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ORIGINAL ARTICLE

An Integrated Assessment of Changes in Brain Structure and Function of the Insula Resulting from an Intensive Mindfulness-Based Intervention

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Abstract Mindfulness training is thought to alter both brain function and structure, yet these effects are almost always investigated and reported independently. In the present study, we combine these two approaches to provide a more complete description of the effects of a 6-week mindfulness-based health and wellness intervention. Region-of-interest analysis of brain structure revealed a significant increase in cortical thickness within the left-hemisphere posterior insula, a region that plays a role in auditory perception and interoception. We then examined changes in the resting-state functional connectivity (rs-FC) of this insula cluster and found two regions with which it displayed increased functional connectivity: one in the right-hemisphere ventrolateral prefrontal cortex (vlPFC) and another spanning parts of the left-hemisphere middle and superior temporal gyri (MTG/STG). Individuals with the greatest improvements in dispositional mindfulness showed the greatest increases in insular thickness as well as greater increases in rs-FC of the posterior insula with vlPFC and MTG/STG. The insula, vlPFC, and MTG/STG comprise a structurally connected network involved in, respectively, early auditory perception, the allocation of attention to changes in sounds, and the detailed analysis of fluctuations in sounds. Accordingly, these findings suggest a mechanistic account of the alterations in brain structure and function that underlie changes in auditory processing following a mindfulness-based intervention.

Keywords Mindfulness · Insula · Cortical thickness · Functional connectivity · Ventrolateral prefrontal cortex

Psychological interventions that lead to measurable changes in cognitive abilities or well-being inevitably elicit some corresponding change in the brain. This could involve changes in brain structure, brain function, or both—ranging from the number of dopamine D4 receptors or the BOLD response of a 5-mm^2 cortical region to large-scale changes in white matter tracts or distributed patterns of brain activity across functional networks. It is never possible—technologically or practically—to examine all possibly affected neural mechanisms, and of course, most studies do not attempt to measure any. When randomized trials of psychological interventions do include measures of brain function or structure, it is common to include just one. For instance, in the rapidly growing field of mindfulness research, there is considerable work examining brain morphometry or functional connectivity, but rarely both (Mooneyham, Mrazek, Mrazek, & Schooler 2016; Fox et al. 2014). This is unfortunate because the combination of measures—including both structural and functional measures—holds great promise in providing a richer characterization of the neural mechanisms underlying effective psychological interventions.

The likelihood that mindfulness training programs in particular lead to both structural and functional changes is very high given the existence of so many empirical reports using these methods in isolation. Not only experienced meditators but also individuals receiving brief mindfulness training show changes in the activation and functional connectivity of brain regions, particularly within default, executive, salience, and sensory networks (Brewer et al. 2011; Wells et al. 2013; Kilpatrick et al. 2011; Mrazek, Mooneyham, Mrazek, & Schooler 2016; Farb, Segal, & Anderson 2012; Mooneyham

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et al. 2016). Similarly, both meditation expertise and training are associated with structural changes in distributed brain regions, including the insula, sensory cortices, prefrontal cortex, and hippocampus (Fox et al. 2014; Hölzel et al. 2011; Lazar et al. 2005). The goal of the present research is to combine structural and functional approaches to understand the neural underpinnings of the effects produced by a mindfulness-based health and wellness intervention.

Although there are many definitions of mindfulness, nearly all converge on emphasizing the importance of being fully present with what one is currently doing (Mrazek, Mooneyham, & Schooler 2014). This presence of mind can be directed to the deliberate recollection of the past or anticipation of the future, but a primary goal of cultivating mindfulness is bringing greater attention to the present moment. Indeed, mindfulness training leads people to be less distracted from tasks and more aware of what is happening in the present (Mrazek, Smallwood, & Schooler 2012; Mrazek, Franklin, Phillips, Baird, & Schooler 2013). Furthermore, mindfulness training can lead to greater perceptual discrimination among subtly different visual stimuli as well as greater coherence between self-reported emotional states and physiological measures (MacLean et al. 2010; Sze, Gyurak, Yuan, & Levenson 2010).

Provided that mindfulness training elicits greater attention to ongoing perceptual experiences, brain networks involved in perception may be particularly likely to show training effects. In one study that examined the impact of mindfulness training on resting-state functional connectivity (rs-FC) while participants listened to the sounds of the magnetic resonance imaging (MRI) scanner, training led mainly to altered rs-FC in perceptual networks, including an auditory network comprising primary auditory cortex, superior temporal gyrus, and posterior insula (Kilpatrick et al. 2011). Another study showed changes in posterior insular function and connectivity linked to interoception as a function of mindfulness training (Farb et al. 2012). The posterior insula is a region of particular interest to the present research because of its implication in auditory and interoceptive processing by both structural and functional neuroimaging research (Bamiou, Musiek, & Luxon 2003; Remedios, Logothetis, & Kayser 2009; Flynn 1999) and due to the previously demonstrated effect of mindfulness training on the activation and functional connectivity of this region (Kilpatrick et al. 2011; Farb et al. 2012; Kirk, Gu, Harvey, Fonagy, & Montague 2014). Changes in cortical thickness of the posterior insula may lead to changes in auditory processing, and the posterior insula may interact with other regions implicated in auditory processing, such as the left middle/superior temporal gyrus (MTG/STG) and right ventrolateral prefrontal cortex (vlPFC) in a network involved in the detection of novel auditory information (Petrides & Pandya 2002; Plakke & Romanski 2014; Rauschecker & Scott 2009; Schönwiesner et al. 2007; Buse & Roessner 2016; Kiehl, Laurens, Duty, Forster, & Liddle 2001). Various forms of meditation are associated with activity in the insula as well as somatomotor cortex, the primary cortical site of tactile information processing (Fox et al. 2016). A meta-analysis by Fox et al. (2014) also points to altered somatomotor cortex structure among long-term meditation practitioners compared to controls, and a correlational study of Zen meditators showed a correlation between hours of meditation experience and gray matter in primary somatosensory cortex (Grant, Courtemanche, Duerden, Duncan, & Rainville 2010). However, to our knowledge, no study has demonstrated changes in somatomotor cortex structure as the result of an experimental meditation intervention.

Experimental Overview

This investigation combined structural and functional measures to provide an integrated characterization of the effects of a 6-week mindfulness-based health and wellness intervention. Specifically, we sought to assess whether a 6-week intensive training program could alter cortical thickness. We first examined the effect of the intervention on cortical thickness across the entire cortical surface and then examined two specific regions of interest—the somatomotor and insular cortices—which have shown consistent structural changes as a function of mindfulness training as well as altered functional connectivity patterns following an intervention highly similar to the one reported here (Fox et al. 2014; Mrazek et al. 2016). Since structural changes likely underlie changes in function (Hermundstad et al. 2013), we next examined whether brain regions exhibiting changes in cortical thickness also exhibited changes in their resting-state functional connectivity (rs-FC) with the rest of the brain. Patterns of rs-FC predict individual differences in cognitive abilities and are thought to reflect the repeated history of co-activation of brain regions (Guerra-Carrillo, Mackey, & Bunge 2014). Training-induced changes in rs-FC often correlate with improvements in performance, indicating that rs-FC provides a meaningful window into the brain dynamics underlying improvements that result for psychological interventions. Finally, we assessed the relationship between improvements in mindfulness and the observed neurophysiological changes.

Methods

Participants

Thirty-eight college undergraduates (22 female; mean age = 20.38, SD = 2.28) from the University of California Santa Barbara participated in the research. All 38 participants completed baseline pre-test neuroimaging before the study began, and 37 participants completed a post-test round of neuroimaging. One participant in the waitlist condition withdrew from the study for personal reasons before the post-test and was therefore excluded from analysis. As a result, the intervention group consisted of 19 participants and the waitlist group consisted of 18 participants. All participants competed pre-testing and post-testing during the same period of time. The program was offered cost-free, and participants received financial compensation at the rate of $10/h for the research testing.

Intervention Program

Using a randomized waitlist controlled design, participants engaged in a 6-week multifaceted intervention that emphasized the cultivation of mindfulness. The intervention group participated in the intervention program for the 6 weeks immediately following the first testing session, and the waitlist group participated in the program for the 6 weeks immediately following the post-testing session. The intervention curriculum and structure were modeled after an extant training program that has been previously shown to increase dispositional mindfulness and alter rs-FC patterns of the insula (Mrazek et al. 2016).

The intervention convened for five and a half hours each weekday over a period of 6 weeks. Each day included 60 min of formal mindfulness practice, 150 min of physical exercise, 30 min of structured small group discussion, and 90 min of lecture or discussion on practical strategies for cultivating mindfulness and wellness during daily life. The formal mindfulness practice included a variety of exercises designed to cultivate sustained attention to a subset of sensory experience (e.g., the sensations of breathing, the sounds of music, or the ambient environment). All activities were presented as an opportunity to practice mindfulness. Participants were encouraged to limit alcohol intake to no more than one drink a day, to eat a diet of primarily whole foods, and to consistently sleep at least 8 h each night. With minor exceptions (e.g., temporary illness), all participants attended every session of the intervention.

Magnetic Resonance Imaging Overview and Acquisition

During the pre- and post-testing scanning sessions, participants completed both a structural scan to examine cortical thickness and a resting-state scan to examine functional connectivity. The pre-testing session took place over the 4 days immediately preceding the beginning of the intervention program, and the post-testing session took place over the 4 days immediately following the program. Participants received the following instructions before the resting-state scan: "Please close your eyes. You do not have to think of anything in particular." Participants received clear indication that this resting-state scan was not a mindfulness task.

MRI images were obtained at pre- and post-testing sessions using a Siemens 3.0-T Magnetom TIM TRIO (SYNGO MR B17) MRI scanner. A high-resolution T1-weighted anatomical scan was first acquired for each subject according to FreeSurfer's recommended MPRAGE specifications for cortical thickness analyses (acquisition time = 6:03; repetition time (TR) = 2530 ms; echo time (TE) = 3.50 ms; TI = 1100 ms; flip angle = 7°; field of view (FOV) = 256 mm; acquisition voxel size = 1 × 1 × 1 mm). This was followed by a T2*-weighted echo-planar imaging (EPI) sequence resting-state scan (TR = 1200 ms; TE = 30 ms; flip angle = 90°; acquisition matrix = 64 × 64; FOV = 192 mm; acquisition voxel size = 3 × 3 × 5 mm; 22 interleaved slices; 480 volumes). The resting-state scan lasted for a duration of 9 min and 34 s.

Resting-State fMRI (Echo-Planar Imaging) Data Preprocessing

The first four volumes of each EPI sequence were removed to eliminate potential effects of scanner instability. Slice timing of the EPI images was performed using AFNI's 3dTshift, followed by motion correction of the images using AFNI's 3dvolreg. Affine co-registration of the mean EPI image and T1 volume was then calculated using FreeSurfer's BBRegister. Brain, cerebrospinal fluid (CSF), and white matter masks were extracted after FreeSurfer parcellation and transformed into EPI space using BBRegister. Co-registered EPI images were then masked using the brain mask. Principal components of physiological noise were estimated using CompCor (Behzadi, Restom, Liau, & Liu 2007), where a joined white matter and CSF mask and voxels of highest temporal variance were used to extract two sets of principal components (i.e., aCompCor and tCompCor); motion and intensity outliers in the EPI sequence were also discovered based on intensity and motion parameters using ArtDetect (http://www.nitrc.org/projects/artifact_detect). All time series data were then denoised using a GLM model with the motion parameters, CompCor components, and intensity outliers used as regressors. Finally, resultant images were smoothed using a 5-mm full-width half minimum (FWHM) kernel, highpass (0.01 Hz) and lowpass (0.1 Hz) filters were applied, and nonlinear normalization warping from subject functional/anatomical space to 2-mm Montreal Neurological Institute (MNI) space was computed using Advanced Normalization Tools (ANTs).

Measures

Self-Reported Mindfulness Dispositional mindfulness was assessed using the Mindful Attention and Awareness Scale

(MAAS; Brown & Ryan 2003). This 12-item scale measures attention to what is occurring in one's present experience (e.g., "I find myself preoccupied with the future or the past"; reverse scored). The MAAS was administered at a separate testing session, and the scanning and survey sessions were administered in counterbalanced order. At post-testing, each participant completed the measures in the same order as they did at pre-testing.

Cortical Thickness Cortical reconstruction and volumetric segmentation were performed with the FreeSurfer image analysis suite (Dale, Fischl, & Sereno 1999; Fischl, Sereno, & Dale 1999; Fischl, Sereno, Tootell, & Dale 1999). In brief summary, this processing stream includes the following steps: (1) motion correction of T1-weighted images, (2) removal of nonbrain tissue, (3) automated Talairach transformation, (4) segmentation of the subcortical white matter and deep gray matter structures, (5) intensity normalization, (6) tessellation of the gray matter/white matter boundary, (7) automated topology correction, and (8) surface deformation following intensity gradients to optimally place the gray/white and gray/CSF borders. The resultant individual cortical thickness maps were then registered to a spherical atlas which utilizes individual cortical folding patterns to match cortical geometry across individuals. The maps were then parcellated into units based on gyral and sulcal structure. FreeSurfer's procedures for the measurement of cortical thickness have been successfully validated against both histological analyses (Cardinale et al. 2014; Rosas et al. 2002) and manual measurements (Kuperberg et al. 2003; Salat et al. 2004).

Insula Functional Connectivity As described in the following, the intervention elicited increased cortical thickness in one brain region: the left posterior insula. We sought to explore whether the region exhibiting changes in cortical thickness also exhibited changes in rs-FC across the intervention. The coordinates of peak cortical thickness change were thus transformed into MNI coordinates, and a 4-mm-radius sphere was drawn around these coordinates. The average time course across voxels within this sphere was extracted for each subject and session, and full-brain rs-FC values (Fisher *r*-to-*z* transformed) were calculated using this seed region for each individual scan using AFNI.

Additional Measures At both pre-testing and post-testing, participants also completed a task of sustained interoceptive attention in the scanner during which they focused on the sensations of breathing. This task followed the resting-state scan reported here, and participants were given clear instructions that differentiated the measures. As previously reported, the intervention led to changes in the dynamic functional connectivity of the executive, salience, and default networks that correspond to increased focused attention during the mindful breathing task (Mooneyham et al., in press).

Other measures not pertinent to the present findings were also collected, including a measure of fluid intelligence and prosociality, as well as several other validated scales. These measures will be reported in full in a forthcoming article.

Statistical Approach

Whole-brain analyses employing analysis of variance (ANOVA) on data from randomized controlled trials carry a risk of producing findings that are not driven by the experimental manipulation but rather by a combination of chance baseline differences and chance divergence between conditions over time (Voss et al. 2010). This risk is reduced by using stringent corrections for multiple comparisons, but can be further minimized using a statistical procedure that first detects regions showing significant changes over time in the intervention condition and then subsequently examines these regions at pre-test and post-test across both conditions (Hölzel et al. 2011; Mrazek et al. 2016). This approach was employed for the following cortical thickness and functional connectivity analyses and is described in detail in the following.

Cortical Thickness To assess changes in cortical thickness over time among the participants completing the intervention, we performed two stages of statistical testing. First, we performed a series of paired *t* tests on voxel-wise cortical thickness values of regions across the entire cortical surface. Correction for multiple comparisons across the cortical surface was implemented by testing statistical results against an empirical null distribution of maximum cluster sizes across 10,000 iterations using Z Monte Carlo simulations as implemented in FreeSurfer, with a multiple comparison-corrected cluster-forming threshold set at $p < .05$ (two-sided). Second, we performed a narrower cortical thickness analysis to specifically examine the somatomotor and insular cortices. To do so, we performed separate paired *t* tests on intervention group cortical thickness values within masks corresponding to the left- and right-hemisphere insular and somatomotor cortices using region-of-interest (ROI) definitions provided by FreeSurfer's cortical parcellation atlas (i.e., the Desikan-Killiany Atlas). To produce a somatomotor mask, we combined FreeSurfer's masks of the post-central gyrus, pre-central gyrus, and paracentral lobule into a single mask for each cerebral hemisphere. Correction for multiple comparisons within each masked surface was implemented in the same fashion as in the whole-surface analysis. Mean values for clusters reaching significance in the initial paired *t* test were then extracted for all subjects from both the pre-test and post-test scans. These values were subjected to a mixed-model ANOVA to determine whether there was a statistically significant interaction between condition (intervention vs.

waitlist control) and testing session (pre vs. post; $p < .05$). Finally, for clusters that demonstrated a significant interaction, follow-up t tests were performed to confirm that there was neither a difference in cluster values between the intervention and waitlist-control groups at pre-testing (two-sample t test) nor a difference within the waitlist-control group from pre-testing to post-testing (paired t test). Subsequently reported results passed all of the described criteria for statistical significance.

Resting-State Functional Connectivity Group-level analysis was conducted using the general linear model (GLM) framework implemented in SPM8 (Wellcome Trust Department of Imaging Neuroscience, University College London, UK). Analysis of the rs-FC maps was performed using the same procedure described in the previous section. Whole-brain rs-FC analyses were conducted, correcting for multiple comparisons using topological false discovery rate (FDR) correction (Chumbley, Worsley, Flandin, & Friston 2010). Cluster-forming threshold was set at $p < 0.001$, and cluster size threshold was set at $p < 0.05$ (FDR corrected).

Correspondence Between Imaging and Mindfulness Measures We examined the relationship between our neuroimaging (i.e., cortical thickness and rs-FC) results and self-reported dispositional mindfulness data to provide converging evidence that the neuroimaging findings reflected meaningful changes in brain structure and function. Given the relatively small sample size for a within condition analysis, we employed a rank order analysis that is more robust to deviations from normality and linearity than the Pearson correlation coefficient (Kasper, Elliott, & Giesbrecht 2012; Mooneyham et al. 2017). This analysis iteratively compared pairs of participants within the intervention group to determine whether the person who improved more in dispositional mindfulness also increased more in either cortical thickness or functional connectivity (depending on the specific test being employed). The rank order process was done for all possible pairs of participants to create an average accuracy, and a jackknife method that left out one participant for each cycle of comparisons was used to compute standard error. Rank order accuracies above chance (0.50) indicate a positive relationship between the changes across both variables. Tests of significance were done for each group using a one-sample t test against a null distribution with a mean equal to 0.50.

Results

Dispositional Mindfulness

The intervention placed considerable emphasis on cultivating the ability to focus attention through mindfulness training. Accordingly, we predicted that the intervention would lead to increases in dispositional mindfulness. Prior to training, no significant differences in dispositional mindfulness were observed between conditions, $p = .773$. Repeated measures analysis of variance (rmANOVA) revealed a significant condition by session interaction. As reported in Mooneyham et al. (2016), relative to the waitlist control condition which did not change over the 6 weeks, the intervention elicited substantial increases in dispositional mindfulness, $F(1,35) = 16.363$, $p < 0.001$.

Cortical Thickness

The whole-surface analysis of change in cortical thickness changes—which used strict correction for multiple comparisons—did not reveal any regions demonstrating a significant change in thickness. However, our a priori ROI analyses did reveal a site of significant change. While no changes were observed within the somatomotor cortices in either hemisphere, a significant increase in cortical thickness was detected in a region within the left-hemisphere posterior insula, $t(18) = 3.78$, $p < .01$. Given this result, we extracted mean cortical thickness values from this surface cluster across all participants (both groups) and sessions. Follow-up analyses revealed the following: (i) a significant group × session interaction effect within a mixed-model ANOVA ($F(1,35) = 11.49$, $p < .01$), (ii) no pre-existing differences in cortical thickness between groups at pre-testing ($t(18) = -0.52$, $p > .05$), and (iii) no changes across sessions within this region among the waitlist-control group ($t(17) = 1.32$, $p > .05$). These follow-up analyses suggest that the increase in insular cortical thickness was caused by the intervention training. Figure 1 displays the location of this insular region and the average cortical thickness values for each group at each session. In support of a significant relationship between the morphometric effects of the intervention and the improvements in mindfulness, pre-test to post-test increases in insular cortical thickness predicted increases in dispositional mindfulness using rank order analysis, $t(18) = 4.47$, $M = 0.53$, $SEM = 0.02$, and $p < .001$.

Insula Functional Connectivity

Knowing the intervention elicited increased cortical thickness within left-hemisphere posterior insula, we next examined the rs-FC of this region. To assess functional changes of the posterior insula cluster, we conducted a seed-based rs-FC analysis using this insula region as the seed (MNI −35, −18, 8). This series of analyses revealed that the intervention elicited increased rs-FC between left posterior insula and two other regions: one residing in right-hemisphere ventrolateral prefrontal cortex (vlPFC; peak-MNI: $t(18) = 5.11$, $p = .041$, FDR-corrected; Fig. 2) and the other spanning the left-hemisphere middle and superior temporal gyri (MTG/STG; peak-MNI:

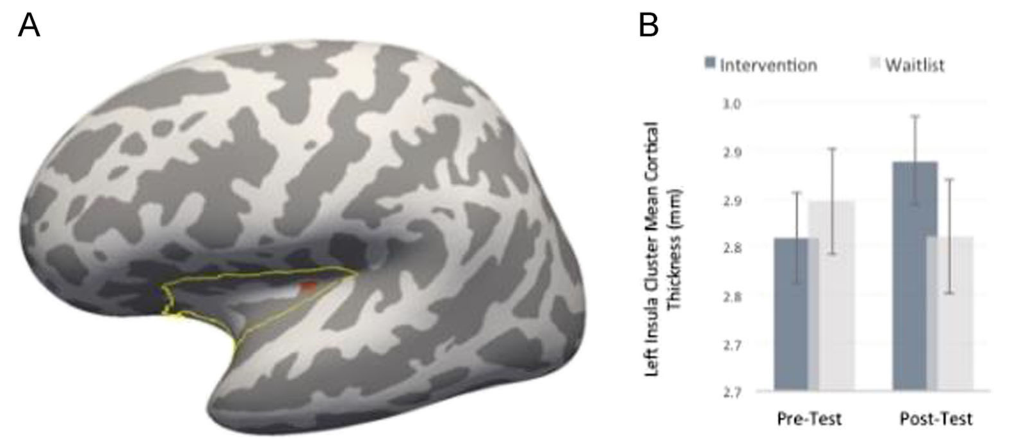

Fig. 1 Cortical thickness effects within the left insula. **a** The location of the left-hemisphere regions demonstrating increased cortical thickness in the intervention group across sessions, displayed on the left-hemisphere inflated surface. The cluster of thickness increase is displayed in *orange*; the left insula mask used in the analysis is outlined in *yellow*. **b** Mean cortical thickness values within the left insula cluster for both groups at each session. *Error bars* represent 95% confidence intervals (Color figure online)

$t(18) = 5.55$, $p = .041$, FDR-corrected; Fig. 3). Pre- to post-testing increases in rs-FC between the insula and the right ventrolateral prefrontal cluster predicted increases in dispositional mindfulness using rank order analysis, $t(18) = 1.90$, $M = 0.51$, $SEM = 0.02$, and $p = .037$; this relationship was also observed for the left temporal cluster, $t(18) = 3.53$, $M = 0.52$, $SEM = 0.02$, and $p = .001$.

Discussion

Investigations into the effects of psychological interventions most often examine influences on brain function and structure separately, but the combination of these approaches can provide a richer characterization of changes within the brain. In the present study, we observed that an intensive 6-week health and wellness intervention emphasizing the cultivation of mindfulness led to both structural and functional changes in the posterior insula. Specifically, we observed an increase in cortical thickness in the left-hemisphere posterior insular cortex—a region associated with auditory perception and interoception (Bamiou et al. 2003; Craig 2011; Flynn 1999; Farb et al. 2012; Kirk et al. 2014). While the participants rested in the scanner, this specific cluster within posterior insula also showed increased rs-FC from pre-test to post-test with vlPFC and MTG/STG—regions also implicated in

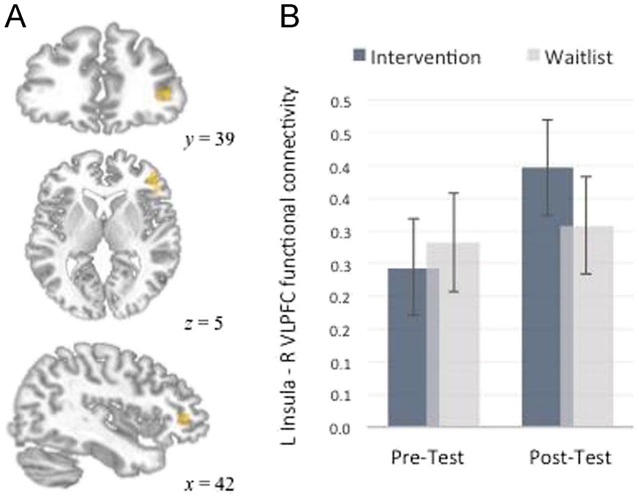

Fig. 2 Increased functional connectivity between posterior insula and ventrolateral prefrontal cortex. **a** The location of the right-hemisphere ventrolateral prefrontal cluster; MNI peak coordinate: (56, 30, 2). **b** Mean Fisher *r*-to-*z* transformed functional connectivity values for the intervention and waitlist groups at pre- and post-testing. *Error bars* represent 95% confidence intervals. $N = 37$

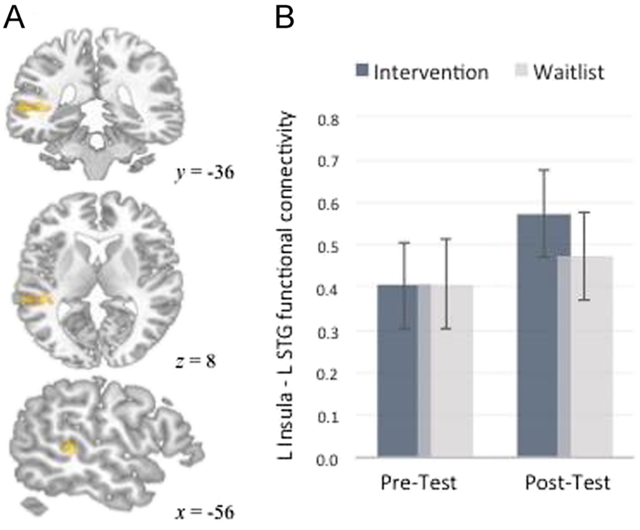

Fig. 3 Increased functional connectivity between posterior insula and middle/superior temporal (MTG/STG) gyrus. **a** The location of the left-hemisphere middle/superior temporal (MTG/STG) cluster; MNI peak coordinate: (−62, −34, 8). Cluster-forming threshold set at $p < 0.001$ and cluster size threshold set a $p < .05$ (FDR corrected). **b** Mean Fisher *r*-to-*z* transformed functional connectivity values for the intervention and waitlist groups at pre- and post-testing. *MTG* middle temporal gyrus. *Error bars* represent 95% confidence intervals. $N = 37$

auditory processing (Mothes-Lasch, Becker, Miltner, & Straube 2016; Schönwiesner et al. 2007; Vossel, Weidner, & Fink 2011). These changes in functional connectivity were greatest for those who showed the largest increases in dispositional mindfulness. Cumulatively, these findings represent a plausible account of neural changes underlying changes in auditory perception after mindfulness training.

Cortical Thickness Changes in the Posterior Insula

The intervention elicited an increase in cortical thickness within a region of the left-hemisphere posterior insular cortex. We predicted both functional and structural changes in the insula based on extensive previous research implicating the insula in the immediate and long-term effects of meditation practice. For instance, correlational studies have shown that meditation experience is associated with insular cortical thickness, gray matter density, and gyrification (Lazar et al. 2005; Hölzel et al. 2008; Luders et al. 2012; but see Hölzel et al. 2011). Yet the insula is not monolithic, and existing work has found morphometric changes in different subregions of the insula. Some research has found increased gray matter density localized in the right anterior insula—a key hub within the *salience network*, a collection of brain regions involved in the detection and evaluation of motivationally relevant stimuli (Hölzel et al. 2008; Seeley et al. 2007). By contrast, the present investigation observed increased cortical thickness within the left posterior insula—a region implicated in auditory processing and interoception (Bamiou et al. 2003; Remedios et al. 2009; Flynn 1999; Farb et al. 2012).

Several lines of evidence suggest that the posterior insula plays a role in relatively early auditory processing. First, the posterior insula is densely connected to both the thalamus and auditory cortex (Jones & Burton 1976; Mesulam & Mufson 1985; Keifer, Gutman, Hecht, Keilholz, & Ressler 2015). Second, the latency of posterior insula responses to auditory evoked potentials is consistent with direct connections from the medial geniculate nucleus of the thalamus, suggesting that the posterior insula receives auditory information in the early stages of sensory processing (Sudakov, McLean, Reeves, & Marino 1971; Guldin & Markowitsch 1984; Keifer et al. 2015). Third, cell staining has revealed that the cytoarchitecture of the posterior insula is consistent with that of primary sensory areas (Rivier & Clarke 1997).

The posterior insula is also implicated in interoception (Flynn 1999), and it sends structural and functional projections to anterior portions of the insula that have more integrative sensory functions. Interoceptive and auditory information represented in the posterior insula is hierarchically re-represented in the mid-insula and ultimately the anterior insula as it is progressively integrated with sensory, social, and emotional information (Craig 2011). The insula is consistently activated by various forms of meditation (Fox et al. 2016), and mindfulness training has been shown to elicit changes in posterior insula function (Farb et al. 2007; Burton 2009). Farb et al. (2012) demonstrated that daily compliance with an 8-week Mindfulness-Based Stress Reduction (MBSR) program (Kabat-Zinn 1990) was associated with increased posterior insula activation during an interoceptive attention task. Farb et al. (2012) also observed that MBSR training led to enhanced functional coupling between posterior insula and more anterior insular regions during an external attention task, whereas their waitlist control group only showed this pattern of connectivity during the interoceptive attention task. Consistent with our finding that mindfulness training led to changes in posterior insular thickness, this suggests that extended mindfulness training may lead to broad enhancements in interoceptive attention, extending beyond tasks that require the explicit direction of attention to internal sensations.

The increase in cortical thickness within the posterior insula may therefore represent an increased functional capacity for early-stage processing of auditory stimuli and interoception. This interpretation is generally consistent with the observed correlation between increased cortical thickness in the left posterior insula and improved dispositional mindfulness—a measure indexing attention to moment-to-moment sensations. It is also consistent with the intervention's emphasis on mindfulness training and the inclusion of specific exercises involving sustained attention to ambient sounds or music. In light of the posterior-to-anterior processing pathway within the insula, it is possible that the training effect on dispositional mindfulness was mediated by greater posterior insular sensory processing, which would in turn increase signaling to the anterior insula and modulate the allocation of attention to sensation. However, interpreting the significance of a structural change within the brain is challenging. The posterior insula has also been implicated in cardiorespiratory regulation (Oppenheimer, Gelb, Girvin, & Hachinski 1992). Lacking insight into to the function of the specific posterior insula cluster identified in this investigation, any conclusions about the functional significance of the increased cortical thickness remain highly speculative. Fortunately, characterizing the nature of a structural change within the brain is much easier when considered in context of that region's functional connectivity changes.

Resting-State Functional Connectivity Changes in the Posterior Insula

To explore the functional significance of the increase in cortical thickness within the posterior insula, we examined training-induced changes in the rs-FC of this region. The posterior insula increased in rs-FC with both the left MTG/STG and right vlPFC. These structurally connected regions have been repeatedly implicated in auditory processing and are thought to comprise a network involved in the detection of novel auditory information (Petrides & Pandya 2002; Plakke & Romanski 2014; Rauschecker & Scott 2009; Schönwiesner et al. 2007; Buse & Roessner 2016; Kiehl et al. 2001).

Specifically, the STG is involved in the detailed analysis of fluctuations in auditory input signals, including the filtering of attended sounds from background noise (Mothes-Lasch et al. 2016). The middle temporal gyrus has been implicated in semantic processing, likely bridging the auditory representations in the STG to conceptual information distributed throughout the cortex (Hickok & Poeppel 2004; Visser, Jefferies, Embleton, & Lambon Ralph 2012). The right vlPFC is involved in phonological processing and allocating attention toward acoustic changes (Burton 2009; Schönwiesner et al. 2007).

As described above, the posterior insula plays an early role in auditory processing. Participants had received clear indication that the resting-state scan was not a mindfulness task, so the increased rs-FC of regions involved in auditory processing suggests a shift in participants' default mental state toward greater monitoring of moment-by-moment fluctuations in ambient sounds. Taken together, the observed increases in rs-FC between a region involved in early auditory processing (the posterior insula) and regions involved in further analysis and monitoring of acoustic and multimodal semantic information (MTG/STG and vlPFC) suggest a mechanistic account of increases in present-moment sensory awareness.

Limitations and Future Directions

The present results provide evidence of structural changes within the posterior insula as a result of the mindfulness-based health and wellness intervention. However, the precise nature of these effects remains unknown because increased cortical thickness may reflect several possible anatomical features, including a greater number of dendrites per neuron, increased volume of glial cells, or increased regional vasculature. While it is not possible using most methods (including ours) to distinguish between these possible contributions to cortical thickness, each of these mechanisms is supportive of increased neural function. Advances in methods and continued research will be necessary to discern the precise underlying anatomical nature of structural changes elicited by psychological interventions.

The present findings suggest that the mindfulness-based intervention strengthened the functional relationships between the insula and other brain regions involved in the processing of auditory input. The available evidence suggests that the insula may be producing downstream effects on the processing of auditory information within the more specialized regions of the vlPFC and MTG/STG. However, this conclusion cannot be confirmed by the present analyses. Because the present study did not include any behavioral tests of auditory perception, we cannot directly relate the observed neural effects to measurable perceptual changes. Effective connectivity analyses designed to detect causal effects from the temporal relationships between the activation patterns of various brain regions may also be informative for future research that addresses the question of how the insula modulates auditory processing.

Many psychological interventions and nearly all mindfulness training programs involve a variety of training elements, making it impossible to precisely determine which element was responsible for observed changes (Mooneyham et al. 2016). The present intervention is also limited in its ability to pinpoint a single element of the intervention that is responsible for changes in brain structure and function. Although (i) the curriculum strongly emphasized mindfulness, (ii) results revealed dramatic increases in dispositional mindfulness, and (iii) changes in brain structure and function were correlated with changes in mindfulness, we cannot definitively conclude that it was the mindfulness training alone that was responsible for the reported findings. Although isolating the effect of a targeted manipulation has indisputable value in establishing causality, this approach inherently leads to the study of variables in relative isolation. Given that most phenomena are the result of many interacting causes, there is a risk of neglecting how multiple influences combine to have greater effects than when they are studied in isolation. Accordingly, the present study aimed to evaluate how a highly effective multifaceted health and wellness program that strongly emphasized mindfulness would—as a whole—change brain function and structure.

Conclusions

Perhaps as much as any other region of the brain, mindfulness-based interventions appear to influence both the structure and function of the insula. The present findings contribute to this growing evidence base, though they are unique in their combination of structural and functional neuroimaging methods as well as their identification of increased rs-FC within a brain network implicated in relatively early auditory processing. As a result of our intervention, the left-hemisphere posterior insula increased in both cortical thickness and rs-FC with regions in the vlPFC and MTG/STG. These increases in cortical thickness and rs-FC were correlated with improvements in dispositional mindfulness, suggesting that these findings represent a plausible mechanistic account of the brain dynamics underlying changes in auditory perception after mindfulness training. These findings also demonstrate the promise of combining structural and functional neuroimaging methods to better characterize the effects of psychological interventions. This combined approach—which will be further bolstered as advances in both structural and functional methods develop in parallel—is ultimately necessary for a full understanding of how the mind and brain improve through training.

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