.2mm) and large global signal change (DVARS > 20) were censored prior to computing correlations (Power et al. 2014). AFNI motion censor intersection files (that flagged volumes with high movement and signal change after wavelet despiking, with thresholds of *fd* = 0.2 and *dvars* = 20) were used to apply motion scrubbing for each subject. Fisher's Z transformation was applied to normalize correlation coefficients.

All network metrics were computed using the Brain Connectivity Toolbox implemented in Matlab (181). Each correlation matrix was binarized prior to computing network metrics in order to represent the presence or absence of a functional connection among a pair of regions (rather than correlation strength). Network metrics calculated from these matrices can vary with the connection density (*d*) in a network, which can be manipulated by varying the number of valid network connections (200). Thus, it is desirable to repeat analyses at different density thresholds to ensure that statistical results obtained are reliable and stable across connection densities (200). Since network topology can be influenced by the density threshold used (184), the correlation matrix for each subject was thresholded at a range of density values ($0.05 < d < 0.15$, with an interval of 0.01) to obtain an undirected binarized graph. For all subsequent analyses, we report the average across these density values. Using the BCT Toolbox, the following calculations were performed: path length (lambda), clustering coefficient, global efficiency, local efficiency, participation coefficient, and node degree. We evaluated the following network metrics: (1) Characteristic path length (L) of a network: the average minimum number of edges linking any two nodes, calculated from the mean of entries in a distance matrix (which gives the distance between any two nodes *i* and *j*, where distance equals the length of the shortest path) (201,202). (2) Clustering coefficient (*C*) of a node: the number of edges between a node's neighbors out of all possible edges; to obtain the clustering coefficient of a network, clustering coefficient is averaged over all nodes in the network (201,202). (3) Global efficiency: calculated as the inverse average shortest path length from a node to all other nodes; essentially, a measure of how close a node is to all other nodes (181,202). (4) Local efficiency: calculated as the inverse average shortest path length of a node's neighbors (closely related to clustering, and an indication of the closeness of a neighbors of a node) (181). (5): Participation coefficient (*P*): the degree to which nodes within a network connect to nodes outside of their network versus connections to nodes within their network (203). (6) Node degree: the number of edges connected to each node (181,202).

Statistical analyses were performed in SPSS. The 264 ROIs defined from Powers et al. (2011) have previously been subdivided into 13 different functional networks using subgraph detection algorithms (184); average values for each network were generated for each network metric output from Brain Connectivity Toolbox calculations. An independent samples t-test was used to test for between-group differences in network metrics for each network at baseline and post-treatment, a protocol utilized in previous RCTs (195).

4.3 RESULTS

The primary focus of this study was on differences in resting state whole-brain network organization after a mindfulness training intervention (compared to a relaxation intervention, which served as an active control). Here, we hypothesized that mindfulness training increases the efficiency of intrinsic neural networks related to regulatory control and salience responding –

specifically, the salience, dorsal, and ventral attention networks. Consistent with hypotheses, at post-treatment, significant between-groups differences were observed within the ventral attention network and the salience network; no significant differences in dorsal attention network properties were observed between the mindfulness intervention group and the control cohort.

Within the ventral attention network (Figure 7), clustering coefficient (averaged across nodes) was greater in the mindfulness intervention group relative to the relaxation control at post-training ($t_{2,24} = 2.032$, $p = 0.053$; Figure 8). No significant between-group differences in clustering coefficient were present at baseline. Greater local efficiency of the ventral attention network (averaged across nodes) was also observed in the mindfulness intervention group relative to the relaxation control ($t_{2,24} = 2.665$, $p = 0.014$; Figure 9). No significant between-group differences in local efficiency were present at baseline ($t_{2,27} = 0.099$, $p = 0.92$). Higher clustering and local efficiency are both indicative of stronger connections and shorter path lengths within ventral attention network nodes (and their immediate neighbors) in mindfulness-trained participants, possibly signifying a greater ability for interconnected VAN nodes to engage in specialized neural processing (181). These changes may represent stronger within-network connectivity, as well as more efficient communication between VAN nodes and their close neighbors.

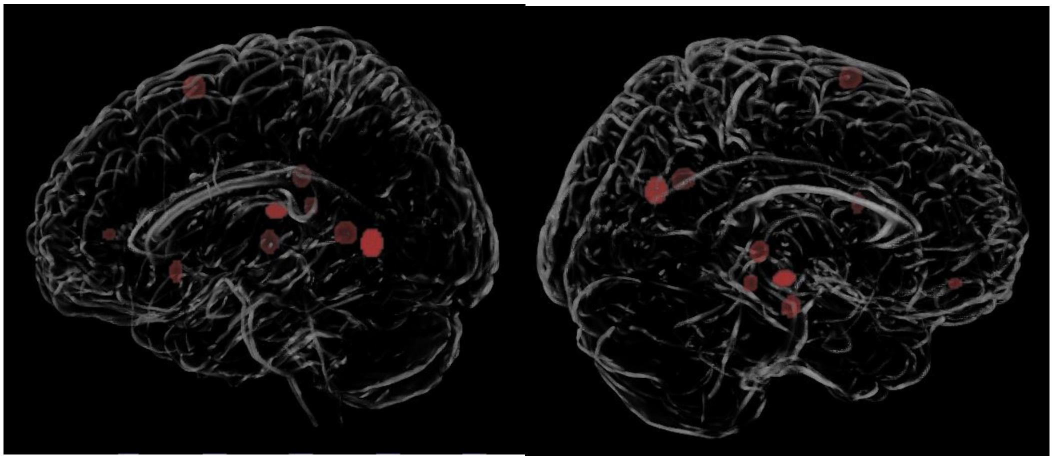


Figure 7. Ventral Attention Network Nodes.

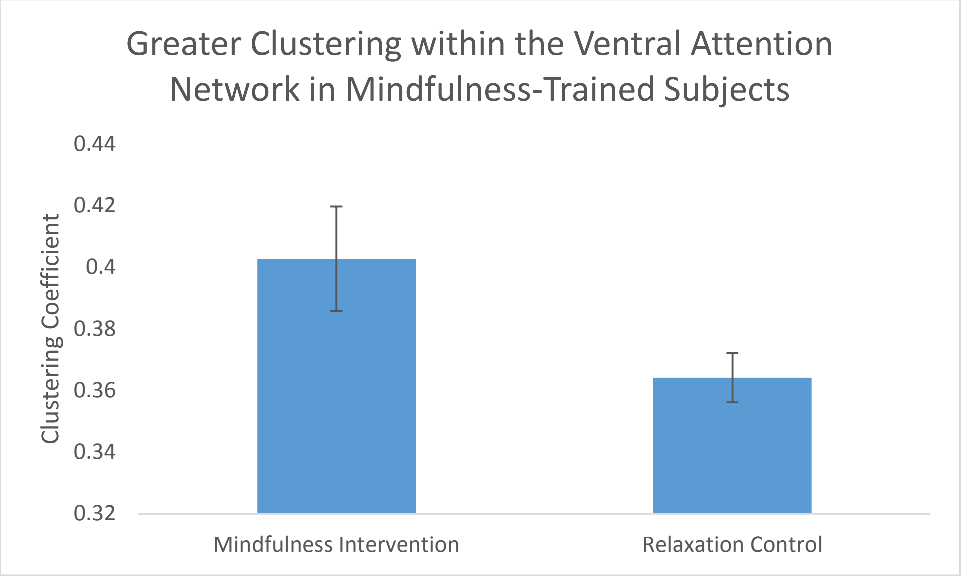


Figure 8. Greater average (+/- SEM) clustering coefficient (C) in the mindfulness training group relative to the control intervention groups at post-treatment.

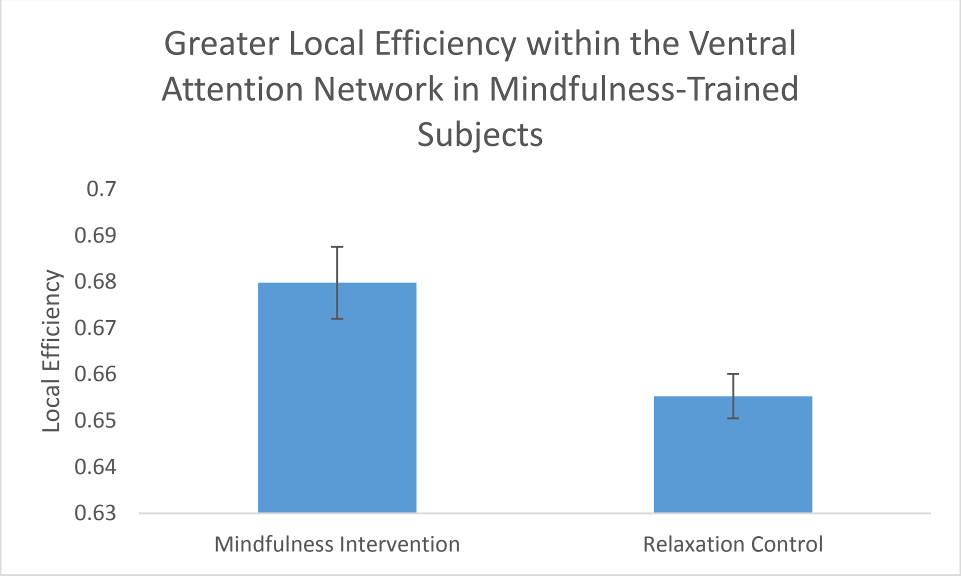


Figure 9. Greater average (+/- SEM) local efficiency in the mindfulness training group relative to the control intervention group at post-treatment.

The salience network (Figure 10) exhibits a significantly smaller degree in the mindfulness intervention group relative to the relaxation control group ($t_{2,24}$ = -2.059, p = 0.051, Figure 11), indicating fewer local connections within nodes in this community in the mindfulness-trained group at post-intervention. This finding indicates weakening of salience network node connectivity with both within-network nodes, and nodes outside of the salience network. Similarly, participation coefficient was smaller in the mindfulness intervention group relative to the relaxation control ($t_{2,24}$ = -2.019, p = 0.05, Figure 12). No significant between-group differences in participation were present at baseline in either degree ($t_{2,27}$ = -1.343, p = 0.19) or participation coefficient ($t_{2,27}$ = 0.805, p = 0.43). Together with the degree difference, the smaller participation coefficient suggests weakening of connections outside the salience network. Within the salience network, three nodes have a particularly strong effect on the smaller connectivity degree observed among mindfulness-trained subjects relative to the control group at post-intervention: left dorsal anterior cingulate (BA32; MNI = -1, 15, 44; $t_{2,24}$ = -2.281, p = 0.032, HER mean = 33.89 ± 3.43, HEM mean = 22.80 ± 3.45), left insula (BA47; MNI = -35, 20, 0; $t_{2,24}$ = -3.50, p = 0.002, HER mean = 32.01 ± 2.62, HEM mean = 19.73 ± 2.34), and right supramarginal gyrus (MNI = 55, -45, 37; $t_{2,24}$ = -2.051, p = 0.051, HER mean = 37.52 ± 4.29, HEM mean = 26.74 ± 3.04) (Table 7). No significant between-group differences in degree were present at baseline for left dorsal anterior cingulate ($t_{2,27}$ = -0.644, p = 0.53, HER mean = 29.44 ± 3.55, HEM mean = 26.81 ± 1.81), left insula ($t_{2,27}$ = 0.722, p = 0.48, HER mean = 24.81 ± 2.48, HEM mean = 27.61 ± 3.01), and right supramarginal gyrus ($t_{2,27}$ = 0.128, p = 0.90, HER mean = 32.64 ± 3.30, HEM mean = 33.18 ± 2.56) (Table 7).

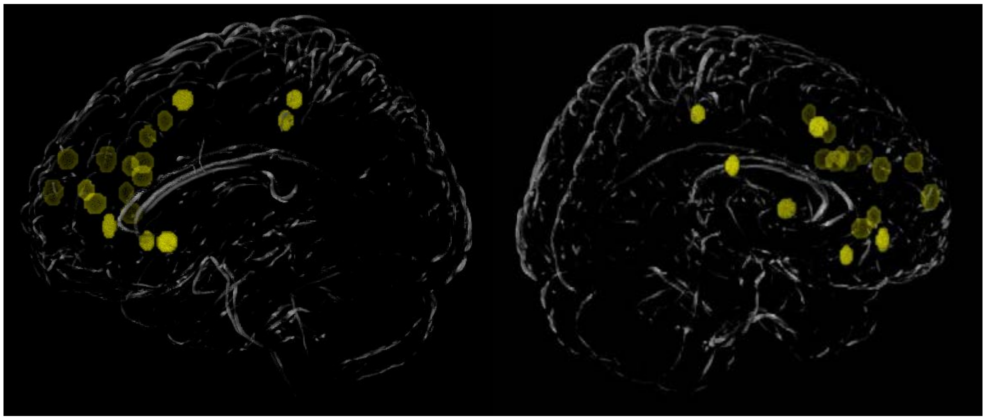


Figure 10. Salience Network Nodes.

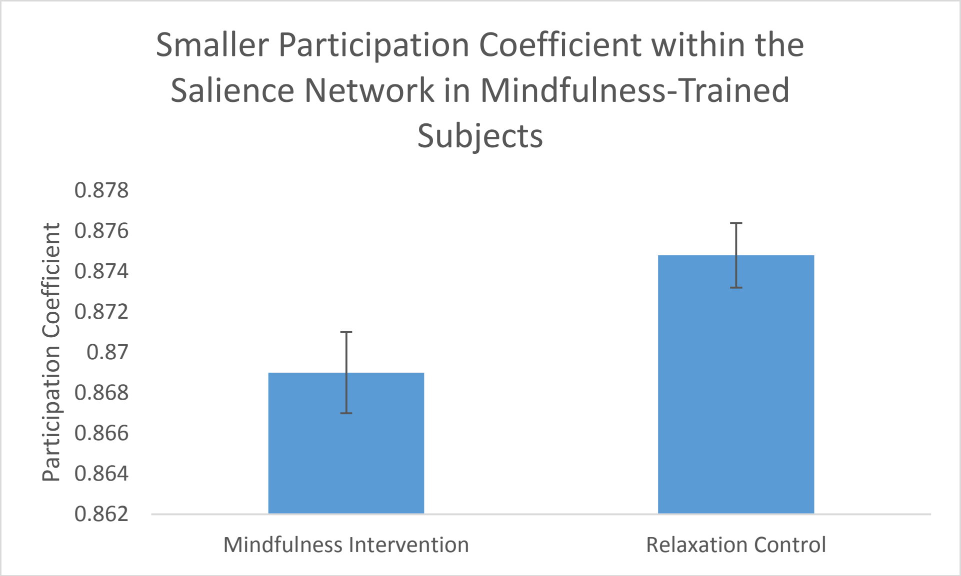


Figure 11. Smaller average (+/- SEM) participation coefficient (P) in the mindfulness training group relative to the control intervention group at post-treatment.

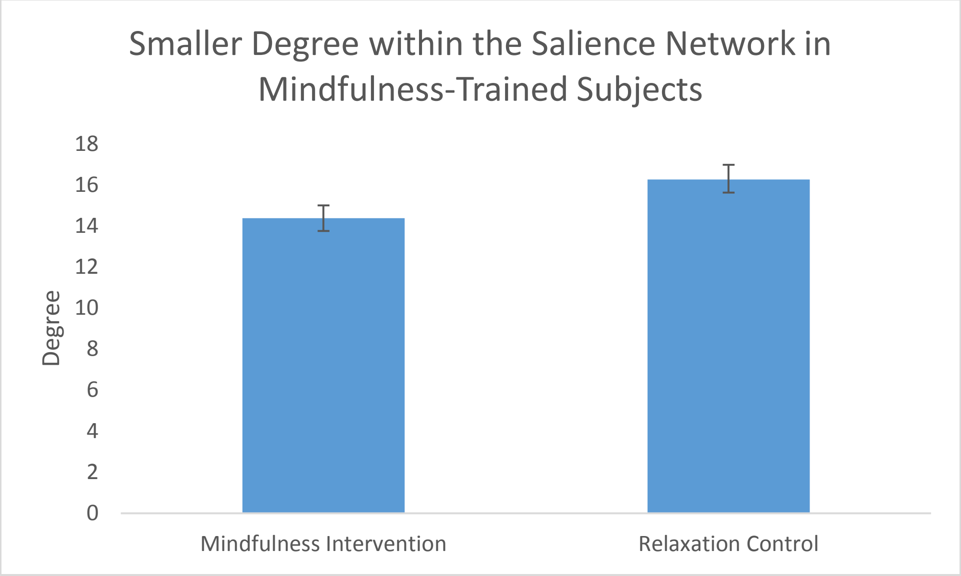


Figure 12. Smaller average (+/- SEM) node degree in the mindfulness training group relative to the control intervention group at post-treatment.

Table 7. Group differences in node degree at time 1 and time 2.

Pre-post within-group comparisons of node degree				
	MNI Coordinates	**Mean (Time 1)**	**Mean (Time 2)**	***P***
Left insula (HEM)	-35, 20, 0	27.62	19.72	0.05
Left Insula (HER)	-35, 20, 0	27.18	25.97	0.15
L dACC (HEM)	-1, 15, 44	26.80	22.79	0.30
L dACC (HER)	-1, 15, 44	29.44	32.54	0.38
R Supramarginal gyrus (HEM)	55, -45, 37	33.18	26.74	0.11
R Supramarginal gyrus (HER)	55, -45, 37	32.64	37.51	0.37

4.4 DISCUSSION

The present study examined differences in resting state whole-brain network organization after mindfulness training (compared to a relaxation intervention, which served as an active control). Consistent with our hypotheses, results indicate that significant differences in attention and salience network properties can be detected after a brief mindfulness intervention – specifically, greater clustering and local efficiency within the ventral attention network (Figure 7), and smaller degree and participation within the salience network (Figure 10) among mindfulness-trained subjects relative to the control group.

Previously, we have reported greater rsFC between right inferior frontal gyrus (a key ventral attention network node) and dlPFC, providing an initial indication that ventral attention network circuitry may be altered by mindfulness training. Here, we report increased clustering coefficient and local efficiency within nodes of the ventral attention network at post-treatment in the mindfulness training group relative to the relaxation control subjects. A greater clustering coefficient and local efficiency in VAN nodes together suggests that this shows greater small-world properties after mindfulness training, consistent with our hypothesis of more efficient within-network information processing. Underlying this difference may be an increase in open monitoring, a flexible, receptive type of attention to one’s internal and external experience specifically trained by mindfulness. Training open monitoring skills may thus create a more efficient ventral attention network, consistent with its role in stimulus-driven attention allocation and reorienting (144).

Higher local efficiency in the VAN is indicative of more short-range connections between nodes. Interestingly, prior work has suggested that such an increase in short-range connections may occur as a functional reorganization response after injury (204), or reflect greater internal organization and fault tolerance after an assault on the system (205). The present study was conducted in a high-stress subject sample; therefore, an increase in VAN local efficiency in our study population could reflect a compensatory mechanism to restore efficient within-network communication in chronically stressed individuals. Indeed, stress-associated network deficits have been associated with decreased white matter integrity, and resting state functional connectivity is strongly associated with underlying structural connectivity architecture (206). Weakened structural, and therefore functional, connections reflect disrupted information exchange between brain regions. The present study provides initial evidence that stress-associated disruptions in connectivity may be at least partially ameliorated by mindfulness training, as demonstrated by greater small-world metrics in mindfulness-trained subjects at post-treatment.

Contrary to hypotheses, we found no significant differences in network properties of the dorsal attention network between the mindfulness-trained group and the control population. Although previous studies have demonstrated changes in seed-based resting state functional connectivity between regions associated with the DAN, we theorize that our intervention may have been too brief to detect network-level changes within the DAN in our subject population. A previous study of resting state brain network plasticity suggests that resting state functional connectivity recovers at different rates for different networks after chronic stress; specifically, functional connectivity in the default mode network, VAN, and sensorimotor networks decreased with recovery from chronic stress, while regions of the DAN continued to display increased stress-associated rsFC (207). Additionally, we did not evaluate between-network changes in this study; it is possible that the DAN network changes that occur over this time course with mindfulness training would be instead demonstrated in an altered balance between DAN strength and that of other networks. Such measures should be evaluated in future mindfulness training studies of network connectivity.

Within the salience network, we report a smaller degree and participation coefficient in the mindfulness-trained group relative to controls at post-treatment, an effect that is driven by a decrease in salience network degree and participation coefficient within the mindfulness group from pre- to post-intervention. It has been suggested that greater participation and degree (the number of connections per node) equates to a greater energetic cost in a network – in other words, an "overcharged network" with a poor balance between information transmission and energy consumption (208). We see a higher degree in the salience network in the control group, suggesting that our high-stress subject sample is characterized by a hyper-connected salience network, which may reflect an overcharged stress-responding system. Importantly, this set of results suggests that a mindfulness training intervention can decrease the wiring cost of a hyper-connected salience network, as demonstrated by the lower degree and participation coefficient at post-treatment in the mindfulness group. Moreover, the smaller degree and participation coefficient suggests weakening of connections outside the salience network, which may indicate functional decoupling of the salience network from other networks in the mindfulness training group.

Of note, three nodes in particular within the salience network showed a highly significantly smaller degree in the mindfulness-trained group at post-treatment: the dorsal ACC, left insula, and right supramarginal gyrus, regions that have all previously received attention in the context of mindfulness and stress. Increases in cerebral blood flow to right ventral prefrontal cortex, left insula, and anterior cingulate have been demonstrated in subjects experiencing high levels of task-induced psychological stress (209), suggesting that persistent activation of these regions may occur in chronically-stressed populations; this repeated neural activation pattern may underlie the higher nodal degree in these regions that we observe in our stressed subject sample. Previous neuroimaging studies of mindfulness meditators have shown increased activation in the insula, thought to be related to greater interoceptive attention and awareness (38,48,172,210). More generally, the insula functions in bottom-up saliency detection, network-switching to access working memory and attention in response to a salient event, and modulation of the physiological response to salient stimuli (211). Additionally, the insula is frequently co-activated with and has strong structural connections to the ACC; this white matter tract carries ascending projections from the spinal cord to communicate information about the physical state of the body (212,213). At the cellular level, insula and ACC are also distinguished by a high population of von Economo neurons, which enable control signals to be rapidly transmitted between insula and ACC and from these two nodes to other connected cortical regions (211,214). The insula-ACC connection is crucial for responding to the degree of stimulus salience in order to quickly guide behavior; it is thought that the insula plays a larger role in detecting salient stimuli, while the ACC is responsible for modulating sensorimotor cortical responses for action selection – together, these functions highlight the insula-ACC role in the integration of bottom-up salience responding/attention allocation with top-down control of behavior (211). Here, we observe a higher nodal degree in both salience network nodes in our high-stress population that decreases after mindfulness training (Table 7), suggesting that mindfulness may reduce hyperconnectivity of these two nodes, allowing more efficient transmission of control signals from insula and ACC.

The right supramarginal gyrus is similarly implicated in integrating and interpreting sensory information, and has been linked to empathy (215), disembodiment (216), attention for behaviorally-relevant sensory stimuli (176), and interoceptive attention and awareness (217). Studies of different meditation practices have associated increased recruitment of right supramarginal gyrus with focused attention meditation and mantra-induced meditation, and have further shown that greater right supramarginal gyrus activation is associated with longer-term meditation experience (218). In contrast, in a study of interoceptive attention and awareness practitioners (which trains attending to breathing sensations), greater supramarginal region activation was seen in novices, while experienced practitioners showed deactivation, suggesting that supramarginal gyrus activity may be a function of how much one is focused on external experience versus internal experience (or needs to continuously reorient attention towards internal sensations) (217). The smaller degree of connectivity in this node in the mindfulness-trained group at post-treatment may therefore reflect a down-regulation of the frequency of neural firing in the right supramarginal gyrus as mindfulness-trained subjects better orient their attention inward.

The present study focused on evaluating network metrics within networks after a mindfulness training intervention, and is the first study to our knowledge to apply graph theory measures to resting state neural networks in the context of mindfulness training. Much room remains for future work in this area; examining the behavior of individual nodes (e.g. the salience network dorsal ACC node) of established importance for mindfulness using measures such as betweenness-centrality can provide additional information on a node’s centrality within a network. Additionally, we looked only at resting state data here, which has the advantage of being task-independent; however, future studies of task-based fMRI network analysis can look at network changes between cognitive states. Finally, as previous studies have shown that structural networks converge with resting state functional network strength (219), future mindfulness training interventions should evaluate structural connectivity (e.g. with diffusion tensor imaging) alongside resting state functional connectivity.

5.0 CONCLUSIONS

This work has focused on the neural circuitry of executive control, stress responding, and mindfulness training. I first showed that right amygdala-subgenual anterior cingulate cortex (sgACC) resting state functional connectivity was positively associated with self-reported stress in a large healthy community adult sample, and that rsFC between right amygdala and sgACC decreased after our mindfulness training intervention in high-stress community adults, indicating that decoupling of this right amygdala-sgACC functional pathway may underlie mindfulness-based stress reduction. I next showed that resting state functional connectivity between distributed brain regions implicated in attention and executive control is strengthened after mindfulness training; specifically, dorsolateral prefrontal cortex – a hub in the frontoparietal control network – shows increased rsFC to regions in lateralized dorsal (supplementary eye fields, middle frontal gyrus, parietal cortex) and ventral (inferior frontal gyrus, middle temporal gyrus) control networks. In my final study, I demonstrated increased small-world properties in the ventral attention network in mindfulness-trained subjects at post-treatment relative to a control group, alongside decreased degree and participation in nodes belonging to the salience network in mindfulness-trained subjects at post-treatment, demonstrating that mindfulness training may decrease the energetic cost of an over-wired salience network in our high-stress sample. Collectively, these results demonstrate that mindfulness training may decrease baseline functional coupling between regions implicated in stress-responding while increasing functional connectivity between regions implicated in executive control, and at the network-level, enhance the efficiency of information transfer between distributed neural circuitry for attentional monitoring and salience-responding.

While stress-responsive circuitry and executive control circuity are dissociable neural networks, the dual finding of decoupled stress-associated regions and more strongly coupled executive control regions after mindfulness training suggests, broadly, a possible framework for thinking about how mindfulness may affect the overall balance of communication between systems in the brain to produce its typical psychological and health outcomes. I posit that by training focused attention, mindfulness enhances rsFC between executive control-associated regions – these strengthened executive connections may, in turn, down-regulate stress-related neural wiring such as the amygdala-sgACC connection. To test this in the future, we would need to look at effective connectivity between these regions (such as dlPFC-right amygdala) to detect a causal influence of one region over the other. Another explanation could be that by training attention and awareness to the present (e.g., open monitoring for internal and external stimuli, which may be reflected by the enhanced rsFC I report between dlPFC and right IFG, a region implicated in attentional orienting and salience responding), mindfulness reduces stress-related psychological processes such as rumination and mind-wandering – and thus stress-related functional coupling decreases. Future research must build upon these themes by linking our neural changes to behavior, e.g. pre-post intervention measures of executive task performance and self-report measures of rumination.

These findings fill an important gap in the mindfulness literature, namely, they build a bigger picture of the intrinsic functional connectivity patterns that evolve from mindfulness training interventions. Whereas early fMRI studies of mindfulness meditators looked at differences in neural activity on cognitive tasks, the field has moved more recently towards recognizing that functional (and structural, e.g. Tang et al. 2011) connectivity between brain regions is important for starting to tease out underlying mechanisms, placing an emphasis on how these regions are communicating with each other, rather than just activating and deactivating in isolation. While previous studies have looked at functional connectivity changes associated with mindfulness (e.g. Brewer et al., 2011), the studies I present here are the first to (a) identify a functional pathway that may link mindfulness training with reduced stress, and (b) show increased baseline functional connectivity between executive control and attention-associated brain regions. Perhaps most significantly, I provide the first mindfulness training study to evaluate neural networks using graph-theoretical analysis techniques. Although the scope of this third study was limited in the larger scheme of network metrics available (for example, future analyses will test for between-network differences, and aim to further tease out how the contributions of individual nodes to these networks changes with mindfulness training (e.g. node centrality, edge-betweenness)), novel findings emerge that add to our understanding of how mindfulness training affects the dynamic role of salience and ventral attention network nodes. These network results may be related back to the seed-based findings presented in Chapter 1 and Chapter 2, respectively. A previous study has suggested that the functional neural activity under psychological stress overlaps most closely with the salience network (53), and we see a roughly similar pattern of decreased rsFC between two specific stress-related regions (right amygdala and sgACC) and decreased degree and participation in the salience network (suggestive of weakening salience network node connectivity within and outside the network). Accordingly, whereas increased rsFC was observed between dlPFC and right IFG (suggesting enhanced executive/ventral attention-associated region connectivity), at the network level, the ventral attention network becomes more small world, indicating a more efficient network. Postulating further, it could be the case that we see salience and ventral attention network changes occurring together in mindfulness training because of their complementary functions, with the ventral attention network responsible for stimulus-driven attention and orienting, and the salience network responsible for the behavioral response to salient events – consistent with the present-centered attention to sensory stimuli that mindfulness fosters.

Importantly, a strength of this body of work is our utilization of a randomized controlled trial of mindfulness training with a matched active control group; in contrast, much of the mindfulness literature to date is cross-sectional correlational, or uses practitioners of varying forms of meditation and length of experience.

Moving forward, several methodological challenges remain for this field; the first is to link mindfulness training-associated neural changes to markers of health and to long-term stress-related disease outcomes. This will necessitate well-controlled longitudinal studies of mindfulness training that track epidemiological data and biological markers such as interleukins, C-reactive protein, tumor necrosis factor alpha (TNF-α), and other pro-inflammatory cytokines. It is becoming increasingly common to see mindfulness training programs used in clinical settings; however, the biological mechanisms linking increased mindfulness to health improvements are still largely unknown. Previous studies have shown that mindfulness may decrease markers of inflammation that are elevated in many stress-related chronic diseases (220) and have also linked systemic inflammation to changes in functional connectivity (221,222) and white matter myelination (223). There is particularly strong evidence for the importance of glucocorticoids in brain function; animal studies show that glucocorticoid receptor signaling stimulates axon myelination (224,225) and that high glucocorticoid levels reduce myelination and oligodendrocyte proliferation (223). These microstructural changes would affect the speed and timing of impulse conduction across neural networks, and could be causing stress-related changes in functional connectivity. In Chapter 2, I present preliminary findings of an association between hair cortisone and right amygdala-sgACC rsFC. One possibility is that mindfulness training may modulate HPA axis activity, fostering decreased levels of inflammation, and thereby produce better health outcomes. Given the neural effects of mindfulness training and the known interactions between brain and immune systems, the possibility that mindfulness could affect neural activity in such a way as to mediate endocrine and immune system activity is an intriguing topic for future investigation.

Second, linking structural and functional neural changes associated with mindfulness interventions will be an important area for moving this field forward. Although previous studies have demonstrated mindfulness-associated structural connectivity differences (e.g. changes in white matter integrity (45)), and functional connectivity patterns identified in this study are consistent with known anatomical projections, the two have not yet been examined in tandem. Questions remain about the degree to which these structure-function changes overlap in the context of mindfulness (and over what time scale of training); for example, would we see changes in fractional anisotropy in the uncinate fasciculus corresponding to amygdala-sgACC functional connectivity changes? Using parallel structural and functional imaging techniques (such as diffusion tensor imaging and resting state fMRI) in a mindfulness training study will allow us to examine microstructural changes in white matter directly alongside baseline changes in coactivation between networked brain regions.

This series of studies pushes us towards a more mechanistic understanding of the underlying neurobiology of mindfulness. By understanding the mechanisms by which mindfulness training works on the brain, we can move towards identifying a way to harness the neuroprotective effects of mindfulness and potentially prevent or treat a variety of physical and psychological ailments. Such mechanistic knowledge may, for example, allow us to identify individuals who will benefit from mindfulness training programs, modify mindfulness training programs to maximize efficacy for patient populations with characteristic neural findings (particularly high-stress patient populations), and finally, determine the amount and frequency of mindfulness practice required to effect particular physiological changes and achieve specific health outcomes.

APPENDIX A

A.1 RIGHT AMYGDALA SEED CONNECTIVITY, WHOLE BRAIN ANALYSIS

Table 8. Right Amygdala Seed Connectivity, Chapter 2. Whole brain analysis.

Decreased pre-post coupling in HEM relative to HER, $p < 0.005$, $k > 30$	Region (aal)	Peak MNI coordinate	Cluster Size
	Superior temporal gyrus	-38 4 24	151
	Left Fusiform	-22 -32 -22	42
	Left Calcarine	4 -90 -2	2328
	Midbrain	4 -16 4	70
	Right Middle Occipital	28 -76 26	273
	Left Superior Occipital	-20 -70 22	137
	Right Paracentral Lobule	16 -32 50	43
	Left Middle Cingulate	-2 -42 52	107
Increased pre-post coupling in HEM relative to HER, $p < 0.005$, $k > 30$			
	Right Cerebellum Crus	38 -58 -42	67
	Vermis	-2 -54 10	59
	Right Insula	44 4 8	424
	Left Middle Frontal Gyrus	-36 42 -2	66
	Left Superior Frontal Gyrus	-22 52 2	90
	Left Putamen	-28 -22 8	61
	Left Inferior Frontal Operculum	-54 10 4	154
	Left Middle Temporal	-58 44 10	50
	Superior Temporal Gyrus	-38 -42 10	67
	Left Middle Frontal Gyrus	-36 42 18	35
	Right Inferior Frontal Operculum	60 10 22	74
	Left Angular Gyrus	-48 -52 28	32
	Postcentral Gyrus	48 -34 36	47

	Left Inferior Parietal Cortex	-50 -44 42	138
	Right Superior Parietal Cortex	26 -58 70	56

A.2 LEFT AMYGDALA SEED CONNECTIVITY, WHOLE BRAIN ANALYSIS

Table 9. Left Amygdala Seed Connectivity, Chapter 2. Whole brain analysis.

Decreased pre-post coupling in HEM relative to HER, $p < 0.005$, $k > 30$	Region (aal)	Peak MNI coordinate	Cluster Size
	Left Thalamus	-8 -6 6	79
	Right Superior Medial Frontal Cortex	8 70 0	94
	Right Middle Orbitofrontal Cortex	32 56 -14	54
	Cerebellum	-18 -84 -32	35
	Posterior Cerebellum	-10 -38 -46	53
	Left Cerebellum	-26 -52 -44	70
Increased pre-post coupling in HEM relative to HER, $p < 0.005$, $k > 30$			
	Postcentral Gyrus	28 -48 64	77
	Middle Frontal Gyrus	40 2 58	72
	Precentral Gyrus	-14 -38 70	107
	Postcentral Gyrus	34 -36 50	284
	Sub-Gyral	-18 0 44	37
	Superior Parietal	24 -68 54	88
	Precentral Gyrus	60 -2 44	346
	Left Cerebrum	-60 -22 48	182
	Precentral Gyrus	62 -10 30	73
	Superior Temporal Gyrus	54 -24 4	97
	Middle Temporal Gyrus	-54 -30 -14	54
	Occipital Lobe	46 -66 -16	84

Table S1. A detailed overview of the 3-day Health Enhancement thru Mindfulness (HEM) and Health Enhancement thru Relaxation (HER) intervention programs.

Table 10. Outline of the 3-day Mindfulness Training RCT.

	Health Enhancement thru Mindfulness		Health Enhancement thru Relaxation
Day 1 10 -12:15 chapel	Welcome and orientation to the guidelines for participation in the program; introduce the concept of mindfulness; centering exercise and individual introductions; mindful raisin-eating exercise; 45-minute body scan exercise (show alternative postures, and start with some stretching). Depending on when program starts, raisin and body scan may be after lunch.	10:00-12:00 Conf area	Orientation, guidelines*, and introductions (pairs first, then big group) How do you manage stress? (keep it somewhat light) (this may generate a list of diverse coping methods, and can be referred back to as the weekend goes on). End session with stream imagery for relaxation.
12:15-1:45	Mindful lunch (not silent) (1/2 hr in cafeteria, 1 hr break) (3 student interviews)	12:00-1:30	Lunch (3 student interviews)
1:45-3:15 Conf area	Continue intros, raisin exercise, body scan as needed, depending on progress prior to lunch. Discussion of body scan/morning, weave in relevant 'attitudes of mindfulness' (non-striving, patience, don't know mind) intro to sitting and postures, sitting meditation with AOB. Review definitions of mindfulness as indicated. Use Mountain meditation or other stabilizing imagery as indicated.	1:30-3:00 chapel	Brief disc of physical activity as method for stress reduction. Stretching / exercise. Discussion what keeps you from exercising, if you don't--list
3:15-4:15	Snack /tea (2 student interviews)	3:00-4:00	Snack / tea (2 student interviews)
4:15-5:45 Chapel	Standing and walking Mindful movement yoga, Intro awareness of pleasant events assignment (to discuss tomorrow)	4:00-5:30 Conf area	Intro to safe place. Demonstration of dyadic work--development of an image of being safe and at ease. Work in pairs, writing down, then

			guiding each other. (comfort and ease imagery) Share feedback in large group
5:45-7:30	Mindful dinner (2 student interviews)	5:30-7:30	Dinner (2 student interviews)
7:30-9 Conf area	Sit (~15 min). Walking. Seated body scan, followed by stretching. Use Mountain imagery as indicated. Reflection and discussion of 1st today's experiences, integrating attitudes of mindfulness. Orientation and instructions for continuing mindfulness practice during the later evening and bedtime, noting pleasant and also unpleasant events, (give calendars) returning wandering mind to present moment awareness. Lying down Body Scan.	7:30 – 9 chapel	Sleep Hygiene: talk about the connection between sleep and health. Ask them how they sleep (this could take a long time!) Give Sleep Hygiene handout and go over it briefly. Nutrition (using Pollen material) What is true; what is helpful here? Reminder: maintain quiet this evening and in early morning (if you talk, keep it fairly quiet) Relaxation exercise for sleep.
9-10:30	Quiet time (4-5 interviews)	9:00-10:30	Quiet time (4-5 interviews)
10:30 pm	Lights out	10:30pm	Lights out
Day 2 7am	Wake up (maintain silence)	Day 2 7am	Wake up (maintain quiet)
7:30-8:15 Chapel	Mindful stretch – on floor, followed by sit with AOB, AO body sensations. Prep for mindful breakfast.	7:30-8:30 Conf .	Movement – gentle calesthenics Reflective writing re: ideas sparked by yesterday's and last night's disc (what do they want to explore, what do they want to remember.)
8:15-9:15	Mindful Breakfast (silence optional)	8:30-9:30	Breakfast
9:15-10:45 Conf area	Brief sit (~15 minutes). Discussion of last evening and this morning's mindfulness practice, including silence; dyads and then group disc/ mindful listening re: pleasant and unpleasant events. Begin disc of what makes an event pleasant/ unpleasant; topic of 'stress/ stress physiology'	9:30-11 Chapel	Physical Exercise 'non-mindfulness' version of yoga stretch/strengthening exercises. Incorporate some HEP exercise activities. Something to think about on your walk: what are your personal strengths?
10:45-11:15	Walking meditation (unguided)	11-11:30	Take a walk

11:15-12 Conf area	Mindful stretch followed by sit. Continue disc of anatomy of stress, stress reactivity /automatic pilot and possibility of responding mindfully to stressful events. Use metaphors as indicated (e.g, 4 story building, waterfall and barrel, etc.)	11:30-12:15 Chapel	Discussion of strengths and values. Who are your role models and what do you admire re: them? Exercise: draw your personal 'values and strengths' coat of arms. Share 'coat of arms' in dyads and then large groups.
12-1	Mindful lunch	12:15-1:15	Lunch
1-2:30 Chapel	Sit with AOB, Body, AOSound, AO Thought (internal image/talk). Disc of participants own stress reactivity patterns (start with dyads) how do they know they are stressed and how do they take care of self. Disc mindful approach to emotions; problem focused and emotion focused coping. Lovingkindness meditation if appropriate. Introduce possibility of silent period (to start ~4:30pm).	1:15-3 Conf area	Demonstration of development of imagery for feeling competent and effective, or strong. They break into dyads to do this. (if you don't have a memory to use, can imagine one – like you are an actor in a scene) Share feedback in large group
2:30-3:30	Snack / tea	3-4	Snack / tea
3:30-5:30 Conf area	Chair yoga followed by sit. Continue discussion of stress reactivity and mindful responding as needed. Disc of upcoming silent time with rationale as a time to be with yourself, focus on yourself in a caring way. If group/individuals seem able to meet this, silence begins (~4:30pm) run this section like MBSR retreat: body scan, mindful movement, sitting, lovingkindness meditation (include choiceless awareness)	4-5:45 Chapel	Stress physiology Stress and Health: JKZ handout, Charting arousal/time and symptom development—the arousal curve and what symptoms shows up at which level of arousal. Do this as a group discussion. What do they experience when they are stressed.. You could have them do this in dyads first, and then expand to the bigger group. This should take approximately 1 hour. Go back to Pollen material if did not finish it yesterday. Earlier today we did imagery exercises around our strengths. What personal strengths do you have? Hobbies and interests – have a go around about favorite hobbies and interests that help them to feel comfortable / good. Create a group list of ideas.

5:30-7:15	Dinner	5:45-7:30pm	Dinner
7:15-9 Chapel	Practice in silence. Variety of formal practices, include choiceless awareness. Poetry reading. Mountain or Lake meditations as indicated.	7:30-9 Conf area	Disc – how do I have / make fun? Demonstration and then guided imagery for fun, breaking into dyads to develop them. Humorous video
9-10:30	Quiet time	9-10:30	Quiet time
10:30	Lights out	10:30	Lights out
Day 3 7am	Wake up	Day 3 7am	Wake up
7:30-8:15 Conf area	Mindful stretching/yoga followed by brief body scan.	7:30-8:30 chapel	Movement – gentle calesthenics - narrative writing – free association or free drawing What am I learning/ do I want to take with me?
8:15-9:15	Mindful breakfast (silence)	8:30-9:30	Breakfast
9:15-10 chapel	Continue formal practices (guided). Seeing meditation. Participants remain in silence.	9:30-10:30 Conf area	Disc of stress and social support. Support can come from the living or the deceased. Even just a small thing from memory can be powerful (Nazi example) Draw circles of intimacy/ mandala of social support system
10-11	Walking unguided. Mindfulness in motion (3 interviews)	10:30-11:30	Quiet Rest or take a walk (3 interviews)
11-12 chapel	Sit. Awareness Exercises for breaking silence (dyads, etc). Group discussion of silence. If we have not engaged in silence, continue discussion of mindful approaches for self care and responding to stress.	11:30-12:15 Conf area	Discussion of support system /mandalas in dyads and in big group. Brief relaxation focusing on feeling competent and connected, Supported.
12-1:30	Mindful Lunch (optional silence) (3 interviews)	12:15-2:00	Lunch (3 interviews)
1:30-3 Conf area	Continue formal practices. Include Mountain meditation as well as other formal practices already introduced. Mindfulness in everyday life disc. Obstacles to applying what you have learned and how you are going to	2:00-3:30 Chapel	Obstacles to applying who you have learned and how you are going to work with them Guided reflections for the future – where do you imagine yourself 5 years from now? Who is there? What are you doing?

	work with them. Formal practice – body scan. Development of personal ‘action plans’		Discuss in small and large groups
3-3:45	Break / tea (2 interviews)	3:30-4:15	Break / tea (2 interviews)
3:45-5:30 chapel	Practice. group led yoga stretches if appropriate. Discussion of personal plans for continuing practice. Final comments and closure exercises. Dyads and whole group.	4:15-5:30 Conf area	Making a personal plan for your self: creation of daily routine, relaxation, etc. Final comments/closing exercises. Drawing—this group and this time here.
5:30-7:30	Final study procedures (5 interviews)	5:30-7:30	Final study procedures (5 interviews)

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